SCIENTIFIC REPORTS

Energy expenditure and body temperature variations in llamas living in the High Andes of Peru

Alexander Riek [1,2], Anna Stölzl[2], Rodolfo Marquina Bernedo[3], Thomas Ruf[4], Walter Arnold [4], Catherine Hambly[5], John R. Speakman [5,6] & Martina Gerken[2]

Some large herbivores exhibit seasonal adjustments in their energy metabolism. Therefore, our aim was to determine if the llama (one of the most extensively kept livestock breeds) exhibits seasonal adjustment of their energy expenditure, body temperature and locomotion, under its natural high altitude Andean habitat. For this purpose, energy expenditure, body temperature and locomotion were measured in seven non-pregnant llama dams for ten months on the Andean High Plateau (4400 m above sea level). Daily energy expenditure was measured as field metabolic rate using the doubly labelled water method at four different measurement times. Additionally, a telemetry system was used to continuously record activity, body temperature (3 min intervals) as well as the position (hourly) of each animal. The results show that llamas adjusted their body temperature and daily energy expenditure according to environmental conditions. Furthermore, llamas under high altitude Andean climatic conditions exhibited a pronounced daily rhythm in body temperature and activity, with low values at sunrise and increasing values towards sunset. Llamas also had remarkably low energy expenditure compared to other herbivores. Thus, despite the domestication process, llamas have not lost the ability to adjust their body temperature and daily energy expenditure under adverse environmental conditions, similar to some wild herbivores.

Endothermic mammals have to invest a substantial amount of energy to keep their species specific body temperature (T_b) within a narrow limit of 37–39 °C especially with changing environmental conditions[1]. Therefore, many small mammals in particular those weighing less than ten kilograms, employ energy saving mechanisms such as torpor or hibernation and thus reduce their T_b and energy expenditure substantially during harsh environmental conditions[2–5]. Larger animals, with the exception of bears and badgers, were thought not to use such metabolic mechanisms to save energy until some studies on cervid species[6] and other larger ruminants[7,8] indicated that they exhibit some form of seasonal adjustment in their metabolism. However, most of these studies were conducted on captive animals using respirometry. In more recent studies, results from free-ranging wild herbivores[9–12] using telemetry and continuous long-term data recording, suggested that these species are also able to reduce their T_b and energy expenditure during unfavorable environmental conditions.

The climate of the Andean Plateau also known as 'Altiplano' (altitude >4000 m above sea level, a.s.l.) in South America can be considered as unfavourable to livestock. It is characterised by low annual precipitation of less than 500 mm per year, low ambient temperatures (T_a) at night falling at times below −20 °C and thus large daily T_a amplitudes exceeding 45 °C on some days. Furthermore, vegetation is scarce and low in energy and protein content. The llama (*Lama glama*) and the alpaca (*Vicugna pacos*) are the largest autochthonous herbivores which have been domesticated in South America 6,000–7,000 years ago from their wild ancestors, the guanaco (*Lama guanicoe*) and the vicuña (*Vicugna vicugna*)[13,14], respectively. Although llamas and alpacas have also been reported to live in lowlands in pre-Columbian times[15], they are typically concentrated in the high Andean regions. There are

[1]Institute of Animal Welfare and Animal Husbandry, Friedrich-Loeffler-Institut, Dörnbergstr. 25/27, 29223, Celle, Germany. [2]Department of Animal Sciences, University of Göttingen, Albrecht-Thaer-Weg 3, 37075, Göttingen, Germany. [3]Centro de Estudios y Promoción del Desarrollo del Sur, Calle Malaga Grenet 678 - Umacollo, Arequipa, Peru. [4]Research Institute of Wildlife Ecology, Department of Integrative Biology and Evolution, University of Veterinary Medicine Vienna, Vienna, Austria. [5]Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB24 2TZ, UK. [6]Institute of Genetics and Developmental Biology, State Key Laboratory of Molecular Developmental Biology, Chinese Academy of Sciences, 100101, Beijing, PR China. Correspondence and requests for materials should be addressed to A.R. (email: Alexander.Riek@fli.de)
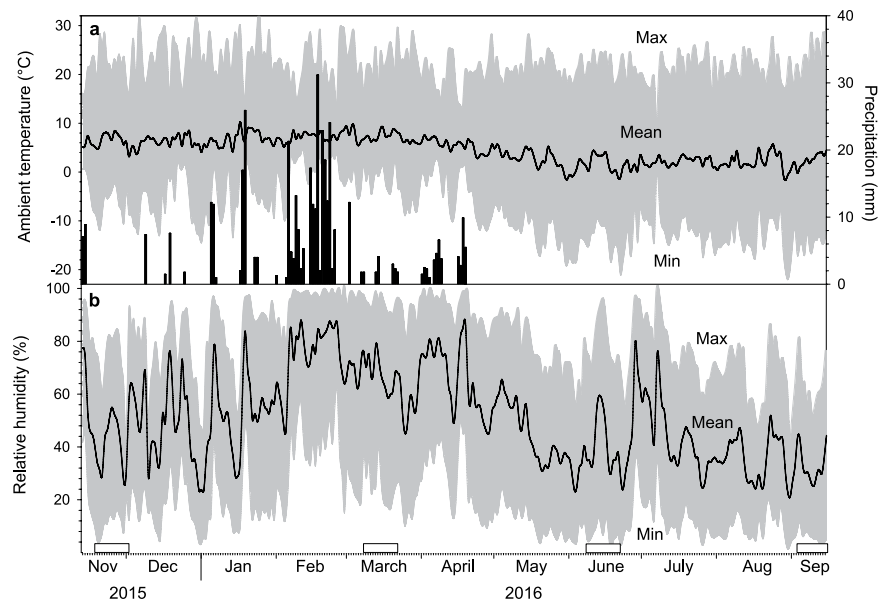
Figure 1. Climatic variables during the study in the Andes of Peru. (**a**) Average daily ambient temperature, (**b**) average daily relative humidity (black lines) with daily maxima and minima (grey shaded area) and precipitation (black bars) during the course of the study (308 days) at the study location in the high Andes of Peru (4400 m a.s.l.). Rectangles denote field metabolic rate measurement periods.

currently about 3.3 million llamas living mainly at the Andean High Plateau of Bolivia and Peru[16] and they are of predominant economic and cultural importance for the rural population[17]. Apart from climatic challenges and feed shortages, llamas and alpacas are also confronted with the impact of high altitude, i.e. reduced atmospheric pressure. Under these conditions, energy efficiency is a prerequisite for survival. In this context, it is noteworthy that South American camelids have been shown to possess an extraordinary high blood oxygen affinity[18].

Although there exists a large body of scientific literature on South American camelids on health, nutrition and reproduction in temperate regions (for review see Fowler 2010[19]), there is still a large gap in scientific knowledge on how these animals adapt to the harsh environment of the high Andes. Therefore, the aim of our long-term study was to determine if the llama, exhibits seasonal and/or daily adjustment mechanisms with regard to energy expenditure and T_b in its natural habitat of the high Andes in South America.

