

RESEARCH ARTICLE

Regulation of heart rate and rumen temperature in red deer: effects of season and food intake

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SUMMARY

Red deer, *Cervus elaphus*, like other temperate-zone animals, show a large seasonal fluctuation in energy intake and expenditure. Many seasonal phenotypic adjustments are coordinated by endogenous signals entrained to the photoperiod. The cues determining variation in the resting metabolism of ungulates remain equivocal, however, largely because of the confounding effects of food intake and thus the heat increment of feeding. To distinguish endogenous seasonal and environmental effects on metabolism, we subjected 15 female red deer to two feeding treatments, 80% food restriction and low/high protein content, over two winter seasons in a cross-over design experiment. We used rumen-located transmitters to measure heart rate and rumen temperature, which provided indices of metabolism and core body temperature, respectively. Our mixed model ($R^2=0.85$) indicated a residual seasonal effect on mean daily heart rate that was unexplained by the pellet food treatments, activity, body mass or air temperature. In addition to an apparently endogenous down-regulation of heart rate in winter, the deer further reduced heart rate over about 8 days in response to food restriction. We found a strong correlation between rumen temperature and seasonal or periodic variation in heart rate. An effect of lowered rumen (and hence core body) temperature was enhanced during winter, perhaps owing to peripheral cooling, which is known to accompany bouts of hypometabolism. Our experimental results therefore support the hypothesis that a reduction in body temperature is a physiological mechanism employed even by large mammals, like red deer, to reduce their energy expenditure during periods of negative energy balance.

Key words: energy expenditure, environmental cues, heart rate, metabolic rate, season, seasonal acclimatisation, thermoregulation, ungulate.

INTRODUCTION

Animals in cold temperate climates often exhibit a wide annual cycle in their rate of energy expenditure (Moen, 1978; Kenagy et al., 1989; Speakman et al., 1999; Arnold et al., 2004; Arnold et al., 2006; Signer et al., 2010a). During summer, abundant food allows energy to be allocated to reproduction and fat storage, whereas during winter, food is scarce and mechanisms to reduce energy expenditure such as the use of torpor are crucial. The seasonal coordination of phenotypic changes is, for many temperate-zone animals, governed by an endogenous timer entrained to the time of year *via* the nocturnal release of melatonin (Paul et al., 2008). This allows physiological adjustments to be made in preparation for the expected conditions in each season. In addition to an underlying endogenous signal, however, short-term changes in environmental conditions, principally food availability and temperature, are important secondary cues that affect the expression of mechanisms relating to energy expenditure (Kenagy, 1986; Humphries et al., 2003). Emergence from hibernation, for example, is determined by a combination of an endogenous timer and external cues such as soil temperature and the timing of spring growth in herbaceous food (Körtner and Geiser, 2000). Distinguishing the relative importance of endogenous seasonal and environmental cues (Kenagy, 1986) is difficult, however, because they typically vary in synchrony. Whereas observational studies of wild animals provide information on seasonal changes in metabolic physiology (e.g. Kenagy et al., 1989; Arnold et al., 2004; Arnold et al., 2006), experimental studies are required to distinguish the explanatory factors and test hypotheses derived from these observations.

In the present study, we aimed to determine the influence of endogenous seasonal and immediate environmental factors on the regulation of metabolism by red deer kept under near-natural conditions. Red deer show a large annual fluctuation in energy expenditure (Arnold et al., 2004), which may to some extent reflect variation in resting metabolic rate (i.e. the minimum energetic cost of maintenance in a fasted animal). Seasonal differences in resting metabolic rate have been indicated for several temperate ungulates, including white-tailed deer (Silver et al., 1969), moose (Regelin et al., 1985), big horn sheep (Chappel and Hudson, 1978), roe deer (Wiener, 1977) and red deer (Pauls et al., 1981). Whether these results support an endogenously controlled adjustment in metabolism has been debated, however, owing to the potential confounding effect of seasonal variation in food intake and therefore the heat increment of feeding, i.e. the heat generated as a by-product of digestion (e.g. Nilssen et al., 1984; Mautz et al., 1992; Mesteig et al., 2000). Manipulation of food intake has a large effect on the metabolic rate of ungulates (Jensen et al., 1999; Mesteig et al., 2000). Even when provided with unrestricted food, a voluntary reduction in food intake could therefore account for a lowered metabolism in winter (Worden and Pekins, 1995).

We designed the current experimental study to test the hypothesis that endogenous signals direct seasonal changes in the metabolic physiology of red deer. We were also specifically interested in the relationship between core body temperature and metabolism and how this may vary seasonally. Fifteen semi-wild female red deer were subjected in a cross-over design experiment

to two food treatments, 80% food restriction and low/high protein content, which varied independent of the time of year. We used newly developed rumen-located transmitters (Signer et al., 2010b) to record heart rate, which provided a qualitative index of metabolic rate, and also rumen temperature. Heart rate is strongly and often linearly correlated with oxygen consumption in free-ranging animals including ruminants, particularly outside periods of strenuous exercise (Butler et al., 2004; Brosh, 2007; Green, 2010). In his review of the use of heart rate to estimate metabolic rate in ruminants, Brosh (Brosh, 2007) found that, over a large range of heart rates, reproductive condition, feeding or cold exposure had either non-significant or relatively minor (<5%) effects on the estimated oxygen pulse per heart beat. The use of heart rate without prior calibration against oxygen consumption, which in our study of semi-wild deer was impractical, therefore provides a robust index of energy expenditure. We analysed these data to quantify the additive and interacting effects of season and food intake, in addition to other biological and environmental factors, on the mean daily heart rate of red deer.

