CIRCADIAN RHYTHM IS MAINTAINED DURING HIBERNATION IN YELLOW-BELLIED MARMOTS

СОХРАНЕНИЕ ЦИРКАДНЫХ РИТМОВ У ЖЕЛТОБРЮХИХ СУРКОВ ВО ВРЕМЯ СПЯЧКИ

LE RYTHME CIRCADIEN EST MAINTENU DURANT L'HIBERNATION CHEZ LES MARMOTTES À VENTRE JAUNE

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Abstract

Circadian rhythms of body temperature were evident during deep torpor in 11 of 12 individuals. The mean period was 23.94 hours. Circadian cycles of oxygen consumption were apparent in five of 14 marmots. Arousal tended to occur during the increase phase of the cycle. This report may be the first to demonstrate cycles of oxygen consumption during deep torpor.

Key-words: *Circadian rhythm, body temperature, oxygen consumption, torpor.*

Резюме

Циркадные ритмы температуры тела были очевидны в течение периода глубокой спячки у 11 из 12 особей. Средний период был 23.94 часа. Циркадные циклы потребления кислорода были очевидны у 5 из 14 сурков. Пробуждение имело тенденцию происходить в течение стадии увеличения цикла. Это сообщение может быть первым, демонстрирующим циклы потребления кислорода в течение глубокой спячки.

Ключевые слова: *циркадные ритмы, температура тела, потребление кислорода, спячка.*

Résumé

Des rythmes circadiens de la température corporelle étaient évidents pendant la torpeur profonde chez 11 individus sur 12. La période moyenne était de 23,94 heures. Des rythmes circadiens de consommation de l'oxygène étaient évidents chez 5 marmottes sur 14. L'éveil tend à se produire au cours de la phase croissante du cycle. Ce rapport pourrait être la première démonstration de l'existence de cycle de consommation d'oxygène pendant la torpeur profonde.

Mots-clés : *Rythme circadien, température corporelle, consommation d'oxygène, torpeur.*

Introduction

Hibernation in ground-dwelling squirrels is characterized by a reduction in body temperature, metabolic rate, and breathing rate, by a markedly reduced heart rate, by a state of torpor more profound than deep sleep (Bartholomew 1982), and by a reduced immune response (Jaroslow & Smith 1961, Sidky et al 1972). This reduction of many physiological functions raises the question of whether circadian rhythms persist during deep torpor; i.e., is 24-hour-periodicity independent of low body temperature (Pohl 1964).

Circadian rhythmicity was evident in the body temperature (T_B) of euthermic *Spermophilus parryi* (Folk & Folk 1964) and *S. richardsoni* (Wang 1972), in activity cycles of euthermic *S. lateralis* (Mrosovsky et al 1976; Zucker et al 1983) and probably occurred in the T_B and metabolic rate (VO_2) of euthermic *S. tereticaudus* (Hudson 1964). Circadian cycles of activity were described during hibernation of *Marmota marmota*, but these cycles occurred only during arousal when the marmots were euthermic (Cochet et al 1992).

A 24-hour periodicity disappeared when the T_B of *S. lateralis* decreased below 10^0C; however, measurable rhythms continued for several days during multi-day torpor before the amplitude decreased and rhythmicity disappeared (Pohl 1964). Circadian rhythmicity was evident in a minority of hibernating *S. parryi* when T_B exceeded 8^0C and was evident during euthermia and daily torpor (Barnes & Ritter 1993). Only in *S. lateralis* have persistent circadian rhythms, in T_B, been demonstrated during multi-day torpor (Grahn et al 1994). Given that arousal during torpor may be timed by a circadian rhythm (Pohl 1967, Strumwasser et al 1967), one would expect circadian rhythms to be present in other *Marmotini*.

In this paper we demonstrate that circadian rhythms of T_B and VO_2 persist during deep torpor in the yellow-bellied marmot (*Marmota flaviventris*). To our knowledge, this is the first report of circadian rhythms during multi-day torpor in a marmot and the second report for *Marmotini*.