Results

Climatic conditions.
The climatic conditions during the time of our study (13 Nov 2015–15 Sep 2016) were typical for the Andean High plateau with very low T_a's during the night and high T_a's during the day (Fig. 1a). Average daily T_a over the entire study period was 4.6 ± 2.7 °C and ranged from -3.7 °C to 10.3 °C. The mean daily minimum T_a during our study was -8.1 ± 6.1 °C and ranged from -22.1 °C to 4.6 °C. During the entire study of 308 days, there were 263 days with frost. Mean daily maximum T_a was 22.2 ± 3.6 °C and ranged from 9.6 °C to 32.7 °C. The amplitude of daily T_a fluctuations, i.e. the difference between daily maximum and minimum T_a during the time of the study averaged 30.1 ± 7.3 °C and ranged from 9.5 °C to 45.2 °C. The mean daily relative humidity (RH) was 50.9 ± 17.6%, mean daily maximum RH was 81.0 ± 14.1% (range 41.8–100.0%) and mean daily minimum RH was 17.7 ± 12.4% (range 0.65–61.4%; Fig. 1b). The total precipitation during our study was 424 mm. Precipitation occurred exclusively during the wet season from November to April on 54 of the 308 study days (Fig. 1a). The highest rainfalls occurred on 18 February (31 mm) and 19 January (25 mm). Rainfall on the remaining days ranged between 1 and 18 mm. Natural daylight during our study ranged from 10 to 12 h per day.

Rumen temperature.
Over the entire study period, we collected over 760,000 rumen temperature (T_r) measurements at 3 min intervals, ranging from 36.25 °C to 41.17 °C. The average daily T_r during our study was 38.46 ± 0.25 °C (Table 1). The T_r followed a diurnal rhythm with the lowest T_r usually just after sunrise and the highest T_r around late afternoon (Fig. 2). Comparing the minimum T_r and maximum T_r between months, the lowest recorded minimum T_r occurred in September (36.25 °C) and the highest maximum T_r in June (40.81 °C). The T_r amplitude, i.e. the difference between daily maximum T_r and daily minimum T_r, was very variable and reached on some days more than 3 °C, increasing from November to September over the entire study. This trend was also evident during the FMR measurements, i.e. the T_r amplitude was significantly ($P < 0.001$) lower in November and March compared to June and September (Table 1, Fig. 2). There was a significant positive relationship between T_r and T_a over the entire study period (T_r, °C $= 38.38 + 0.02 * T_a$, °C; $R^2 = 0.39$, $F_{1,6} = 47.5$, $P < 0.01$, $n = 51744$, Fig. 3). An example of the adjustment of T_r to T_a is given in Fig. 4. The figure shows that on days with low T_a amplitudes for high Andean conditions as it was the case in March with only 23 °C, T_r amplitudes decreased as well even though locomotor activity (LA) was high. Contrarily, on days with large T_a fluctuations of more than 37 °C such as in September during the dry season, T_r decreased at night much further compared to March.

Variable	Entire study (308 days)	Periods of FMR measurements (15 days each)				SEM	$F_{(3, 17)}$-value	Months p-value
		November	March	June	September			
Average daily T_r	38.46 ± 0.25	38.47[a]	38.49[a]	38.49[a]	38.31[b]	0.06	4.51	0.018
Range	37.79–40.11	38.27–38.98	38.04–38.77	38.05–39.02	37.85–38.95			
Average daily T_r minimum	37.75 ± 0.35	37.83[a]	37.86[a]	37.83[a]	37.54[b]	0.08	3.72	0.030
Range	36.25–39.00	37.06–38.44	36.75–38.19	36.94–38.62	36.88–38.31			
Average daily T_r maximum	39.19 ± 0.34	39.05	39.12	39.24	39.13	0.09	2.05	0.147
Range	38.49–41.17	38.69–39.88	38.68–39.94	38.81–41.17	38.62–40.12			
Average daily T_r amplitude	1.44 ± 0.41	1.23[c]	1.27[c]	1.40[b]	1.61[a]	0.10	7.96	<0.001
Range	0.62–3.44	0.75–2.50	0.63–2.44	0.75–3.22	0.83–3.06			

Table 1. Rumen temperatures in llamas in the high Andes of Peru. Rumen temperature (T_r, °C) variables in llama dams ($n = 7$) during the entire study of 308 days and during the field metabolic rate measurements of 15 days each in November, March, June and September under Andean climatic conditions in Peru. Values are means ± sd for the entire study and LS-Means for the months adjusted for repeated measurements of seven animals with the corresponding *SEM*, *F*- and *p*-value. [a,b,c]Means within a row between month not sharing the same superscript differ by $P < 0.05$.
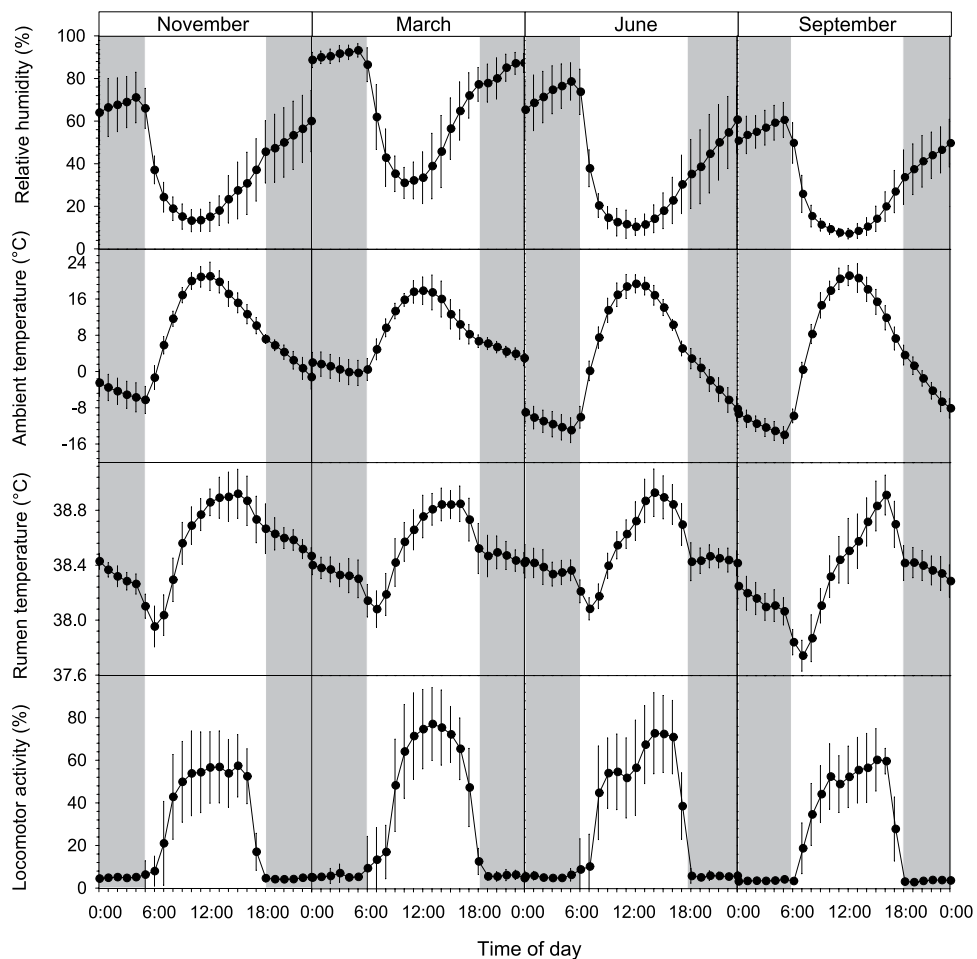

Figure 2. Average diurnal rhythms of relative humidity, ambient temperature, rumen temperature and locomotor activity. Data were collected during the FMR measurements of 15 days each in November, March, June and September in llamas ($n = 7$) under high Andean climatic conditions in Peru (4400 m a.s.l.). Values are hourly means ± *se*. Grey shaded areas indicate night-phase.

