Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*)

II. Energy expenditure

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Summary

Many large mammals show pronounced seasonal fluctuations of metabolic rate (MR). It has been argued, based on studies in ruminants, that this variation merely results from different levels of locomotor activity (LA), and heat increment of feeding (HI). However, a recent study in red deer (*Cervus elaphus*) identified a previously unknown mechanism in ungulates – nocturnal hypometabolism – that contributed significantly to reduced energy expenditure, mainly during late winter. The relative contribution of these different mechanisms to seasonal adjustments of MR is still unknown, however. Therefore, in the study presented here we quantified for the first time the independent contribution of thermoregulation, LA and HI to heart rate (f_H) as a measure of MR in a free-roaming large ungulate, the Przewalski horse or Takhi (*Equus ferus przewalskii* Poljakow).

f_H varied periodically throughout the year with a twofold increase from a mean of 44 beats min^{-1} during December and January to a spring peak of 89 beats min^{-1} at the beginning of May. LA increased from 23% per day during December and January to a mean level of 53% per day during May, and declined again thereafter. Daily mean subcutaneous body temperature (T_s) declined continuously during winter and reached a nadir at the beginning of April (annual range was 5.8°C), well after the annual low of air temperature and LA.

Lower T_s during winter contributed considerably to the reduction in f_H. In addition to thermoregulation, f_H was affected by reproduction, LA, HI and unexplained seasonal variation, presumably reflecting to some degree changes in organ mass. The observed phase relations of seasonal changes indicate that energy expenditure was not a consequence of energy uptake but is under endogenous control, preparing the organism well in advance of seasonal energetic demands.

Key words: hypometabolism, hypothermia, metabolic rate, winter adaptation, body temperature regulation.

Introduction

Warm-blooded herbivores living in habitats with harsh wintering conditions face a twofold problem. The energy requirements for maintaining a high body temperature at low ambient temperatures in winter are considerably higher, and yet the availability and quality of plant forage is substantially decreased. An efficient strategy used by small mammals to cope with this twofold challenge is daily torpor or hibernation, i.e. the reduction of endogenous heat production (Geiser and Ruf, 1995; Hiebert, 1990; Kayser, 1964; Ruf and Heldmaier, 1992). However, to abandon a high body temperature bears an enormous cost – the impairment or even complete loss of manoeuvrability. Small and medium-sized mammals solve this problem by seeking shelter in burrows, crevices or caves before entering the torpid state. Large mammals typically lack such

reactions, except for bears. Therefore, one would expect that hypometabolism as a strategy to withstand severe food shortage and low ambient temperature is absent in non-burrowing large mammals. Instead, these species might cope with harsh wintering conditions solely by improving body insulation, by reducing locomotor activity (LA), and by using body energy reserves to fuel metabolism. In other words, an over-wintering strategy without a reduction in basal metabolic rate (BMR) seems feasible because of the low conductance and the proportionally large amount of fat reserves that can be stored in a large body.

Nevertheless, pronounced seasonal fluctuations in metabolic rate (MR) are known to occur in ruminants with a winter nadir in northern species (Arnold et al., 2004; Nilssen et al., 1984a; Renecker and Hudson, 1985) and with a summer nadir in desert

species (Ostrowski et al., 2006; Williams et al., 2001). It has been argued that this seasonal variation in MR does not reflect a change in BMR but results from different levels of LA or of the heat increment of feeding (HI) (Mautz et al., 1992; Mesteig et al., 2000; Jiang and Hudson, 1993; Worden and Pekins, 1995). However, a recent study of red deer (*Cervus elaphus*) identified a previously unknown mechanism in ungulates – nocturnal hypometabolism – that contributed significantly to reduced energy expenditure, mainly during late winter (Arnold et al., 2004).

It is unknown whether this type of hypometabolism is also used by other large ungulates. Furthermore, it is unclear as to what degree these different mechanisms determine MR; up until now, the crucial physiological and behavioural parameters have never been measured simultaneously over a sufficient period of time. In this study, we quantified for the first time the independent contribution of thermoregulation, LA and HI to MR in a free-roaming large ungulate, the Przewalski horse or Takhi (*Equus ferus przewalskii* Poljakow). As an indicator for field MR that can be measured over long periods of time, we recorded heart rate (f_H) (Butler et al., 2004). Like northern ruminants, Przewalski horses reduce voluntary food intake during winter, suggesting that energy expenditure is also decreased (Kuntz et al., 2006). We hypothesized that the same reactions that decrease winter MR of ruminants also exist in Przewalski horses and enable this species to cope with severe energetic bottlenecks.