Materials and methods

Yellow-bellied marmots were live-trapped in the Upper East River Valley, Gunnison County, Colorado in August of 1995 and 1996. Because marmots in this area have been studied since 1962 (Armitage 1991), the age, sex, and relatedness was known for each animal. The marmots were transported to The University of Kansas where a temperature-sensitive radio transmitter (Mini-Mitter VM-FH) was surgically implanted in the abdominal cavity of each animal. Animals were housed singly, except for two groups of three littermate young each, in standard rabbit cages placed in temperature-controlled walk-in environmental rooms. Animals were given at least two weeks to recover from surgery before conditions for hibernation were initiated. During this period room temperature was maintained at 15^0C and photoperiod was 12L:12D. Each cage was supplied with a metal nest box and paper towels for nest material. Purina lab chow and water were provided *ad libitum*. Conditions for hibernation were established by decreasing the room temperature to either 10^0C or 6^0C, removing food and water, and maintaining constant darkness. The marmots were monitored daily with the aid of a red light to determine hibernation status. Status was recorded as alert (eyes open, head up) or torpid (body curled, head tucked under).

We kept one group of young, (two females and one male), two female young, three male young, two adult males, and three adult females at 10^0C and one group of young, (two males and one female), one female young, one male young, and two female adults at 6^0C. Individuals are identified by their left tag number and age and sex (Y = young, A = adult, F = female, M = male).

When T_B and VO_2 were measured, the nest box with the torpid marmot was placed in a plexiglass metabolism chamber which was placed in an environmental growth chamber (see Armitage & Salsbury 1992, for details of measuring system and procedures) maintained at the hibernating temperature. T_B and VO_2 were measured through one entire torpor bout for each animal or group of three. In addition, some animals were measured at ambient temperatures lower than the hibernating temperature. Because of the length of time of a complete torpor bout, usually only one torpor bout was measured for each animal or group of three, but a few animals were repeated. T_B and VO_2 were recorded every five minutes with a Data Quest III data analysis system. All VO_2 values were converted to standard pressure. Hourly means were calculated from the recorded data and the hourly means were used to determine if circadian cycles occurred.

The presence of circadian cycles was determined by examining the data for peaks in VO_2 and T_B and measuring the time between peaks. Amplitude of a cycle was measured as the vertical distance between the trough and peak of each individual period. Means for period and amplitude were calculated for each animal during multi-day torpor and compared using a t-test.

Results and discussion

Complete torpor bouts were recorded for all animals except for an adult male that died at the beginning of the study in 1995, and an adult female that completed hibernation before a torpor bout was measured. A juvenile male died in the spring of 1997 during his second run. Both males had symptoms suggestive of pneumonia. T_B was not recorded for two marmots because of transmitter failure, but oxygen consumption was recorded for all animals.

Body temperature

Circadian rhythms of T_B were evident in 11 of 12 individuals (Fig. 1). A circadian rhythm was not discernable in AF1234 (Fig. 2). It is unknown whether she may have exhibited a circadian clock in other torpor bouts that were unrecorded. Although AF993 was maintained for two weeks at 6^{0}C, she remained euthermic with no evidence of a circadian pattern (Fig. 3). This behavior occurred in May when marmots at our study site typically terminate hibernation. The wide swings in T_B indicate that she may have been in a transition stage between hibernation and arousal. Physiological mechanisms invoked in achieving euthermy may have obscured underlying circadian patterns.

The mean period of the circadian rhythm was 23.94 hrs ± 4.795 SD, n = 11. Only 2 individuals maintained an approximate 24-hour period for the entire bout (SD < 2 hrs). Period length ranged from 12.5 to 73 hours. A period greater than 48 hrs occurred four times in three animals: 73, 51.5, 48, and 54 hrs. Most long periods were divisible by 24, which suggest a 24-hour clock was still operating. For example, Group 97 approximated a 24-hour cycle, but only when the first period of 54 hrs is divided by 2 and a value of 27 is used to calculate the mean. During these long periods, temperature peaks were either too small to detect, were too numerous to discern a single peak, or no peak was evident.

