

Dietary Fatty Acid Composition and the Hibernation Patterns in Free-Ranging Arctic Ground Squirrels

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ABSTRACT

Laboratory experiments have demonstrated that the amount of polyunsaturated fatty acids (PUFAs) in the diet before hibernation influences patterns of mammalian torpor. The hibernation ability of ground squirrels is greatest (longest torpor bouts, greatest number of animals entering torpor) when the PUFA content of their fall diets is 33–74 mg/g, under laboratory conditions. The extent to which natural fall diets both (a) vary in PUFA content and (b) influence the torpor patterns of free-ranging populations of hibernating mammals is unknown, however. We conducted a 3-yr study on the diet PUFA contents and subsequent hibernation patterns of free-ranging arctic ground squirrels (*Spermophilus parryii*) in the Brooks Range of Alaska. We found that the PUFA contents of fall diets varied more than threefold among individuals. Our study also revealed that arctic ground squirrels that consumed a moderate-PUFA (33–74 mg/g) diet had (a) longer torpor bouts, (b) fewer arousals from torpor, (c) shorter arousal periods, (d) more days in torpor, and (e) greater probability of persisting in the population than those that consumed a high-PUFA (>74 mg/g) diet during the fall. No animals were demonstrated to have consumed a diet representing low-PUFA (<33 mg/g) values. Our study is therefore the first to demonstrate that estimated dietary PUFA levels of a free-ranging hibernator influence subsequent torpor patterns.

Introduction

Mammals and birds are endotherms, capable of maintaining a constant body temperature (T_b) over a wide range of ambient

temperatures (T_a) through a high metabolic rate (Willmer et al. 2000). The prolonged periods of high metabolic heat production by mammals and birds require high rates of food intake. Food availability in the wild often fluctuates, and consequently the energetic costs of maintaining a high T_b (32°–42°C) via endothermy can become prohibitive during certain situations. This is one reason why not all mammals and birds are constantly homeothermic but instead enter torpor in anticipation of unreliable food availability (Geiser 2004). Torpor is a period when physiological and biochemical processes, including metabolic rate, are greatly reduced. Torpor in mammals and birds is not a state in which endothermy/thermoregulation is abandoned (Geiser 2004). It involves the regulation of T_b at a new and substantially lower level, with a new critical minimum T_b being maintained. Minimum metabolic rates during mammalian torpor can be 2%–10% of basal metabolic rate (Kayser 1965), with minimum T_b as low as -2°C (Barnes 1989). Mammals and birds generally employ one of two common patterns of torpor, depending on species: multiday torpor during hibernation and daily torpor. Hibernation is seasonal, usually from late summer/fall to spring. Hibernators do not remain hypometabolic throughout the hibernation season; instead, bouts of torpor last from days to weeks, interrupted by brief (<36 h) periods of high metabolic rates and high T_b (euthermy). Hibernation is the most common pattern of torpor found in mammals (Geiser and Ruf 1995; Geiser 2004).

Torpor is known to occur in about 75 different species of mammals, and it is common in many groups. About 270 extant species of marsupials (infraclass *Metatheria*) currently exist (Feldhamer et al. 1999), and torpor occurs in at least 33 (>12%) of them, with species employing torpor being found in six of the seven marsupial orders (Geiser 1994). Placental mammals (infraclass *Eutheria*) are classified into 18 orders (Feldhamer et al. 1999), six of which contain species that utilize torpor (Mzilikazi et al. 2002; Geiser 2004). Torpor either is an ancestral trait that has been retained in many lineages or it has appeared repeatedly, frequently, and independently throughout the course of mammalian evolution, which leads to a fundamental question: What are the ecological benefits and costs of torpor in mammals that have favored the evolution of this strategy in some species but not in other groups? The primary benefit associated with mammalian torpor is the conservation of energy and food stores, and these savings were well quantified more than 40 yr ago (Kayser 1965). The potential ecological constraints on mammalian torpor that may have restricted the evolution of this strategy, however, have only recently begun to be elucidated.

One possible ecological constraint on mammalian tor-

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por may be the concentration of polyunsaturated fatty acids (PUFAs) in the natural diets of free-ranging mammals. A PUFA has more than one carbon-carbon double bond per molecule, as opposed to a saturated fatty acid, which contains no carbon-carbon double bonds, or a monounsaturated fatty acid, which contains only one such bond per molecule. Mammals can synthesize saturated and monounsaturated fatty acids, but they are incapable of producing PUFAs. Two of the most common PUFAs produced by plants are linoleic acid (18 carbon atoms, two double bonds [18:2]) and α -linolenic acid (18 carbon atoms, three double bonds [18:3]). When mammals consume PUFAs, these fatty acids are incorporated into their cell membranes and storage lipids (Gunstone 1996). Laboratory experiments with chipmunks (*Tamias amoenus*), two ground squirrel species (*Spermophilus saturatus* and *Spermophilus lateralis*), two species of prairie dogs (*Cynomys ludovicianus* and *Cynomys leucurus*), and marmots (*Marmota flaviventris*) revealed that dietary levels of linoleic acid during fattening influence their ability to hibernate (Geiser and Kenagy 1987, 1993; Frank 1992; Florant et al. 1993; Thorp et al. 1994; Harlow and Frank 2001). Similar results were found in studies with mice and two species of marsupials (Geiser 1991; Geiser et al. 1992; Withers et al. 1996). A total of 17 laboratory experiments detailing the effects of diet linoleic acid levels on mammalian torpor have been conducted (reviewed by Munro and Thomas [2004]). It thus appears that diet PUFA levels influence the torpor patterns of mammals.

