An hourglass mechanism controls torpor bout length in hibernating garden dormice

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Author contributions
TR conceived the ideas and led the writing of the manuscript. KG and SG collected the data. KG
managed the data and computed variables, SG provided the experimental animals and equipment.
GS and HG implanted transmitters. TR analysed the data. All authors contributed critically to the
drafts and gave final approval for publication.

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Summary statement

In hibernating garden dormice, torpor bout length depends on oxygen consumption. This indicates that torpor duration is determined by accumulation of a metabolic imbalance, which is cleared during periodic rewarming.

Abstract

Hibernating mammals drastically lower their rate of oxygen consumption and body temperature ($T_b$) for up to several weeks, but regularly rewarm and stay euthermic for brief periods (< 30 h). It has been hypothesized that these periodic arousals are driven by the development of a metabolic imbalance during torpor, that is, the accumulation or the depletion of metabolites or the accrual of cellular damage that can be eliminated only in the euthermic state. We obtained oxygen consumption (as a proxy of metabolic rate) and $T_b$ at 7-minute intervals over entire torpor-arousal cycles in the garden dormouse (*Eliomys quercinus*). Torpor bout duration was highly dependent on mean oxygen consumption during the torpor bout. Oxygen consumption during torpor, in turn, was elevated by $T_b$, which fluctuated only slightly in dormice kept at ~ 3-8°C. This corresponds to a well-known effect of higher $T_b$ on shortening torpor bout lengths in hibernators. Arousal duration was independent from prior torpor length, but arousal mean oxygen consumption increased with prior torpor $T_b$. These results, particularly the effect of torpor oxygen consumption on torpor bout length, point to an hourglass mechanism of torpor control, i.e., the correction of a metabolic imbalance during arousal. This conclusion is in line with previous comparative studies providing evidence for significant interspecific inverse relationships between the duration of torpor bouts and metabolism in torpor. Thus, a simple hourglass mechanism is sufficient to explain torpor/arousal cycles, without the need to involve non-temperature-compensated circadian rhythms.

Introduction

Hibernation in mammals is characterized by a profound reduction of metabolic rate, often to a level of $\leq$ 5% of basal metabolic rate (BMR) (Ruf and Geiser, 2015). Typically, this decrease of metabolic rate is accompanied by a reduction of body temperature ($T_b$) to values just above ambient temperature ($T_a$). However, most hibernators do not maintain low metabolic rate and $T_b$ throughout winter. Apart from a few species that can continually hibernate at $T_b$s of approximately 30°C or even above that (Dausmann et al., 2004; Tøien et al., 2011), hibernating mammals regularly rewarm from the torpid to the euthermic state during so-called spontaneous arousals (Fig. 1). The maximum duration of torpor bouts is a species-specific trait and varies from ~3 to 98 days (Ruf and Geiser,
Rewarming and subsequent intervals of interbout euthermia (IBE) are responsible for at least 70% of the total energy expenditure over winter (Wang, 1979). However, since the first discovery of these spontaneous “periodic changes” of T_b (Hall, 1832), their function has remained unclear. Among other hypotheses, it has been suggested that hibernators rewarm in order to sleep (Daan et al., 1991; Trachsel et al., 1991), to combat pathogens (Prendergast et al., 2002), or to restore enzymes required for cardiac function at low T_b (Ruf and Arnold, 2008). However, the “warming up for sleep” hypothesis has been refuted (Larkin and Heller, 1998; Strijkstra and Daan, 1998), and the other hypotheses remain speculative. Similarly, the clock mechanisms that control the timing of torpor and arousal within the hibernation season are entirely unknown, and even their fundamental nature is a question of debate (Malan, 2010; Ruf and Geiser, 2015).