Each individual had a unique pattern in T_B fluctuations during its torpor bout (Table 1). Individual periods did not significantly differ from 24 hrs. P values ranged from 0.10 to 0.94 (one-sample t-test, $\mu = 24$). P values of AF1234 and YF2236 approached significance. When an extreme value was removed, AF1234 had a period significantly shorter than 24 hrs ($p < 0.005$). AF1234 is not considered to have exhibited a circadian rhythm (Fig. 2). YF2236 exhibited a 24-hour cycle only for the first five days, after which her period length ranged from 12.5 to 15 hrs with one period of 24 hrs. When combined, the smaller periods approximated 24 hrs, which suggests that peaks are caused by stimuli other than the circadian clock. Such stimuli may induce partial arousals with a subsequent return to torpor (Lyman & O'Brien 1969).

Table 1.

Range, mean and standard deviation of the period (hours) of body temperature cycles for marmots housed at 6 and 10^{0}C. n = number of cycles. T_A = ambient temperature.

Period					
T_A	Individual	Range	Mean	SD	n
10^{0}C	AF1234	13-32	18.20	7.88	5
	Group96	13.5-30	23.57	6.03	7
	YGF2245	13-26.5	21.80	5.32	5
	YGF2297	12-36.5	25.43	9.55	7
	YM2247	17.5-30.5	24.21	4.24	7
	YM2194	18-73	24.39	4.24	11
	YM2038	23-27	24.42	1.59	6
6^{0}C	YF2236	12.5-27.5	20.65	5.84	10
	YF2275	20-30	25.62	4.92	4
	YM2255	23-27	24.7	1.48	5
	AF1227	18.5-51.5	24.91	3.96	8
	Group97	21-54	23.90	2.79	5
	Grand mean	12.5-73	23.940*	4.795	74*

*does not contain values from AF1234

The amplitudes of T_B fluctuations varied both within a torpor bout and between animals. T_B amplitudes of animals at 10^0C were significantly lower than those of animals at 6^0C ($p < 0.05$). However, the higher amplitudes in 6^0C animals resulted from two animals (YF2275 and YM2255) with unusually high amplitudes of 1 to 2^0C. When YF2275 and YM2255 were excluded from the analysis, amplitudes for 10^0C animals had a higher mean than that of marmots housed at 6^0C. Amplitudes of rhythms of torpid *S. lateralis* were independent of ambient temperature (Grahn et al 1994). Because our data were strongly affected by small sample size and individual variability, we conclude that there is no evidence to support an effect of temperature on amplitude.

Oxygen

The mean period for the 14 animals was 22.18 hrs 7.17 (Table 2). Circadian cycles of VO_2 were apparent in five of the 14 marmots (Fig. 1). We know of no other report of circadian cycles in VO_2 during deep torpor. Although the data are scanty, they suggest that a circadian system may be present. Possibly a circadian clock of oxygen consumption may drive the circadian rhythm of T_B (Fig. 1). For animals for which we have T_B data, VO_2 began to increase prior to that of T_B as often as T_B increased before VO_2. However, other factors affect VO_2 (Lyman & O'Brien 1969, Heldmaier et al 1993) and the relationship between rhythms of VO_2 and T_B is unclear.

Table 2.

Range, mean and standard deviation of the period (hours) of oxygen consumption cycles for marmots housed at 6 and 10^0C. n = number of cycles. T_A = ambient temperature.

