

Torpor patterns, arousal rates, and temporal organization of torpor entry in wildtype and UCP1-ablated mice

R. Oelkrug · G. Heldmaier · C. W. Meyer

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Abstract In eutherian mammals, uncoupling protein 1 (UCP1) mediated non-shivering thermogenesis from brown adipose tissue (BAT) provides a mechanism through which arousal from torpor and hibernation is facilitated. In order to directly assess the magnitude by which the presence or absence of UCP1 affects torpor patterns, rewarming and arousal rates within one species we compared fasting induced torpor in wildtype (UCP1^{+/+}) and UCP1-ablated mice (UCP1^{-/-}). Torpor was induced by depriving mice of food for up to 48 h and by a reduction of ambient temperature (T_a) from 30 to 18°C at four different time points after 18, 24, 30 and 36 h of food deprivation. In most cases, torpor bouts occurred within 20 min after the switch in ambient temperature (30–18°C). Torpor bouts expressed during the light phase lasted 3–6 h while significantly longer bouts (up to 16 h) were observed when mice entered torpor during the dark phase. The degree of hypometabolism (5–22 ml h⁻¹) and hypothermia (19.5–26.7°C) was comparable in wildtype and UCP1-ablated mice, and both genotypes were able to regain normothermia. In contrast to wildtype mice, UCP1-ablated mice did not display multiple torpor bouts per day and their peak rewarming rates from torpor were reduced by 50% (UCP1^{+/+}: $0.24 \pm 0.08^\circ\text{C min}^{-1}$; UCP1^{-/-}: $0.12 \pm 0.04^\circ\text{C min}^{-1}$). UCP1-ablated mice therefore took significantly longer to rewarm from 25 to 32°C (39 vs. 70 min) and required 60% more energy for

this process. Our results demonstrate the energetic benefit of functional BAT for rapid arousal from torpor. They also suggest that torpor entry and maintenance may be dependent on endogenous rhythms.

Keywords Hypometabolism · Hypothermia · Brown adipose tissue

Abbreviations

BAT	Brown adipose tissue
CET	Central European time
NST	Non-shivering thermogenesis
UCP	Uncoupling protein
T_a	Ambient temperature
T_b	Body temperature

Introduction

Endotherms employ daily torpor to reduce their daily energy expenditure on a day to day basis (Geiser 2004; Heldmaier et al. 2004). In daily heterotherms, such as short day acclimated Djungarian hamsters (*Phodopus sungorus*), or mouse lemurs (*Microcebus murinus*), daily torpor is observed as an obligatory behaviour during seasonal acclimation, e.g. in response to decreasing day length. Daily torpor also occurs as a facultative behaviour in response to acute energy shortfalls. An example is the mouse (*Mus musculus*), in which torpor is only displayed when animals are confronted with food deprivation or a combination of food deprivation and cold (Hudson and Scott 1979; Webb et al. 1982; Swoap et al. 2006; Dikic et al. 2008; Brown and Staples 2010).

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R. Oelkrug · G. Heldmaier · C. W. Meyer (✉)
Department of Animal Physiology, Faculty of Biology,
Philipps-Universität, Karl-von-Frisch Strasse 8,
35043 Marburg, Germany
e-mail: meyer@staff.uni-marburg.de

Arousal from torpor is usually accompanied by a rapid rewarming of body temperature. In order to rewarm from hypothermia, small mammals can recruit heat by two major thermogenic mechanisms, shivering thermogenesis (ST) and non-shivering thermogenesis (NST). It is well known that a large proportion of the latter is derived from the dissipation of heat mediated by uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) mitochondria (Cannon and Nedergaard 2004), although other tissues may also contribute to NST (Foster and Frydman 1978; Heldmaier and Buchberger 1985; Granneman et al. 2003; Ukropec et al. 2006; Shabalina et al. 2010). In contrast to shivering muscle, BAT is considered a highly efficient site of heat production for rewarming from torpor because of its central location in the cervicothoracic region. The heat generated in BAT is readily distributed in the body core while peripheral temperatures can still be low, thereby facilitating the full rewarming process (Jansky 1973).

Daily torpor and hibernation are also observed in monotremes or adult marsupials which lack classical BAT (Rowlatt et al. 1971; Hayward and Lisson 1991; Jastroch et al. 2008; Nicol et al. 2008). In members of these taxonomic groups rewarming to normothermia cannot be supported by UCP1-mediated thermogenesis, raising the question on the significance of BAT in the context of torpor (Lyman and O'Brian 1986; Geiser and Baudinette 1990; Stone and Purvis 1992). A comparison of deep torpor in the short beaked echidna (*Tachyglossus aculeatus*; Monotremata, no BAT) with the similar sized marmot (*Marmota marmota*, BAT present) suggested that the peak rewarming rates in echidnas were 50% lower than in marmots (Nicol et al. 2009). Rewarming from torpor through mechanisms other than BAT UCP1-mediated NST, therefore, appears to be less efficient.