Over several decades, it had been assumed repeatedly that the torpor-arousal cycle is driven by a so-called hourglass mechanism. This hypothesis assumes the development of a metabolic imbalance during torpor, that is, the accumulation or depletion of metabolites or the accrual of cellular damage that can be eliminated only in the euthemic state (Carey et al., 2003; Fisher, 1964; French, 1985; Galster and Morrison, 1976; Lyman et al., 1982; Martin and Epperson, 2008; Strijkstra, 1999; Twente and Twente, 1968; Van Breukelen and Martin, 2002b). It seems, however, that at the low body temperatures of deep torpor the continued degradation and depletion of metabolites is much more likely than its energy consuming production and accumulation. An effect of metabolism on torpor bout length also seemed to be supported by a study by French (1985) that pointed to a decrease of torpor bout duration with body mass - and, by inference, oxygen consumption increase - albeit in a limited sample of mammals. A more recent comparative study showed that torpor bout duration among mammals in fact decreases as minimum oxygen consumption in torpor increases (Ruf and Geiser, 2015). This result is fully compatible with the idea of a metabolic imbalance, such as a metabolite deficiency, that is formed faster if torpor oxygen consumption is high. Also, the hourglass hypothesis seemed to be supported, for instance, by the observation that in ground squirrels an increase in oxygen consumption during torpor, due to animals defending a setpoint T_b at very low T_a, is associated with a shortening of torpor bout duration (Buck and Barnes, 2000; Geiser and Kenagy, 1988). However, the conclusiveness of these studies was somewhat limited by the fact that measurements were either restricted to certain time points during torpor episodes (Geiser and Kenagy, 1988), or T_b and oxygen consumption were obtained from different individuals than torpor bout duration (Buck and Barnes, 2000).
The main reason why the existence of such an hourglass mechanism was dismissed in the past, was the complete absence of an effect of body mass on torpor duration, when hibernating animals were compared (Geiser and Ruf, 1995; Malan, 2010; Ruf and Geiser, 2015). Malan (2010) argued that the absence of an effect of body mass on torpor bout duration is reason to refute the hourglass hypothesis. Indeed, as metabolic rate is usually strongly affected by body mass, it seems logical to assume that independence of torpor bout duration from body mass also should reflect independence of the torpor-arousal cycle from metabolism. Instead of an hourglass mechanism, Malan (2010) therefore proposed the existence of a specialized, non-temperature compensated circadian clock that governs torpor-arousal cycles.

Therefore, here we obtained data on entire torpor-arousal cycles in the garden dormouse (*Eliomys quercinus*), a medium sized hibernator (~100 g). We hypothesised that, if an hourglass is the governing mechanism, torpor bout duration should decrease with increasing mean torpor oxygen consumption measured over the entire torpor bout in the same individual. We also aimed to test whether torpor bout duration is affected by the previous arousals, e.g., by their duration or oxygen consumption during arousals. We further hypothesized that the duration or oxygen consumption during arousals may be affected by the previous torpor bout, if an hourglass mechanism is at work. Alternatively, if the torpor-arousal cycle was governed by a non-temperature-compensated circadian clock, the duration of torpor bouts should not be affected by oxygen consumption.

**Methods**

*Animals and housing*

The garden dormouse (*Eliomys quercinus*) is a nocturnal, arboreal and omnivorous rodent widely distributed in Europe. Garden dormice show deep hibernation with oxygen consumption depression down to <2% compared with their euthermic state while T<sub>b</sub> reaches 1 °C. The maximum duration of torpor bouts in this species is 20 days, the average during midwinter is 14 days (Ruf and Geiser, 2015).

The adult garden dormice used in this study were bred and raised at the Research Institute of Wildlife Ecology (FIWI), Vienna, Austria (latitude 48°15′ N, longitude 16°22′ E). Animals were reared in outdoor enclosures under natural variations of photoperiod and T<sub>a</sub>. Prior to hibernation, dormice were housed separately in polycarbonate cages (60×40×40 cm) and had access to food (Altromin 7024, Altromin GmbH & Co. KG, Lage, Germany) and water *ad libitum*. The dormice were also fed
with sunflower seeds and dry insects twice a week. During the experiments the animals had no access to food to simulate a natural situation of winter hibernation. The experiments involved 22 garden dormice with body masses ranging from 75 g to 169 g prior to hibernation.

**Experiments**

Experiments were carried out between November 2014 and April 2016. The rate of oxygen consumption (abbreviated VO\(_2\) in tables and figures) was measured in garden dormice during torpor by indirect calorimetry. Dormice were kept in an individually ventilated Perspex respiratory chamber (volume 5.4 l) supplied with fresh air. Respirometry chambers were placed inside refrigerators set to +5 °C, but fluctuated with the refrigerator control (range 2.9-7.8 °C: SD 0.67 °C). The temperature inside the refrigerators was measured with small (~2 g) temperature-loggers (custom made and calibrated at the Research Institute of Wildlife Ecology; accuracy ±0.1°C). Core \(T_b\) of each animal was continuously recorded with transmitters (model: TA-10TA-F20, 1.75 cc, 3.8 g, accuracy: 0.15 °C; Data Sciences International, Saint Paul, MN, USA). Transmitters were calibrated prior to implantation between 0 and 40 °C in a temperature-controlled water bath. The transmitters were surgically implanted under anaesthesia as described in detail elsewhere (Giroud et al., 2018). A receiver board (RPC-1; Data Sciences International, Saint Paul, MN, USA) was positioned under each individual cage to collect the radio frequency signals from transmitters. \(T_b\) was recorded for 10 s every 5 min. Each animal was held in the respiratory chamber until at least two arousal phases were recorded. The animals measured were removed from the refrigerators as part of another experiment (Huber et al., 2021). Therefore, we obtained a varying number of measurements from each animal. We obtained 114 records of IBE together with the complete preceding or subsequent torpor bout from 22 dormice. All animals were weighed before and after the experiment (but before re-feeding) to the nearest 0.1 g (CS 200, Ohaus, Parsippany, NJ, USA).