Period					
T_A	**Individual**	**Range**	**Mean**	**SD**	**n**
10^0C	AF1234	25-28.5	26.75	2.47	2
	AM2055	10-25	19.71	4.66	12
	YGF2245	11-38	22.86	8.55	7
	YGF2297	24-35	28.12	5.11	4
	YM2247	19-39	27.67	7.12	6
	YM2194	10-40	20.40	10.14	27
	YM2038	13-30	19.9	5.70	20
	AF245	7-21.5	15.00	7.37	3
6^0C	YF2236	13-39	23.65	8.27	10
	YF2275	20-28	24.75	3.59	4
	YM2255	21.5-28	24.2	2.75	5
	AF1227	14.5-32	23.5	5.72	11
	Group97	11-26	19.75	5.75	8
	Grand mean	7-40	22.18	7.165	97

Function of the circadian cycle

The circadian cycle could control the length of the hibernation bout through a temperature-sensitive clock running slower at lower body temperature (Heller et al 1989) or by a temperature-compensated clock (Pohl 1967, 1987) where bout length is a function of the number of circadian cycles (Grahn et al 1994). In hibernating *S. lateralis*, *S. columbianus*, and *S. tridecemlineatus*, the length of the period of uninterrupted hibernation is linearly related to environmental temperature (Twente et al 1977). This observation is consistent with a temperature-sensitive clock, but could also be consistent with a temperature-compensated clock if the number of circadian cycles is affected by temperature. In contrast, the lack of any difference in period length in marmots at 6^0C and 10^0C (Table 1) supports a temperature-compensated biological clock.

The length of the bout of deep torpor consists of multiples of 24-hour cycles (Grah et al 1994). Thus, arousal should occur on the increase phase of a cycle. Unlike Grahn et al (1994), our data were insufficient to predict the time of arousal, but like Grahn et al, arousals tended to occur during the increase phase of the T_B cycle. Arousals occurred 16.69 " 7.52 hrs (range 8 to 36.5 hrs) after the peak prior to arousal. Three of 12 animals aroused during the decrease phase of the T_B cycle, less than 12 hrs after the previous peak. Thus, our data are consistent with the interpretation that the circadian cycle controls the length of a hibernation bout by determing the time of arousal.

Conclusions

This study demonstrates that the circadian pacemaker of the yellow-bellied marmot is active during deep torpor and probably operates throughout the hibernating season. It is unclear if VO_2 is controlled by the circadian system, but there is clear evidence of its existence in some marmots. Although arousal tended to occur during the increase phase of the cycle, data were insufficient to use the circadian cycle to predict arousals. There were no clear differences in amplitude or period between marmots at 6^0C and 10^0C, which suggests that the circadian clock is temperature-compensated.