Previous laboratory studies with golden-mantled ground squirrels (*S. lateralis*) show that hibernation ability is greatest when the linoleic acid (18:2) content of the diet is at least 33 mg/g but less than 74 mg/g. Squirrels fed a 33–74-mg linoleic acid/g diet (1) were more likely to hibernate, (2) spent less time fasting before the onset of torpor, (3) had lower metabolic rates during torpor, and (4) had longer torpor bouts than those maintained on diets containing either less or more linoleic acid (Frank 1992, 2002; Frank and Storey 1995, 1996; Frank et al. 1998). Subsequent laboratory experiments have demonstrated that the amount of α -linolenic acid (18:3) in the diet influences the torpor patterns of *S. lateralis* in a manner identical to that for linoleic acid (Frank et al. 2004).

The goal of this study was to determine whether natural variations in the total amount of PUFAs (linoleic and α -linolenic acids) in the fall diets of free-ranging hibernators influence their hibernation patterns. We predicted that free-ranging ground squirrels ingesting fall diets with total PUFA (18:2 and 18:3) contents outside of the 33–74-mg/g range would (a) have shorter torpor bouts during hibernation, (b) spend less time in torpor during hibernation, and (c) have a greater rate of disappearance from a population than those with fall diets that have total PUFA contents between 33 and 74 mg/g. We tested these predictions in a 3-yr field study on the diet composition and subsequent hibernation patterns of adult arctic ground squirrels (*Spermophilus parryii*) in the northern Brooks Range of Alaska.

Material and Methods

Study Species and Location

Arctic ground squirrels (*Spermophilus parryii*) are omnivores that hibernate for 6–8 mo each year (Karels et al. 2000). Body mass greatly increases for 5–7 wk before immergence into hibernation, and adult body fat contents reach 30%–41.5% of total mass when animals are last trapped in the fall (Buck and Barnes 1999a). Fat and lean mass decrease over winter; both triacylglycerols and carbohydrates derived from protein are thought to be important substrates for thermogenesis and metabolism for arctic ground squirrels during hibernation (Buck and Barnes 1999a, 2000). The hibernation season of ground squirrels is characterized by 1–3-wk intervals of torpor separated by brief (<36 h) arousal episodes during which animals spontaneously rewarm to near-euthermic body temperatures of 34°–36°C for 15–24 h (Young 1990; Michener 1992; Barnes and Ritter 1993). Our study site was near the Toolik Field Station operated by the Institute of Arctic Biology (University of Alaska–Fairbanks) in northern Alaska (68°38'N, 149°38'W, elevation 809 m). It encompassed an area of about 50 ha and was tundra dominated by dry heath (*Eriophorum vaginatum*), with dwarf shrubs of willow (*Salix* spp.) and birch (*Betula* spp.).

Adult female *S. parryii* at Toolik begin fattening during July, with maximal body mass and the onset of hibernation attained by mid-August, whereas adult males begin fattening in August, with maximum body mass and hibernation achieved by late September (Buck and Barnes 1999a). Adult *S. parryii* were live-trapped for the field study during the last 2 wk of August in 1998, 1999, and 2000. A total of 57 individuals were collected. Ground squirrels were brought to a laboratory at the Toolik Field Station after capture, where they were anesthetized using Metaflane vapors. A 1–2-g sample of white adipose tissue (WAT) was removed from an abdominal fat pad via laparotomy, and a small temperature-sensitive data logger was inserted into the abdominal cavity before the incision was sutured closed. WAT samples were frozen at –80°C for later analyses. Data loggers were modified Onset Tidbit temperature loggers (model TBI32-20+50; Pocasset, MA) programmed to begin core body temperature readings ($\pm 0.2^\circ\text{C}$) at 30-min intervals on August 31 of each year. Details of logger preparation are in Long et al. (2007). Each ground squirrel was allowed to recover in captivity for 24 h after surgery, marked with a standard rodent ear tag, and then released at the site of capture. Spring emergence from hibernation is in early April for males and mid-April to early May in females at this study site (Buck and Barnes 1999a). We trapped during the last 3 wk of May during 1999, 2000, and 2001 in an attempt to retrieve all the implanted squirrels. The logger was surgically removed from each squirrel after capture, and all squirrels were released at the site of capture after a 24-h recovery period.

For an additional experiment using semisynthetic diets of different PUFA levels (see “Dietary Fatty Acid Analyses”), 38 adult *S. parryii* were collected from areas adjacent to our study site during July 1999 and transported to the Animal Care Fa-

cility of the University of Alaska–Fairbanks. They were divided into four diet groups, housed individually in standard cages, and maintained at $T_a = 22^\circ\text{C}$ on a natural (fall) photoperiod for the next 5 wk. An approximately 1-g biopsy of WAT was collected via laparotomy from fat pads in the abdomen of each squirrel at the end of the feeding/fattening period (September) and stored at -80°C for later fatty acid analysis. All animal care and experimentation was conducted under the approval of the University of Alaska–Fairbanks Animal Care and Use Committee.