Field metabolic rate and water turnover. The field metabolic rate (FMR) varied between the four different measurement periods of 15 days each (Table 2). The lowest and highest individually recorded FMR were 11.6 MJ d^{-1} and 28.3 MJ d^{-1}, respectively. In June, during the dry season, when average T_a amplitudes were high
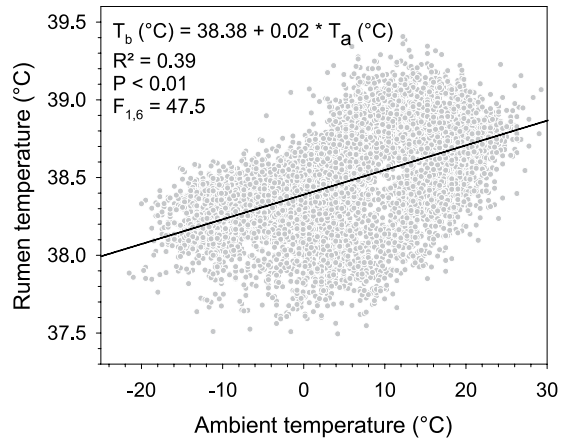
Figure 3. Relationship between rumen temperature and ambient temperature. Data are hourly means from seven adult non-pregnant llama dams ($n = 51744$) under high Andean climatic conditions (rumen temperature was taken at 3 min intervals during 308 days of sampling). Slope and intercept are adjusted for repeated measurements of individual animals (see text for details).
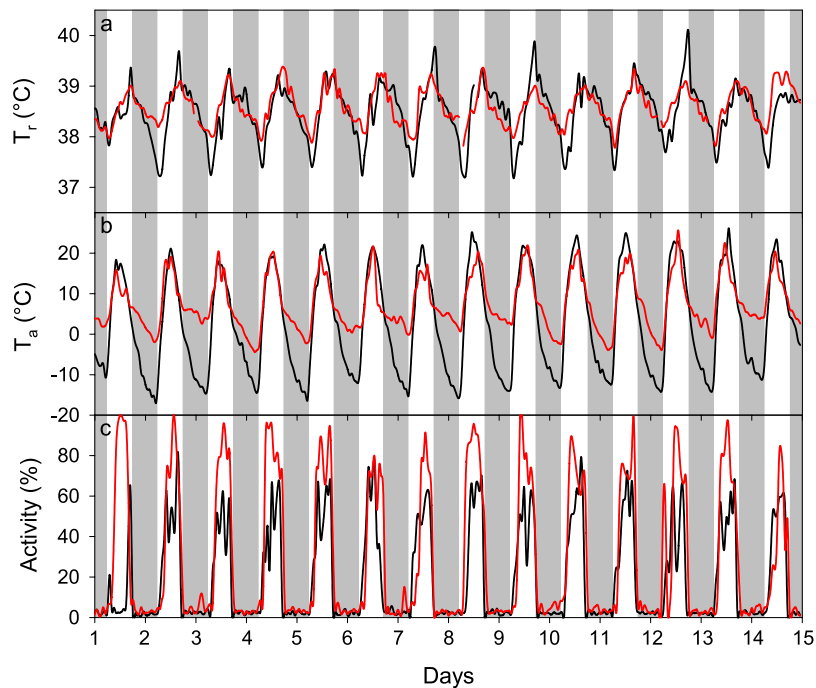

Figure 4. Examples of the diurnal rhythm of the (**a**) mean rumen temperature (T_r), (**b**) ambient temperature (T_a) and (**c**) locomotor activity. Data are from adult non-pregnant llama dams ($n = 7$) during the field metabolic rate measurements in March (red line) and September (black line). Grey shaded areas indicate night-phase.

($35.35 \pm 2.67\,°C$) and animals traveled on average longer daily distances ($5.83 \pm 0.28\,km$), FMR was significantly higher (26.22 ± 1.48 MJ d^{-1}) compared to the measurements during the wet season, i.e. November (13.15 ± 1.77 MJ d^{-1}) and March (15.43 ± 1.84 MJ d^{-1}). The FMR values measured during the wet season in November and March did not differ ($P = 0.13$), however they did differ ($P < 0.001$) between the two measurements during the dry season (i.e. June and September). In general, FMR values were higher during the dry than during the wet season (Table 2). Mixed model analysis revealed that daily distances travelled ($P < 0.001$, $F_{1,6} = 36.74$, Fig. 5), average T_a ($P < 0.01$, $F_{1,6} = 17.44$), average minimum T_a ($P < 0.05$, $F_{1,6} = 7.81$) and average maximum T_a ($P < 0.01$, $F_{1,6} = 15.46$) had significant effects on FMR.

Total body water of individual animals ranged from 56 to 71% of body mass. Average total body water was significantly lower in November ($61.4 \pm 5.35\%$) compared to March ($66.8 \pm 4.0\%$), but did not differ between all other measurement months (Table 2). Similarly, total water intake (TWI) in September was 3.75 ± 0.51 L d^{-1}, significantly lower compared to all other measurement months, while TWI did not differ between November, March and June.

Variable		November	March	June	September	SEM	$F_{(3, 17)}$-value	Months p-value
Body mass	(kg)	125.4ᵃ	109.9ᵇ	117.2ᵃᵇ	125.5ᵃ	5.16	5.62	0.007
Body condition score	(points)	2.48	2.27	2.46	2.66	0.18	1.74	0.195
Field metabolic rate	(MJ d⁻¹)	13.15ᶜ	15.43ᵇᶜ	26.22ᵃ	16.19ᵇ	0.79	61.91	<0.001
Total body water	(%)	61.44ᵇ	66.75ᵃ	65.85ᵃᵇ	64.45ᵃᵇ	1.52	3.40	0.041
Total water intake	(L d⁻¹)	4.65ᵃ	5.00ᵃ	5.20ᵃ	3.75ᵇ	0.20	19.23	<0.001
Daily activity 24 h	(%)	25.05ᵇ	29.71ᵃ	28.07ᵃ	23.05ᵇ	0.80	26.33	<0.001
Day	(%)	43.69ᵇ	53.10ᵃ	50.26ᵃ	42.91ᵇ	1.92	20.15	<0.001
Night	(%)	4.82ᵇ	6.30ᵃ	5.53ᵃ	3.56ᵇ	0.11	30.24	<0.001
Distance travelled[1]	(km d⁻¹)	4.34ᵇ	4.50ᵇ	5.83ᵃ	4.84ᵇ	0.17	23.24	<0.001
Daily rumen temperature	(°C)	38.47ᵃ	38.49ᵃ	38.49ᵃ	38.31ᵇ	0.06	4.51	0.018
Daily ambient temperature	(°C)	6.71 ± 1.21	7.46 ± 2.85	1.99 ± 1.79	5.28 ± 1.30			
Daily minimum temperature	(°C)	−6.74 ± 2.87	−1.20 ± 2.22	−13.52 ± 2.52	−14.46 ± 1.71			
Daily maximum temperature	(°C)	23.30 ± 2.72	21.96 ± 2.85	21.83 ± 2.32	23.16 ± 3.37			
Daily temperature amplitude	(°C)	29.93 ± 4.58	23.17 ± 4.12	35.35 ± 2.67	37.62 ± 3.87			