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FIGURES / *РИСУНКИ*

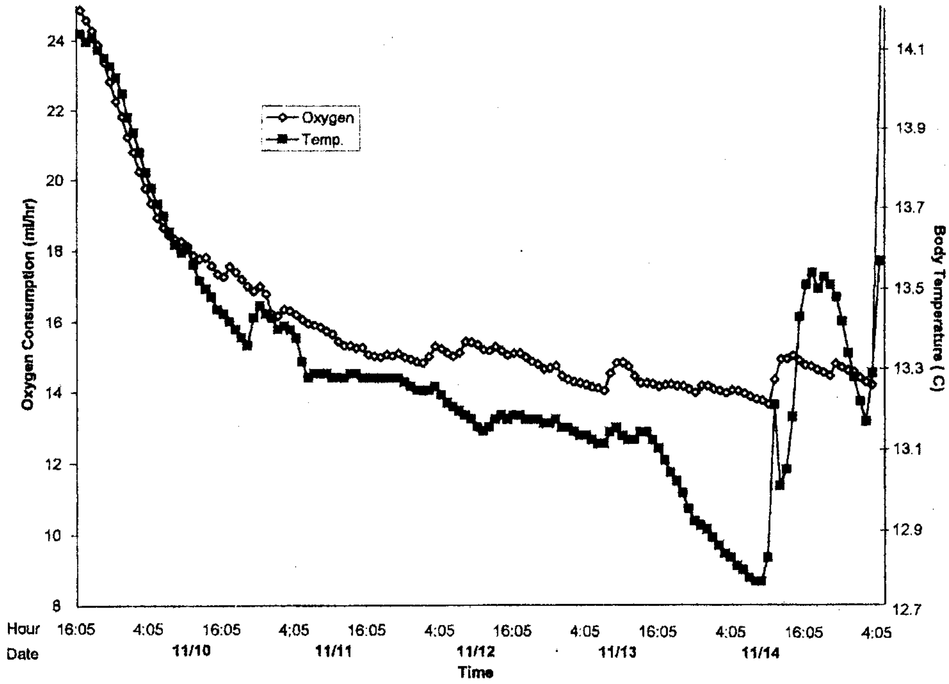

Fig. 1. Body temperature and oxygen consumption of YM2255 during deep torpor at 6^{0}C ambient temperature in constant darkness.

Рис. 1. *Температура тела и потребление кислорода у особи YM2255 в течение глубокой спячки при температуре 6^{0}С в постоянной темноте.*

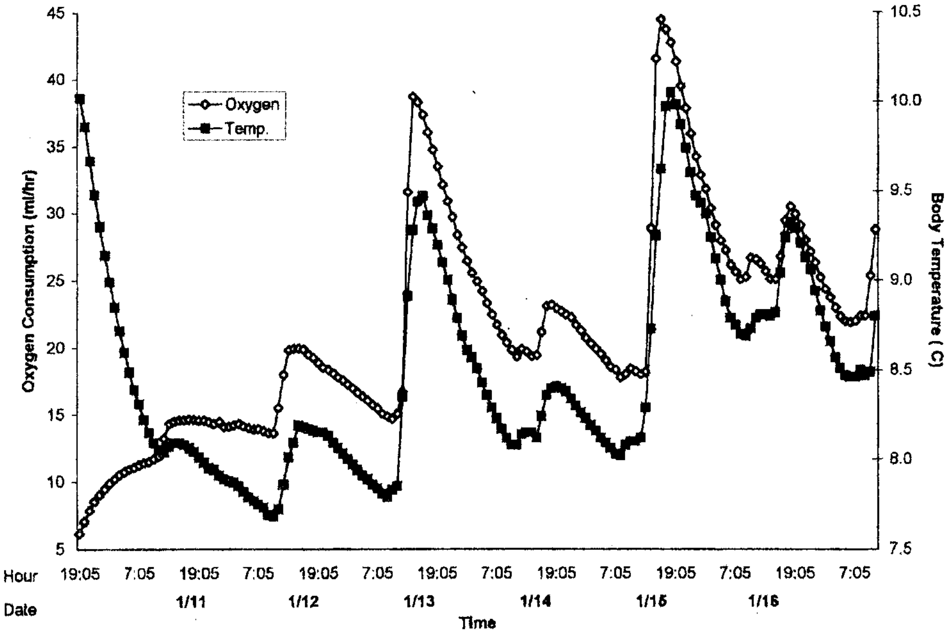

Fig. 2. Body temperature and oxygen consumption of AF1234 during deep torpor at 10^{0}C ambient temperature in constant darkness.

Рис. 2. *Температура тела и потребление кислорода у особи AF1234 в течение глубокой спячки при температуре 10^{0}С в постоянной темноте.*