The total time and effort required for arousal, however, depends upon body mass and overall thermogenic capacity and it may be facilitated by high burrow temperature, insulative properties of the burrow and the presence of burrow mates. Torpid animals may also seek warmer microclimates or even bask to support arousal (Ortmann et al. 1997; Lovegrove et al. 1999; Mzilikazi et al. 2002; Warnecke and Geiser 2010). All of these parameters may conceal the specific extent by which BAT and UCP1-mediated NST assists in rewarming. In order to directly assess the contribution of UCP1-mediated NST for arousal from shallow torpor we investigated mice that genetically lack functional brown adipose tissue (UCP1-ablated mice, UCP1^{-/-}; Enerback et al. 1997). The examination of wildtype and UCP1-ablated mice allows a direct comparison of torpor behaviour and arousal in the presence or absence of UCP1-mediated NST within the same species. We hypothesized that UCP1-ablated mice should be reluctant to express torpor,

or rewarm from torpor at substantially lower rates as compared to wildtype mice.

For torpor induction, warm acclimated mice (30°C) were deprived of food for up to 48 h and in addition, the ambient temperature (T_a) was reduced from 30 to 18°C on the second day of fasting. Torpor behaviour appears tightly linked to the circadian system in most species studied so far (Heldmaier and Steinlechner 1981; Ortmann et al. 1997; McKechnie and Lovegrove 2001; Ehrhardt et al. 2005). Therefore, the reduction of ambient temperature was started at four different experimental time-points. We thereby aimed to elucidate if torpor entry and its duration is an immediate (facultative) response to the cold stimulus, or if this behaviour depends on time of day.

Materials and methods

Mice and maintenance

Wildtype and UCP1-ablated mice (genetic background C57Bl/6J) were derived from our breeding colony at the Philipps-Universität of Marburg. In the founder animals (originally provided by L. Kozak, Pennington Medical Research Centre, Baton Rouge, USA) the UCP1 gene had been inactivated by homologous recombination with a deletion vector in which exon 2 and parts of exon 3 had been replaced with a neomycin resistance gene (Enerback et al. 1997). Genotypes were identified using a PCR-based strategy (Oelkrug et al. 2010) and only female UCP1^{+/+} and UCP1^{-/-} mice were included in the experiments.

Mice were bred and raised at an ambient temperature of 24°C. Except for the days on which torpor was induced, mice always received food (Ssniff 1534, Soest, Germany) ad libitum. They were kept on a 12:12 h light:dark cycle (lights on 6:00 CET). At the age of 2–6 months, they were surgically implanted with temperature sensitive transmitters (series 3000; model XM-FH; Mini Mitter Company, Inc., USA) for the monitoring of abdominal core body temperature (T_b). Following surgery mice were maintained in individual cages at an ambient temperature of 24°C. Hereafter they were warm acclimated at 30°C for at least 3 weeks before the onset of the first torpor trial.

Experimental set-up and protocol

For the metabolic measurements, individual mice were transferred to metabolic cages (modified home cages, ~ 5 l volume) which were connected to an open circuit respirometry system for continuous monitoring of oxygen consumption (VO_2) and carbondioxide production (VCO_2). The basic set-up of the respirometric system has been described previously (Heldmaier and Ruf 1992). In parallel, body

temperature was recorded using TR 3000 receiver plates running on the Vital View software (Mini Mitter Company Inc., USA). The metabolic parameters (VO_2 , VCO_2 , T_b) were determined in each mouse during 24 h at 5 min intervals.

In $n = 7$ wildtype and $n = 5$ UCP1-ablated mice four successive torpor trials were performed, each separated by a 3-day recovery period. For torpor induction, mice were initially deprived of food at 12:00 CET and then continued to be maintained at 30°C T_a . After 18, 24, 30 or 36 h of food deprivation at 30°C (i.e. at 6:00, 12:00, 18:00 and 24:00 CET), the ambient temperature was lowered to 18°C for different periods of time (Fig. 1a). Trials were terminated after a maximum of 48 h without food, and mice still torpid at this time point were induced to arouse by vibration and acoustic stimulation (knocking on the cage). Individuals clearly unable to regain normothermia by themselves were removed from the cages and re-warmed using an infrared lamp. All experimental procedures were approved by the German Animal Welfare Authorities.

Arousal heat production, peak rewarming rates and energy expenditure during rewarming

For the calculation of arousal heat production, oxygen consumption data (VO_2) from the beginning of an arousal to the highest oxygen consumption value were obtained. A mouse was considered torpid if its oxygen consumption decreased below 25 ml h⁻¹ for more than 2 h. This threshold was chosen to distinguish torpor episodes from periodic fluctuations in metabolic rate (Dikic et al. 2008). In case of multiday torpor, only the first bout of hypometabolism was considered for the evaluation, because UCP1-ablated mice frequently failed to arouse from the second bout of torpor. Altogether 41 torpor episodes were observed (Fig. 1a, dark fillings) from which 39 arousal periods could be analyzed (excluding one disturbed registration and 6 arousals from second bouts in the 12:00 h trial). For each arousal period VO_2 -data were converted to HP rates (J s⁻¹) using the respiratory quotient (RQ; volumes of CO₂ produced/volumes of O₂ consumed) and the caloric equivalents given by (Heldmaier 1975): $HP (J s^{-1}) = (4.44 + 1.43 \times RQ) \times VO_2 (ml h^{-1})$. Cumulative HP (J) was calculated from summated HP rates (J s⁻¹) and the total time (s) required for rewarming or arousal.

