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Torpor patterns of hibernating eastern chipmunks *Tamias striatus* vary in response to the size and fatty acid composition of food hoards

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Summary

1. Many endotherms employ torpor during periods of resource scarcity, but this state of substantially reduced body temperature and metabolism appears to impose significant physiological costs. Accordingly, individuals can be expected to vary the expression of torpor according to the size of their energy reserves.
2. Although dietary polyunsaturated fatty acids (PUFAs) are important for maintaining the fluidity of membrane phospholipids and depot fats at low body temperatures, they are also prone to autoxidation, which can result in significant somatic damage. Dietary PUFA may thus influence the depth and duration of torpor during hibernation.
3. We evaluated the hypothesis that both an increase in the size of the burrow food hoard and an elevation of its PUFA composition can cause chipmunks to reduce their use of torpor both by reducing the time spent torpid and by maintaining higher body temperature during torpor.
4. We provided individual chipmunks with equicaloric natural-PUFA and high-PUFA supplements 10 days prior to autumn immergence. We measured seven parameters that characterize the depth and duration of torpor used by hibernating chipmunks using temperature-sensitive data loggers mounted on neck collars. We compared torpor patterns for the natural-PUFA, high-PUFA and control groups at a study site in southern Quebec, Canada. We also compared control animals from Quebec with unsupplemented controls from a more southerly site in Pennsylvania, USA characterized by higher food availability and less severe winters.
5. Chipmunks provided with natural-PUFA supplements spent less than half as much time in torpor as control animals at the same study site, and when in torpor they exhibited skin temperatures almost twice as high as controls. Chipmunks provided with high-PUFA supplements significantly reduced the depth and duration of torpor bouts compared with animals provided with natural-PUFA supplements. The torpor patterns of unsupplemented chipmunks at the southern site approximated those of natural-PUFA chipmunks at the main study site.
6. Our results provide clear evidence that chipmunks adjust the depth and duration of torpor expression according to both the size and the composition of their energy reserves. Furthermore, both the extent and the nutritional form of environmental energy availability are important determinants of the cost and benefits of torpor expression by free-ranging endotherms.

Key-words: hibernation, hoarding behaviour, oxidative stress, rodent, temperature logger.

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Introduction

Winter presents a severe energetic challenge to endothermic animals. Just as food availability drops to its annual minimum, low ambient temperatures (T_a) increase thermoregulatory costs and hence food requirements. Many mammals escape this energetic bottleneck by accumulating energy reserves prior to winter, either in the form of body fat or as a food reserve, and by subsequently expressing bouts of torpor during which they depress body temperature (T_b) well below their active 'normothermic' level. Reduction in T_b and the $T_b - T_a$ gradient substantially reduces both resting metabolism and the cost of thermoregulation, which can reduce metabolic rate by up to 99% (Wang & Hudson 1971; Thomas *et al*. 1990).

The depression of T_b and suppression of metabolic rate that characterize hibernation have an obvious adaptive value when energy reserves are limited and feeding opportunities are sporadic or non-existent. If the expression of torpor were free of any constraints, hibernators would be expected to employ the deepest possible torpor bouts for the longest possible periods. However, the expression of deep and prolonged torpor bouts appears to be either constrained by physiological processes or actively regulated by hibernators. All hibernators studied to date arouse periodically, increasing T_b and metabolic rate to levels characteristic of the active, normothermic state. Although the precise role of these arousals remains unclear, it is thought that they serve to restore physiological processes (homeostasis) inhibited by torpor (Thomas & Geiser 1997; Humphries *et al*. 2003a). The fact that arousals and the ensuing bouts of normothermy are energetically costly and account for 80–90% of energy expenditure over the hibernation period (Kayser 1965; Thomas *et al*. 1990) suggests that they cannot be avoided. These episodes may thus be viewed as a cost that hibernators must pay to re-establish homeostasis during hibernation.

A variety of physiological costs have been attributed to torpor. Torpor depresses immune response and Prendergast *et al*. (2002) suggested that animals arouse to restore immune function. Torpor may cause sleep deprivation (Daan *et al*. 1991; Trachsel *et al*. 1991) and partial memory loss (Millesi *et al*. 2001), or result in dehydration stress (Thomas & Geiser 1997). Torpor also leads to somatic damage caused by reactive oxygen species (ROS). At low T_b, the low rates of tissue perfusion reduce the circulation of antioxidant enzymes and low molecular mass antioxidants such as vitamins E (α-tocopherol) and C (ascorbate), resulting in significant oxidative damage (Carey *et al*. 2000). Furthermore, during arousal from deep torpor, the explosive increase in metabolic rate, accompanied by the perfusion of tissues with highly oxygenated blood, increases the generation of ROS which may temporarily overwhelm the antioxidant defences and result in severe oxidative stress (Buzadzic *et al*. 1990; Toien *et al*. 2001).