Table 2. Average physiological and behavioural variables in llamas in the high Andes of Peru. Data are averages from seven llama dams during 15 days in four different months under Andean climatic conditions in Peru. Values are LS-Means with the corresponding *SEM*, *F*- and *p*-value. Additionally average ambient temperature variables for the respective time periods are given (means ± *sd*). [1]For November and June averages are from six animals. ᵃ,ᵇ,ᶜMeans within a row not sharing the same superscript differ by $P < 0.05$.
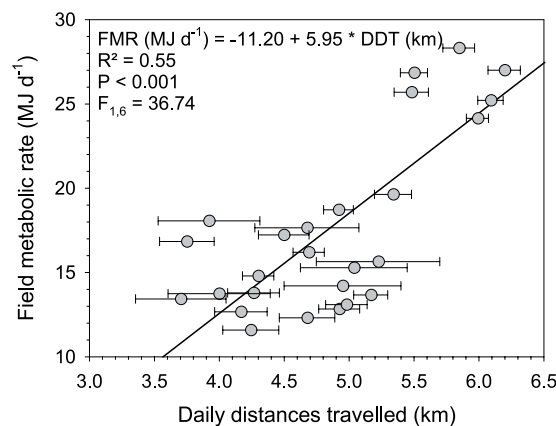

Figure 5. Relationship between field metabolic rate (FMR) and daily distances travelled (DDT). Data are means ± *se* from adult non-pregnant llama dams at four different measurement periods of 15 days each under high Andean climatic conditions (4400 m a.s.l.; $n = 26$; 6 animals in November, 7 in March, 6 in June and 7 in September). Slope and intercept are adjusted for repeated measurements of individual animals (see text for details).

Locomotor activity and distances covered. In our study, animals were herded to (07.00 h) and from (17.00 h) the grazing grounds every day approximately at the same time, thus LA followed a strong diurnal pattern over the entire study period, similar to T_a. An example of that pattern for LA as well as for T_a and T_r is depicted in Fig. 4. During the FMR measurements average daily LA was significantly higher (P < 0.001) during March (29.71 ± 1.80%) and June (28.07 ± 1.38%), compared to November (25.05 ± 1.59%) and September (23.05 ± 2.75%). The same trend was evident when dividing the data into night (i.e. when animals stayed in the corral) and day (i.e. when animals were out grazing; Table 2).

Average daily distances traveled by the animals varied over the study period. Over the entire study the mean daily distance travelled was 4.67 ± 1.41 km and ranged from 1.03 km to 12.06 km. During the FMR measurements the daily distances travelled in June were significantly (P < 0.001) higher compared to all other FMR measurement months, but no differences (P > 0.05) were detected between November, March and September (Table 2).
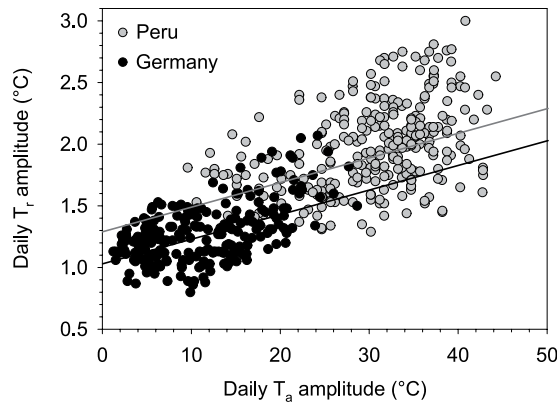
Figure 6. Comparison of temperature amplitudes in llamas between two study locations. Relationship between daily rumen temperature (T_r) and daily ambient temperature (T_a) amplitudes at the two different study locations in Germany (black dots, black line: Daily T_r amplitude = 1.03 + 0.02 * daily T_a amplitude, $R^2 = 0.25$, $F_{1,6} = 12.84$, $P < 0.01$) and Peru (grey dots, grey line: Daily T_r amplitude = 1.29 + 0.02 * daily T_a amplitude, $R^2 = 0.22$, $F_{1,6} = 10.79$, $P < 0.05$). Data are means of seven animals and the respective T_a amplitude of that day (Germany, 365 days; Peru, 308 days). Slopes and intercepts are adjusted for repeated measurements of individual animals (see text for details).

Discussion

Our study is the first measuring FMR using the doubly labelled water method in the llama in its natural habitat of the Peruvian high Andes. Furthermore, we combined FMR data with data from a telemetry system measuring T_r, activity and distances traveled by GPS. These are the first continuously recorded long-term T_r and activity measurements for a camelid in the high Andes. Our data show that llamas spend substantially more energy when traveling long distances. However, compared with other ruminants and herbivores llamas have a lower FMR. Furthermore, considering the harsh climate of the Andes, llamas seem to adjust their T_b according to T_a to save energy.

Our present data on FMR in llamas kept in their natural habitat of the Andes are similar to results reported recently for llamas measured in a temperate lowland environment[20], which ranged from 17.48 to 25.87 MJ d^{-1}. However, considering the much larger daily T_a fluctuations in the high Andes (Fig. 1), the present FMR values suggest that llamas adjusted their FMR according to T_a. Several studies have reported reductions in FMR in domestic and wild ungulates during adverse environmental conditions[10–12,21]. Our results from llamas in the Andes support these findings. The range of T_a in which T_b is regulated by sensible heat loss and thus does not require additional energy for thermoregulation is called the thermal neutral zone (TNZ). Although the TNZ of the llama has not been measured, results from guanacos, which is the wild ancestor of the llama, suggest that the TNZ lies somewhere in the range of −15.5 to 20 °C[22,23], i.e. −15.5 °C being the lower critical temperature and 20 °C the upper critical temperature outside which the animal needs additional energy to regulate T_b. Assuming a similar TNZ for the llama, animals in our study were outside their TNZ for some portions of the day during all FMR measurement periods when average T_a increased above 20 °C (Table 2). Thus, the increased FMR measured in June and September can be partially explained by the increased average T_a amplitudes as evidenced by correlations between the FMR and T_a variables. However, it needs to be emphasised that these are average T_a variables over FMR measurement periods of 15 days each. On some individual days during the FMR measurements T_a ranged between −19 °C and 28 °C and thus were even further outside the suggested TNZ. The FMR measured in June (26.22 ± 1.48 MJ d^{-1}) was nearly 100% higher than that in November (13.15 ± 1.77 MJ d^{-1}). This can partly be explained by the longer distances the animals travelled in June compared to all other measurement periods (Table 2, Fig. 5). However, FMR was significantly affected by T_a and thus animals seemed to have increased their energy expenditure not only due to the longer distances traveled but also due to differences in T_a.

The course of daily T_a in our study was typical for the High Andean climate with very low T_a at night and moderate to high T_a during the day (Figs 1 and 2). Thus daily T_a amplitudes reached 45 °C on some days. With increasing T_a amplitudes, T_r amplitudes increased as well, similar to results found in a previous study on llamas kept in a temperate environment[20]. However, the daily T_a and T_r fluctuations in the previous study were much smaller compared to the present results. Although a comparison between both locations has to be treated with caution (due to random effects etc.), the data show that T_r and T_a amplitudes were correlated in both studies (Fig. 6). The results from the high Andes, however, suggest a higher flexibility in regulating T_r according to T_a in llamas kept at these altitudes (~4400 m a.s.l.).