In order to compare the efficiency of rewarming from torpor, we used two different approaches. One approach was to calculate peak rewarming rates (PWR, °C min⁻¹) from the body temperature time course during an arousal, assuming a four-parameter sigmoid curve (for details see Nicol et al. 2009). For these calculations, the Regression Wizard function of SigmaPlot was used (SigmaPlot 10 for Windows, Systat Software Inc, San Jose, CA, USA). Average R_{adj}^2 was 0.995 for all tested trials with SD 0.003 ($n = 36$

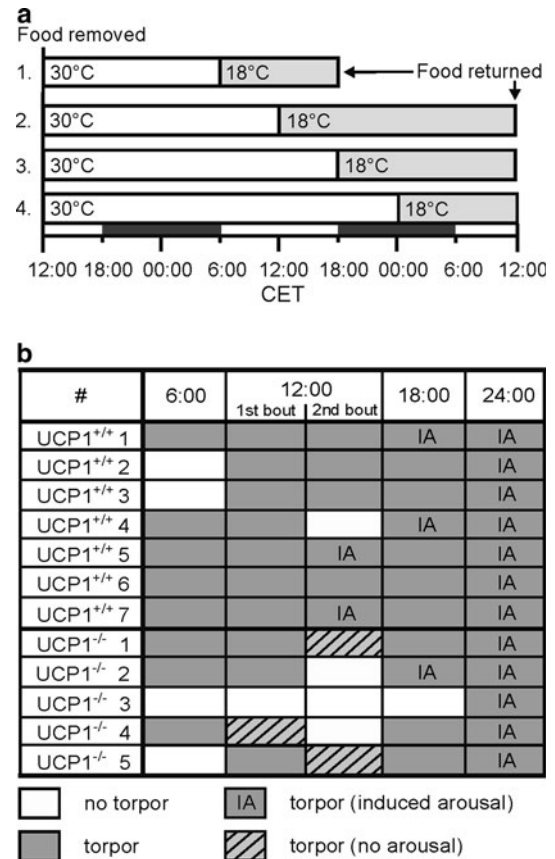


Fig. 1 a The experimental time schedule for torpor induction in wildtype and UCP1-ablated mice. Mice were food deprived for up to 48 h and additionally challenged with cold at different experimental time points (switch from 30 to 18°C, as indicated by the shaded areas). Periods of lights off are indicated by the dark bars at the bottom. **b** Individual torpor responses to different schedules of food deprivation and cold (switch from 30 to 18°C) in 7 wildtype (UCP1^{+/+}) and 5 UCP1-ablated mice (UCP1^{-/-}). The top row indicates the time of day (CET, central European time) when ambient temperature was changed. When the cold period started at 12:00 CET on the second day of food deprivation, the wildtype mice displayed two bouts of torpor during the subsequent 24 h

torpor bouts; T_b was not available for three bouts from a wildtype mouse). In the second approach, we selected the arousal period during which each mouse rewarmed from 25 to 32°C T_b . This body temperature range was chosen because minimal body temperature (T_b min) in all but two torpor bouts was below 25°C, and $T_b = 32°C$ is often used as the threshold temperature below which an animal is called torpid (Barclay et al. 2001). Oxygen consumption during rewarming from 25 to 32°C was converted to HP rates and cumulative HP as outlined above.

Statistical analyses

All data are expressed as mean ± standard deviation (SD). Differences in metabolic parameters between wildtype and UCP1-ablated mice were analyzed using Student’s *t* test.

Results

Metabolic responses to food deprivation and cold

Figure 2 shows representative individual tracings of body temperature (T_b) and oxygen consumption in wildtype and UCP1-ablated mice challenged with food deprivation and a switch to cold ambient temperatures (18°C) at different experimental time points (at 6:00, 12:00, 18:00 and 24:00 CET, i.e. after 18, 24, 30 and 36 h of FD, exemplified in a–d). In all four trials and in both genotypes, clear bouts of hypometabolism and hypothermia could be observed. Altogether, we obtained 24 full torpor bouts from the wildtype mice and 15 full torpor bouts from the UCP1-ablated mice during which $\dot{V}O_2$ decreased below 23 ml h⁻¹ and T_b was below 28°C. An overview on the individual torpor responses is given in Fig. 1b. In all trials, the body mass of the wildtype and UCP1-ablated mice was comparable (UCP1^{+/+}: 24.1 ± 1.3 g; UCP1^{-/-}: 24.2 ± 1.4 g). On average, mice lost between 1.8 and 7.0 g of body mass during each trial, with no differences between genotypes (UCP1^{+/+}: -4.5 g; UCP1^{-/-}: -4.4 g).