The fatty acid composition of membrane phospholipids and depot fats probably play an important role in determining both the degree of T_b depression during torpor and the level of oxidative stress. Diets with lower than normal levels of polyunsaturated fatty acids (PUFAs) have been shown to reduce the depth and duration of torpor bouts in a variety of hibernators, presumably because a certain level of PUFAs are necessary to maintain the fluidity of membranes and depot fats at low T_b (see Munro & Thomas 2004 for a review). However, PUFAs have the disadvantage of being much more sensitive to ROS than saturated and monounsaturated fatty acids. In the presence of ROS, PUFAs undergo autoxidation to produce toxic lipid peroxides (Mead *et al*. 1986), and this effect may be exacerbated by the high levels of oxidative stress associated with deep torpor and subsequent arousals. Thus, at low T_b the sensitivity of PUFAs to ROS may counteract their beneficial effects on membrane fluidity, such that either a lack of PUFA or an excess of PUFA can inhibit deep torpor expression. Therefore, the depth of torpor and the frequency of arousals also depend on the PUFA composition of membrane phospholipids and depot fats.

These arguments suggest that the expression of torpor during hibernation should be viewed in the light of a cost–benefit trade-off. The benefit of deep and prolonged torpor bouts is a substantial energy saving that allows animals with limited energy reserves to survive long winters. The cost of torpor is the inhibition of certain physiological processes and the increased risk of somatic damage that may impair physiological integrity and, ultimately, survival. Hibernators should thus regulate the depth and duration of torpor as a function of the size and fatty acid composition of their energy reserves. Hibernating mammals that rely on depot fats to sustain winter energy requirements typically have a maximum reserve size ≤ 40% of total body mass (Humphries *et al*. 2003a). This severely constrains their energetic options during hibernation, necessitating the expression of deep torpor by all individuals. In contrast, mammals that accumulate a food hoard in burrows prior to hibernation have much larger potential reserve sizes and, as a result, a wider range of energetic options (Humphries *et al*. 2003a,b). If the expression of torpor is indeed regulated by a cost–benefit trade-off, the outcome of which is shaped by the size of the energy reserve, then food-storing species should have the energetic flexibility to allow them to adjust the depth and duration of torpor bouts in response to changes in the size of their food reserves.

The strongest evidence that hibernators adjust the expression of torpor according to the size of their energy reserve was provided by recent laboratory and field studies on *Tamias striatus* (French 2000; Humphries *et al*. 2003b). These studies showed that individuals reduce both the depth and the duration of torpor bouts when provided with supplemental food in the form of sunflower seeds. However, sunflower seeds contain 65% linoleic acid (USDA 1984) and are far richer in PUFAs than the natural diet of chipmunks (Table 1).

Table 1. Percentage of 18 carbon chain fatty acids in the fat portion of two natural food items (acorns and beech nuts), of raw peanuts, sunflower seeds and of the mix of raw peanuts and sunflower seeds; data from (USDA 1984). DBI (double bond index) is calculated as the sum of the number of double bonds multiplied by the respective percentage for each fatty acid, and is calculated for 18 carbon chain fatty acids only. Total unsaturated fatty acids not mentioned here are below 7% for beech nuts and below 1·4% for other nuts

	Acorn	Beechnuts	Peanuts	Sunflower seeds	50/50 peanuts and sunflower seeds mix
18 : 0	1·06	2·37	2·23	4·44	3·33
18 : 1	63·34	37·7	48·24	18·9	33·57
18 : 2	19·26	36·8	31·6	65·83	48·7
18 : 3	0	3·4	0·006	0·14	0·073
DBI	101·86	121·5	111·5	151·0	131·3

For this reason, it is possible that the observed reduction in torpor was a response to high dietary PUFA intake rather than the size of the food reserve *per se*.

The purpose of our study was twofold. First, we determined experimentally the effect of variation in energy reserve size and PUFA content on the expression of torpor by hibernating *T. striatus*. To discriminate the effects of hoard size and PUFA content, we provided groups of free-ranging chipmunks with two equicaloric supplements that varied in PUFA content and compared their torpor patterns with an unsupplemented control group. Secondly, we examined the influence of natural gradients in food availability and winter length (latitude) by comparing torpor expression of our control animals in southern Quebec, Canada with that of a group of chipmunks in north-eastern Pennsylvania, USA where food was much more abundant, temperatures were milder and winter was shorter.