The Przewalski horse is a typical steppe herbivore (Van Dierendonck and Wallis de Vries, 1996) and its original distribution probably covered the whole Eurasian steppe belt. However, in the 1960s the species became extirpated in the wild (Boyd and Houpt, 1994). Przewalski horses have been successfully saved from total extinction by breeding in captivity, and since the middle of the 1990s they have been reintroduced into their natural habitat in Mongolia. The species' recent natural refuge, the Dzungarian Gobi, is a desert or semi-desert with a continental climate. Rain falls mostly during the summer months, but mean rainfall amounts to not more than 100 mm per year, and temperatures are extreme with a maximum of 40°C in summer and a minimum of –40°C in winter (Volf, 1996). The reintroduction of an almost extinct species into such an extraordinary seasonal environment requires in-depth knowledge of its physiological capabilities, seasonal adjustments and factors governing these changes. Only this knowledge allows us to identify suitable habitats or the reasons for fluctuations in the mortality or reproductive success of released horses. Hence, the results of our study will not only help to better understand over-wintering strategies of large ungulates but also to develop the most appropriate strategy for a successful reintroduction of the Takhi.

Materials and methods
Study site and animals

The study was performed between May 2002 and November 2003 in an approximately 3 km^2 enclosure in the Nationalpark Neusiedlersee-Seewinkel in Austria (47°45′N, 16°48′E). At the northern end of the completely open, unwooded area there was an artificial water supply and an open shelter (5 m✕9 m) to provide shade during the typically hot and dry summer. Physiological and behavioural measurements were performed on seven Przewalski horses (four adult and two 1–2-year-old mares, and one adult stallion) feeding exclusively on natural vegetation. Air temperatures and wind speed (km h^{-1}) were recorded every 15 min at a weather station located approximately 500 m south of the enclosure. For details on the study site and herd, see Kuntz et al. (Kuntz et al., 2006).

Telemetric measurements

For remote measurements of physiological parameters and LA, a self-constructed telemetry system was used. The system consisted of an implanted transmitter and a receiver and storage unit located in a collar. The microprocessor-controlled transmitter (50 g, 65✕35✕11 mm) was encapsulated in physiologically inert medical-grade silicone rubber and surgically implanted under the skin in the ventro-lateral neck region. f_H was extracted from the QRS complexes of the electrocardiogram (ECG) detected by two subcutaneously implanted electrode plates (surgical steel, 8 mm diameter). Electrodes were subcutaneously connected to the transmitter with a coiled silicone rubber-insulated wire of multi-stranded stainless steel fitted in silicone rubber tubing to form an elastic and flexible lead. Subcutaneous temperature (T_s) was recorded in the implant by a thermistor. Prior to surgical implantation of the device, the temperature dependence of the sensor output was calibrated in a waterbed between 20°C and 40°C at 5°C intervals. Recalibration after explantation of the transmitters yielded virtually identical results (drift over the measurement period <0.1°C).

Data were collected at two-minute intervals. During each of these measurement periods f_H was determined for 60 s, T_s once. Both parameters were transmitted at 100 KHz *via* a short-range telemetry data link to the receiver in the collar. Resolution of T_s measurements was 0.1°C, and resolution of the f_H measurements was 1 beat min^{-1}. Furthermore, movements of experimental animals were detected by a vibration sensor that was located in the collar and which measured accelerations in three axes. The proportion of time with LA during a two-minute measurement cycle was defined as the proportion of seconds with sensor activation, counted by a built-in microcontroller. All measured values were stored in the collar and retrieved at the end of the experiment.