Dietary Fatty Acid Analyses

The average amounts of monounsaturated and polyunsaturated fatty acids in natural *S. parryii* diets during the entire fall feeding/fattening period were estimated by measuring the concentrations of these fatty acids in abdominal WAT samples collected just before hibernation (August). This method was previously developed and used successfully to determine the fatty acid compositions of the fall diets of free-ranging golden-mantled ground squirrels (*Spermophilus lateralis*) in alpine areas (Frank et al. 1998, 2004) because rodents cannot be induced to vomit, and therefore diet composition during the entire 5–7-wk fall feeding period cannot be determined by repeatedly collecting stomach contents from the same animals (see Frank 1994). The concentrations of both MUFAs and PUFAs in mammalian WAT depend on the amount of these fatty acids in the diet during fattening (Mead et al. 1986).

The semisynthetic diets fed to captive ground squirrels varied only in fatty acid composition and were produced by the Test Diets Division of Purina Mills (Richmond, IN). A mixture of 90% Purina 5001 Chow and 10% plant oil was produced in each case; the diets were then homogenized and pressed into 1-g pellets. All diets were thus 20% protein, 51% carbohydrate, 16.5% lipid, 7% ash, and 5.3% fiber. A different commercial plant oil was used in each diet to vary fatty acid composition; thus, all fatty acids were in a natural (triacylglycerol) form. Most of the fatty acids in the diets were 18 carbon atoms long: oleic (18:1), linoleic (18:2), and α -linolenic (18:3) acids. Linoleic acid was the most common PUFA in these diets because it accounts for more than half of the PUFAs found in the diets of free-ranging ground squirrels (Frank 1991, 1994). The fatty acid composition of the low-PUFA diet was 14.3 mg/g of 18:1, 13.1 mg/g of 18:2, and 1.2 mg/g of 18:3. The medium-low-PUFA diet contained 79.4 mg/g of 18:1, 33.0 mg/g of 18:2, and 3.0 mg/g of 18:3. The medium-PUFA diet had 37.4 mg/g of 18:1, 51.2 mg/g of 18:2, and 5.6 mg/g of 18:3. The high-PUFA diet consisted of 33.0 mg/g of 18:1, 78.4 mg/g of 18:2, and 3.5 mg/g of 18:3.

The fatty acid compositions of both the semisynthetic diets and the WAT samples were determined by gas-liquid chromatography. All lipids were first extracted from diet samples using a chloroform-methanol procedure (Folch et al. 1957). All fatty acids were isolated from the extracted lipids by transesterification with 1.0 N methanolic HCl, producing fatty acid

methyl esters (Christie 1989). Fatty acid methyl esters were identified and quantified using a model 5890 gas-liquid chromatograph (Hewlett Packard, Palo Alto, CA) fitted with a 30-m-long model DB-23 capillary column (J & W Scientific, Folsom, CA). The column was initially held at 110°C for 3 min, then raised to 160°C at $20^\circ\text{C}/\text{min}$, and finally brought to 210°C at a rate of $4^\circ\text{C}/\text{min}$. The carrier gas was helium flowing at a rate of $30\text{ cm}^3/\text{min}$. This system permitted the identification and quantification of all fatty acids types that were 12–22 carbon atoms in length. The fatty acid compositions of WAT samples collected from the captive *S. parryii* were examined by least squares regression to quantify the relationship between dietary oleic acid content (mg/g) and the amount of oleic acid (%) in the WAT sample, as well as that between dietary linoleic acid content (mg/g) and the level of 18:2 (%) in WAT (Fig. 1).

Separate comparisons were made for oleic and linoleic acids because mammals can synthesize MUFAs but not PUFAs. The equation describing the relationship between dietary and WAT levels of oleic acid ($y = 31.85 + 0.37x$) was used to estimate the oleic acid contents of natural fall diets for *S. parryii* included in the free-ranging hibernation study from the levels of oleic acid found in the WAT sample collected from each individual before hibernation. Oleic acid was the only MUFA considered because it is the only MUFA produced by most plant species (Mead et al. 1986) and the only MUFA found in the diets of free-ranging ground squirrels (Frank 1994; Frank et al. 1998). The equation summarizing the relationship between levels of linoleic acid in the diet (mg/g) and in WAT ($y = 0 + 0.38x$) was used to estimate dietary linoleic acid content (mg/g) from the concentration of linoleic acid (%) measured in the WAT collected from free-ranging *S. parryii* during August. Commercial plant oils do not contain significant amounts of α -linolenic acid (Gunstone 1996); thus, semisynthetic diets varying in α -linolenic acid content could not be produced for the laboratory experiments. Mammals absorb and accumulate lin-