A reduction of ambient temperature from 30 to 18°C occurring at 6:00, 12:00, or 24:00 CET, induced a bout of hypometabolism and hypothermia in most mice within ~20 min after the onset of cold (Figs. 2a, b, d, 3a). When the cold exposure started at 18:00 CET (30 h without food), i.e. the time around *lights off*, torpor entry was delayed by 6 h (i.e. to midnight) relative to the cold onset and all mice entered torpor spontaneously (Figs. 2c, 3a). Nocturnal torpor bouts usually lasted longer than 6 h (up to 16 h) and on the next morning, we frequently induced an arousal by knocking on the cage in order to terminate the experiment (Fig. 3b). The longest bout from which an individual rewarmed by itself lasted almost 14 h in a wildtype mouse and 15 h in an UCP1-ablated mouse.

In the trial during which cold onset occurred at 12:00 CET (i.e. after mice had been without food for 24 h; Fig. 2b) clear genotype differences in the patterns of oxygen consumption and body temperature were evident. Initially, all wildtype mice and four UCP1-ablated mice readily entered a short bout of hypometabolism and hypothermia in acute response to cold. With the exception of one UCP1-ablated mouse, all individuals were able to arouse from torpor. After resuming normometabolism and normothermia all but one wildtype mouse spontaneously exhibited another long second bout of torpor around 9–12 h later (i.e. entry around midnight). In contrast, either the UCP1-ablated mice did not enter a second torpor bout, or they were clearly unable to arouse from a second torpor bout on their own (Figs. 1b, 2b). The fifth UCP1-ablated mouse did not enter any torpor and instead displayed a constant shallow body temperature (T_b ~ 33°C) without pronounced fluctuations in metabolic rate.

Arousal heat production, peak rewarming rates and energy expenditure during rewarming

The minimal oxygen consumption values observed in torpor ranged from 22.2 to 5.1 ml h⁻¹ in wildtype and 22.0 to 4.8 ml h⁻¹ in UCP1-ablated mice (Fig. 3c). Minimal body temperatures were 21.8 ± 2.4 and 21.2 ± 2.1°C, respectively (Fig. 3d).

The comparison of cumulative heat production during the full arousal period revealed that this process was energetically more costly in UCP1-ablated mice (UCP1^{+/+}: 2.33 ± 0.67 kJ; UCP1^{-/-}: 2.94 ± 0.90 kJ; $P = 0.019$; Fig. 4a). The time required to increase heat production from the onset of arousal to terminal values took significantly longer in UCP1-ablated mice compared with wildtype mice (UCP1^{+/+}: 127 ± 59 min; UCP1^{-/-}: 190 ± 65 min; $P = 0.004$; Fig. 4b).

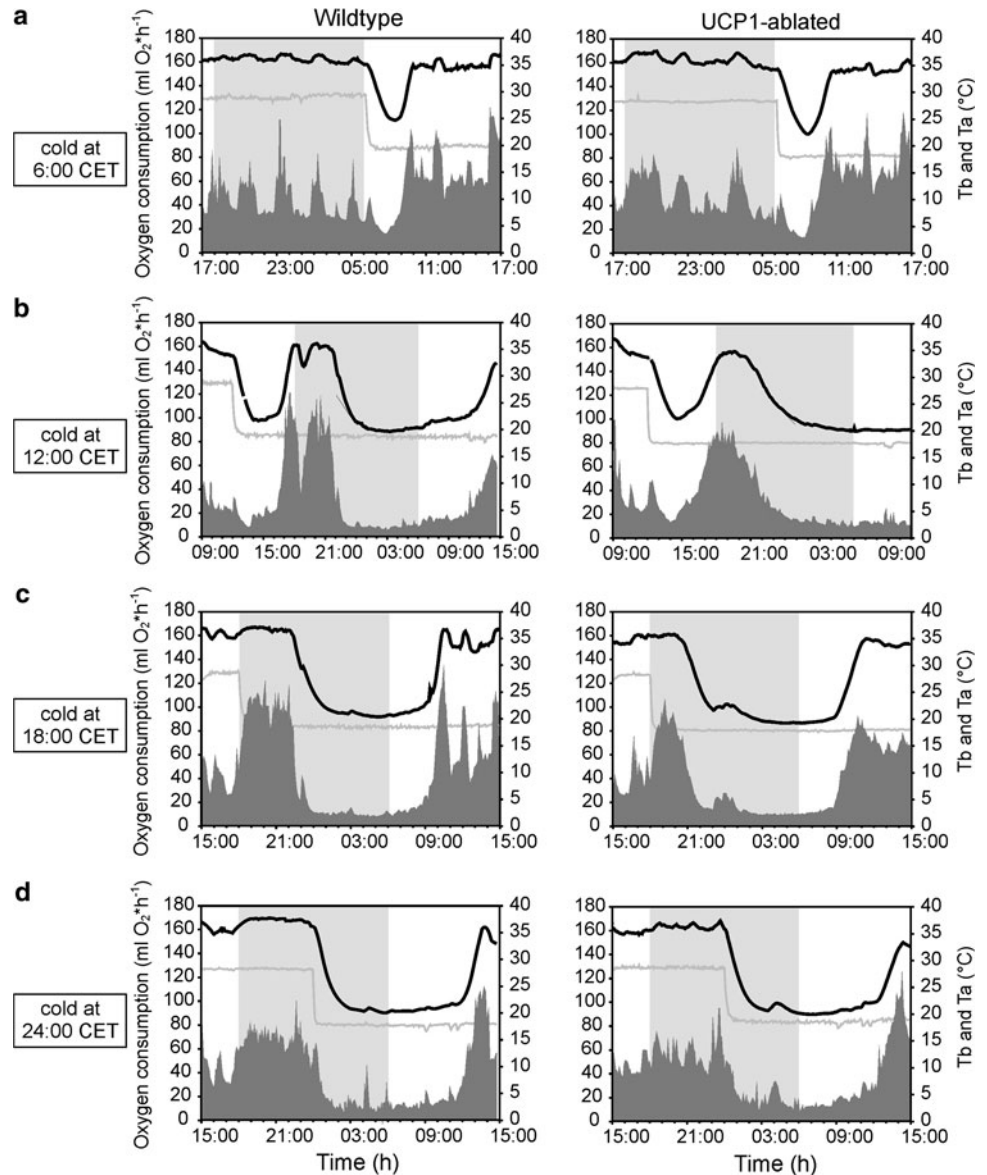
Peak rewarming rates (PWR) were 0.24 ± 0.08°C min⁻¹ in wildtype mice, while UCP1-ablated mice reached rewarming rates of only 0.12 ± 0.04°C min⁻¹ (Fig. 4c). Body temperature during PWR was significantly lower in UCP1-ablated (28.2 ± 0.1°C) mice than in wildtype mice (29.6 ± 0.9°C; $P < 0.001$).