Anaesthesia and surgery

Przewalski horses were immobilized by intramuscular injection of a mixture of 2.45 mg etorphine and 10 mg azepromazine (1 ml Immobilon®; Novartis, Herts, UK), plus 10 mg butorphanol (1 ml Butomidor®; Richter Pharma AG, Wels, Austria), plus 10 mg detomidine (1 ml Domosedan®; Pfizer Co., Vienna, Austria) per adult animal with dart projectors. Immobilized animals were carried to the open shelter where a field surgical facility had been established.
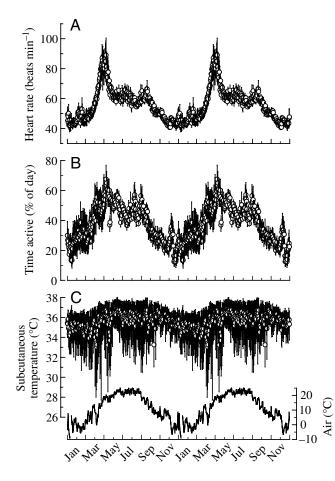
Fig. 1. Seasonal changes in physiological parameters and behaviour (daily means; measures of variation reflect differences between individuals). Data are double plotted to ease visualization of the annual pattern. Labels on the x-axis indicate the first month of a two-month interval. Only complete data sets, i.e. those with information about all parameters are plotted. (A) Daily means of heart rate (means ± s.e.m.). (B) Daily means of locomotor activity (means ± s.e.m.). (C) Top: daily means and range of subcutaneous temperature; bottom: daily means of air temperature.

After fixation with ropes on an inflatable surgery bed the animals were intubated. Anaesthesia was maintained with a mixture of 1000 mg ketamin, 15 mg midazolam and 1 mg detomidin in 500 ml NaCl, administered intravenously, at a dosage of 1.2 ml h^{-1} and kg body mass. Body mass was estimated or measured to the closest kg using a portable balance.

Surgery was performed by a veterinarian, an anaesthetist and two assistants. A ventral midline neck incision was made through the skin. Subcutaneous tissue was separated by blunt dissection to form a pouch on the left-ventro-lateral aspect of the neck. Two further incisions were made through the skin along the caudal ventral midline of the pouch for the ECG electrodes. Electrodes were subcutaneously pulled from the transmitter pouch through a subcutaneous tunnel prepared with a sterile catheter. The transmitter and electrodes were fixed to

the subcutis and the skin incisions were closed with sutures of absorbable material (Arnold et al., 2004). For further details regarding surgical procedures and veterinary care, see Giacometti et al. (Giacometti et al., 2001). Owing to technical problems, it was necessary to immobilize animals and to remove the transmitter in one case and to replace collar and transmitter in another case. All procedures performed conformed to the current laws regulating animal welfare and experiments with animals in Austria (GZ 68.205/9-Pr/4/2002; GZ 68.205/68-BrGT/2003).

Estimating the heat increment of feeding

From July 2002 until January 2004 information on the daily dry matter intake (DMI) and its nutrient content was available for approximately every other month for some of the study horses (Kuntz et al., 2006). Furthermore, information regarding the annual course of body condition was available from a visually obtained body-score index that correlated significantly with body mass (Kuntz et al., 2006). HI was estimated from these data to explore the energetic consequences of seasonal variation in DMI and fattening. The period from April to October, with a trend of increasing mean body condition, was considered as a time of above-maintenance metabolism, and the period from November to March with decreasing mean body condition as a time of below-maintenance metabolism. For each DMI measurement (kg), we assumed a digestibility of 50% [table 3.5 in Blaxter (Blaxter, 1989)] and estimated HI (MJ) from the proportions of various nutrients, their gross energy content and the proportions of metabolizable energy converted to HI by non-ruminant herbivores [table 12.1 in Blaxter (Blaxter, 1989)]:

$$
\begin{aligned}
\text{HI}_{\text{(below maintenance)}} = \\
\text{DMI} \times 0.5 \times [(\% \text{ crude protein} \times 23.8 \times 0.24) \\
+ (\text{crude fat} \times 39.6 \times 0.02) \\
+ (\text{crude fibre} \times 17.4 \times 0.1) \\
+ (\text{nitrogen-free extract} \times 17.4 \times 0.1)] ,
\end{aligned} \tag{1}
$$

and

$$
\begin{aligned}
\text{HI}_{\text{(above maintenance)}} = \\
\text{DMI} \times 0.5 \times [(\text{crude protein} \times 23.8 \times 0.5) \\
+ (\text{crude fat} \times 39.6 \times 0.21) \\
+ (\text{crude fibre} \times 17.4 \times 0.36) \\
+ (\text{nitrogen-free extract} \times 17.4 \times 0.36)] .
\end{aligned} \tag{2}
$$