**Metabolic rate measurements**

Metabolic rate was measured as the rate of oxygen consumption determined by a dual channel oxygen analyser (Moxzilla, Sable Systems, Las Vegas, USA). The analyser was calibrated using a high precision gas-proportioning pump (H. Wösthoff, Bochum, Germany, type 55A27/7a). Flow rates through airtight respirometry chambers were measured with calibrated mass flow meters (FMA 3100, Omega Engineering, Stamford, CT, USA). A gas multiplexer enabled switching airflow between 6 animal chambers at 1-minute intervals. A 7\(^{th}\) empty respirometry chamber supplied with fresh air was recorded to continually correct for drift. Thus, a VO\(_2\) reading was recorded per animal at 7-minute intervals. Air leaving the respirometry chambers was not dried but relative humidity was
measured (RH300, Sable Systems, Las Vegas, USA) and corrected for. All recordings were interfaced to a computer (Labjack U6, Lakewood, Colorado, USA) and VO$_2$ was calculated by a self-written Python program based on equations given in Lighton (2008).

Data analysis
To determine torpor bout and arousal length we used a threshold of 30 ml O$_2$ h$^{-1}$, slightly above torpor oxygen consumption in all animals (chosen by visual inspection; Fig. 1). From the times when oxygen consumption crossed this threshold, we calculated arousal duration as well as torpor bout duration. We also computed mean oxygen consumption, $T_b$, and $T_a$ for these phases. Consequently, these variables include a small number of points during transitions, i.e., rewarming from and entrance into torpor (Fig. 1). We included these points because oxygen consumption during these transitions may well affect a putative hourglass mechanism. We also recorded hibernation duration as the time between first placing the animals in cold chambers and the onset of each torpor bout.

Due to rapid rewarming, arousals always started with a burst of oxygen consumption (Fig. 1, inset graph). Partly, the animals also displayed a second burst of oxygen consumption towards the end of arousals prior to re-entrance into torpor. We measured the amplitude of the peaks in oxygen consumption (PEAKlate) by averaging the three highest values during the second half of an arousal. Dormice deplete body fat reserves during hibernation. Therefore, the body masses used in statistical analyses were computed by linearly interpolating body mass between the onset of the hibernation season and termination of measurements at the time of each arousal. We also computed means of $T_b$ ($T_{b, \text{pre}}$, $T_{b, \text{sub}}$) during torpor (in previous and subsequent bouts relative to the arousal) as well as $T_a$ during prior torpor bouts ($T_{a, \text{pre}}$) and during arousal ($T_{a, \text{AR}}$). Due to collinearity (VIF~8) we entered only one $T_a$ measurement in the statistical analysis.

The data comprised multiple individual torpor-arousal cycles from the same animal, ranging from 1 to 9 torpor-arousal cycles per animal (Fig. 2). Therefore, we used generalized linear mixed models (GLMMs), with separate intercepts for individuals to adjust for repeated measurements. GLMMs were computed using R 4.0.2 (R Core Team, 2019), specifically the packages ‘brms’ (Bürkner, 2017; Bürkner, 2018) and ‘rstan’ (Stan Development Team, 2020). The Bayesian GLMM approach implemented in these libraries has the advantage that it can readily estimate random effects even when data are only partly obtained as repeated measures. This data structure often causes singularities and prevents random effect estimates with other methods. Also, Bayesian analysis provides inferences that are conditional on the data and are exact, without reliance on asymptotic
approximation (SAS Institute Inc., 2018). We provide posterior parameter distributions, their mean as well as 95% credible intervals, and a Bayesian version of R² for regression models (Table 1).