Materials and methods

The main portion of this study was conducted at Mont-Orford Provincial Park, Quebec, Canada (45°25′ N, 71°40′ W) in a mature mixed deciduous forest dominated by American beech (*Fagus grandifolia*), but also containing sugar maple (*Acer saccharum*), ash (*Fraxinus* spp.) and poplar (*Populus* spp.). This site, which we identify as QC-beech, produced a small beech mast during the fall of 2002 and chipmunks actively hoarded beech seeds during late summer and early fall in preparation for hibernation. We captured chipmunks using Sherman live traps placed where we observed chipmunks foraging. We marked each individual with a permanent ear-tag and clipped a pattern in the dorsal fur for visual identification following Clarke & Kramer (1994). Over the course of the 2002 active season, reproduction occurred only during summer and juveniles first emerged from the natal burrow during September and were identified on the basis of body mass using an upper limit of 75 g at first capture (Smith & Smith 1971). To identify burrow location, we offered peanuts to marked chipmunks and followed them to the entrance of their burrow.

To manipulate the size and PUFA composition of energy reserves, we assigned randomly a total of 34 chipmunks to control (n = 12), natural-PUFA supplement (n = 11) and high-PUFA supplement (n = 11) treatments. We provided the two food-supplemented groups with equicaloric supplements differing in PUFA content. The natural-PUFA supplement consisted of raw unshelled peanuts, which have a PUFA content intermediate between beech nuts and acorns, two staples in the diet of *T. striatus* (Table 1). The high-PUFA supplement combined equal amounts of peanuts and sunflower seeds to create a diet with PUFA content substantially higher that the natural diet of chipmunks (Table 1). Food-supplemented chipmunks were allowed to transfer approximately 31 500 kJ of food into their burrow hoard from a box placed near their burrow entrance (Humphries *et al*. 2003b). This amount should provide enough energy for chipmunks to remain normothermic throughout the hibernation season (Humphries *et al*. 2003b). To ensure that our supplement increased the size of the natural hoard, we allowed animals to forage normally from August until early October and then provided the food supplement over a 10-day period immediately preceding autumn immergence. We observed the boxes periodically during the provisioning period to ensure that only treatment chipmunks were transferring food from the box to their burrow. We removed the boxes as soon as the 31 500 kJ target had been transferred. No food was provided to the 12 control animals, which were occasionally observed harvesting beech nuts.

To examine how torpor expression of unsupplemented chipmunks varied across natural, environmental gradients, we established two secondary study sites. One was a forest dominated by sugar maple, located 2 km from the main Mont-Orford site (QC-maple). We could not find data on the fatty acid composition of maple seeds in the literature; therefore, we presumed that both PUFA composition and seed abundance may differ between main site and QC-maple. For this reason, 12 animals were fitted with collars at this site to determine whether reliance on maple seeds would modify torpor patterns when beech nuts were not available. The other secondary site was a mature oak forest in the Hawk Mountain Sanctuary, Schuylkill County, PA (40°38′ N, 76°00′ W), approximately 600 km southwest of the Quebec study sites. This mature oak forest (PA-oak) produced an abundant acorn mast during the fall of 2002 that apparently saturated the chipmunks'

requirements, because considerable mast remained on the ground after the autumn immergence of chipmunks. The shorter winter and extremely high food abundance at the PA-oak site contrasted with a longer winter and lower food abundance at the QC-beech and QC-maple sites, allowing us to examine how chipmunk torpor patterns varied as the ratio of food supply to overwinter cost changed.

We used temperature-sensitive data-loggers mounted on neck-collars to measure chipmunk torpor patterns over the entire hibernation period. Each collar consisted of a model DS-1921 Thermochron (Dallas Semiconductors, Dallas, TX, USA) that we removed from its original housing, trimmed, and fitted with a 30 mAh lithium battery. After potting in epoxy and mounting on a plastic-coated wire collar, the final mass of the collar was 2·5 g, which represents approximately 2–3% of chipmunk body mass. We programmed the thermochron circuit to read the temperature at a 128-min interval which allowed it to store temperature data for 183 days. Intensive live trapping near burrow entrances around the time of spring emergence allowed us to recover 33 collars (high-PUFA group: 4; natural-PUFA group: 6; QC-beech control group: 6; QC-maple control group: 6; PA-oak control group: 11).