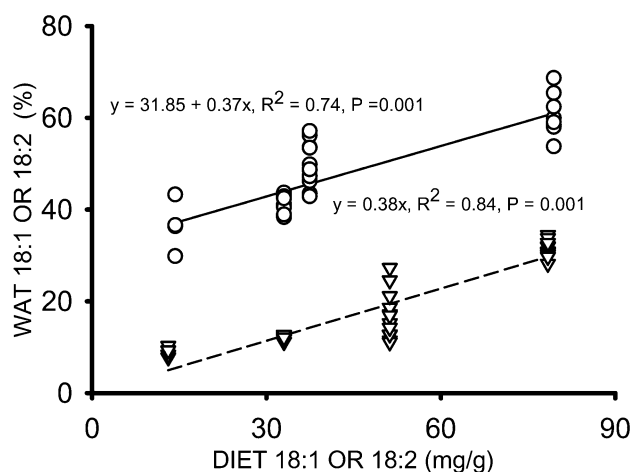


Figure 1. Relationships between dietary and white adipose tissue (WAT) levels of oleic acid (circles) and linoleic acid (triangles). The line drawn through each set of points represents the least squares linear regression.

oleic and α -linolenic acids equally well in their WAT (Gunstone 1996), however; therefore, the linoleic acid equation was also used to estimate dietary α -linolenic acid content (mg/g) from the concentration of α -linolenic acid present in the WAT samples collected from free-ranging *S. parryii* during August.

Because the level of α -linolenic acid in the diet influences torpor in a manner virtually identical to that for linoleic acids (Frank et al. 2004) and because these were the only two PUFA types found in the diet (WAT) of free-ranging *S. parryii* (see "Results"), the estimated levels of linoleic and α -linolenic acids were summed (18:2 + 18:3) to calculate the total PUFA content for the average fall diet of each free-ranging individual. A review of all laboratory studies on the effects of dietary PUFA levels on mammalian torpor indicates that dietary PUFAs affect the torpor patterns of most sciurid (chipmunks, ground squirrels, and marmots) hibernators to the same extent (Munro and Thomas 2004). The relationship between dietary PUFA content and subsequent torpor patterns has been most extensively detailed for the golden-mantled ground squirrel (*S. lateralis*). Interpreting the results of several studies together indicates that hibernation is most likely when the diet has a PUFA content of at least 33 mg/g but no more than 74 mg/g (Frank 1992, 2002; Frank and Storey 1995, 1996; Frank et al. 1998). Consequently, free-ranging *S. parryii* with a total dietary PUFA content of 33–74 mg/g during the fall were placed in the moderate-PUFA group, whereas those with fall diets that had PUFA contents above 74 mg/g were placed in the high-PUFA group. None of the free-ranging squirrels had a fall diet with a PUFA content below 33 mg/g (Fig. 2); thus, there was no low-PUFA (<33 mg/g) group.

Field Studies on Hibernation Patterns

Adult female *S. parryii* hibernate for an average of 20 d more than adult males (Buck and Barnes 1999a) in the same population. The hibernation patterns of male and female ground squirrels were analyzed separately using the T_b data collected by each implanted logger retrieved. An individual was considered to be in torpor when $T_b < 30^\circ\text{C}$ because euthermic *S. parryii* normally maintain T_b above 30°C (Long et al. 2005). The following parameters were measured for each squirrel. The durations of the first and last torpor bouts, as well as the average duration of all torpor bouts observed for each individual during hibernation, were calculated. The total number of torpor bouts and the number of torpor bouts lasting <24 h during hibernation were also measured. The duration of the last bout of euthermia ($T_b > 30^\circ\text{C}$) observed before spring emergence from hibernation was also determined. Finally, the duration of the entire hibernation season (the time spanning the first onset of torpor to the end of the last torpor bout) and the total amount of time spent in torpor during hibernation were measured. We also examined the proportions of adult *S. parryii* recaptured that were previously collected and implanted with loggers during August 1998 and 1999 for a possible relationship between diet PUFA content and the probability of disappearing from

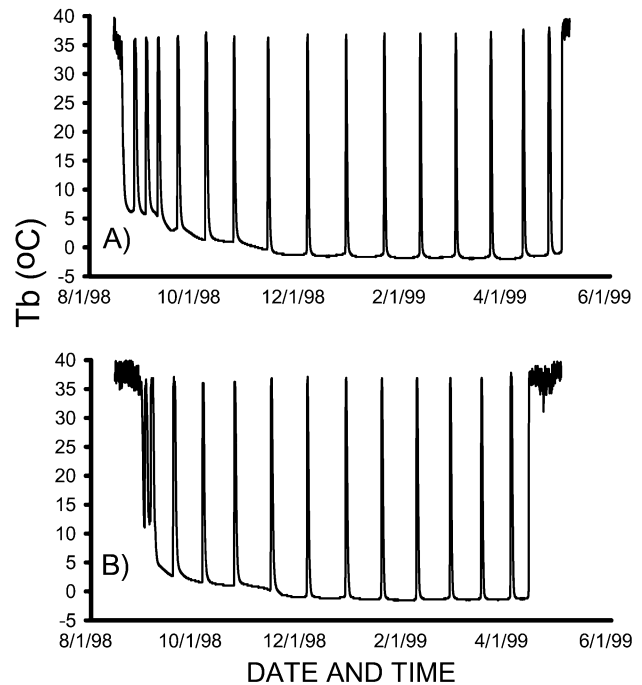


Figure 2. Body temperatures (T_b) for (A) a single female free-ranging *Spermophilus parryii* that ingested a moderate-PUFA diet and (B) another female that consumed a high-PUFA diet before the onset of the 1998–1999 hibernation season.

the study site. A total of 44 (25 male, 19 female) adult squirrels were included in this analysis, and an individual was classified as missing if it was not recaptured during any of the three to five subsequent trapping periods at this site. Trapping periods were 4–8 d, with 60–80 traps rotated among all the known burrow systems within the study site (Buck and Barnes 1999b).