All GLMMs samples were drawn with the No-U-Turn-Sampling (NUTS) algorithm using 4 chains and 4000 iterations (including 2000 iterations per chain for warmup). We visually inspected MCMC chain plots and only report models for which the convergence diagnostic, Rhat, was 0.95-1.05. The response variables and corresponding full models are given in Table 1. Because the evaluation of all possible predictors was computationally not feasible, we reduced models in a stepwise procedure. Terms were eliminated to determine the model that maximized the expected predictive accuracy (ELPD) using the function ‘loo_compare’, which is based on leave-one-out cross validation (Vehtari et al., 2018). However, in all models containing oxygen consumption, we kept body mass as a fixed predictor in order to adjust MRs for mass effects while avoiding the use of indices (Fernández-Verdejo et al., 2019). We used only weakly informative priors, (the default priors in brms), as we had no prior information on expected slopes, and to avoid bias on the resulting posterior distributions (Gelman et al., 2014; Kruschke, 2015).

To assess the relative importance of explanatory variables (within each response variable), models were recomputed with scaled variables (i.e., after subtracting the mean and dividing the standard deviation). We did not assess interactions between predictor variables, as this would have resulted in severe overfitting of the limited data set. All response variables were approximately normally distributed as confirmed by quantile-quantile plots, and we used family “gaussian” for brms fits.

Data will be made available on Phaidra (https://www.vetmeduni.ac.at/en/bibliothek/infoservice/phaidra/).

Ethics Statement

All procedures were approved by the institutional ethics committee and the national Austrian authority according to § 26 of Law for Animal Experiments, Tierversuchsgesetz 2012 – TGV 2012 (BMBWF-68.205/0137-WF/V/3b/2014).
Results

Torpor bout duration
Torpor bout length increased progressively and torpor oxygen consumption declined over the hibernation season (Fig. 2). A regression of these variables showed a strong decrease of torpor bout duration as torpor bout oxygen consumption increased, and torpor bout oxygen consumption was the dominating variable determining the duration of torpor episodes (Table 1; Figs. 2, 3). Torpor T\textsubscript{b} also remained in the best model of torpor bout duration, but the 95% CI of the T\textsubscript{b}-effect overlapped 0. Body mass was kept as a fixed factor, but its 95% CI also included 0 (Table 1). Mean torpor bout duration was 10.34 \pm 3.69 d. The variation in torpor bout duration intercepts among individuals was moderate: the marginal R\textsuperscript{2} (fixed effects only) of the best model was 0.60, the conditional R\textsuperscript{2} (including the random part) was 0.73.

Torpor oxygen consumption
As expected from the above relationship, torpor oxygen consumption was negatively related to torpor bout duration (Table 1). Torpor oxygen consumption also tended to rise with mean torpor T\textsubscript{b}, with the 95% credible interval for the slope just overlapping 0 (Fig. 4). Torpor oxygen consumption also was associated with average oxygen consumption during the previous arousal, which showed large differences between individuals (see below; Table 1). Expectedly, higher body mass also elevated total torpor oxygen consumption (Table 1). As indicated by the R\textsuperscript{2} (Table 1), approximately 20% of the variance was due to individual differences in torpor bout oxygen consumption levels.

Arousal duration
Arousals, including the re-warming and re-entrance phases, lasted from 8.2 to 16.7 h and were longer if oxygen consumption during the arousal episode was high (Fig. 5a). Higher bursts of oxygen consumption during the last half of the arousal (PEAKlate) also prolonged its duration (Fig. 5b), while increased body mass had a shortening effect (Table 1). The mean duration of arousals was 11.10 \pm 0.15 h, and was completely independent from the prior torpor bout. Including the random factor individual had a moderate effect, as the marginal R\textsuperscript{2} of the best model was 0.62, whereas the conditional R\textsuperscript{2} was 0.83.

Arousal oxygen consumption
Variation in arousal oxygen consumption was dominated by individual differences, as indicated by the conditional R\textsuperscript{2} (0.78) that was much greater than the marginal R\textsuperscript{2} (0.17). However, even slight fluctuations of temperature affected arousal oxygen consumption. Interestingly, the average T\textsubscript{b}
during the previous torpor bout also had a positive effect on arousal oxygen consumption (Fig. 6; Table 1).

**VO₂ peaks during arousals**
The mean maximum height of oxygen consumption during euthermia (Tₜ > 34 °C) was 1.18-fold the mean oxygen consumption during the second half of arousal. Only in 30% of arousals was maximum oxygen consumption more than 20% greater than mean oxygen consumption during that period, i.e., constituted clearly visible peaks (e.g., Fig. 1).