In our study T_r decreased during the night and increased during the day. These daily T_r fluctuations were higher during the dry season (May–September) than during the wet season (November–April) and similar to the T_a fluctuations (Figs 2 and 4), suggesting that animals followed a shallow daily hypometabolism. Reducing the metabolic rate to save energy has been known for a long time to be employed by many small mammals weighing less than 10 kg (for review see Heldmaier et al.[3]; Ruf and Geiser[5], Geiser[24]) but not for larger animals with the exception of bears and badgers. But there is increasing evidence, that also larger mammals such as red deer[10], ibex[11] and horses can reduce their metabolic rate to save energy. The average daily T_r fluctuations we report

$$FMR\ (MJ\ d^{-1}) = 0.54\ BM^{0.84\pm0.16}$$
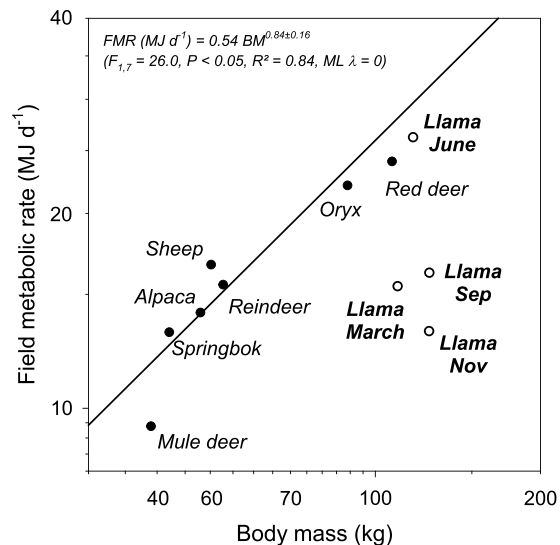$$(F_{1,7} = 26.0,\ P < 0.05,\ R^2 = 0.84,\ ML\ \lambda = 0)$$

Figure 7. Relationship between field metabolic rate (FMR, measured using the doubly labelled water method) and body mass in herbivores. Data are from seven herbivores (black dots) published elsewhere (see text for details) and from the llama of the present study (black circles) at four different measurement periods under high Andean climatic conditions (4400 m a.s.l.). The regression line was derived using the phylogenetic least square approach, excluding the data from the llama. However, the maximum likelihood (ML) of lambda was 0 and thus the regression line represents an ordinary least square regression.

here were lowest in November (1.44 °C) and highest in September (1.61 °C, Table 2). These values are in the range of previously reported T_b amplitudes for zebras (1.7 °C)[25] alpacas (1.5 °C)[26], angora goats (1.4 °C)[27], blesbok (1.4 °C)[26], impalas (1.1 °C)[28] and pronghorn (1.0 °C)[29]. However, these values and our results are means of several animals over a number of days. The highest individual T_r amplitudes in our study over a period of ten months ranged from 2.50–3.44 °C (Table 1). Even higher amplitudes of 4–7 °C have been found for the Arabian oryx[8], springbok[30] and camel[31]. The daily T_r fluctuations observed in our study were larger than the circadian variations of llamas under temperate conditions (37.5–38.6 °C)[32] and suggest that the animals used heterothermy, possibly to reduce energy expenditure. Furthermore, these daily T_r fluctuations followed the daily photoperiod and daily T_a cycle over the entire study period as evidenced by the correlation between T_r and T_a (Fig. 3). Similar results have been also found for ibex[11], red deer[10] and horses[9,33–35]. Because animals were herded every morning at around the same time to the pastures, activity increased sharply at that time and thus possibly resulted in an increase of T_r. In earlier studies on herbivores, T_b or T_r fluctuations decreased with decreasing average T_a[10,11]. In the present study, however, daily T_r fluctuations increased with decreasing average T_a and higher T_a amplitudes (Tables 1 and 2, Figs 2 and 4). The increased T_r amplitudes could be explained by a decrease in pasture quality during the dry season. Thus, energy needs might have been compromised, which could have led to increased heterothermy by lowering the minimum T_r and thus increasing the T_r amplitude[26]. However, our body mass and body condition score data do not support this suggestion (Table 2). Therefore, it is more likely that animals lowered their T_r at night to increase the capacity to store heat during the day and thus reducing energetic costs as has been shown in a number of herbivores such as the eland[36], Arabian oryx[37], giraffe[38], Arabian sand gazelle[39], Thompson's gazelle, Grant's gazelle[40] and the Asian elephant[41].

In an earlier study[20] llama FMR measured in a temperate European environment was compared with the FMR of other herbivores published so far measured using the doubly labelled water method under natural conditions (Mule deer, *Odocoileus hemionus*[42]; reindeer, *Rangifer tarandus*[43]; springbok, *Antidorcas marsupialis*[44]; red deer, *Cervus elaphus*[45]; Arabian oryx, *Oryx leucoryx*[8]; sheep, *Ovis aries*[46]; alpacas, *Lama pacos*[47]). Based on these data, a phylogenetic corrected regression equation was derived (FMR, MJ d^{-1} = 1.23 BM$^{0.63\pm0.12}$) from which a predicted FMR of 28.9 MJ d^{-1} for the llama could be computed. The predicted FMR was about 10% and 30% higher than the actual measured FMR in that study in summer and winter, respectively. In the present study, we derived a separate phylogenetic corrected regression equation (Fig. 7). The resulting regression line predicted FMR values for llamas of 31.34 MJ d^{-1}, 28.05 MJ d^{-1} and 31.37 MJ d^{-1} for November, March and September, respectively. These predicted values were 138%, 81% and 93% higher compared to the actual measured FMR values for November, March and September, respectively. The measured FMR in June (the highest of the four measurements) however was with 26.22 MJ d^{-1} just 11% lower compared to the predicted FMR from the regression line (29.53 MJ d^{-1}). Thus, the three measurements from November, March and September were exceptionally low, compared to values from other herbivores, with the exception of the mule deer. As already suggested in previous studies[48], camelids in general and the llama in particular seem to have exceptionally low energy expenditures compared to other herbivores, which might be an adjustment to the harsh Andean climatic conditions and low food supply at high altitudes. An even lower FMR has been reported for the giant panda[49]. Contrarily, predicted FMR values from phylogenetic corrected regression equations for alpacas did not deviate much from actual values (Fig. 7). The relative higher FMR in alpacas compared to llamas might be due to their additional metabolic requirements for

fine fibre production. In this context studies on high altitude adaptation of oxygen transport properties of blood and circulation could give further insight into the energy metabolism in camelids. Among other features, a high blood oxygen affinity assures a sufficient blood saturation. Interestingly, many of the special blood and circulation properties found in South American camelids are also described for camels[18,19]. However, camels do not live in high altitudes, but Old and New world camelids share their capability to survive in arid climates.

The TWI calculated during the FMR measurement periods did not differ between November, March and June but was significantly lower in September, i.e. at the end of the dry season (Table 2). Interestingly T_r amplitudes were highest at the end of the dry season in September and FMR decreased during the dry season from June to September (Table 2), suggesting that animals not only conserved energy but also water towards the end of the dry season. This is in agreement with previous studies suggesting that animals, especially camelid species with a pronounced low metabolism living in resource poor environments have an adaptive advantage because not only less energy resources are required but also less water is lost during respiration[48].