All diet compositions were compared to each other with a one-way ANOVA (general linear models) procedure. The torpor patterns for each diet group within a sex were compared with Student's t -tests. All statistical methods were performed using SYSTAT software, version 11. Proportions of squirrels disappearing from the population in each group were statistically compared using the extension of Fisher's exact test developed by Freeman and Halton (1951).

Results

Dietary Fatty Acid Compositions

A total of 57 adult *Spermophilus parryii* were captured and implanted with loggers, 16 in August 1998, 28 in August 1999, and 13 in August 2000. Ten of the squirrels implanted during August 1998 were eventually recaptured, 17 of those implanted during August 1999 were recovered, and only two of those implanted during August 2000 were found during the following spring. The loggers in five of the recovered squirrels failed, however; thus T_b data were collected for a total of 24 (13 male, 11 female) squirrels during the course of the 3-yr study. The

fatty acid compositions of WAT samples collected from these 24 squirrels, along with the estimated dietary fatty acid contents indicated, are summarized in Table 1. Six types of fatty acids were present in the WAT, with the only PUFAs being linoleic (18:2) and α -linolenic (18:3) acids. Linoleic acid accounted for more than half of all PUFAs present, on average. Total dietary PUFA contents varied more than threefold among individuals (Table 1), with five male and eight female squirrels having moderate-PUFA (33–74 mg/g) diets and eight male and three female squirrels consuming high-PUFA (>74 mg/g) diets during the fall.

The mean (\pm SE) dietary oleic acid contents for male squirrels in the moderate- and high-PUFA categories were 37.8 ± 4.9 and 13.6 ± 5.4 mg/g, respectively, whereas those of the females in the moderate- and high-PUFA groups were 49.3 ± 6.4 and 30.1 ± 5.7 mg/g, respectively. The diets of both male and female arctic ground squirrels in the moderate-PUFA groups contained significantly more oleic acid than those in the high-PUFA group ($F_{3,20} = 7.61$, $P = 0.001$), but there were no significant differences in oleic acid contents between male and female squirrels within the same diet category. The mean (\pm SE) dietary linoleic acid levels for male squirrels in the moderate- and high-PUFA categories were 36.7 ± 2.7 and 59.9 ± 5.2 mg/g, respectively, while those of the females in the moderate- and high-PUFA groups were 39.7 ± 2.9 and 54.2 ± 5.2 mg/g, respectively. The mean linoleic acid contents of both male and female *S. parryii* in the moderate-PUFA groups were significantly less than those in the high-PUFA groups ($F_{3,20} = 7.19$, $P = 0.002$), and sex did not significantly influence diet linoleic acid content within a category. The mean (\pm SE) dietary α -linolenic acid contents for male squirrels in the moderate- and high-PUFA categories were 25.2 ± 2.8 and 43.1 ± 3.9 mg/g, respectively, whereas those of the females in the moderate- and high-PUFA groups were 26.2 ± 3.2 and 38.2 ± 1.2 mg/g, respectively. The male and female squirrels in the moderate-PUFA category ingested fall diets with significantly lower mean α -linolenic acid contents than those feeding on high-PUFA diets during this period ($F_{3,20} = 6.6$, $P = 0.003$), with no significant differences between the sexes.

Hibernation Patterns

The body temperatures recorded for two different female *S. parryii* throughout the hibernation season are summarized in Figure 2 to illustrate the typical patterns observed. The mean durations of the first torpor bouts during hibernation were not significantly different between the moderate- and high-PUFA groups for male *S. parryii* ($t = -1.40$, $df = 11$, $P = 0.19$; Fig. 3), but the mean torpor duration for female squirrels in the moderate-PUFA group was more than 4 d greater ($t = -2.78$, $df = 8$, $P = 0.02$) than that for females in the high-PUFA group (Fig. 3). The mean individual average length of torpor bouts for male squirrels that consumed a moderate-PUFA diet (Fig. 3) was more than 3 d greater than that for males on a high-PUFA diet ($t = -2.68$, $df = 11$, $P = 0.02$). The mean individual average lengths of torpor bouts for female squirrels that ingested moderate- and high-PUFA diets were not significantly different ($t = -1.15$, $df = 9$, $P = 0.28$; Fig. 3), however. The mean length of the last torpor bout for male squirrels that ingested a moderate-PUFA diet (Fig. 3) was not significantly different from that for males that fed on a high-PUFA diet ($t = -1.48$, $df = 11$, $P = 0.17$), and likewise the female squirrels that consumed moderate- and high-PUFA diets had last torpor bouts that were statistically equivalent ($t = 0.57$, $df = 9$, $P = 0.64$) in duration (Fig. 3). The mean amount of time that male squirrels remained euthermic ($T_b > 30^\circ\text{C}$) during the last arousal period between torpor bouts before the cessation of hibernation was 0.6 d less (Fig. 3) for male squirrels that ingested a moderate-PUFA diet during the fall than that for males that consumed a high-PUFA diet ($t = 3.19$, $df = 11$, $P = 0.009$). The mean duration of the last arousal periods for female squirrels that fed on a moderate-PUFA diet was not significantly different (Fig. 3) from that for females that ingested a high-PUFA diet ($t = 0.67$, $df = 9$, $P = 0.52$).