The thermochron collars provided us with a detailed record of temperatures spanning the period prior to autumn immergence until just after the initiation of spring activity. Temperature-sensitive transmitters mounted in contact with the skin have been shown previously to provide a reliable index of T_b, with accuracy usually approaching *c.* 2 °C, e.g. Audet & Thomas (1996) and Kortner & Geiser (2000). We refer to temperatures as T_{skin}, but emphasize that these closely approximate T_b (see Results). Because T_{skin} never dropped below 30 °C for animals that we knew to be active, we define torpor as $T_{skin} \leq 30$ °C and normothermy as $T_{skin} > 30$ °C in the following analyses. For each animal, we calculated the total time spent in torpor during the hibernation season (total time spent torpid), the duration of hibernation from the onset of the first to the end of the last torpor episode (hibernation length), and the last day of torpor expression (last torpor day, expressed as Julian days since 1 January 2003). We also report the longest torpor bout, defined as the longest period when T_{skin} remained ≤ 30 °C, and the minimum T_{skin}, defined as the lowest temperature recorded by the collar during the winter. To compare torpor patterns between groups, we also used the five longest consecutive bouts of torpor and normothermy to calculate the mean torpor bout duration, mean normothermic bout duration and mean torpor T_{skin}.

We compared our measures of torpor used by QC-beech and QC-maple control groups using ANOVA. The lack of any significant differences allowed us to pool these two groups as QC-control. To compare control, natural-PUFA and high-PUFA treatments for Quebec, we used a factorial general linear model with Student–Neumann–Keul *post-hoc* tests, including age class, sex and treatment as fixed effects and as interaction terms. We identified significant variables using a backwards stepwise selection. To compare the QC-control with the PA-oak (control) group we used the same approach, excluding age class as a fixed effect because we were unable to reliably identify the age of captured animals in Pennsylvania. Because a general linear model (GLM) revealed no significant effect of sex or its interaction with region, we reduced this analysis to a simple *t*-test between independent samples. Results are presented as mean ± SE.

Results

T_{SKIN} AS AN INDEX OF T_B

T_{skin} measurements for animals during mid-winter normothermic intervals (36·3 ± 0·15 °C, max = 39·5 °C, n = 10 animals), when burrow temperatures were < 10 °C, were similar to those measured immediately prior to and following the hibernation season (36·8 ± 0·13 °C, max = 40·5 °C, n = 10 animals) and while testing collars in summer 2002 (37·5 ± 0·22 °C, max = 41 °C, n = 6 animals), when prevailing burrow temperatures were much warmer. During arousals, T_{skin} averaged 1–2 °C less than the mean T_b of normothermic chipmunks measured with implanted radio transmitters in the laboratory (38 °C; Wang & Hudson 1971). T_{skin} never dropped below 30 °C for active chipmunks. We conclude that T_{skin} provides a reasonable index of T_b and used 30 °C to define the transition between normothermy and torpor.

TORPOR PATTERNS FOR CONTROL, NATURAL-PUFA AND HIGH-PUFA GROUPS

None of the seven parameters measured for unsupplemented control chipmunks differed between the QC-beech and QC-maple sites (ANOVA; $P > 0{\cdot}43$ in all cases). Therefore, we pooled these two groups and refer to them collectively as QC-control (n = 12). All chipmunks in the QC-control group expressed deep (T_{skin} < 15 °C) and prolonged (duration > 48 h) torpor bouts during hibernation. On average, QC-controls were in torpor for 69% of the period between their first and last torpor bout. Torpor bouts averaged 78·5 ± 1·2 h and T_{skin} averaged 8·9 ± 1·2 °C during the five longest bouts of torpor (Table 2). The torpor patterns of QC-controls were characterized by three recognizable phases roughly corresponding with autumn, winter and spring (Fig. 1), although the timing of onset and duration of phases varied considerably between chipmunks.

Our food supplementation experiments provided strong evidence that both hoard size and PUFA content influence torpor expression by chipmunks (Tables 2 and 3). The natural-PUFA group, which we consider to represent a manipulation of hoard size only, spent less than half as much time in torpor during winter as QC-controls (natural-PUFA: 42·4 days;

Table 2. Mean (± SE) values for parameters describing hibernation of chipmunks in Quebec. Torpor bout length, normothermic bout duration and torpor T_{skin} were calculated using the five longest torpor bouts; the values thus represent the deepest part of the hibernation period rather than a general mean. Last torpor bout is expressed as Julian date. Different superscript letters represent significant differences