In order to elucidate the efficiency of BAT during rewarming, we analyzed the period of increase in body temperature from 25 to 32°C (highlighted Fig. 5a) which was available from all but two torpor bouts ($n = 34$). Rewarming from 25 to 32°C took almost twice as long in the UCP1-ablated mice (UCP1^{+/+}: 38.5 ± 12.5 min; UCP1^{-/-}: 70.1 ± 24.0 min, Fig. 5b) and required an additional 590 Joules (UCP1^{+/+}: 0.969 ± 0.209 kJ; UCP1^{-/-}: 1.59 ± 0.317 kJ; Fig. 5c, shaded area in Fig. 5a). Mean heat production (J s⁻¹) during rewarming from 25 to 32°C was not significantly different between wildtype and UCP1-ablated mice (UCP1^{+/+}: 0.376 ± 0.049 J s⁻¹; UCP1^{-/-}: 0.367 ± 0.063 J s⁻¹; Fig. 5d). Assuming a linear increase in heat production (J s⁻¹) with the time required for rewarming from 25 to 32°C (s), the rate of heat production change (J s⁻²) during this process was significantly higher in the wildtype as compared to the UCP1-ablated mice (UCP1^{+/+}: 0.123 ± 0.055 mJ s⁻²; UCP1^{-/-}: 0.051 ± 0.030 mJ s⁻²; $P \leq 0.001$; Fig. 5e).

Discussion

The comparison of wildtype and UCP1-ablated mice allowed us to investigate the role of UCP1-mediated NST during induced torpor behaviour. Clearly, absence of UCP1 (absence of functional BAT) did not impair the full expression of a single bout of torpor. Peak rewarming rates from torpor, however, were 50% lower in UCP1-ablated mice. The magnitude by which absence of uncoupled BAT

Fig. 2 Representative 24 h tracings of oxygen consumption (*dark area plot*) and body temperature (*black line*) from individual wildtype and UCP1-ablated mice in response to different experimental schedules of food deprivation (FD) and cold (see Fig. 1). **a** Mice had previously experienced 18 h of FD, and ambient temperature (T_a) was reduced to 18°C at 6:00 CET, as indicated by the *grey line*. **b** Mice had been held without food for 24 h and T_a was reduced to 18°C at noon. **c, d** The reduction of T_a occurred at 18:00 CET and 24:00 CET, respectively. Each experimental treatment induced bouts of hypometabolism and hypothermia in both genotypes. The *shaded area* represents the dark phase of the light–dark cycle (*lights off*: 18:00 CET, *lights on*: 6:00 CET)



thermogenesis compromised rewarming from torpor in the artificial system of the UCP1-ablated mouse is within the range of what has been previously suggested from suppression of NST through beta adrenergic blockade in wildtype mice (–30%; Swoap and Weinschenker 2008) or in hibernating bats (–50%; Heldmaier 1970). A 50% difference in peak rewarming rates could also be observed from the rates of emergence from torpor during the hibernation season in the echidna (no BAT; PWR: $\sim 0.1^\circ\text{C min}^{-1}$) compared with the marmot (BAT present; PWR: $0.2^\circ\text{C min}^{-1}$) (Nicol et al. 2009).