**Discussion**

Our analysis shows that, during hibernation, torpor oxygen consumption was the single most important variable determining the duration of torpid state. This is fully in line with the assumption that animals must arouse from torpor early, whenever an elevated metabolism facilitates a metabolic imbalance. Hence these data, obtained from continuous measurements over entire torpor bouts, strongly support the idea of an underlying hourglass mechanism.

One argument against such an hourglass mechanism used in the past was the absence of body mass effects on torpor duration (Geiser and Ruf, 1995; Malan, 2010; Ruf and Geiser, 2015). The problem with all arguments involving body mass effects is, however, that minimum oxygen consumption during deep torpor in fact is virtually independent of body mass among hibernators. In comparative studies, depending on the data set analysed, the slope of the regression of torpor oxygen consumption versus body mass is either indistinguishable from zero (Heldmaier et al., 2004) or minute, compared with the body mass dependency of basal metabolic rate in euthermic animals (Ruf and Geiser, 2015). In accordance with these comparative studies, the present data show that within a species, mass-specific oxygen consumption in torpor is indeed virtually independent of body mass (slope estimate: 0.0002 ± 0.0001), while mean oxygen consumption strongly increased with smaller body mass in euthermic dormice (slope: -0.015 ± 0.001; Fig. 7).

Thus, a simple hourglass mechanism is sufficient to explain torpor/arousal cycles, without the need to involve non-temperature-compensated cycles, which would be an unusual feature of a circadian rhythm (Rawson, 1960; Zimmerman et al., 1968). More importantly, there is increasing evidence for the central circadian pacemaker being arrested during hibernation (Hut et al., 2002; Ikeno et al., 2017; Revel et al., 2007; Williams et al., 2012). The problem with the alternative mechanism, the
gradual development of some sort of metabolic imbalance is that we know nothing about its nature. It seems likely however, that the hourglass mechanism may involve protein turnover. Protein synthesis – with a few exceptions – is strongly depressed in hibernation (review in Storey, 2003), while their degradation is small, but still ongoing (Van Breukelen and Martin, 2001; Yacoe, 1983). For example, it has been suggested that periodic arousals are due to the need to synthetize SERCA 2a, the calcium-pump which is essential to maintain cardiac function in the torpid state (Ruf and Arnold, 2008). Alternatively, it has been proposed that periodic arousals are required for antibody production, i.e., to boost the immune system (Prendergast et al., 2002).

A common feature of these suggested targets is that the critical process is the loss of a substance during a torpor bout. We are not aware of any mechanism that would lead to the accumulation of a protein. At the same time we know that protein synthesis completely ceases in deep torpor (Van Breukelen and Martin, 2001). Only at high body temperature during interbout euthermia are gene products restored (van Breukelen and Martin, 2002a; Van Breukelen and Martin, 2001). There are strong alterations over a torpor/arousal cycle, such as an almost complete depletion of circulating lymphocytes that is reversed rapidly upon arousal (Bouma et al., 2011). In fact, metabolomics have revealed a multitude of molecular changes, such as of amino acids, the metabolism of purine and pyrimidine, that of enzyme co-factors as well of various lipids, with observed metabolites reduced during torpor and increased upon arousal (Nelson et al., 2009).

Whatever the specific target, some insights can be gained for the relationships of torpor and arousal characteristics within a species. Notably, torpor bout duration in garden dormice was strongly dependent on torpor oxygen consumption. Torpor oxygen consumption was in turn slightly correlated with torpor $T_b$ (Fig. 4). Hence, it seems that $T_b$ acts on bout duration via increasing torpor oxygen consumption. This is in line with well-known effects of $T_b$ on arousal frequency (e.g., Bieber and Ruf, 2009). Possibly, hibernators may use $T_b$ as a proxy for metabolic rate, and for the speed by which a metabolic imbalance is approached. This is in agreement with measurements in ground squirrels which led to the conclusion that $T_b$ per se also contributes, along with oxygen consumption, to determining the length of torpor bouts (Geiser and Kenagy, 1988). A role of $T_b$ in controlling torpor bout duration was also indicated by experiments in arctic ground squirrels, in which a decline in $T_a$ and $T_b$ down to ~0°C leads to increasingly longer maximum torpor bouts, while mean oxygen consumption showed only little variation (Buck and Barnes, 2000).
In the present experiments on garden dormice torpor oxygen consumption was associated with oxygen consumption during the previous arousal (Table 1). It is likely that this correlation merely reflects individual differences in oxygen consumption, both during torpor and arousal, which were strong (Table 1). Oxygen consumption during arousals clearly increased with $T_b$ in the previous torpor bout, and this was the strongest of all effects on arousal oxygen consumption (Table 1, Fig. 6). This finding suggests that any metabolic imbalance, e.g., metabolite depletion, that occurs faster at elevated $T_b$ during torpor leads to increased oxygen consumption during the subsequent arousal.