Parameter	Control $n = 12$	natural-PUFA $n = 6$	high-PUFA $n = 4$
Total time spent torpid (days)	$104{\cdot}3^{a}$ (6·5)	$42{\cdot}4^{b}$ (9·1)	$12{\cdot}6^{c}$ (11·2)
Duration of hibernation (days)	$150{\cdot}5^{a}$ (6·6)	$112{\cdot}1^{b}$ (19·6)	$88{\cdot}1^{b}$ (8·9)
Torpor bout length (h)	$78{\cdot}5^{a}$ (1·2)	$28{\cdot}1^{b}$ (1·3)	$14{\cdot}8^{b}$ (1·3)
Normothermic bout duration (h)	$17{\cdot}1^{a}$ (1·08)	$27{\cdot}9^{b}$ (1·5)	$34{\cdot}8^{b}$ (2·29)
Torpor T_{skin} (°C)	$8{\cdot}9^{a}$ (1·18)	$16{\cdot}3^{b}$ (1·67)	$22{\cdot}4^{c}$ (2·05)
Lowest torpid T_{skin} (°C)	$5{\cdot}1^{a}$ (0·02)	$10{\cdot}4^{b}$ (0·04)	$19{\cdot}0^{c}$ (0·1)
Longest torpor bout (h)	$109{\cdot}2^{a}$ (12·6)	$46{\cdot}6^{b}$ (12·2)	$21{\cdot}9^{b}$ (5·9)
Last torpor bout (days)	$86{\cdot}5^{a}$ (8·5)	$85{\cdot}3^{a}$ (12·0)	$33{\cdot}7^{b}$ (14·7)

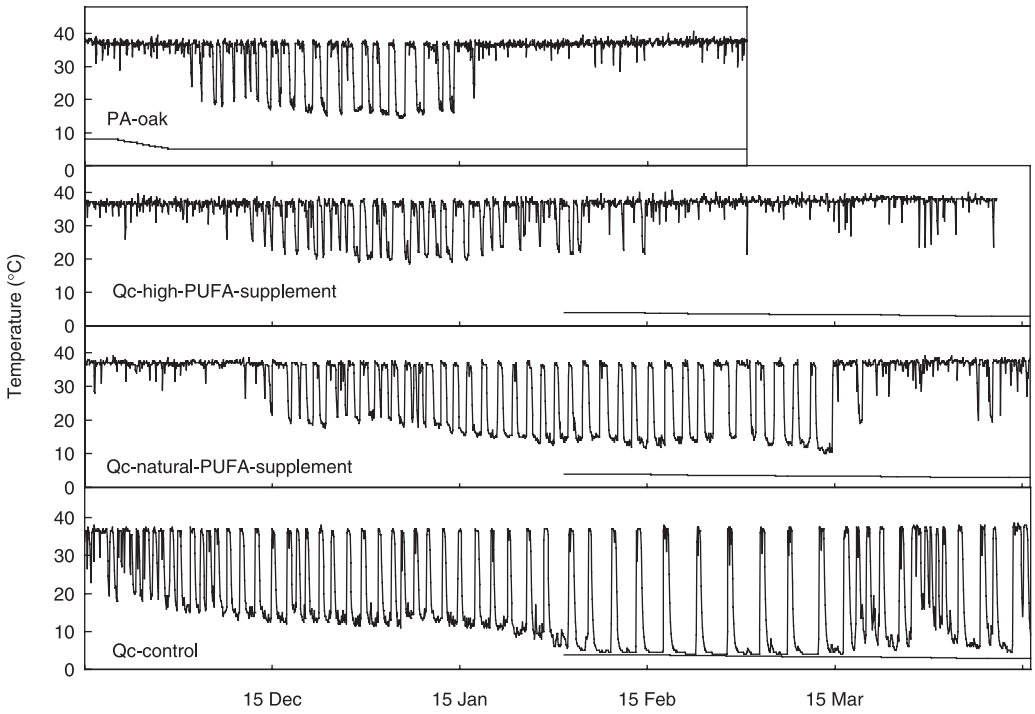

Fig. 1. Skin temperature recordings for a representative chipmunk from each treatment over the course of the hibernation period. Soil temperature is indicated by the lower line on each graph whenever data are available. Note that clear autumn, winter and spring phases, as reported by French (2000), are visible only for the QC-control. In this particular case, the winter phase seems to be subdivided into a first shallower part and a second deeper part during which T_{skin} closely approached T_a.

control: 104·3 days; Tables 2 and 3; Fig. 2), and when in torpor were characterized by T_{skin} almost twice as high (natural-PUFA: 16·3 °C; control: 8·9 °C; Tables 2 and 3; Fig. 3). Chipmunks provided with a high-PUFA supplement, which we consider to represent a manipulation of both hoard size and dietary PUFA content, further reduced their use of torpor compared with the natural-PUFA group (Tables 2 and 3, Figs 2 and 3). The total time spent in torpor over the winter was only 12·6 days for the high-PUFA group, compared to 42·4 days for the natural-PUFA group. Mean T_{skin} during the five longest torpor bouts was 22·4 °C for the high-PUFA group compared to 16·3 °C for the natural-PUFA group. Animals provided with a high-PUFA supplement also terminated hibernation earlier than the natural-PUFA group (Julian date; natural-PUFA: 85·3; high-PUFA: 33·7).