MATERIALS AND METHODS

Study area, design and animals

This study was conducted over 1.5 years (including two winters) in a 45 ha (450,000 m²) enclosure adjacent to the Research Institute for Wildlife Ecology, Vienna (48.21°N, 16.37°E). The procedures were carried out in accordance with the Austrian legislation as assessed and approved by the institutional ethics commission of the University of Veterinary Medicine Vienna. In total, 22 red deer (*Cervus elaphus* L.) were living in this enclosure under close to natural conditions, including a dominant stag, a subordinate stag and 20 female deer. Fifteen adult females had been trained to enter an automated feeding station where they received a daily amount of pellet food specific to the individual and were weighed while standing on the feeding platform. We fitted these deer with rumen-located transmitters and collar repeater units to continuously measure their heart rate, rumen temperature and activity while they were subjected to an experimental feeding schedule and experienced natural climatic conditions during the study. We recorded air temperature every 10 min at a weather station located in the animal enclosure. We tested for the effect of two diet treatments: food quantity and protein content. Both factors are important in determining the energetic cost of digestion and may also act as zeitgebers for circannual rhythms in gut morphology and physiology, activity and metabolism (Arnold et al., 2004).

Experimental feeding treatments

The experimental deer were trained to enter and feed at a computer-controlled feeding station that delivered either a measured amount under unrestricted supply or a programmed daily restricted amount of one of two types of food pellets (Altromin, Lage, Germany) to each individual. Animals were identified by transponders located in ear tags (Dalton, Bocholt, Germany). A two-gate system ensured that only a single animal at a time could enter the feeding station. When an animal's transponder was detected by a reading antenna at the feeding trough, the system delivered pellets at a rate of 95 g every 40 s. Pellet delivery stopped when an animal raised its head. The animals usually emptied the feeding trough before leaving, enabling us to accurately measure the mass of pellets eaten. We automatically measured and recorded the mass of each deer every 3 min while they were at the feeding trough (usually at least once per day) using a scale located under the feeding platform (Schauer, Prambachkirchen, Austria).

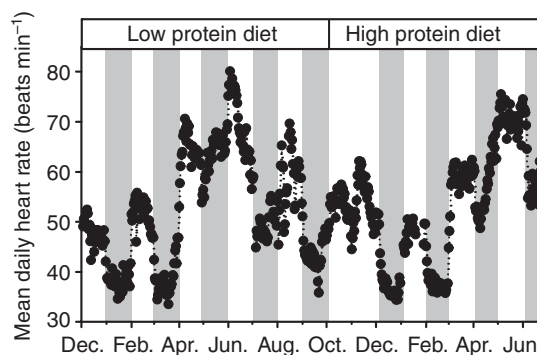


Fig. 1. Mean daily heart rate of one female red deer over the study period. Individuals were given either the low or the high protein diet in the first winter and then switched to the other diet in the second winter (top; initial conditioning phase not shown), and also switched monthly between the unrestricted or 80% restricted (shaded) daily quantities of pellet food.

The deer received one of two types of pellet diet in the two winters according to their feeding group (e.g. Fig. 1). One type of pellet contained 30.9% dry organic matter crude protein (high protein diet), whereas the other contained 17.4% crude protein (low protein diet). These levels reflected the actual protein content measured in the natural diet eaten by red deer in July (summer) and January (winter), respectively (Arnold et al., 2004). The diets were nearly isocaloric – high protein diet: gross energy 21.5 kJ g⁻¹, fibre 21.5% (dry organic matter), nitrogen-free extract (nfe) 40.6%, fat 6.9%; low protein diet: energy 19.5 kJ g⁻¹, fibre 31.8%, nfe 47.4%, fat 3.4%.

The deer were also subjected to an alternate monthly schedule of food restriction. During each day of food restriction, an individual received only 20% of the daily mean mass of food pellets it consumed during the preceding month when it was allowed unrestricted intake. We knew from preliminary trials that this level of temporary food restriction does not cause unabated long-term mass loss or prevent the deer from returning to the feeding station. Deer could also forage on natural vegetation in the enclosure, which contained a meadow (~3 ha, 30,000 m²) surrounded by mixed beech and oak forest. Of course, during winter, there was much less natural forage available in the enclosure compared with that in the summer and we presumed that any foraging by deer was not sufficient to compensate for the restricted availability or protein content of the pellets. Foraging on natural vegetation may have been more significant during summer, particularly for deer on restricted pellet rations.

For 6 months prior to the beginning of the experiment, the experimental animals were assigned to dietary groups and fed either high or low protein pellets. Dietary groups were switched at the beginning (3rd December) and again half-way through the experiment (1st October). This preconditioning and switching of dietary groups ensured a comparable nutritional state of the deer entering a new diet regime.