There was a positive association between arousal duration and arousal oxygen consumption (Fig. 5a). In terms of the hourglass mechanism this would mean that, within this species, increasing metabolic imbalances are not corrected by merely increasing arousal duration, but also by intensifying arousal oxygen consumption. Partly, arousal duration was prolonged by additional peaks of oxygen consumption in the second half of the arousal. (Fig. 5b). However, these peaks were not a prerequisite for torpor, as they were clearly identifiable only in 30% of all arousals.

There can be no doubt that the results of the present study, namely the dominant effect of metabolism on bout duration (Fig. 3) clearly indicate the oxygen consumption dependent development of an imbalance that is largely eliminated during arousal. The fact that both arousal and torpor bout duration, apart from some seasonal changes, are species-specific traits with limited variance (Ruf and Geiser, 2015), argues for an almost complete reset of this imbalance prior to the subsequent torpor bout. Only if the elimination of an imbalance during arousal is complete, we can expect a more or less constant torpor bout duration (after its initial seasonal lengthening).

As outlined before, the missing allometric relation of bout duration and oxygen consumption in torpor is only one of the reasons why an hourglass mechanism has not been universally accepted before. A simple further cause might be a lack of studies that gathered and analysed oxygen consumption and $T_b$ throughout the entire torpor/arousal cycle, and not just by punctual measurements, possibly from different individuals. Thirdly, the relationship between bout duration and oxygen consumption is not always simple. Whenever hibernators maintain a large body-to-environment temperature gradient, i.e., thermoregulate in torpor, the resulting shortening of bout duration is smaller than expected from oxygen consumption at higher temperatures, when they keep minimal gradients (Buck and Barnes, 2000; Geiser and Kenagy, 1988). However, this may be readily explained if that fraction of metabolism allocated to pure heat production, in contrast to “basal” torpor MR, does not contribute equally to the formation of an imbalance during torpor. A fourth reason is that torpor/arousal cycles, especially when they are relatively short but constant,
when plotted like actograms, may resemble free-running circadian rhythms (e.g., Daan, 1973). As circadian rhythms are ubiquitous it seems natural to assume their involvement in the temporal control of hibernation too. However, it is now commonly accepted that torpor is an ancestral trait (Grigg et al., 2004; Kayser, 1961; Lovegrove, 2012a; Malan, 1996; Ruf and Geiser, 2015). There also seems to be a prevailing view that daily torpor is the ancient trait, whereas prolonged hibernation is considered an advanced, secondary adaptation (Grigg et al., 2004; Lovegrove, 2012b; Malan, 1996; Ruf and Geiser, 2015). In that case, the transition from daily to multiday torpor, often under conditions of constant darkness, i.e., hibernation, requires that any circadian rhythmic signal is either suppressed or its generation is even shut off. Apparently, this is exactly what happens at the onset of hibernation in fall, and is reversed in spring (Hut et al., 2002; Ikeno et al., 2017; Revel et al., 2007; Williams et al., 2012). This seems to be true for the central circadian pacemaker at least, but to the best of our knowledge hibernation is a centrally controlled phenomenon too (e.g., Florant and Heller, 1977; Ruby, 2003).

It has been argued, on the other hand, that the simplest path to evolving a timer for hibernation cycles is via adaptation of an existing timing mechanism, the circadian system (van Breukelen and Martin, 2015). This led to the view mentioned above that a torpor/arousal cycle is considered a single non-temperature compensated circadian day (Malan 2010; van Breukelen and Martin, 2015). Indeed, the period of the circadian clock can be significantly modified by various metabolites, including mTOR signaling (Cao, 2018; Zhang et al., 2009). Circadian periods are lengthened up to a few hours by these signals, typically to 27-28 h. However, in a free-living edible dormouse, for example, a circadian day would have to be lengthened from ~24 h to ~832 h on average during midwinter, as determined by temperature loggers (Hoelzl et al., 2015). Estimates for maximum torpor bout duration in certain bats are even above 2000 h (Ruf and Geiser 2015). Thus, given these high natural torpor bout lengths and the also the evidence that circadian rhythms are in fact well temperature compensated in hibernators and ectotherms (Rawson 1960; Zimmerman et al. 1968) we consider this scenario rather unlikely.