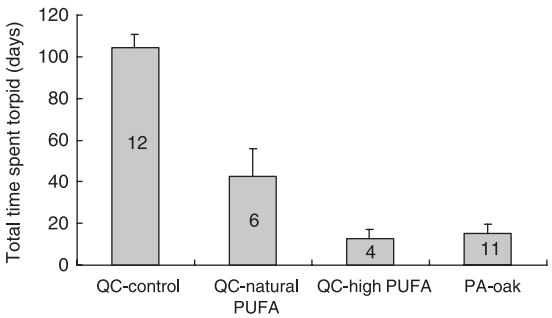

Fig. 2. Total time (mean ± SE) spent torpid by chipmunks from the four treatment groups over the course of the hibernation period. An animal was considered torpid when the collar reading was below 30 °C. Sample sizes are indicated within the bars. The two control groups differ significantly ($t = 10{\cdot}8$, $P = 0{\cdot}000$) and all treatment groups for Quebec differ significantly (see Table 3).

Table 3. General linear model for the parameters describing hibernation of chipmunks in Quebec. Means for torpor bout duration, torpor T_{skin} and normothermic bout duration were calculated using the five longest torpor bouts. Only significant variables are presented

Parameter	d.f.	*F*-value	*P*-value
Total time spent torpid			
Treatment	2	31·81	0·000
Sex	1	4·59	0·046
Error	18		
Duration of hibernation			
Treatment	2	8·17	0·004
Error	19		
Torpor bout length			
Treatment	2	13·92	0·000
Sex	1	6·03	0·024
Error	18		
Normothermic bout duration			
Treatment	2	33·06	0·000
Sex	1	14·17	0·002
Treatment × sex	2	5·32	0·018
Error	15		
Torpor T_{skin}			
Treatment	2	18·25	0·000
Sex	1	7·24	0·015
Error	18		
Lowest torpor T_{skin}			
Treatment	2	26·15	0·000
Sex	1	7·48	0·014
Error	18		
Longest torpor bout			
Treatment	2	12·33	0·001
Error	19		
Last torpor bout			
Treatment	2	5·21	0·016
Sex	1	4·91	0·040
Error	18		

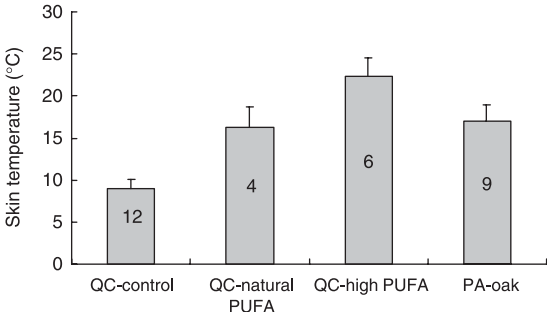

Fig. 3. Mean (± SE) T_{skin} of chipmunks from the four treatment groups during the five longest torpor bouts of the hibernation period. Sample sizes are indicated within the bars. The two control groups differ significantly (t = 3·66, P = 0·002) and all treatment groups for Quebec also differ significantly (see Table 3).

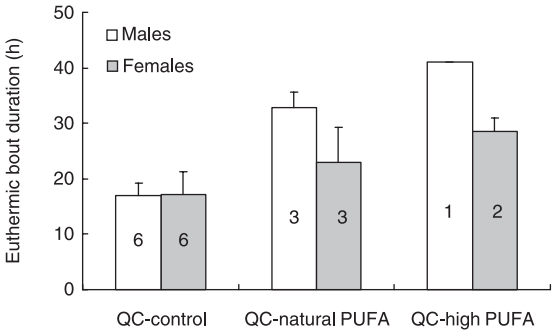

Fig. 4. Normothermic bout duration (mean ± SE) as a function of sex across treatments for chipmunks in Quebec. Sample sizes are indicated within the bar (see Table 3 for model).

In addition to these treatment effects, gender had a significant effect on six of the seven torpor variables (Table 3). Males had consistently shorter torpor bouts, higher T_{skin} and longer normothermic intervals than females across the three treatments. Furthermore, males increased the duration of normothermic intervals in response to supplementation to a greater extent than females (Fig. 4). Age class (juveniles vs. adults) had no significant effect on any of the eight torpor variables, either as a main effect or as an interaction.