The mean total number of multiday (>24 h in duration) torpor bouts for male squirrels that consumed a moderate-PUFA diet was not significantly different from the mean for those that ingested a high-PUFA diet ($t = 0.74$, $df = 11$, $P = 0.48$). Female squirrels that consumed a moderate-PUFA

Table 1: Mean (\pm SE) fatty acid compositions of white adipose tissue (WAT) from free-ranging *Spermophilus parryii* and the corresponding dietary fatty acid content

Fatty Acid Type	Notation ^a	WAT Concentration (%) ^b	Dietary Content (mg/g)
Palmitic acid	16:0	$17.0 \pm .7$ (9.5–26.7)	...
Palmitoleic acid	16:1	$5.6 \pm .8$ (0–11.6)	...
Stearic acid	18:0	$1.4 \pm .2$ (0–3.3)	...
Oleic acid	18:1	43.8 ± 1.7 (27.1–60.8)	32.6 ± 4.2 (0–78.2)
Linoleic acid	18:2	18.0 ± 1.1 (10.4–31.8)	47.6 ± 2.9 (27.4–83.7)
α -linolenic acid	18:3	$12.5 \pm .9$ (5.9–23.0)	33.1 ± 2.4 (15.6–60.6)
Total PUFAs		30.5 ± 1.8 (16.5–50.2)	80.7 ± 4.7 (43.5–132.0)

Note: PUFA = polyunsaturated fatty acid. Ranges for each mean are given in parentheses.

^a The number to the left of the colon indicates the number of C atoms, and the number to the right denotes the number of C-C double bonds.

^b $N = 24$ for each mean.

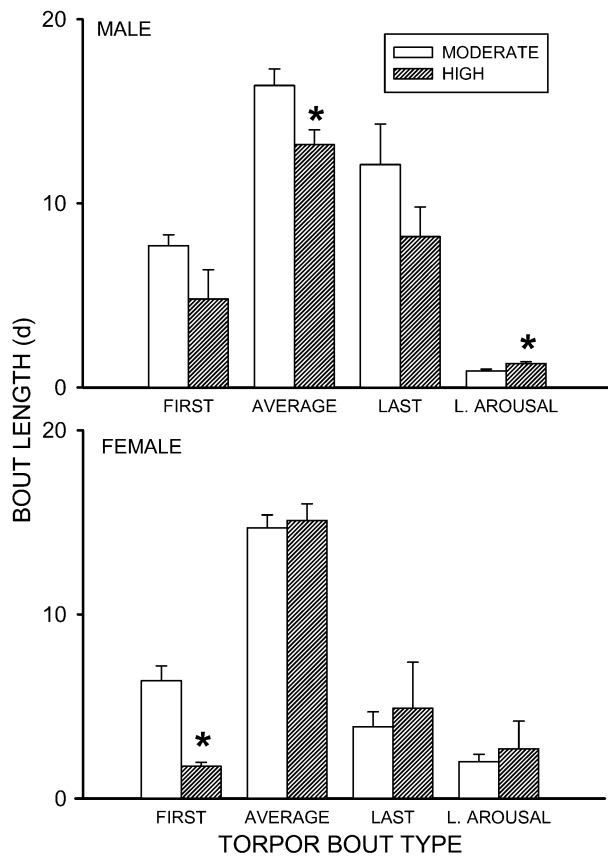


Figure 3. Mean (\pm SE) torpor and arousal bout lengths during hibernation by free-ranging *Spermophilus parryii* that consumed moderate- or high-PUFA diets during the previous fall. An asterisk indicates that the high-PUFA group is significantly different from the corresponding moderate-PUFA group at the $P < 0.05$ level. For males, $N = 5$ for each moderate-PUFA diet mean and $N = 8$ for each high-PUFA diet mean. For females, $N = 8$ for each moderate-PUFA diet mean, and $N = 3$ for each high-PUFA diet mean.

diet had a significantly greater number of multiday torpor bouts during hibernation ($t = -2.79$, $df = 6.7$, $P = 0.03$) than females in the high-PUFA diet group (Table 2). None of the male squirrels in the moderate-PUFA group had short (<24 h in length) torpor bouts during the hibernation season, whereas male squirrels in the high-PUFA dietary category had a mean (\pm SE) of 1.9 ± 0.4 short torpor bouts per hibernation season, and this was significantly greater than 0 ($t = 4.26$, $df = 7$, $P = 0.004$). Consequently, the mean total number of torpor bouts (short and multiday) for male squirrels that consumed a moderate-PUFA diet was also less than the mean total torpor bouts for males in the high-PUFA group ($t = 2.56$, $df = 11$, $P = 0.03$; Table 2). Except for a single short torpor bout observed during the hibernation of one individual in the moderate-PUFA group, none of the females squirrels in either the moderate- or high-PUFA categories had short torpor bouts. The mean minimum T_b maintained during torpor by male squirrels in the moderate-PUFA category was not significantly

different from the mean minimum T_b for those in the high-PUFA group ($t = 1.87$, $df = 11$, $P = 0.09$; Table 2). Similarly, the mean minimum T_b during torpor for female squirrels that fed on moderate- and high-PUFA diets did not significantly differ ($t = 1.53$, $df = 9$, $P = 0.16$; Table 2).