Is an hourglass mechanism, that is, a rhythmic phenomenon caused by a process of accruing and subsequently decreasing physiological debts, unique among regulatory systems? Certainly not. A prime example is the sleep/wake cycle controlled by an increasing sleep deprivation during wakefulness, which is relieved during sleep. Whereas the current model of sleep regulation, which of course constitutes another hourglass mechanism, also involves circadian thresholds (Daan et al., 1984), such a circadian component is neither necessary nor desired in hibernation control of animals.
in constant darkness in underground burrows. Continued entrainment could be beneficial only in the rare hibernators that overwinter above ground.

Given the interspecific, highly significant, relationship between torpor oxygen consumption and bout duration (Ruf and Geiser, 2015) it seems that the hourglass mechanism determining the length of hibernation bouts is ubiquitous. Also, minimum torpor oxygen consumption is apparently subject to natural selection that deceases, while mean and maximum bout length increase in species living at higher latitudes under harsher conditions (Ruf and Geiser, 2015). The interspecific comparison also provides interesting insights into arousal duration. If arousal serves to correct a metabolic imbalance generated in torpor, e.g., to synthetize a crucial substance at high Tb, this task should take longer if oxygen consumption during arousals is low. This is exactly what has been observed: the duration of interbout euthermia is sharply lengthened as body mass increases and basal metabolic rate declines. IBE duration ranged from just 1.5 h in a 5 g bat to >28 h in a 3400 g alpine marmot (Ruf and Geiser, 2015).

Conclusion
We conclude that control of torpor duration in hibernating garden dormice is governed by a progressively increasing metabolic imbalance that is eliminated during periodic arousals. Our data suggest that arousal from deep torpor may be activated once this imbalance, e.g., the depletion of a crucial metabolite, reaches a critical threshold. Comparative data suggest that this mechanism may be ubiquitous among hibernators. The threshold for arousal could be constant or under circadian fluctuation depending on the winter ecology of a species.

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References


Figures

**Fig. 1.** Example of oxygen consumption during torpor and two arousals (with interbout euthermia) in a garden dormouse. The duration torpid phases and of arousals was determined from MR crossing a threshold of 30 mL O$_2$ h$^{-1}$ (dashed blue line; set by visual inspection). Mean metabolic rates were computed from all data points falling above (arousal; red triangles) or below (torpor; blue circles) this threshold. The **inset graph** shows the first arousal on an amplified time scale. Arousals were characterized by an initial burst of oxygen consumption. In this case, there was a second burst of oxygen consumption prior to torpor entrance (during the second half of the arousal), with a height termed PEAKlate.

**Fig. 2.** Torpor bout duration (a) and rate of mean oxygen consumption in torpor (b) as a function of the time since onset of hibernation in garden dormice. While torpor duration gradually increased, torpor oxygen consumption decreased. Same colours indicate data from the same animals. Data from 94 torpor-arousal cycles in 22 animals.
Fig. 3. Torpor bout duration decreased as mean torpor oxygen consumption increased; results of a Bayesian GLMM. Blue shaded areas indicate the 95% credible intervals of the predicted values at each value of torpor oxygen consumption. The inset graph shows the posterior distribution of the slope estimate, its mean (solid line), and its 95% credible interval (shaded). The dashed line indicates a slope of zero. Data from 94 torpor-arousal cycles in 22 animals.

Fig. 4. Torpor oxygen consumption as a function of body temperature. Results of a Bayesian GLMM. Blue shaded areas indicate the 95% credible intervals of the predicted values. The inset graph shows the posterior distribution of the slope estimate, its mean (solid lines), and 95% credible intervals (shaded). The dashed line in the inset graphs mark a slope of zero. Data from 94 torpor-arousal cycles in 22 animals.
Fig. 5. Arousal (AR) duration as a function of (a) mean oxygen consumption during the same arousal and (b) of the height of the metabolic burst in the second half of the arousal; results of a Bayesian GLMM. Blue shaded areas indicate the 95% credible intervals of the predicted values. The inset graph shows the posterior distribution of the slope estimate, its mean (solid line), and 95% credible intervals (shaded). Data from 94 torpor-arousal cycles in 22 animals.
Fig. 6. Mean oxygen consumption during arousal as a function of mean body temperature during the prior torpor bout; results of a Bayesian GLMM. Blue shaded areas indicate the 95% credible intervals of the predicted values. The inset graph shows the posterior distribution of the slope estimate, its mean (solid line), and its 95% credible interval (shaded). The dashed line indicates zero. Data from 94 torpor-arousal cycles in 22 animals.