Chipmunks in Pennsylvania (PA-oak) resorted to torpor to a much lesser extent than did control chipmunks in Quebec (QC-control). Two PA-oak animals never entered torpor (i.e. never had T_{skin} < 30 °C) and two expressed only a few sporadic, shallow torpor bouts. The eight remaining chipmunks expressed regular torpor bouts, but spent much less time in torpor and had significantly higher T_{skin} than QC-controls (Table 4, Figs 2 and 3). The reduced time

Table 4. Comparison (means ± SE) between hibernation by control groups of Quebec (QC-control) and Pennsylvania (PA-control) chipmunks. In Pennsylvania, two chipmunks who did not enter torpor were included in the calculation of total time spent torpid and duration of hibernation with a value of 0, but were excluded from the calculation of the remaining variables. Two PA-control chipmunks who only expressed sporadic torpor bouts were also excluded from calculation of normothermic bout length because of the absence of any clear transitions between bouts of torpor and normothermy

Parameter	QC-control	PA-oak	d.f.	*t*	*P*-value
Total time spent torpid (days)	104·3 ± 6·5 (n = 12)	15·1 ± 4·7 (n = 11)	21	10·76	0·000
Duration of hibernation (days)	150·5 ± 6·6 (n = 12)	34·8 ± 8·4 (n = 11)	21	10·99	0·000
Torpor bout length (h)	88·1 ± 1·2 (n = 12)	32·3 ± 8·4 (n = 9)	19	3·48	0·003
Normothermic bout duration (h)	17·1 ± 2·1 (n = 12)	27·4 ± 2·7 (n = 7)	17	3·61	0·002
Torpor T_{skin} (°C)	8·9 ± 1·4 (n = 12)	17·0 ± 1·7 (n = 9)	19	3·66	0·002
Lowest torpor T_{skin} (°C)	5·4 ± 1·3 (n = 12)	13·6 ± 1·5 (n = 9)	19	4·12	0·001
Longest torpor bout (h)	109·2 ± 11·8 (n = 12)	43·0 ± 13·6 (n = 9)	19	3·69	0·002

spent in torpor by Pennsylvania chipmunks resulted from both a shorter hibernation season and reduced time spent in torpor during the hibernation season.

Discussion

Our study presents clear evidence that food supplementation with natural levels of PUFA (in this case, 31 500 kJ of raw peanuts) leads to a reduction in the depth and duration of torpor for free-ranging chipmunks. The total time spent torpid by the natural-PUFA group was reduced to less than half the value for controls, while the mean T_{skin} during the five longest torpor episodes was *c.* 8 °C higher. These results corroborate studies of captive animals by French (2000) and of free-ranging animals by Humphries *et al.* (2003b), which also found torpor expression by *T. striatus* to be reduced by food supplementation. However, our data significantly advance these earlier studies by (1) demonstrating that torpor is reduced in response to supplements that approximate the PUFA composition of natural diets, (2) demonstrating that diets with PUFA levels exceeding that of the natural diet further reduce the expression of torpor in free-ranging animals and (3) achieving continuous sampling of body temperature patterns to permit identification of which components of the torpor cycle are affected by hoard size and composition. In fact, all components of the torpor cycle are affected – chipmunks with large energy reserves have significantly shorter torpor bouts, warmer torpor temperatures and longer normothermic intervals than control chipmunks from the same region. Levels of dietary PUFA exceeding natural levels lead to additional effects on most torpor components.

There is no reason to believe that the hoard size effect is an experimental artefact, because natural variation in the torpor patterns expressed by chipmunks across gradients in food availability and latitude are generally consistent with the results of experimental food supplements. Control animals from a more southerly locality with higher food availability (PA-oak) were characterized by substantially reduced torpor expression, similar to experimentally supplemented chipmunks in Quebec. Although the shorter hibernation period of Pennsylvania chipmunks probably resulted from a shorter winter at lower latitudes, this cannot account for the differences that we found within the hibernation period. All evidence indicates that reduced torpor expression during hibernation results from greater food abundance and a reduced need to conserve energy. In support of this interpretation, the mid-winter torpor bout duration and mean T_{skin} during torpor for this group was closer to the values for the natural-PUFA group than to any other of the treatment groups. This suggests that any increase in food availability, whether natural or experimental, reduces the depth and duration of torpor bouts used by chipmunks.

We interpret the response of chipmunks to our supplementation experiments as indicating that hoard size and dietary PUFA levels act in concert to determine the degree of expression of torpor during the hibernation period. However, two alternate explanations should be considered. We do not know how chipmunks evaluate the size and the energetic value of the food hoard in their burrow. Although our high-PUFA and natural-PUFA supplements had the same caloric value, they differed in the number and type of foods. It is possible that chipmunks perceived the high-PUFA supplement, which consisted of both sunflower seeds and peanuts, as more energetically valuable than the natural-PUFA supplement, which consisted of peanuts alone, independent of their PUFA composition. Perhaps chipmunks perceive a food hoard consisting of a single food type in general, or of peanuts in particular, as more susceptible to spoilage than a hoard consisting of both peanuts and sunflower seeds. If this were the case, chipmunks given single-species or peanut supplements would express deeper and longer torpor bouts in order to spare food and account for the possibility of greater spoilage of their food hoard during the winter. Alternatively, if chipmunks perceive hoard size based on the time they are engaged in loading the cheek pouches and transporting food to the burrow, then supplements containing sunflower seeds may be perceived to be larger because the energy return rate (kJ/time) is lower than for larger items such as acorns and peanuts (Munro, Humphries, personal observations). Thus, at present we cannot reject the possibility that what we interpret here as a PUFA effect may, in fact, simply be a variation of the hoard size effect.