Heart rate, body temperature and activity telemetry

We used a newly developed rumen telemetry system that allows heart rate and rumen temperature to be detected in free-ranging ruminant animals without the need for surgery. After restraining a trapped animal in a crush cage, a transmitter (22×80 mm cylinder, 100 g) was introduced into the pharynx with an applicator and then swallowed by the deer into its reticulum. Some animals that did not

enter the trap were first anaesthetised with a combination of tiletamine–zolazepam (Zoletil, Virbac SA, Louvain la Neuve, Belgium) and xylazine (Rompun[®], TS Bayer, Leverkusen, Germany) applied *via* a dart projector. Anaesthesia was reversed by intramuscular administration of the specific antagonists atipamezole (Antisedan[®], Farnos, Turku, Finland) and sarmazenil (Sarmazol[®], Dr E. Graeb AG, Bern, Switzerland) and the transmitter was then introduced during the early recovery period, as soon as the swallowing reflex resumed. The transmitters remained permanently in the reticulum without causing harm and had a battery life of 24 months.

The rumen transmitters contained a biaxial micro-accelerometer to detect vibrations caused by the beating of the heart. An in-built microprocessor amplified, filtered and processed the acceleration signal to distinguish a regular pulse of vibrations identical to the heart rate [for details about the transmitters and their calibration, see Signer et al. (Signer et al., 2010b)]. Heart rate was not detectable during vigorous movement or contractions of the rumen. A microcontroller in the transmitter switched every 3 min to measure temperature *via* a pre-calibrated thermistor. Signals were transmitted *via* unique sequences of UHF radio waves to the collar repeater unit, which both stored the data in solid state memory and transmitted a powerful VHF encoded signal to a receiver station, where the data were automatically stored on computer. The collar unit also detected head movements using a triaxial acceleration sensor and we scored the duration of locomotor activity, estimated *via* a pre-calibrated algorithm of directional movements (Signer et al., 2010b), every 3 min. These data were stored in solid-state memory by the collar unit.

Data filtering and analysis

To distinguish genuine heart rate measurements from noise, we first removed data that were beyond expected physiological limits (>120 beats min^{-1}) and then applied specific filtering algorithms using the statistical package 'R' (R Development Core Team, 2009). In the raw data, heart rate signals were partially obscured by other detected vibrations, such as during rumination or movement activity. We took advantage of the fact that genuine heart beats are generally expected to generate sequences of data points with high periodicity. The filtering program first identified 3 min intervals containing sequences of high periodicity within the expected natural beat-to-beat variation in heart rate. When we found such sequences, a permutation procedure was applied to assess the probability of finding this degree of periodicity by random chance and data points were retained only if this probability was <0.025 . Subsequently, a few remaining obvious outliers were removed by visual inspection of the data. These filtering methods were conservative and provided a high degree of confidence in the remaining data set. The remaining heart rate data originate from animals at rest or moving slowly and we therefore refer to these filtered data as the 'stationary' heart rate ($f_{H,s}$) (Signer et al., 2010a).

For rumen temperature, the measurements contained brief periods when the rumen cooled rapidly for up to several hours at a time, presumably because of ingested cold water, snow or food pellets. We applied a filter that detected these rapid decreases in rumen temperature and removed subsequent data until the temperature returned to the previous baseline level. All continuous variables were averaged first hourly and then daily within individuals prior to analysis. Our statistical analyses are based on these daily means.

We tested for effects of season, food treatments and other explanatory variables on $f_{H,s}$ using mixed-effects models (function 'lme' in the statistical package R) with 'individual' included in all

models as a random intercept factor (Pinheiro and Bates, 2000). This method accounts for the repeated measures within individuals and allows for the unequal size of data sets and possible differences in the mean levels of $f_{H,s}$ among individuals. We tested for a seasonal effect by including month of year as a factor. Binomial factors designated periods when individuals were subjected to restricted or unrestricted pellet intake (in alternate months) and high protein 'summer' or low protein 'winter' pellet diets (in alternate winters). In full models, we included the following explanatory variables measured in individual deer: rumen temperature ($^{\circ}\text{C}$), body mass (kg), age (years), activity (% of day when 'activity' was detected) and lactation (approximate period of lactation for those animals giving birth in each year). Day of giving birth was either observed or determined from a sudden drop of body mass. We also included air temperature ($^{\circ}\text{C}$). To test for the possible variation in effects of food restriction and food type among the seasons (i.e. with the factor 'month of the year'), we added two-way interaction terms for these variables. Also, because our model showed that rumen temperature had a strong effect, we tested for its interaction with season. To account for temporal correlation detected in the residual error term in our models, we included a first-order auto-regressive correlation structure (i.e. corAR1 in R) (Pinheiro and Bates, 2000).

We followed the principle of model simplification by removing terms to achieve a model with the smallest value for Akaike's information criterion (AIC), which is a test of the goodness of fit of competing models penalised by the number of included parameters. Models were fitted by maximum likelihood during selection but the final model was fitted by restricted maximum likelihood (Pinheiro and Bates, 2000). We also calculated Akaike weights using 'AICcmodavg' in R (Mazerolle, 2010) to help judge the level of support for the top-ranking models (Burnham and Anderson, 2002). By fitting mixed effects models, we could simultaneously analyse all of the data without first averaging for individuals, which otherwise is necessary to avoid pseudoreplication. A consequence of this approach, however, is that the inclusion of some variables can improve the model and be statistically significant in an ANOVA even though their effect size is biologically trivial. To overcome this, we deliberately focused our analysis on estimating the effects of the main variables of interest, namely season, food treatment, rumen temperature and their interactions.