The parallel recording of oxygen consumption enabled us to calculate total heat production during the rewarming period. In the absence of UCP1-mediated NST, arousal was less efficient, as rewarming for the same increase in body temperature (here: from 25 to 32°C T_b) required an

additional 590 Joules (+60%) in UCP1-ablated mice. One possible explanation for the increment in thermogenesis in the absence of functional BAT is the need to compensate increased heat loss to the environment arising from thermogenesis of peripheral muscles. As we observed no difference in minimal metabolic rate or T_b min during torpor, and as the mean heat production during rewarming from 25 to 32°C did not differ between genotypes (Fig. 5c), our results do not support increased thermal conductance in UCP1-ablated mice. If anything, the UCP1-ablated mice reduce heat loss (decrease thermal conductance), e.g. by restricting blood flow to the tail (Wang et al. 2006). We conclude that heat dissipation rates outside BAT cannot fully compensate for the loss of uncoupled thermogenesis. As a result, the arousal process is less rapid, and it becomes energetically more costly because in addition to the

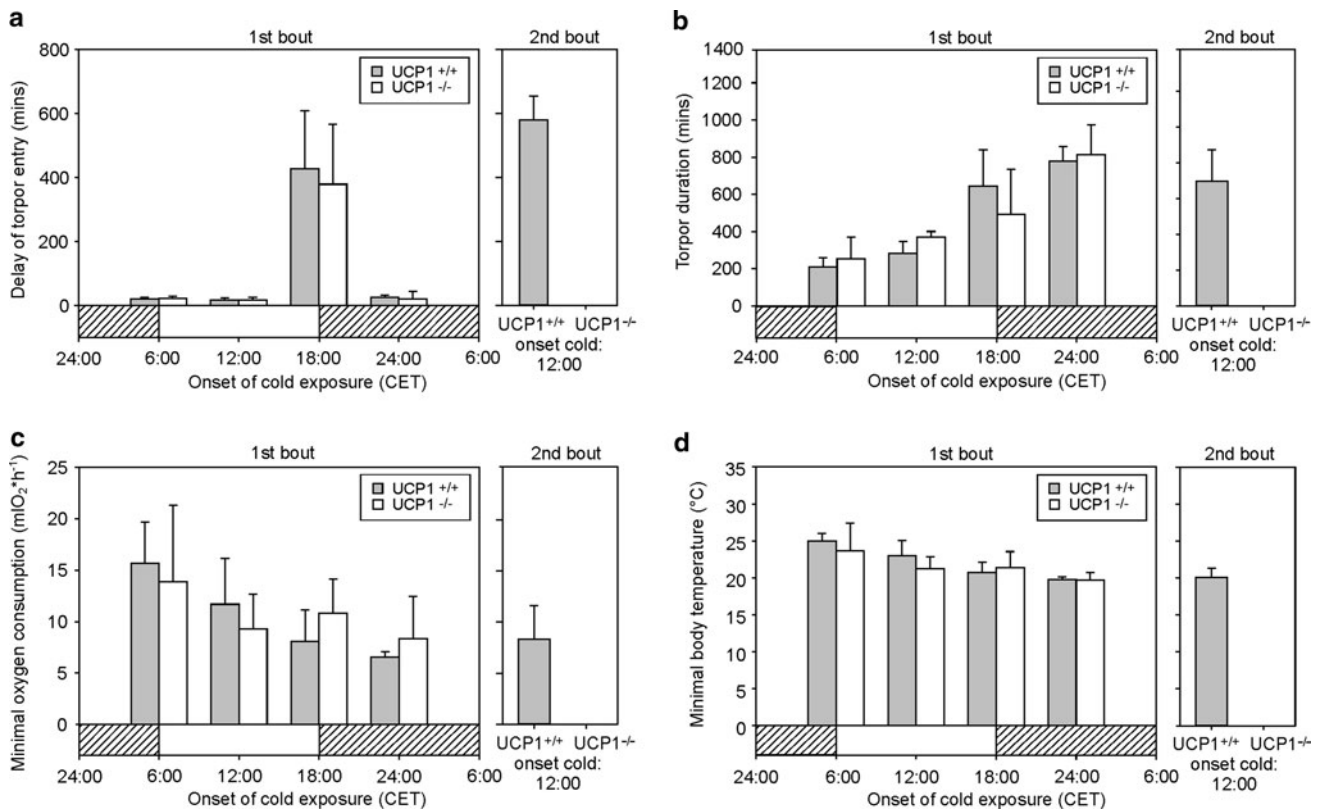


Fig. 3 Onset of torpor relative to the onset of cold (a), duration of induced torpor (b), minimal rates of oxygen consumption during torpor (c) and minimal body temperature in torpor (d) recorded from

previously food deprived wildtype (UCP1^{+/+}) and UCP1-ablated mice (UCP1^{-/-}). Each bar represents the mean \pm SD of 3–7 mice