Male arctic ground squirrels that consumed a moderate-PUFA diet during the fall had a hibernation season with a mean length that did not significantly differ from that for males in the high-PUFA group ($t = -0.90$, $df = 11$, $P = 0.39$; Fig. 4). The mean hibernation season length for female squirrels that consumed a moderate-PUFA diet, however, was nearly 17 d longer than the mean hibernation season length of females in the high-PUFA group ($t = -2.4$, $df = 9$, $P = 0.045$; Fig. 4). Further analyses of female hibernation seasons were thus conducted, and they revealed that the mean (\pm SE) Julian date for immergence into hibernation for females in the moderate-PUFA group was 237.8 ± 4.6 , whereas that for those in the high-PUFA group was 261.0 ± 9.6 , significantly later ($t = 2.514$, $P = 0.04$). The mean (\pm SE) Julian dates for emergence from hibernation for the moderate- and high-PUFA groups were 122.7 ± 3.2 and 116.3 ± 6.9 , respectively, and they did not significantly differ ($t = -0.971$, $P = 0.364$). The mean total amounts of time torpid during hibernation by male squirrels in the moderate- and high-PUFA groups were not significantly different ($t = -1.00$, $df = 11$, $P = 0.34$; Fig. 4), but female squirrels that consumed a moderate-PUFA diet spent an average of 16 more days in torpor during hibernation than females in the high-PUFA group ($t = 6.8$, $df = 6.8$, $P = 0.04$; Fig. 4). A total of 44 (25 male, 19 female) adult squirrels were collected, implanted with loggers, marked, and released during August 1998 and 1999, 27 of which (15 male, 12 female) were eventually recaptured. Of the original 44 squirrels, 18 ingested a moderate-PUFA diet during the fall, whereas the remaining 26 consumed a high-PUFA diet during this period. No arctic ground squirrels had a natural diet that resulted in their classification into a low-PUFA diet. Only 22% (4/18) of the squirrels that fed on a moderate-PUFA diet were not recaptured during multiple (three to five) subsequent trapping periods, while the proportion of squirrels in the high-PUFA group that were not recaptured was 50% (13/26), significantly greater (Fisher exact test: Fisher index = 3.38, $P = 0.03$).

Discussion

The results of this study clearly support our hypotheses that free-ranging ground squirrels with fall diets containing PUFA levels outside of the 33–74-mg/g range (*a*) have shorter torpor bouts and (*b*) spend less time in torpor than those consuming fall diets with PUFA contents of 33–74 mg/g, although the particular effects on torpor associated with diet PUFA level depend on gender. The total PUFA contents of fall diets consumed by free-ranging arctic ground squirrels at Toolik varied more than threefold between individuals during our 3-yr study. Male squirrels that ingested high-PUFA (>74 mg/g) fall diets had (1) shorter torpor bouts, (2) more torpor bouts, and (3)

Table 2: Mean (\pm SE) numbers of torpor bouts and minimum body temperatures during hibernation by free-ranging *Spermophilus parryii* with moderate- and high-PUFA fall diets

Hibernation Parameter	Male <i>S. parryii</i>		Female <i>S. parryii</i>	
	Moderate PUFA	High PUFA	Moderate PUFA	High PUFA
Multiday torpor bouts	10.4 \pm .4	10.9 \pm .4	16.4 \pm .6	14.0 \pm .6 ^a
Total torpor bouts	10.4 \pm .4	12.8 \pm .7 ^a	16.4 \pm .6	14.0 \pm .6 ^a
Minimum T_b ($^{\circ}$ C)	-1.9 \pm .1	-1.6 \pm .1	-1.9 \pm .1	-1.7 \pm .1

Note: PUFA = polyunsaturated fatty acid; T_b = body temperature. For males, $N = 5$ for each moderate-PUFA diet mean and $N = 8$ for each high-PUFA diet mean. For females, $N = 8$ for each moderate-PUFA diet mean, and $N = 3$ for each high-PUFA diet mean.