To examine the temporal response of deer to food restriction, we fitted segmented linear regressions to changes in daily mean physiological variables plotted as a function of days since the beginning of each period of restricted or unrestricted food intake ('segmented' in R) (Muggeo, 2003). This technique attempts to estimate a new model with two (or more) regression slopes, separated by a 'break-point', and uses the Davies' test to examine whether the segmented regressions have significantly different slopes. All data are presented as the mean and s.e.m. of daily mean values for each individual. Additionally, we calculated the coefficient of variation for annual changes in daily means of variables.

RESULTS

Both season and food restriction strongly affected the mean daily $f_{H,s}$ of individual deer (e.g. Fig. 1). The timing of a pronounced seasonal fluctuation in $f_{H,s}$ and rumen temperature differed from the sinusoidal annual pattern in ambient temperature, with the peak in $f_{H,s}$ and rumen temperature occurring earlier in the spring (Fig. 2). Daily mean $f_{H,s}$ increased steeply in late March and April to reach approximately 65–70 beats min^{-1} in May, remained at an intermediate level of 50–60 beats min^{-1} over the summer and autumn

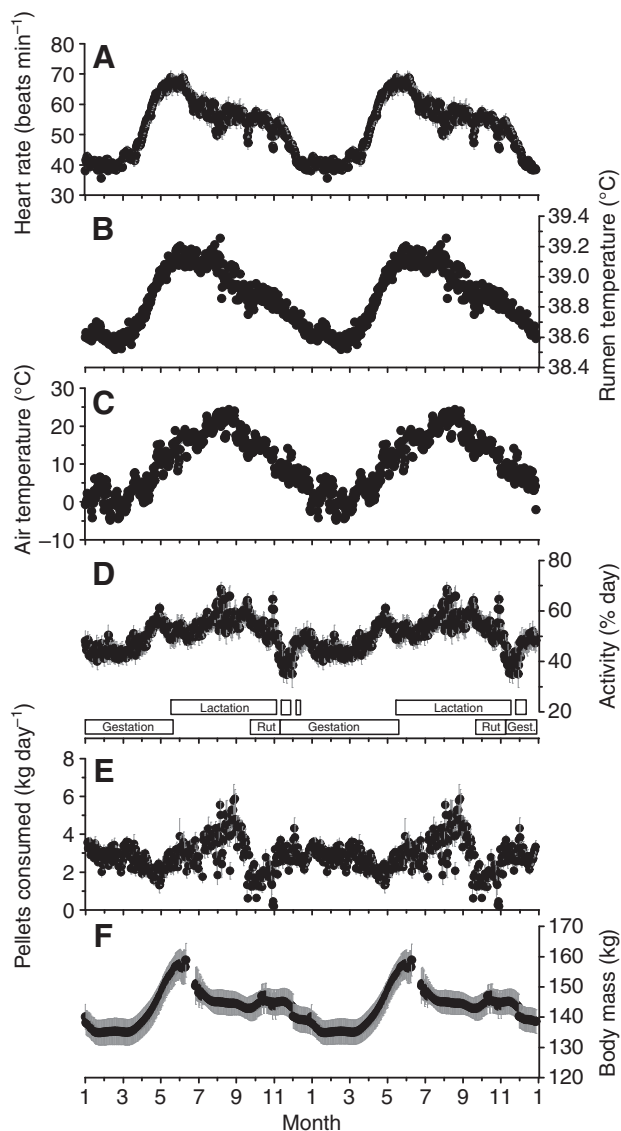


Fig. 2. Seasonal variation in daily mean (A) heart rate, (B) rumen temperature, (C) air temperature, (D) time spent active, (E) mass of pellets consumed during periods of unrestricted access and (F) body mass (missing values indicate malfunctioning of the scales). Also shown is the approximate timing of reproductive phases in red deer (D). Symbols and error bars represent the mean and s.e.m. of individual daily mean values. Data are double plotted to ease visualisation of seasonal patterns.

months, and declined in late November to approximately 40 beats min⁻¹ over the winter months (annual range ~30 beats min⁻¹, coefficient of variation CV=0.176). Rumen temperature and body mass followed a similar annual pattern, with rumen temperature ranging from approximately 39.1 to 38.6°C (annual range ~0.5°C, CV=0.005) and body mass ranging from approximately 155 to 135 kg (annual range ~20 kg, CV=0.041) from spring to winter. Despite the alternate monthly access to unrestricted pellet food, body mass decreased by approximately 10 kg, on average, among individuals over the winter months (i.e. December, January, February). Activity increased slightly during April to reach generally higher levels during summer than during winter (annual range 30%, CV=0.123). Pellet consumption during periods of unrestricted access began increasing several weeks after the spring increase in

Table 1. Analysis of variance (type III) results for our best mixed-effects model explaining daily mean heart rate of the female red deer

Random effect	s.d.		
Individual (intercept)	6.33		
Fixed effects	d.f.	F-value	P-value
(Intercept)	1, 4400	1.986	0.168
Month	11, 4411	11.4	<0.0001
Air temperature	1, 4400	5.7	0.017
Age	1, 4400	20.1	<0.0001
Body mass	1, 4400	125.3	<0.0001
Activity	1, 4400	82.3	<0.0001
Rumen temperature	1, 4400	2.9	0.089
Lactation	1, 4400	23.3	<0.0001
Food restriction	1, 4400	59.3	<0.0001
Pellet type	1, 4400	4.1	0.044
Month×food restriction	11, 4400	10.5	<0.0001
Month×pellet type	11, 4400	6.8	<0.0001
Month×rumen temperature	11, 4400	11.6	<0.0001

$f_{H,s}$, and reached a peak in late summer. A considerable decrease in pellet consumption coincided with the time of the rut, a phenomenon that previously was only known for male deer (Bobek et al., 1990).