In conclusion, our study provides evidence that llamas kept at the Andean High Plateau have an exceptionally low energy expenditure compared to other ruminants. Furthermore, llamas seem to adjust their T_b according to T_a which must involve some trade-offs that allow them to save energy instead of keeping their T_b constant. Understanding these trade-offs may provide further insights into the adaptations of animals allowing them to survive in extreme environments such as the high Andes.

Methods

Animals and study site.
Procedures performed in our study were in accordance with the Peruvian animal ethics regulations and approved by the Peruvian National Ministry for Health (SENASA 2016-0009809). The study was conducted for 308 days from November 2015 to September 2016 at the research station Toccra (Centro de Desarrollo Alpaquero de Toccra) of the non-governmental organisation DESCOSUR (Centro de Estudios y Promoción del Desarollo del Sur, Arequipa, Peru) at an altitude of 4400 m a.s.l., approx. 80 km to the North of the city of Arequipa in the Andes of Southern Peru (15°44′21″S, 71°26′33″W). The study area is characterised by a semi-arid climate with an average annual rainfall of 400–500 mm and T_a ranging from as low as −25 °C at night to as high as 30 °C during day time. The average year is divided into a wet season (November–April) when nearly all of the annual rainfall occurs and a dry season (May–October).

Study animals originated from a large female llama herd of 210 animals kept under a traditional Andean herding system, i.e. animals were led to pasture in the morning shortly after sunrise at approx. 07:00 h and were herded back into a corral before sunset at approx. 17:00 h where they stayed throughout the night partly to protect them from their only predator, the nocturnal puma (*Puma concolor*). During the day animals roamed freely on the pasture of the High Andean plateau consisting mainly of the ecosystems *pajonal* (dry with tall bunch grasses) and *bofedal* (wet with grasses and herbs). The *bofedales* are formed by impenetrable stone and clay layers upon which melting water accumulates. No additional feeding was practiced and water was available throughout the year by natural surface water. For the present study a total of seven non-pregnant adult llama dams with an average age of 5.7 ± 1.5 years and a mean body mass of 125.4 ± 15.2 kg were randomly chosen and kept together with the rest of the herd.

Measurements.
Climate. The T_a (resolution: 0.0625 °C) and RH (resolution: 0.04%) were recorded continuously throughout the study with miniature data loggers at 30 min intervals at approx. 1.5 m above the ground (i-Button, DS1923#F5, Maxim Integrated Products, Sunnyvale, CA, USA). Precipitation data were obtained from a nearby weather station at approx. 10 km distance to the farm (15° 58′43″S, 71° 12′48″W).

Telemetry and body condition score. We equipped seven animals with a telemetry system (GPS Plus-3 Store on Board collar, Vectronic Aerospace GmbH, Berlin, Germany) described in detail elsewhere[50]. In brief, the telemetry system consists of two units, a ruminal unit (22 × 80 mm, 100 g) and a collar unit (450 g). The ruminal unit was administered *perorally* after animals were immobilized with an anaesthetic drug (Xylacin, Rompun®; Bayer HealthCare, Leverkusen, Germany, 4 mg/100 kg body mass). The ruminal unit measured T_r every 3 min, which was transmitted via short-distance UHF link to a data logging system located in the collar unit[50]. Furthermore, LA was also recorded every 3 min with two different activity sensors and expressed in % of the maximum value recorded. All data were recorded for 308 days and stored in the collar unit and downloaded via a laptop. Additionally the position of each animal was recorded every 60 min using a GPS device located on the back of the collar (GPS Plus-3 Store on Board collar, Vectronic Aerospace GmbH, Berlin, Germany). The body condition score, a palpable and visual assessment of the degree of fatness of individual animals was recorded during the four FMR measurement times according to a point system (scale: 0 = emaciated to 5 = obese) described in detail elsewhere[51].

Field metabolic rate. The FMR, total body water and TWI were determined during 15 days at four different time periods during the study i.e. 17 November–1 December 2015, 7–21 March 2016, 7–21 June 2016 and 2–15 September 2016, for each animal using the doubly labelled water (DLW) method[52,53]. At the beginning and at the end of the FMR measurements, body mass was recorded for each llama using a mobile scale (Weighing System MP 800, resolution: 0.1 kg, Patura KG, Laudenbach, Germany) and a blood sample of 5 ml was drawn from the *Vena jugularis* of every animal to estimate the background isotopic enrichment of ^{2}H and ^{18}O in the body fluids (method D; Speakman and Racey[54]). After taking the background sample, each llama was injected intravenously with approximately 0.16 g of DLW per kg body mass, (65% ^{18}O and 35% ^{2}H). The individual dose of each llama was determined prior to the injection according to its body mass. The actual dose given was gravimetrically measured by weighing the syringe before and after administration to the nearest 0.01 g (Digital Scale LS200, G&G GmbH, Neuss, Germany). The llamas were then held in a corral 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 7 and 15 days to estimate the isotope elimination rates.

All blood samples were drawn into EDTA blood tubes. Whole blood samples were transported to the city of Arequipa and were pipetted into 1 ml glass vials and stored at −20 °C until determination of [18]O and ^{2}H enrichment. Samples were sent from Peru to Europe by airmail. Blood samples were vacuum distilled[55], and water from the resulting distillate was analysed using a Liquid Isotope Water Analyser (Los Gatos Research, USA) at the University of Aberdeen, Aberdeen, Scotland, UK. Samples were run alongside five lab standards for each isotope and IAEA International standards (SMOW, GISP and SLAP) to correct for daily machine variations and delta values were converted to ppm. Isotope enrichments were converted to values of CO_2 production using a two pool model as recommended for this size of animal[56]. We chose the assumption of a fixed evaporation of 25% of the water flux, since this has been shown to minimize error in a range of applications[57,58]. Specifically carbon dioxide production rate (r_{CO2}) per day in mols was calculated using equation A6 from Schoeller *et al.*[59]. The daily amount of energy expended measured as FMR was calculated from carbon dioxide production by assuming a respiration quotient of 0.85. Total body water (mols) was calculated using the intercept method[53] from the dilution spaces of both oxygen and hydrogen under the assumption that the hydrogen space overestimates total body water by 4% and the oxygen-18 space overestimates it by 1%[59]. The TWI (l/day) that consists of drinking water, preformed water ingested in food and metabolic water was estimated as the product of the deuterium space and the deuterium turnover rate[60].

Statistical Analysis. The measurements of T_r had declines that could be attributed to the ingestions of water and cold food. These data points were removed by visually checking the raw data. In this cleaned data set, T_r values ranged from 36.25 to 41.17 °C. In total 2156 individual days were available for data analysis of LA and T_r. For each animal, hourly and daily means were calculated using R 3.5.0[61].

To compare T_r (Table 1) and various physiological and behavioural variables (Table 2) during the time of FMR measurements a mixed model was used with animal as a random factor to adjust for repeated measurements and month (i.e. FMR measurement periods) as a fixed factor using the MIXED procedure in SAS version 9.4 (SAS, Inst. Inc., Cary, NC). An integrated post-hoc test (Tukey) was used to detect differences between means with a 5% significance level. Data are expressed as LS-Means or means ± sd where appropriate. To adjust for repeated measurements in all other analysis we included animal ID as a random factor in a mixed model using the MIXED procedure in SAS. Thus, slopes and intercepts in Figs 3, 5 and 6 are adjusted for repeated measurements. Additionally we included body mass as a covariate and month as a fixed factor in a mixed model analysis to test whether various variables had an effect on FMR. Daily distances between continuous GPS locations for each animal were calculated with the program package 'geosphere'[62] in R version 3.5.0[61].