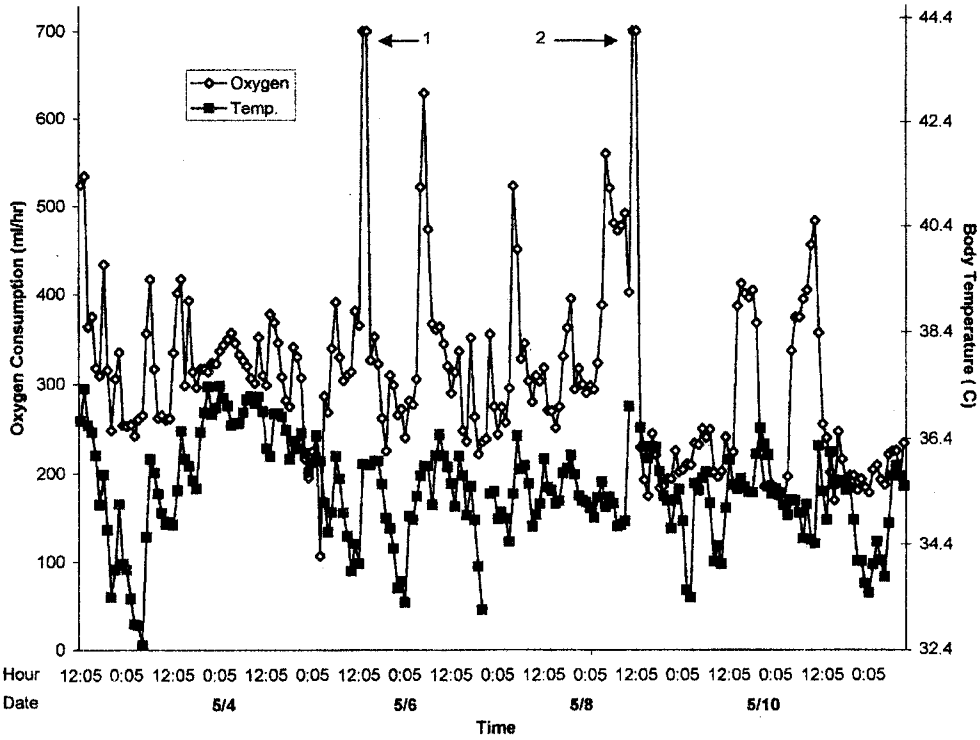

Fig. 3. Body temperature and oxygen consumption of AF993 during deep torpor at 6^0C ambient temperature in constant darkness. The two arrows indicate data points that were lowered to fit them on the graph; actual values exceeded 700 ml/hr.

Рис. 3. *Температура тела и потребление кислорода у особи AF993 в течение глубокой спячки при температуре 6^0C в постоянной темноте. Две стрелки указывают на точки, не уместившиеся в поле графика – реальные значения более 700 мл в час.*

Translated into Russian by I.V. Rymalov
Перевод на русский язык И.В. Рымалова

Введение

Спячка сусликов характеризуется уменьшением температуры тела, уровня метаболтизма и интенсивности дыхания, заметным снижением частоты сердцебиений, оцепенением телда, более глубоким, чем во время сна (Bartholomew, 1982), и уменьшением иммунного статуса организма (Jaroslow, Smith, 1961, Sidky et al., 1972). При такой редукции интенсивности физиологических процессов у зимоспящих животных возникает вопрос - сохраняются ли циркадные ритмы организма во время глубокого оцепенения, т.е. зависят ли 24-х часовые циклы от пониженной температуры тела во время гибернации (Pohl, 1964).

Циркадные ритмы температуры тела во время спячки (Тт) отмечены у зимоспящих *Spermophilus parry* (Polk, Folk, 1964) и *S. richardsoni* (Wang, 1972), в активном состоянии такие циклы описаны у *S. lateralis* (Morosovsky et al., 1975; Zucker et al., 1982). Возможно, что циркадные ритмы температуры тела (Тт) и изменения метаболизма (VO_2) есть у зимоспящего суслика *S. tereticaudus* (Hadson, 1964).

УДК 599.322.2

Holarctic Marmots as a Factor of Biodiversity (K.B. Armitage & V.Yu. Rumiantsev Eds). - *Proceedings of The 3d International Conference on Marmots, Cheboksary, Russia, 25-30 August 1997*. - Moscow: ABF P.H., **2002**, 411 p.

The results of researches on marmots carried out mainly during the 1990th in Russia and abroad are discussed: theoretical and practical problems of marmots' ecology and ethology, their protection and population management.

The book may be useful for wide range of zoologists, specialists on animals' protection and management, students of biology, etc.

Editors: **Kennet B. Armitage & Vadim Yu. Rumiantsev**

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