The linear model providing the best fit (Table 1) and explaining 85.3% of the variation in daily mean $f_{H,s}$ contained the fixed terms: $f_{H,s} = \text{Mth} + \text{PT} + \text{FR} + T_r + T_a + \text{BM} + \text{L} + \text{Age} + \text{Mth} \times \text{FT} + \text{Mth} \times \text{Tr}$. In this model, Mth is month of the year, PT is pellet type (high or low protein content), FR is food restriction (unrestricted or 80% restricted daily pellet ration), T_r is rumen temperature, T_a is air temperature, BM is body mass, L is period of lactation and Age is age of the animals. A term designating individuals was included as a random intercept effect. This model also included a defined autocorrelation structure. We fitted another model without the interaction terms because this allowed us to visualise the effects of the main explanatory variables as partial residual plots.

Both season and rumen temperature explained a relatively large proportion of the variance in $f_{H,s}$, in addition to the effects of pellet food intake, pellet protein content or other explanatory variables (Table 1). The seasonal effect alone in a model without interactions accounted for a change in $f_{H,s}$ of approximately 14 beats min⁻¹ (Fig. 3), representing 37% of the unexplained variance in $f_{H,s}$ (i.e. the partial correlation coefficient). Because food restriction had its maximum effect on $f_{H,s}$ only after 9 days, we also ran a model using data restricted to time periods beginning after deer had been on the diet treatments for 9 days or more. This had a negligible effect on the strength of the seasonal effect (results not shown), and we subsequently used the full data set in all analyses. Food restriction and pellet type had relatively moderate and mild effects, respectively, and both showed significant interactions with season (Figs 4 and 5). Food restriction over a 1 month period reduced $f_{H,s}$ by 7.5 beats min⁻¹ on average among individuals during the winter months (December to February), compared with 3.8 beats min⁻¹ during the summer months (June to August). The high protein diet increased $f_{H,s}$ on average by 2.1 beats min⁻¹ during summer and by 0.5 beats min⁻¹ in winter. Food protein content had no detectable effect on the timing or level of the spring increase in $f_{H,s}$ (Fig. 5).

Rumen temperature had a relatively large effect in our model of $f_{H,s}$. In a model without interactions, for every 0.1°C decrease in rumen temperature, $f_{H,s}$ dropped on average by 3.5 beats min⁻¹ (calculated *via* reduced major axis regression of the partial residuals), resulting in an effect size of 24.5 beats min⁻¹ over the annual range

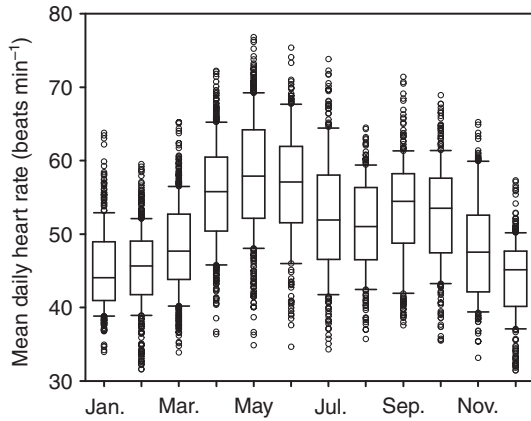


Fig. 3. Partial residual plot showing the independent effect of season on the overall daily mean heart rate as predicted from the linear mixed effects model fitted without any interactions. Box plots show the median, 25th and 75th percentiles (box), 10th and 90th percentiles (whiskers) and outliers (circles).

of rumen temperature. The best model included an interaction between rumen temperature and season (Table 1). The slope of the relationship between $f_{H,s}$ and rumen temperature differed significantly between winter (December to February) and summer (June to August) months (likelihood-ratio test, $D: 21.4, P < 0.0001$) and, within winter only, between periods of unrestricted and restricted food intake ($D: 128.9, P < 0.0001$) (Fig. 6).