Fig. 7. Mass specific oxygen consumption as a function of body mass in euthermic and torpid Garden dormice; results of Bayesian GLMMs. Blue shaded areas indicate the 95% credible intervals of the predicted values. Data from 94 torpor-arousal cycles in 22 animals.
Table 1 Results of Bayesian generalized mixed models for 4 response variables. For each model, estimates for the slope of each predictor and their 95% credible intervals from the best models according to their ELPD are given. Standard deviations of model intercepts show random effects (i.e., differences between individuals). For fixed predictors, scaled estimates are the slopes of scaled predictors, which are directly comparable for each model. $R^2$ are estimates for the mean variance explained as well as their 95% credible intervals. Variables investigated were previous and subsequent torpor bout duration (TBDpre, TBDsub, relative to each arousal), torpor rate of $O_2$ consumption (TVO$_2$), torpor body temperature ($T_b$sub and $T_b$pre), ambient temperature during arousal and torpor ($T_a$ar, $T_a$pre), arousal duration (ARD), mean arousal rate of $O_2$ consumption (ARVO$_2$) and the height of VO$_2$ during the second half of each arousal (PEAKlate). In addition, models contained the variable body mass (BM) whenever they also contained a VO$_2$ measurement. For each response variable, the full GLMM model is given as a subscript.

| Response          | Predictor/Term          | Estimate | l-95% CI | u-95% CI | Scaled Est. | $R^2$ cond. | marg. |
|-------------------|-------------------------|----------|----------|----------|-------------|-------------|
| Torpor duration$^1$ | SD Intercept            | 1.41     | 0.37     | 0.79     |              | 0.73        | 0.60  |
|                   | TVO$_2$                 | -0.70    | -0.88    | -0.53    | -1.90       |             |       |
|                   | $T_b$                   | -0.46    | -1.63    | 0.57     | -0.29       |             |       |
|                   | BM                      | -0.01    | -0.05    | 0.02     | -0.37       |             |       |
| Torpor VO$_2$$^2$  | SD Intercept            | 1.49     | 0.80     | 2.52     |              |             | 0.81  | 0.62 |
|                   | TBD                     | -0.57    | -0.73    | -0.41    | -1.47       |             |       |
|                   | $T_b$                   | 1.00     | -0.06    | 2.30     | 0.63        |             |       |
|                   | ARVO$_2$                | 0.03     | 0.01     | 0.05     | 0.62        |             |       |
|                   | BM                      | 0.05     | 0.02     | 0.08     | 1.27        |             |       |
| Arousal duration$^3$ | SD Intercept            | 1.32     | 0.91     | 1.90     |              | 0.83        | 0.62  |
|                   | ARVO$_2$                | 0.06     | 0.04     | 0.07     | 1.15        |             |       |
|                   | PEAKlate                | 0.01     | 0.01     | 0.02     | 0.65        |             |       |
|                   | BM                      | -0.04    | -0.06    | -0.02    | -0.92       |             |       |
| Arousal VO$_2$$^4$  | SD Intercept            | 17.80    | 12.08    | 25.76    |              | 0.78        | 0.17  |
|                   | $T_a$ar                 | 25.72    | -12.24   | 63.38    | 4.32        |             |       |
|                   | TBDpre                  | 13.93    | 5.86     | 22.28    | 8.77        |             |       |
|                   | BM                      | -0.10    | -0.49    | 0.26     | -2.42       |             |       |

$^1$ TBD$\sim$TVO$_2$+BM+TA$+$TB$+$ARD+ARVO$_2$+PEAKlate+(1$|$ID)
$^2$ TVO$_2$$\sim$TBD$+$BM$+$TA$+$TB$+$ARD+ARVO$_2$+PEAKlate+(1$|$ID)
$^3$ ARD$\sim$TBD$+$TVO$_2$pre$+$BM$+$TAAar$+$TBDpre$+$ARVO$_2$+PEAKlate+(1$|$ID)
$^4$ ARVO$_2$~ TBD$+$TVO$_2$pre$+$BM$+$TAAar$+$TBDpre+(1$|$ID)