To test for the generality of the relation between body mass and FMR in herbivores, published data and our results were assessed using the PGLS approach in order to account for the potential lack of independence between species, because of their shared evolutionary history. The statistical procedure has been described in detail elsewhere[63–67]. In brief, the phylogeny was derived from a published mammalian supertree which includes 4510 species with updated branch lengths derived from dated estimates of divergence times[68]. The supertree for mammals was pruned to include only the species of concern, i.e. herbivores ($n = 8$), using the 'Analysis in phylogenetics and evolution' package (APE[69]) and the 'Analysis of evolutionary diversification' package (GEIGER[70]) in R. The method of PGLS was implemented for the trait data using the 'Comparative analyses of phylogenetics and evolution' package (CAPER[71]) in R using Pagel's branch length transformations (lambda, λ)[72].

Data Availability

The data analysed during the current study are available from the corresponding author on reasonable request.

References

1. Schmidt-Nielsen, K. *Animal Physiology - Adaptation and Environment.* (Cambridge University Press, 1997).
2. Geiser, F. Reduction of metabolism during hibernation in mammals and birds: temperature effect or physiological inhibition? *J. Comp. Physiol.* **158**, 25–37 (1988).
3. Heldmaier, G., Ortmann, S. & Elvert, R. Natural hypometabolism during hibernation and daily torpor in mammals. *Respir. Physiol. Neurobiol.* **141**, 317–329 (2004).
4. Heldmaier, G., Steinlechner, S., Ruf, T., Wiesinger, H. & Klingenspor, M. Photoperiod and thermoregulation in vertebrates: body temperature rhythms and thermogenic acclimation. *J. Biol. Rhythms* **4**, 251–65 (1989).
5. Ruf, T. & Geiser, F. Daily torpor and hibernation in birds and mammals. *Biol. Rev.* **90**, 891–926 (2015).
6. Mauget, C., Mauget, R. & Sempéré, A. Metabolic rate in female European roe deer (*Capreolus capreolus*): incidence of reproduction. *Can. J. Zool.* **75**, 731–739 (1997).
7. Lawler, J. P. & White, R. G. Seasonal changes in metabolic rates in muskoxen following twenty-four hours of starvation. *Rangifer* **17**, 135–138 (1997).
8. Williams, J. B., Ostrowski, S., Bedin, E. & Ismail, K. Seasonal variation in energy expenditure, water flux and food consumption of Arabian oryx *Oryx leucoryx*. *J. Exp. Biol.* **204**, 2301–2311 (2001).
9. Kuntz, R., Kubalek, C., Ruf, T., Tataruch, F. & Arnold, W. Seasonal adjustment of energy budget in a large wild mammal, the Przewalski horse (*Equus ferus przewalskii*) I. Energy intake. *J. Exp. Biol.* **209**, 4557–65 (2006).
10. Arnold, W. *et al.* Nocturnal hypometabolism as an overwintering strategy of red deer (Cervus elaphus). *Am. J. Physiol. Integr. Comp. Physiol.* **286**, R174–R181 (2004).
11. Signer, C., Ruf, T. & Arnold, W. Hypometabolism and basking: the strategies of Alpine ibex to endure harsh over-wintering conditions. *Funct. Ecol.* **25**, 537–547 (2011).
12. Brinkmann, L., Gerken, M., Hambly, C., Speakman, J. R. & Riek, A. Saving energy during hard times: energetic adaptations of Shetland pony mares. *J. Exp. Biol.* **217**, 4320–4327 (2014).
13. Marín, J. C., Romero, K., Rivera, R., Johnson, W. E. & González, B. A. Y-chromosome and mtDNA variation confirms independent domestications and directional hybridization in South American camelids. *Anim. Genet.* **48**, 591–595 (2017).
14. Kadwell, M. *et al*. Genetic analysis reveals the wild ancestors of the llama and the alpaca. *Proc Biol Sci* **268**, 2575–2584 (2001).
15. Bonavia, D. *Los camélidos sudamericanos: una introducción a su estudio.* (Institut français d'études andines, 1996).

16. Food and Agricultural Organization of the United Nations. Domestic Animal Diversity Information System (DAD-IS). Food and Agricultural Organization of the United Nations (2018). Available at, http://www.fao.org/dad-is/browse-by-country-and-species/en/. (Accessed: 4th July 2018).

17. Göbel, B. The symbolism of llama breeding in North-Western Argentina. In *Progress in South American camelids research. Proc. 3rd European Symposium on South American Camelids and SUPREME European Seminar* (eds Gerken, M. & Renieri, C.) 175–180 (Wageningen Press, 2001).

18. Jürgens, K. D., Pietschmann, M., Yamaguchi, K. & Kleinschmidt, T. Oxygen binding properties, capillary densities and heart weights. *J. Comp. Physiol. B* **158**, 469–477 (1988).

19. Fowler, M. *Medicine and surgery of camelids.* (Wiley-Blackwell, 2010).

20. Riek, A. *et al.* Seasonal changes in energy expenditure, body temperature and activity patterns in llamas (*Lama glama*). *Sci. Rep.* **7**, 7600 (2017).

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

22. Riek, A. & Geiser, F. Allometry of thermal variables in mammals: consequences of body size and phylogeny. *Biol. Rev. Camb. Philos. Soc.* **88**, 564–572 (2013).

23. de Lamo, D. A. *Temperature regulation and energetics of the guanaco* (*Lama guanacoe*). (University of Illinois, 1989).

24. Geiser, F. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu. Rev. Physiol.* **66**, 239–274 (2004).

25. Fuller, A., Maloney, S. K., Kamerman, P. R., Mitchell, G. & Mitchell, D. Absence of selective brain cooling in free-ranging zebra in their natural habitat. *Exp. Physiol.* **85**, 209–217 (2000).

26. Hetem, R. S., Maloney, S. K., Fuller, A. & Mitchell, D. Heterothermy in large mammals: Inevitable or implemented? *Biol. Rev.* **91**, 187–205 (2016).

27. Hetem, R. S. *et al.* Effects of desertification on the body temperature, activity and water turnover of Angora goats. *J. Arid Environ.* **75**, 20–28 (2011).

28. Kamerman, P. R., Fuller, A., Faurie, A. S., Mitchell, G. & Mitchell, D. Body temperature patterns during natural fevers in a herd of free-ranging impala (*Aepyceros melampus*). *Vet. Rec.* **149**, 26–27 (2001).

29. Hebert, J. *et al.* Thermoregulation in pronghorn antelope (*Antilocapra americana*, Ord) in winter. *J. Exp. Biol.* **211**, 749–756 (2008).

30. Fuller, A. *et al.* A year in the thermal life of a free-ranging herd of springbok *Antidorcas marsupialis*. *J. Exp. Biol.* **208**, 2855–2864 (2005).

31. Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A. & Houpt, T. R. Body temperature of the camel and its relation to water economy. *Am. J. Physiol.* **188**, 103–112 (1957).