To examine the physiological response of the deer to food restriction, we analysed temporal changes in measured variables since the beginning of periods of restricted or unrestricted food intake. This was done using data for the winter months (December to February) only, because deer were most reliant on pellet food during this time period. Segmented regression revealed significant ($P < 0.001$) breakpoints in the slopes of the relationship between $f_{H,s}$, rumen temperature, body mass and activity with days since the start of restricted or unrestricted pellet food intake (Fig. 7). During food restriction, $f_{H,s}$ decreased over the first 7.9 days before stabilising

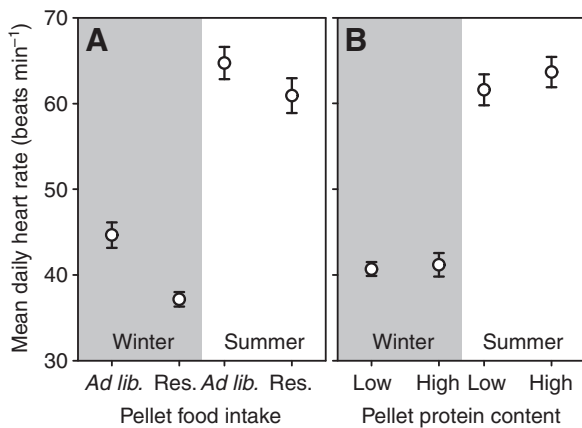


Fig. 4. Heart rate of red deer during periods of (A) unrestricted (*ad lib.*) or 80% restricted (Res.) pellet food intake and (B) low or high protein content pellets over the winter (December to February) or summer (June to August) months. Shown are the mean and s.e.m. of individual daily mean values. Mixed models indicated significant interactions between season (i.e. winter vs summer) and food restriction ($F_{1,2693}=67.9, P < 0.0001$), and between season and pellet type ($F_{1,2818}=5.2, P = 0.022$).

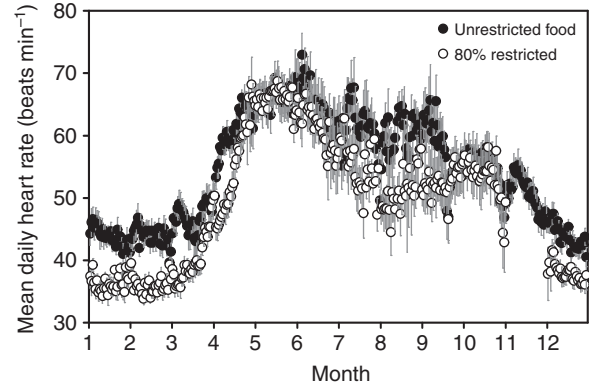


Fig. 5. Seasonal variation in heart rate of female red deer during periods of unrestricted or 80% restricted pellet intake. Symbols and error bars represent the mean and s.e.m. of individual daily mean values.

at a lower value, whereas rumen temperature, body mass and activity decreased over 16.7, 19.2 and 3.5 days, respectively, before also stabilising. With unrestricted food intake, $f_{H,s}$, rumen temperature and body mass increased until reaching significant breakpoints at 3.4, 18.8 and 15.7 days, respectively. Activity was generally lower during periods of unrestricted intake. Food protein content made no significant difference to these temporal patterns.

DISCUSSION

The aim of our experiment was to disentangle the effects of season and food intake on the regulation of resting metabolic rate and body temperature by female red deer. We found a strong residual effect of season in our model of $f_{H,s}$. Because of the cross-over design of the pellet food treatments, this result provides strong evidence of an endogenously controlled annual rhythm in $f_{H,s}$ (i.e. resting metabolism). In addition to an underlying seasonal influence, food restriction caused a further gradual reduction in $f_{H,s}$. Our model also indicated a strong correlation between variation in rumen temperature and $f_{H,s}$, which suggests that adjustments in thermoregulatory heat production are a major determinant of variation in the resting metabolism of red deer. The resulting lowered energy expenditure was sufficient to prevent an ongoing decline in body mass despite an 80% reduction in food intake during winter.

Seasonal variation in metabolism

We found an approximately twofold variation in daily mean $f_{H,s}$ of female red deer between spring and winter, despite alternate months of unrestricted feeding. In winter, $f_{H,s}$ even of deer consuming large amounts of high protein food was approximately 30% lower than that in summer. This unexplained seasonal effect, suggested by our model to account for around a third of the variation in $f_{H,s}$, approximately matched the timing of changes in abundance of natural forage. However, owing to our experimental manipulation of pellet availability and protein content, we can rule out a direct effect of variation in the availability of natural forage (i.e. the heat increment caused by eating additional natural forage). Over the study, deer had unrestricted access to high protein ‘summer’ pellets at all times of the year. Moreover, food restriction still had a moderate effect during summer, demonstrating the importance of the pellet diet throughout the year. The enormous spring increase in $f_{H,s}$ therefore occurred with little if any change in the daily intake of food. Rather, it can be concluded from our results that an endogenous seasonal adjustment in $f_{H,s}$ occurs, as