For 17 estimates of HI, daily means of f_{H}, T_{s} and LA of the respective horses were available for exactly the same day. For 5 further estimates of HI, daily mean f_{H}, T_{s} and LA were measured close enough to justify the inclusion of these data in the analysis (≤12 days apart; mean=6, s.e.m.=1.7).

Data analyses

Telemetrically obtained raw data contained obviously false values because of electronic noise during the transmission. We therefore removed all values outside of the physiological range for horses, i.e. subcutaneous temperatures of 0–42°C and f_{H}s of 10–250 beats min^{-1} from the database. T_{s} remained slightly elevated for 8–14 days after transmitter implantation. These
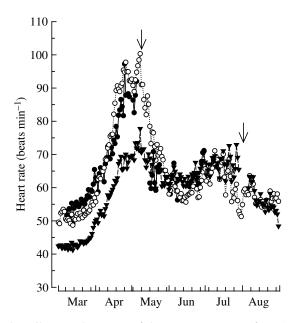
Fig. 2. Daily mean heart rate of three pregnant mares from 1 March 2003 to 31 August 2003. Different symbols represent different individuals, arrows represent the time of parturitions.
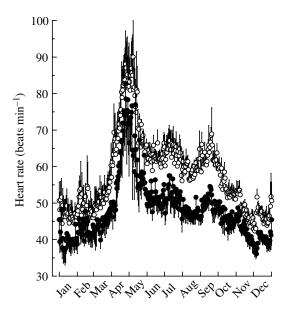

Fig. 3. Daily mean heart rate during locomotor activity (simultaneously measured locomotor activity >10%; open circles, means + s.e.m.) and at rest (locomotor activity ≲10%; closed circles, means – s.e.m.).

days were discarded from statistical analyses. For calculating daily means of f_H, LA and T_s, only days with at least 60 datasets (i.e. two sampling hours) within each quartile of a day were used to obtain the means of these parameters that were unbiased by circadian changes. Altogether, 29 046 h of telemetric measurements remained for final analyses, representing 29 days of measurement from the stallion, 224, 251, 289 and 322 days from the four adult mares, and 33 and 253 days from the two young mares, respectively.

To compare f_H at rest with f_H during LA, we categorized LA according to simultaneous observations of the animals' behaviour (Kuntz et al., 2006). LA values ≲10% represented two-minute intervals of continuous lying or standing motionless and were categorized as intervals spent at rest. Intervals with LA >10% were categorized as intervals with LA.

Statistics

Statistical tests were performed using S-Plus 6.2 for Windows (Insightful Corporation, Seattle, WA, USA). Linear mixed-effect models with the random effect 'individual' were used to analyse data repeatedly sampled from the same individuals (Pinheiro and Bates, 2000). To compare regression models, we used Akaike's Information Criterion (AIC) (Akaike, 1973), which is proportional to the residual sum of squares penalized by the number of parameters in the model.

Significance of seasonal variation was tested by entering a sine (*t*) and cosine (*t*) term into the linear model, with *t* representing the day of the year in radians. Sums of squares and the degrees of freedom of these terms were added to obtain a single *F*- and *P*-value for the periodic function. Coefficients of the sine and cosine term were then used to algebraically compute the amplitude (*A*) and phase (ɸ) of the periodic function. Phase differences between two periodic fits were tested for significance by determining ɸ for the first fit and recalculating both models with sine (*t*–ɸ) and cosine (*t*–ɸ). This transformation of the *x*-axis results in a parameter estimate of 0 for either sin (*t*–ɸ) or cos (*t*–ɸ) for the first model. Both periodic fits have a significantly different ɸ if the term with a parameter estimate of 0 in the first model significantly deviates from zero in the second model.