32. Bligh, J., Baumann, I., Sumar, J. & Pocco, F. Studies of body temperature patterns in South American Camelidae. *Comp Biochem Physiol A* **50**, 701–708 (1975).

33. Brinkmann, L., Gerken, M. & Riek, A. Adaptation strategies to seasonal changes in environmental conditions of a domesticated horse breed, the Shetland pony (*Equus ferus caballus*). *J. Exp. Biol.* **215**, 1061–1068 (2012).

34. Piccione, G., Caola, G. & Refinetti, R. The circadian rhythm of body temperature of the horse. *Biol. Rhythm Res.* **33**, 113–119 (2003).

35. Brinkmann, L., Gerken, M. & Riek, A. Adaptation strategies of Shetland ponies (*Equus ferus caballus*) to seasonal changes in climatic conditions and food availability. *Zuchtungskunde* **85**, 58–73 (2013).

36. Taylor, C. R. & Lyman, C. P. A Comparative study of environmental physiology of an East African antelope: Eland and Hereford steer. *Physiol. Zool.* **40**, 280 (1967).

37. Hetem, R. S. *et al.* Variation in the daily rhythm of body temperature of free-living Arabian oryx (*Oryx leucoryx*): Does water limitation drive heterothermy? *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **180**, 1111–1119 (2010).

38. Langman, V. A. & Maloiy, G. M. O. Passive obligatory heterothermy of the giraffe. *J. Physiol.* **415**, P89 (1989).

39. Ostrowski, S. & Williams, J. B. Heterothermy of free-living Arabian sand gazelles (*Gazella subgutturosa marica*) in a desert environment. *J. Exp. Biol.* **209**, 1421–1429 (2006).

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

41. Weissenböck, N. M., Arnold, W. & Ruf, T. Taking the heat: Thermoregulation in Asian elephants under different climatic conditions. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **182**, 311–319 (2012).

42. Nagy, K. A., Sanson, G. D. & Jacobsen, N. K. Comparative field energetics of 2 macropod marsupials and a ruminant. *Aust. Wildl. Res.* **17**, 591–599 (1990).

43. Gotaas, G., Milne, E., Haggarty, P. & Tyler, N. J. C. Energy expenditure of free-living reindeer estimated by the doubly labelled water method. *Rangifer* **20**, 211–219 (2000).

44. Nagy, K. A. & Knight, M. H. Energy, water, and food use by springbok antelope (*Antidorcas marsupialis*) in the Kalahari Desert. *J. Mammal.* **75**, 860–872 (1994).

45. Haggarty, P. *et al.* Estimation of energy expenditure in free-living red deer (*Cervus elaphus*) with the doubly-labelled water method. *Br. J. Nutr.* **80**, 263–272 (1998).

46. Munn, A. J. *et al.* Field metabolic rate and water turnover of red kangaroos and sheep in an arid rangeland: an empirically derived dry-sheep-equivalent for kangaroos. *Aust. J. Zool.* 23–28 (2008).

47. Riek, A., van der Sluijs, L. & Gerken, M. Measuring the energy expenditure and water flux in free-ranging alpacas (*Lama pacos*) in the peruvian andes using the doubly labelled water technique. *J. Exp. Zool. Part A Ecol. Genet. Physiol.* **307A**, 667–675 (2007).

48. Dittmann, M. T. *et al.* Characterising an artiodactyl family inhabiting arid habitats by its metabolism: Low metabolism and maintenance requirements in camelids. *J. Arid Environ.* **107**, 41–48 (2014).

49. Nie, Y. *et al.* Exceptionally low daily energy expenditure in the bamboo-eating giant panda. *Science* **349**, 171–174 (2015).

50. Signer, C. *et al.* A versatile telemetry system for continuous measurement of heart rate, body temperature and locomotor activity in free-ranging ruminants. *Methods Ecol. Evol.* **1**, 75–85 (2010).

51. Gauly, M., Vaughan, J. & Cebra, C. *Neuweltkameliden: Haltung, Zucht, Erkrankungen*. (Enke, 2010).

52. Lifson, N. & McClintock, R. Theory of use of the turnover rates of body water for measuring energy and material balance. *J. Theor. Biol.* **12**, 46–74 (1966).

53. Speakman, J. R. *Doubly labelled water: theory and practice.* (Chapman & Hall, 1997).

54. Speakman, J. R. & Racey, P. A. The equilibrium concentration of O18 in body-water - Implications for the accuracy of the doubly-labeled water technique and a potential new method of measuring RQ in free-living animals. *J. Theor. Biol.* **127**, 79–95 (1987).

55. Nagy, K. A. The doubly labeled water (3HH 18O) method: a guide to its use. (Laboratory ofBiomedical and Environmental Sciences, University of California, 1983).

56. Speakman, J. R. How should we calculate CO_2 production in doubly labeled water studies of animals? *Funct. Ecol.* **7**, 746–750 (1993).

57. Visser, G. H. & Schekkerman, H. Validation of the doubly labeled water method in growing precocial birds: The importance of assumptions concerning evaporative waterloss. *Physiol. Biochem. Zool.* **72**, 740–749 (1999).

58. Van Trigt, R. *et al.* Validation of the DLW method in Japanese quail at different water fluxes using laser and IRMS. *J. Appl. Physiol.* **93**, 2147–2154 (2002).

59. Schoeller, D. A. *et al.* Energy expenditure by doubly labeled water - validation in humans and proposed calculation. *Am. J. Physiol.* **250**, R823–R830 (1986).

60. Oftedal, O. T., Hintz, H. F. & Schryver, H. F. Lactation in the horse: milk composition and intake by foals. *J Nutr* **113**, 2096–2106 (1983).
61. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria Available at, https://www.r-project.org/ (2018).
62. Hijmans, R. J. Geosphere: Spherical Trigonometry (2016).
63. Garland, T. & Ives, A. R. Using the past to predict the present: Confidence intervals for regression equations in phylogenetic comparative methods. *Am. Nat.* **155**, 346–364 (2000).
64. Rohlf, F. J. Comparative methods for the analysis of continuous variables: Geometric interpretations. *Evolution (N. Y).* **55**, 2143–2160 (2001).
65. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).
66. Garland, T., Harvey, P. H. & Ives, A. R. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**, 18–32 (1992).
67. Freckleton, R. P., Harvey, P. H. & Pagel, M. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712–26 (2002).
68. Fritz, S. A., Bininda-Emonds, O. R. P. & Purvis, A. Geographical variation in predictors of mammalian extinction risk: Big is bad, but only in the tropics. *Ecol. Lett.* **12**, 538–549 (2009).
69. Paradis, E., Claude, J. & Strimmer, K. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
70. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E. & Challenger, W. GEIGER: Investigating evolutionary radiations. *Bioinformatics* **24**, 129–131 (2008).
71. Orme, D. *et al.* CAPER: Comparative analyses of phylogenetics and evolution in R (2012).
72. Pagel, M. D. A method for the analysis of comparative data. *J. Theor. Biol.* **156**, 431–442 (1992).

Acknowledgements

Author Contributions

A.R. conceived the experiment, A.S. and A.R. conducted the experiment in Peru, R.M.B. contributed to the organization and execution of the experiment, T.R. and W.A. contributed to the analysis of the telemetry data, J.R.S. and C.H. conducted the analysis of the doubly labelled water samples. M.G. helped with organizing the field trip. A.R. wrote the manuscript and all authors reviewed the manuscript.

Additional Information

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.