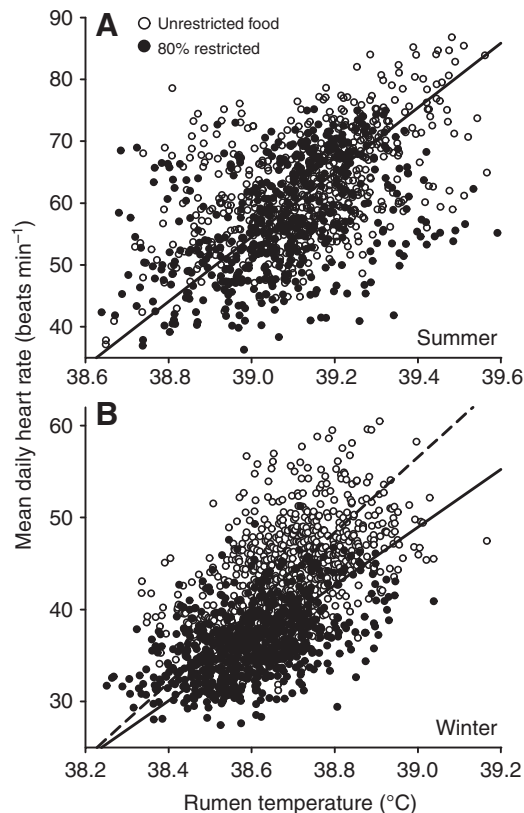


Fig. 6. Heart rate of female red deer as a function of rumen temperature during periods of access to unrestricted or 80% restricted pellet intake in (A) summer (June to August) and (B) winter (December to February) months. The slope of this relationship differed significantly between the seasons (likelihood-ratio test $D: 21.4, P < 0.0001$), and, within winter only, between periods of unrestricted (dashed line) or restricted (solid line) pellet intake ($D: 128.9, P < 0.0001$). Please note the different scales in each panel.

expected, in synchrony with the timing of changes in plant production. Seasonal variation in energy expenditure is a ubiquitous feature of temperate and arctic animals, as exemplified by hibernating mammals (e.g. Kenagy et al., 1989), and has been recorded in both wild and captive ungulates (Silver et al., 1969; Moen, 1978; Regelin et al., 1985; Mautz et al., 1992; Mesteig et al., 2000; Arnold et al., 2004; Theil et al., 2004; Arnold et al., 2006). Our results provide experimental support for a further role of an endogenous annual rhythm in modulating seasonal changes in the resting metabolism of red deer.

Ungulates in cold temperate habitats alternate between contrasting states of energy balance over the year (Parker et al., 2009). In spring and summer, the ruminant system must process a large amount of forage and transfer this energy into offspring and replenishing fat reserves, whereas an up-regulated digestive system is unnecessary during winter, when energy conservation becomes a priority. The metabolism of the gastrointestinal tract and liver constitutes approximately half of the entire resting energy expenditure in domestic ruminant animals (Ferrell, 1988; Burrin et al., 1990; Johnson et al., 1990). In sheep, after 21 days of restricted maintenance feeding, the relative contribution of portal-drained viscera to whole-body oxygen consumption decreased from 41 to 22% (Burrin et al., 1990). Thus, a decrease in the mass or mass-specific metabolism of these organs may provide a large overall

energy saving. The visceral organ mass is highly flexible in many vertebrate animals (Piersma and Lindström, 1997) to match the level of food intake (Webster et al., 1975; Hofmann, 1989; Burrin et al., 1990). There is only limited evidence, however, supporting an endogenous seasonal change in the digestive function of ungulates (Freudenberger et al., 1994; Rhind et al., 2002; Lawler and White, 2003; Asano et al., 2007). Thus, changes in visceral metabolism are unlikely to explain the seasonal variation in $f_{H,s}$ observed in our experimental deer, which occurred irrespective of the amount and protein content of consumed pellet food.

Response to food restriction

Our results indicate a secondary role of food intake in triggering a reduction in $f_{H,s}$ and rumen temperature. This effect was not caused simply by the heat increment of feeding, which is minimal after approximately 8 h in a large ruminant (e.g. Lawler and White, 2003). In contrast, $f_{H,s}$ continued to decrease over 8 days following food restriction in our experimental red deer. A similar gradual decline in resting metabolism with prolonged food restriction has been documented in several other ungulates. Reindeer (*Rangifer tarandus tarandus*) reduced their resting heart rate over 8 days while on a restricted lichen diet (Nilsson et al., 2006). Bedouin goats (*Capra aegagrus hircus*) provided with 40% of their unrestricted food intake decreased their metabolic rate by approximately 40% over 30 days whilst maintaining a similar body mass (Choshniak et al., 1995). Oryx (*Oryx leucoryx*) fed for 5 months on 30% of their unrestricted intake had a 16% lower mass-specific metabolic rate and a 1°C lower body temperature compared with control animals (Ostrowski et al., 2006b). As stated above, a reduction in the mass of visceral organs with long-term food restriction could underlie the reduction in whole-body metabolism. Over periods of days or weeks, however, a reduction in tissue metabolism is a more probable mechanism. A decrease in mitochondrial density in the gastrointestinal tract, for example, was hypothesised to explain the lowered resting metabolism of sand gazelles during starvation (Ostrowski et al., 2006a). Reductions in the oxygen consumption of skeletal muscle have also been shown in goats in response to food restriction (Choshniak et al., 1995). In our study, a concurrent decline in rumen temperature indicates a similar general down-regulation of metabolic activity. Indeed, a reduction in resting metabolism accompanied by a lowered body temperature is a common response to starvation in mammals, including humans (Keys et al., 1950; McCue, 2010). As we discuss below, the strong effect of rumen temperature in our model suggests that thermoregulatory heat production is an important mechanism in ruminants for adjusting energy expenditure.