Results

Heart rate

Mean daily f_H varied considerably throughout the year (Fig. 1A). f_H increased exponentially during March and April to the annual peak in May, remained, after a rapid decay, on a medium level from June to September, and declined during autumn to reach a mean of 44 beats min^{-1} during December and January. The total annual change was approximately twofold, with a maximum of 89 beats min^{-1} at the beginning of May.

Reproducing mares had significantly higher f_Hs during late gestation and early lactation (Table 1A). A pronounced peak of f_H occurred in spring, coinciding with the time of parturition by two of the study mares (9 May 2003 and 10 May 2003, Fig. 2). However, during the same time period a peak in f_H was observed in the mare that delivered only on 4 August 2003, although at a much lower level. In contrast to the situation in spring, the f_H of this mare was only slightly higher when foaling in early August compared with the two mares nursing foals now already three months old (Fig. 2).

Table 1. *Factors associated with seasonal variation of daily mean heart rate (coefficients and test statistics from repeated-measurement multiple-regression analysis; the response-variable daily mean heart rate was inversely transformed to achieve normal distribution of residuals and multiplied by –1 to maintain the directions of predictor effects)*

Fixed effect	(A) Regression model without heat increment of feeding					(B) Regression model including heat increment of feeding				
	Coefficient ($\times 10^{-4}$)	s.e.m. ($\times 10^{-4}$)	Standardized coefficient[c]	$F_{1,1293}$	P-value	Coefficient ($\times 10^{-4}$)	s.e.m. ($\times 10^{-4}$)	Standardized coefficient[c]	$F_{1,10}$	P-value
Reproduction[a]	19.574	1.621		145.7	<0.001	1.091	8.308		0.40	0.898
Daily mean locomotor activity	0.639	0.056	0.276	130.3	<0.001	0.513	0.331	–0.193	2.4	0.152
Daily mean subcutaneous temperature	7.356	0.818	0.184	80.8	<0.001	19.176	5.823	2.221	10.8	0.008
Daily mean air temperature	–0.951	0.162	–0.179	34.7	<0.001	–4.349	2.124	–0.056	4.2	0.068
Daily mean wind speed	0.260	0.079	0.003	10.9	0.001	–0.928	1.073	–0.230	0.7	0.407
Heat increment of feeding						1.630	0.659	0.037	6.1	0.033
Amplitude of unexplained seasonal effect [cos(Julian day/365$\times$2π–ɸ)][b]				301.9	<0.001				12.0	0.002

[a]The time period of the last two months of gestation and the first month of lactation was entered into the regression model as factor 'reproduction'. This time period is known to be the most energetically challenging for the reproducing mare (Boyd and Houpt, 1994; National Research Council, 1989).

[b]No common coefficient computable for this fit of phase and amplitude of a periodic function; d.f., 2,1293 (A) and d.f., 2,10 (B), respectively, for the tested combined term.

[c]Computed for numeric predictors only.

Locomotor activity

Daily mean LA also varied considerably throughout the year, with a more than twofold change (Fig. 1B). LA increased from a mean of 23% per day during December and January to a mean level of 53% per day during May and declined again thereafter. The annual pattern of LA closely resembled seasonal variation of f_H, and LA had, after the factor 'reproduction', the second-strongest influence on daily mean f_H (Table 1A). Nevertheless, LA only explained the profound seasonal change in f_H to some degree. A clear seasonality was also evident in f_H measured in horses at rest. Mean f_H at rest was 15.6% lower (s.e.m.=0.25) than during LA, but showed a similar seasonal change (Fig. 3).

Body temperature

Daily mean T_s increased rapidly during April and May from the late-winter low and remained high and roughly constant from June to October with a mean of 36.2°C (s.e.m.=0.03). The annual range of variation in daily means was 5.8°C. From early November onwards, T_s decreased continuously to reach the annual nadir at the beginning of April, long after the annual low of daily mean air temperature (T_a) and LA (Fig. 1C). Nevertheless, the overall correlation between T_s and T_a was relatively high ($r=0.55$, $F_{1,1303}=458.6$, $P<0.001$). In contrast, the correlation of the daily minimum of T_s (T_{min}) with T_a was considerably weaker ($r=0.13$, $F_{1,1303}=6.6$, $P=0.010$). T_{min} was lower from June to October when the horses had a summer fur. However, T_{min} was more variable than daily mean and maximum T_s throughout the year and was distributed with a strong skew towards lower temperatures (the median was 34°C; interquartile range was from 32.7°C to 34.9°C). This skew arose from bouts of particularly low T_s (<32°C) lasting for up to 570 min. T_{min} during these bouts reached values as low as