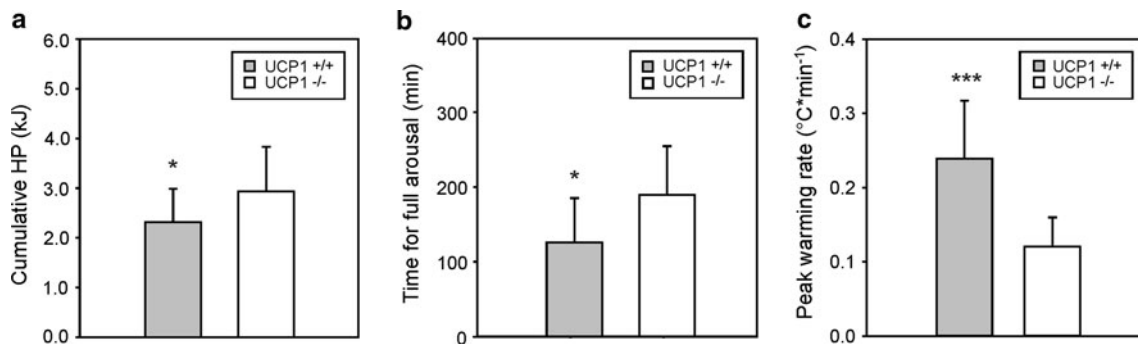


Fig. 4 Cumulative heat production (a), time required for arousal (b) and peak rewarming rates (PWR; c) during arousal from torpor in wildtype (UCP1^{+/+}) and UCP1-ablated mice (UCP1^{-/-}). Bars

represent mean \pm SD from $n = 24$ torpor bouts (21 in c) obtained from $n = 6$ or 7 wildtype mice and 15 torpor bouts from $n = 5$ UCP1-ablated mice. *** $P < 0.001$; * $P < 0.05$

metabolic effects for rewarming the body twice the amount of maintenance metabolism is required (Fig. 5a). Thereby, our results support previous predictions that rapid rewarming rates from torpor should be less expensive in terms of energetic costs than slow rewarming rates (Stone and Purvis 1992; McKechnie and Wolf 2004).

Another transgenic murine model in which BAT thermogenesis is severely impaired is the β -hydroxylase knockout (Dbh^{-/-}) mouse which lacks the ability to synthesize adrenalin and noradrenalin (Swop et al. 2006).

Unexpectedly, these mice failed to enter torpor when fasted, revealing the necessity for an intact sympathetic nervous system for full torpor expression. Dbh^{-/-} mice regained their ability to enter torpor when treated with L-3,4-dihydroxyphenylserine (DOPS) a synthetic precursor restoring central adrenalin synthesis, but the maximal rates of temperature gain during emergence from torpor were 75% lower ($0.06^{\circ}\text{C min}^{-1}$) as compared to wildtype controls ($0.26^{\circ}\text{C min}^{-1}$). It is likely that the slower rewarming rates in Dbh^{-/-} mice relative to UCP1-ablated mice may

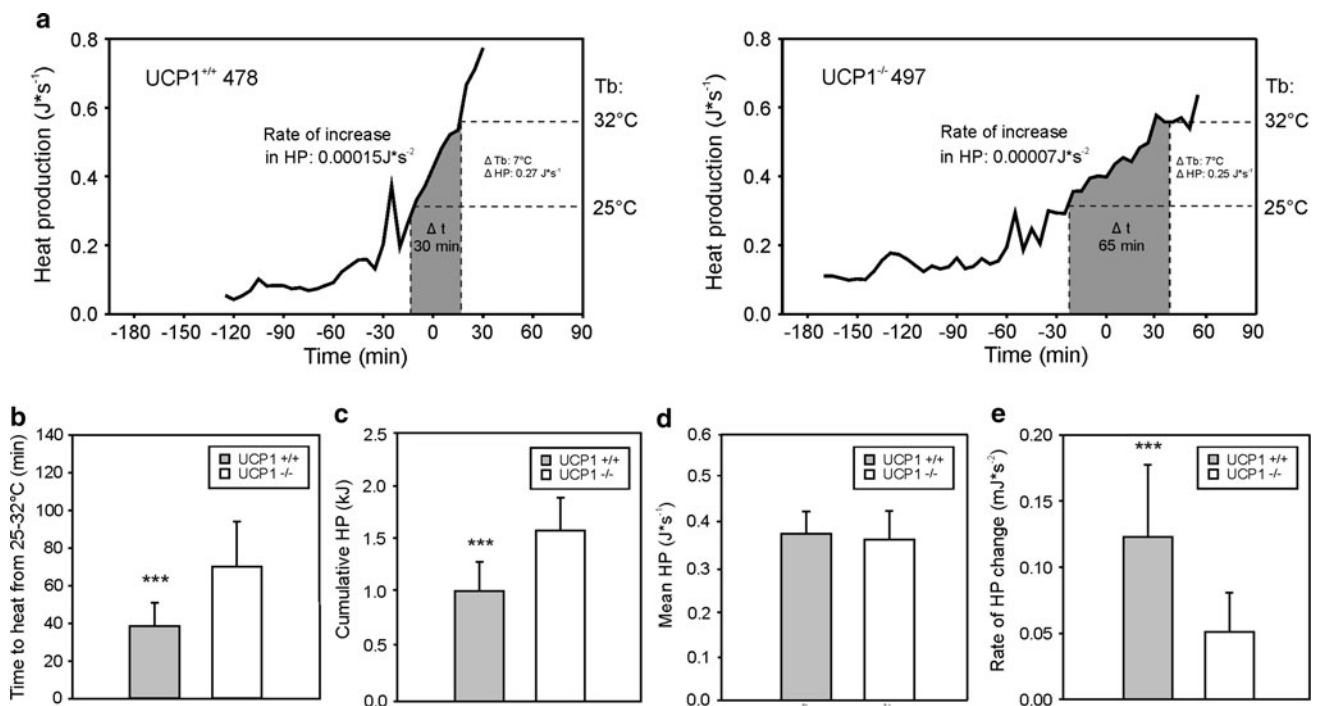


Fig. 5 **a** Body temperature and heat production rates during arousal from torpor in a wildtype (*left*) and a UCP1-ablated mouse (*right*). Minimal body temperature (T_b) in torpor was 19.6°C in the wildtype mouse and 19.8°C in the UCP1-ablated mouse. Minimal HP in torpor was 0.035 J s⁻¹ (6.2 ml O₂ h⁻¹) and 0.038 J s⁻¹ (6.7 ml O₂ h⁻¹), respectively. Data were aligned according to the point of peak