Regulation of thermoregulatory heat production by a large mammal

Our study is one of few to simultaneously measure heart rate and rumen temperature over a year or more in a large temperate-zone ungulate (but see Signer et al., 2010a). Rumen temperature is usually slightly higher than body temperature, but this differential is not affected by moderate changes in food intake (Beatty et al., 2008). A decrease in this differential with starvation occurs rapidly, within 24 h (Dale et al., 1954). Thus, we interpret the gradual changes in mean daily rumen temperature in our experiment to reflect adjustments in the set-point of core body temperature. Our model therefore suggests that a decrease in thermoregulatory heat production was the major cause of the reduced $f_{H,s}$ and hence metabolism of red deer. Previously, a

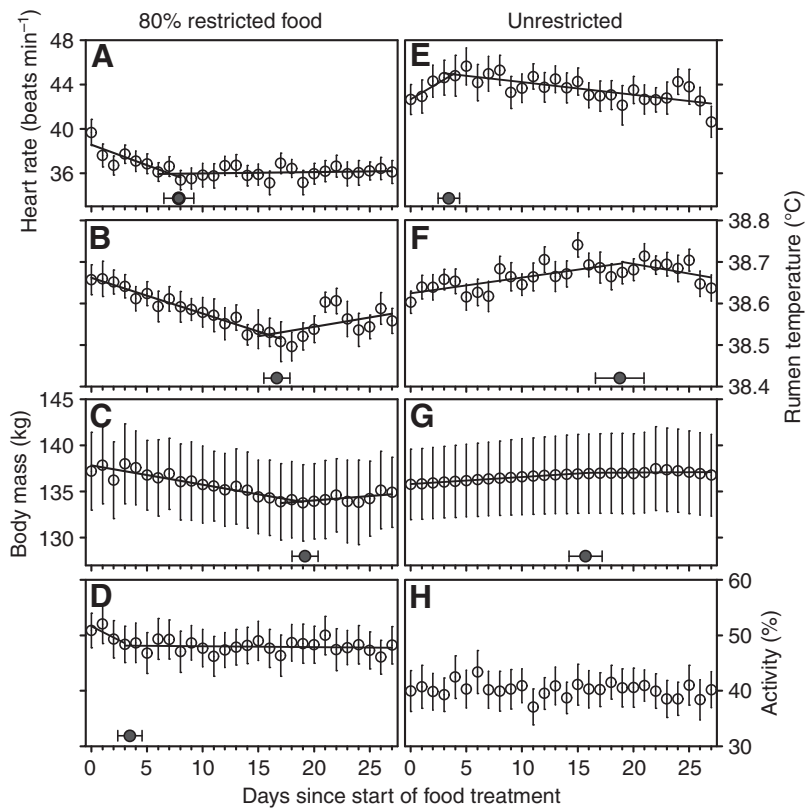


Fig. 7. Daily changes in physiological variables (means \pm s.e.m. of individual daily means) measured in female red deer during months with 80% restricted (A–D) or unrestricted (E–H) pellet food intake. Data are only from winter (December to February), when pellets were the major food source. Also shown are significant breakpoints (filled symbols; error bars are s.e.) in the slopes of temporal relationships estimated from segmented regression analyses.

change in thermal conductance was considered to be the primary mechanism available to large mammals for reducing their thermoregulatory energy expenditure (Scholander et al., 1950; Heldmaier, 1989; Lovegrove, 2005). Relatively small reductions in body temperature, however, may also have biologically meaningful effects on metabolic rate in large endotherms (McCue, 2010). The effect of rumen (i.e. body) temperature estimated by our model is greater than expected using the Newtonian equation of thermoregulatory heat production. A likely explanation for this discrepancy is that whereas this simplified calculation assumes a uniform body temperature, we know that red deer allow substantial peripheral cooling in winter, particularly during nocturnal bouts of hypometabolism (Arnold et al., 2004). Assuming a gradient in body temperature, the small reduction measured in rumen temperature may indicate a much greater reduction in the mean temperature of the entire body mass. We propose that red deer achieve a substantial reduction in metabolism by reducing core body temperature and allowing a gradient in body temperature (i.e. peripheral cooling). The significantly flatter slope of the relationship between $f_{H,S}$ and rumen temperature caused by food restriction in winter (see Fig. 6) suggests that peripheral cooling may be greater during these most energetically stressful conditions. In addition, the relative importance of a reduction in peripheral and core body temperature may differ over time. Peripheral cooling may provide a rapid means to lower thermoregulatory costs, whereas, with ongoing energy stress, a gradual reduction in the set-point of core body temperature may be needed to maintain the energy savings. This scenario could explain the staggered timing of a reduction in $f_{H,S}$ and rumen temperature in response to food restriction in this experiment (see Fig. 7). It should also be kept in mind that a reduction in both basal metabolic rate and the set-point of core body temperature is needed to achieve an overall reduction in thermoregulatory cost at cold temperatures.

This was recently shown to be important for Arctic foxes (*Alopex lagopus*) during winter (Fuglestad et al., 2006).

There is increasing evidence that large mammals employ broadly similar mechanisms of metabolic energy conservation (i.e. a reduction in thermoregulatory heat production) to those already well studied in small mammals. A larger body size, however, allows for adjustments in total body conductance and gradients in body temperature that are not possible in much smaller mammals. Consequently, large mammals can achieve a substantial reduction in metabolic rate with only a minor decrease in core body temperature, probably by coupling such reductions to peripheral cooling. Our study suggests this is an important physiological mechanism in red deer for energy conservation during winter and in response to food restriction.

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