24.4°C. Bouts with $T_s<32$°C usually started in the early morning hours (median onset time was 04.42, s.e.m.=0.015) and principally occurred at any time of the year. However, in August these bouts lasted for a mean of 91 min (s.e.m.=18.6, $N=29$) but for a mean of 181 min during April (s.e.m.=25.9, $N=32$).

Altogether, the energetic consequences of thermoregulation and weather conditions contributed significantly to the seasonal changes in f_H (Table 1A). Daily mean f_H decreased with daily mean T_s with an effect size comparable to that of LA. The independent effect of T_a on f_H was relatively small and negative, indicating a slightly increasing energetic cost of defending a high body temperature at lower T_a. Similarly, f_H increased slightly with daily mean wind speed (Table 1A).

Previous studies of thermoregulatory reactions to cold exposure of winter-acclimatized domestic horses found a zone of thermoneutrality between 10°C and –15°C and an increase in MR to 142% of BMR during periods of acute cold exposure (up to –40°C) (McBride et al., 1985). In contrast to this result, we found the same correlations as reported in Table 1A when calculating the regression model for T_a only in the range –15°C to +10°C.

Heat increment of feeding

Although all of the above mentioned energetically relevant factors contributed independently and significantly to the seasonal variation of f_H, much of this variation remained unexplained. A periodic term added to the multiple regression model further improved the fit (AIC decrease=300, $P<0.001$) and turned out to be the most important predictor (Table 1A). We hypothesized that this unexplained periodic variation of f_H was because of a seasonally changing HI and tested this hypotheses with data from Kuntz et al. (Kuntz et al., 2006).
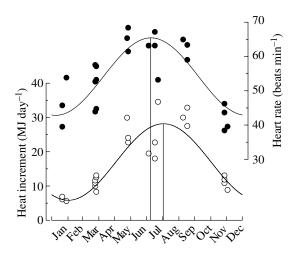
Fig. 4. Upper graph: daily mean heart rate (closed circles) during or close to the day of measurement of heat increment of feeding. Lower graph: estimate of the heat increment of feeding (open circles) and its seasonal change calculated from data presented in Kuntz et al. (Kuntz et al., 2006). Lines represent periodic fits to the data, vertical lines represent the acrophases.

Indeed, HI varied periodically throughout the year {test for difference to zero for the amplitude of the periodic fit {[cos(Julian day/365× 2π−ɸ)], $F_{2,17}$=70.3, P<0.001, Fig. 4, lower graph}. However, predicting f_H with HI resulted in a poorer regression model ($F_{1,18}$=27.8, P<0.001) than using a periodic fit to f_H ($F_{2,17}$=94.0, P<0.001, AIC difference between both models=17, P<0.001). The acrophase of the periodic fit to f_H was on 9 July, and that of the periodic fit to HI on 2 August, representing a significant phase delay (Fig. 4; $F_{1,17}$=11.9, P=0.003; see Materials and methods for details). Furthermore, in a multiple-regression model containing HI in addition to the factors considered so far, a periodic fit remained a strong predictor of f_H, together with T_s, T_a and HI, despite the considerably smaller sample size (Table 1B). The other predictors in this model did not achieve or maintain statistical significance, either because of low-effect size or, as in the case of the factor 'reproduction', because this time period of high MR was insufficiently represented in this smaller sample.

Discussion

Seasonal changes in MR and underlying causes

Substantial changes in MR from summer to winter have been reported for several wild Artiodactyla (reviewed in Arnold et al., 2004). Here, we demonstrate for the first time a similar seasonal adaptation in a perissodactyl species. MR of Przewalski horses living under close to natural conditions varied considerably over the year, as indicated by a twofold change in mean daily f_H. A change in f_H is the cardiovascular system's main response to varying oxygen demand and therefore represents a reliable index of energy turnover rates (Butler et al., 2004). f_H telemetry has already been successfully used to monitor field MR in several species (e.g. Froget et al., 2004; Renecker and Hudson, 1985; Woakes et al., 1995).