rewarming rate. In each panel, the *shaded areas* highlight the specific amount of heat production necessary for rewarming the body from 25 to 32°C (+50% in the UCP1 ablated mouse). **b–d** Bars represent mean ± SD of 23 torpor bouts obtained from $n = 6$ wildtype mice and 14 torpor bouts from $n = 5$ UCP1-ablated mice. *** $P < 0.001$

result not only from their inability to activate BAT, but also because WAT derived lipolysis is compromised, and additionally the sympathetic control of heat loss is impaired.

The onset of fasting induced torpor in mice is usually observed during the second half of the night, irrespective of the duration of previous food deprivation (Hudson and Scott 1979; Webb et al. 1982; Gavrilova et al. 1999; Dikic et al. 2008; Jethwa et al. 2008; Brown and Staples 2010). When we challenged the fasted mice with cold onset during the light phase, we were, however, able to dissociate torpor entry from its normal timing. Fasted mice which experienced the transition from thermoneutral ambient temperature to cold at 6:00 CET (after 18 h FD) or at midday (12:00 CET; after 24 h FD) entered torpor within ~20 min, i.e. torpor entry could be “switched on” in the light phase. The diurnal bouts were shorter (3–6 h) and T_b was less deep, yet the minimal oxygen consumption values in torpor were on average ~16 ml h⁻¹ (Fig. 3b, approximately –50% BMR; Golozoubova et al. 2006) and therefore justify the classification as torpor. In contrast, the mice did not enter torpor when the change in T_a coincided with the timing of *lights off*, indicating that the circadian onset of the nocturnal activity period could not be overridden.

The ability of cold exposed mice to actively suppress metabolic rate below BMR (operational: resting metabolic rate (RMR) at thermoneutrality) as a direct response to decreasing T_a was unexpected because endotherms normally recruit thermoregulatory heat production in order to maintain normothermia (Scholander et al. 1950). The differential responses to the interaction of cold and time of day have led us to speculate that torpor may also be functionally linked to an ultradian rhythm, which facilitates the entry into a state of suspended animation. The hypothesis that an ultradian rhythm may be permissive for torpor entry is supported by two independent findings. (1) It has been observed that following ablation of the SCN, the pacemaker of the circadian rhythm, food restricted Djungarian hamsters (*Phodopus sungorus*) lose their distinct circadian activity rhythm but they continue display torpor. The occurrence of these torpor bouts is irregular, and multiple torpor bouts per day could be observed, indicating that an ablation of the circadian pacemaker does not inhibit fasting induced torpor behaviour but abolishes its timing (Ruby and Zucker 1992). (2) In the same species, peripheral sympathetic inhibition by 6-hydroxydopamine (6-OHDA) not just suppressed torpor for 1 week, but hamsters also lost their ultradian rhythm of body

temperature for 2–6 days (Braulke and Heldmaier 2010). These results not just imply that an intact adrenergic system is a prerequisite for body temperature fluctuations, but the sequence of events also indicated that restoration of (daily) torpor was linked to the re-establishment of ultradian T_b rhythmicity. The interrelations of ultradian rhythmicity, circadian system, and metabolism remain to be elucidated. Interestingly, ultradian rhythms of body temperature fluctuations in the rat have recently been associated with rhythmic BAT thermogenesis (Ootsuka et al. 2009). The significance of this finding in the context of torpor is, however, debatable as rats do not enter torpor (Exner et al. 2000).

During hypothermia which is frequently associated with hibernation or daily torpor, the heat produced in BAT can be rapidly distributed to warm up vital organs such as the brain and the heart and thereby facilitate a coordinated return to normothermic body temperatures and agility. Nevertheless, birds, monotremes and some adult marsupials do not possess BAT, and yet they are able to rewarm from (deep) hypothermia. In species from these three groups rewarming to normothermia is largely achieved through shivering thermogenesis and may also be assisted by selection a warmer thermal environment. Our study shows that although UCP1-mediated NST constitutes a most potent mechanism of endogenous heat production in small mammals ($0.3\text{--}0.4\text{ J s}^{-1}\text{ g}^{-1}$ tissue mass in the Djungarian hamster *Phodopus sungorus*; Puchalski et al. 1987), its presence is not essential for the expression of a single torpor bout. In the absence of UCP1-mediated NST, however, the rate of heat production (efficiency of rewarming) is lower and more energy is required for arousal. The latter may account for the inability of UCP1-ablated mice to display multiple torpor bouts per day (Fig. 2b).

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