We found that males expressed shallower and shorter torpor bouts, exhibit longer normothermic intervals, cease hibernation earlier and increased the duration of normothermic intervals more in response to food supplementation than did females. Similar differences in hibernation patterns between sexes are reported for *Spermophilus richardsonii* (Richardson's ground squirrels); males of this species exhibit longer normothermic intervals and terminate hibernation earlier than females (Michener 1992), perhaps to facilitate males' testicular recrudescence in anticipation of spring reproduction (Michener 1998; see also Barnes *et al.* 1986). Similarly, male chipmunks might exploit their food reserves to a greater extent during hibernation to ensure maximum reproductive capacity for early spring mating (Barnes 1996). In contrast, females may benefit from maintaining deeper torpor to conserve their food hoard for pregnancy and lactation in the spring, while fresh food is not yet available.

One view of hibernation is that the expression of deep and prolonged torpor bouts is the hibernator's optimal strategy and that higher T_b and frequent arousals represent a deviation from this optimum that is imposed by physiological constraints. 'Colder is better' because low T_b allows animals to spare their energy reserves during winter, possibly to maintain a residual reserve to face uncertain conditions in spring. This 'energy minimization' framework is not entirely without

support. Hibernation *is* energetically demanding and some animals *do* face energy shortfalls that result in mortality before spring emergence (Murie & Boag 1984). Presumably these animals would have increased their chances of survival had they been able to regulate their average metabolic rate at a lower level, through some combination of reduced T_b and longer torpor bouts. However, an energy minimization framework does not explain adequately all patterns observed during hibernation. The results that we obtained using a food-storing hibernator as a study model suggest that the precise organization of hibernation, namely the depth and duration of torpor and the proportion of time allocated to normothermy, is under individual control. Food-storing hibernators appear to regulate the expression of torpor primarily as a response to both the size and the quality of their energy reserve and to a lesser extent as a response to their gender-specific reproductive requirements. An experimental increase in the size of their energy reserve provides food-storing hibernators with the energetic flexibility that allows them to increase T_b and to reduce the time spent in torpor, and so reduce the costs associated with torpor. Because they do not need to minimize energy expenditure, there appears to be no fitness benefit to employing deep and prolonged torpor bouts. Simultaneously increasing energetic flexibility (by increasing hoard size) and the risk of oxidative stress (by increasing dietary PUFA content) further reduces the expression of torpor by not only reducing the need for energy economy, but also increasing the physiological costs of torpor. The pattern shown by chipmunks is consistent with the view that the expression of torpor is determined by the balance between costs and benefits. Changing the costs or the benefits or both alters the cost–benefit balance and the optimal hibernation strategy.

Food-storing hibernators such as *T. striatus* may show greater flexibility in their torpor patterns than fat-storing hibernators due to the greater energetic flexibility that the food hoard provides. However, several lines of evidence suggest that fat-storing hibernators also adjust torpor patterns in response to a cost–benefit balance, albeit to a lesser degree. Fat-storing *S. lateralis* (golden-mantled ground squirrels) exhibit a programmed mass loss through winter, whereby animals adjust the rate of fat depletion to meet a target fat reserve by the end of hibernation (Mrosovsky 1976). Fatter animals experience a greater over-winter mass loss than leaner animals, indicating that the two groups regulate either T_b and metabolism in torpor or the frequency and duration of arousals in response to the size of their fat reserve at the onset of hibernation. Additional studies on captive *S. beldingi* (fat-storing ground squirrels) show that males with large pre-emergent energy reserves emerge earlier in spring (French 1982) and in better reproductive condition (Barnes 1984) than males with smaller reserves. Furthermore, hibernating bats (*Rhinolophus ferrumequinum* and *Myotis daubentoni*) select ambient temperatures in hibernacula that are positively correlated with fat reserves (Ransome 1990; Kokurewicz 2004), with the result that T_b and metabolic rate are positively correlated with the size of the energy reserve, as they are for chipmunks. Although no studies have examined the torpor patterns of fat-storing hibernators in the light of a cost–benefit balance, we argue that the current evidence supports the hypothesis that all hibernators regulate the expression of torpor and normothermy either actively, through the endogenous production of heat to regulate T_b (as with chipmunks) or passively, through the selection of hibernaculum's microclimate (as with bats).

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