Among endotherms, low MR during winter and resumption of high metabolic activity in spring are well known from hibernators and species exhibiting daily torpor. The reduction is mainly achieved by a dramatic decrease in endogenous heat production and tolerance of substantially lower body temperatures (Geiser and Ruf, 1995; Kayser, 1964; Ruf and Heldmaier, 1992). Earlier reports regarding a similar reduction in BMR in ungulates during winter were initially refuted on experimental grounds, but found new support with the discovery of nocturnal hypometabolism in *Cervus elaphus* during winter (Arnold et al., 2004). Disentangling the relative contribution of energetically relevant factors to the seasonal changes in MR is only possible with long-term measurement of MR together with simultaneous measurement of critical variables influencing MR. It further seems necessary to study animals in the wild, or kept under almost natural conditions, in order to see undisturbed reactions. Our study with *Equus ferus przewalskii* fulfilled these preconditions.

Reproduction

The most likely candidate causing a spring peak in MR is reproduction. As is typical for precocial mammals, reproducing mares have highest energy expenditure during the last part of gestation and during the first weeks of lactation (Boyd and Houpt, 1994). Wild horses foal predominantly in spring when the growing vegetation provides optimal nutrition (Duncan, 1992). However, high energy expenditure for reproduction does not entirely explain the pronounced increase in daily mean f_H found in spring. Comparison of the f_H courses of the three reproducing mares revealed that the mare that foaled in August 2003 had peak f_H at the same time as the two mares foaling in May 2003, albeit at a lower level (Fig. 2). Nevertheless, reproduction in spring apparently increased daily mean f_H considerably during the most energetically demanding weeks around foaling. Interestingly, the increase in f_H around birth in the mare that foaled on 4 August 2003 was much smaller. This result is compatible with a reduced ability to increase energy expenditure later in the year. It might well be that mothers foaling late in the year are unable to adequately provide for their offspring. Indeed, foals born outside of the main foaling season are known to grow more slowly and are less likely to survive (Duncan, 1992), a phenomenon also reported for red deer calves (Clutton-Brock et al., 1982).

Physical activity

Lower levels of LA during winter contributed to a halving of f_H during winter. LA increased in spring by as much as f_H and simultaneously with f_H (Fig. 1). During a typical day in December, 56% of all available two-minute data sets represented resting behaviour, but at the beginning of June only 3% of the data sets had an activity level ≲10%, which was typical for uninterrupted lying or standing motionless (Kuntz et al., 2006). Furthermore, the intensity of LA might also have increased but remained undetected. The sensor in the collar would have been constantly activated and hence have recorded the maximum of 100% LA if a horse walked slowly throughout

a 2 min measurement interval, but also if it fought furiously with a conspecific. There is no doubt that the latter is energetically more costly as indicated by higher f_H (Kubalek, 2005), but this difference could not be detected with our measurement of LA. Since agonistic interactions are comparatively rare during winter (Kubalek, 2005), the increase in energy expenditure because of changes in the intensity and type of various activities in spring is presumably higher than indicated by the increase in our measure of LA. Nevertheless, the rapid increase in f_H in spring was also found during periods at rest (Fig. 3), corroborating the existence of other energetically relevant changes occurring in spring.

Thermoregulation

The horses showed a distinct seasonal rhythm of peripheral body temperature with considerably lower T_s during winter. T_s and T_a correlated closely, as to be expected from the classical model of thermoregulation (Scholander et al., 1950b; Scholander et al., 1950a). Peripheral vasoconstriction lowers T_s and attenuates heat loss from the body core and should therefore occur more at lower T_a. However, several lines of evidence from our data are incompatible with the classical model. T_s continued to decline during late winter but T_a had already increased (Fig. 1C). At the same time, lowest T_{min} were observed. T_s correlated positively with f_H independent of all other thermoregulatory-relevant factors considered (Table 1), and the same correlation was found at T_a within the thermal-neutral zone of winter-acclimatized domestic horses (McBride et al., 1985). These results indicate that a reduction in endogenous heat production accompanied by a reduction in the set point of body temperature regulation was the major reason for lower T_s. Apparently, this strategy to reduce energy expenditure was increasingly employed by the horses as body energy reserves became depleted as the course of T_s during winter matched the decline of body mass with coinciding annual nadirs in April [cf. fig. 6 from Kuntz et al. (Kuntz et al., 2006) with Fig. 1C].

Low T_{min} during summer might also have reflected a strategy to reduce the energetic cost of thermoregulation. A larger decrease in body temperature during the night increases the capacity to store heat during the hot day and reduces the amount of heat that must be dissipated through evaporation. This reaction is a well-known strategy of desert ungulates to reduce their water requirements (Ostrowski et al., 2003; Ostrowski and Williams, 2006; Schmidt-Nielsen et al., 1957). It could well be that during hot summer days our study animals showed a similar thermoregulatory reaction despite having water available *ad lib.*, because such a reaction is adaptive in their natural desert habitat.

Fasting and fattening

All northern ungulates studied so far eat less during winter, even when provided with food *ad lib.*, and fuel metabolism to some degree with body fat reserves accumulated during the previous summer (reviewed in Kuntz et al., 2006). Przewalski horses also reduce food intake during winter and deplete body fat reserves (Kuntz et al., 2006; Scheibe and Streich, 2003).

Processing and metabolizing food is energetically costly. This cost is mostly determined by the amount of food intake, the crude protein content of food plants and is higher during metabolism above maintenance level (Blaxter, 1989). Thus, considerably higher HI is to be expected during summer. Therefore, the argument that higher MR of large herbivores during the summer months just reflects the intense use of abundantly available protein-rich food and the cost of building up body energy stores rests on solid grounds (Jiang and Hudson, 1993; Mautz et al., 1992; Mesteig et al., 2000; Nilssen et al., 1984b; Pekins et al., 1992; Worden and Pekins, 1995). However, direct measurements and a quantification of the contribution of HI to MR in wild ungulates were lacking so far.

At first glance, our data support the view that there was no seasonal change in BMR but only an HI-induced increase in MR in summer. However, the increase in MR in spring clearly preceded the increase in estimated HI (Fig. 4), possibly reflecting preparation by the organism for the upcoming task of processing large amounts of high-quality food. In line with this interpretation, residual f_H still varied periodically after adding HI to the regression model (Table 1B). To some degree, a likely cause of this residual variation in MR could be a seasonal change in the size and energy requirements of the gut and visceral organs. Atrophy of the digestive tract and splanchnic organs during phases of low or absent use is a widespread phenomenon (e.g. Hume et al., 2002; Hume and Biebach, 1996; Kamler, 2001; Karasov and McWilliams, 2005; Piersma and Drent, 2003; Piersma and Lindström, 2000; Secor and Diamond, 1998; Starck, 1999). Rebuilding the capability to take up energy at a higher rate requires the synthesis of enzymes of the intermediary metabolism, growth of the gut, its absorption capacity, of splanchnic organs, and most importantly, maintenance of a higher mass of metabolically active tissue, i.e. high MR.

An endogenous seasonal cycle of MR

Like many northern mammals, during winter *Equus ferus przewalskii* showed a bundle of reactions known as physiologic adaptation to starvation (Hoffer, 2006). In order to cope with lower food intake during winter, MR was throttled by the absence of advanced gestation or peak lactation, by reduced physical activity, presumably by reduced body mass and organ size and by tolerating lower body temperature. Metabolic heat production was a major factor contributing to f_H and our data support the view that its reduction is a ubiquitous adaptation of endotherms to cope with the energetic challenge of winter (Arnold et al., 2004). However, the reactions found differ in one important aspect from the classical starvation syndrome. They were not merely the result of an inadequate energy uptake, as corroborated by the phase relations of the annual cycles of f_H, LA, T_s and HI. Thus, the seasonal changes in physiological and behavioural parameters found in Przewalski horses are evidently under endogenous control, preparing the organism well in advance for predictable seasonal changes of climate and of availability and quality of food.

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