^a Significantly different from the corresponding moderate-PUFA group at the $P < 0.05$ level.

longer last bouts of euthermic arousal during hibernation than those that consumed a moderate-PUFA (33–74 mg/g) diet before hibernation, as predicted. Female squirrels that fed on a high-PUFA diet during the fall, in contrast, had a 17-d-shorter hibernation period, with about 16 fewer total days in torpor, and fewer torpor bouts than those that ingested a moderate-PUFA diet during the fall. Female squirrels that ingested the high-PUFA diet entered hibernation on average 23.2 d later than those that fed on a moderate-PUFA diet during the fall. Our study is thus the first to demonstrate that the naturally occurring variations in dietary PUFA content influence the subsequent torpor patterns of a free-ranging hibernator and that the types of these effects on torpor patterns vary with gender. Our study also suggests that because of differences in overall hibernation season lengths, dietary PUFA contents may affect the hibernation patterns of adult females to a greater extent than those of adult males.

The results also support our hypothesis that squirrels ingesting fall diets with PUFA contents outside of the 33–74-mg/g range will have a greater rate of disappearance from the population than those with fall diets that have PUFA contents within the 33–74-mg/g range. The proportion of arctic ground squirrels that consumed high-PUFA diets during the fall of 1998 or 1999 and subsequently disappeared from the study site was more than twice that observed for squirrels that ingested moderate-PUFA diets during this same period. A field study on 101 free-ranging *Spermophilus parryii* in the Yukon revealed that more than 90% of the adult mortality in this population occurred during hibernation (Karels et al. 2000). It is therefore possible that most of the missing squirrels died during hibernation, which, in turn, would indicate that squirrels feeding on a high-PUFA diet during the fall have a higher overwinter mortality rate than those that consumed a moderate-PUFA diet. Alternatively, animals experiencing high-PUFA diets may have emigrated from the study population. Laboratory experiments with *Spermophilus lateralis* revealed that ground squirrels consuming a high-PUFA (>74 mg/g) diet during the fall were much less likely to enter hibernation than those that consumed a moderate-PUFA (33–74 mg/g) diet, and the members of the high-PUFA group that did hibernate started at a significantly later time during the fall (Frank 2002). Female *S. parryii* that fed on a high-PUFA diet also delayed their entrance into hi-

bernation; consequently, it is possible that the apparent increased overwinter mortality of the high-PUFA group may be due to a lower propensity to enter hibernation. In addition, most of the energy (WAT) utilized during hibernation is consumed to support the metabolic demands associated with periods of euthermic arousal from torpor (Kayser 1965). The reduced torpor bout lengths and greater number of torpor bouts cause more frequent arousals from torpor for male squirrels that consumed a high-PUFA diet and therefore suggest that the animals may have depleted their body fat reserves at a greater rate during hibernation than those that ingested a moderate-PUFA diet during the fall. This, in turn, suggests that squirrels feeding on a high-PUFA diet may also have a greater overwinter mortality rate because of the premature depletion of their body fat stores before spring. This would be consistent with the findings of Bruns et al. (2000), who observed that the rate of body mass depletion during hibernation by alpine marmots (*Marmota marmota*) was correlated with the proportion of PUFAs in their WAT.

The PUFAs in mammalian cells undergo lipid peroxidation

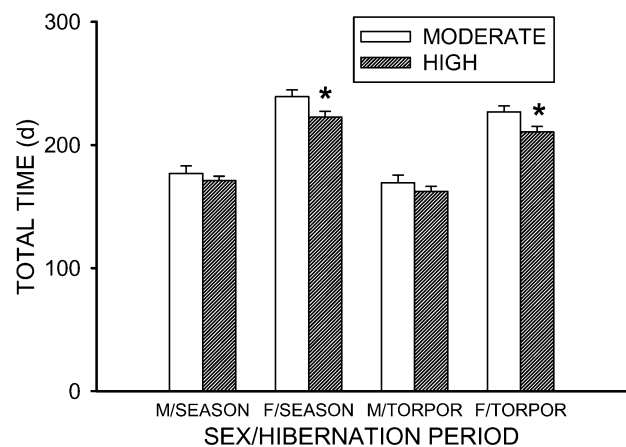


Figure 4. Mean (\pm SE) number of days of hibernation and number of days in torpor during hibernation by free-ranging *Spermophilus parryii* that consumed moderate- and high-PUFA diets during the previous fall. An asterisk indicates that the high-PUFA group is significantly different from the corresponding moderate-PUFA group at the $P < 0.05$ level. See Figure 3 for samples sizes.

more readily and rapidly than either the saturated fatty acids or the MUFAs (Gunstone 1996). Lipid peroxidation is a self-sustaining chain reaction between PUFAs and reactive oxygen species, and it produces lipid peroxides that are highly toxic to cells (Frankel 1995). Laboratory hibernation experiments revealed that the rate of lipid peroxidation in the small intestine increases during torpor (Carey et al. 2000). Laboratory experiments have also demonstrated that when the PUFA content of the fall diet is increased, the rate of lipid peroxidation and subsequent cell damage during torpor increases in brown adipose tissue as well (Frank and Storey 1995). The shorter torpor bouts observed for *S. parryii* consuming high-PUFA diets during the fall, as well as the 17-d-shorter hibernation period for female *S. parryii* that ingested a high-PUFA diet, are thus probably due to relatively greater levels of tissue damage through lipid peroxidation during torpor in those squirrels than in those that consumed a moderate-PUFA diet before hibernation.

It is also possible that most of the ground squirrels that disappeared from the study site simply dispersed to another location before they could be recaptured. Habitat favorable for arctic ground squirrel colonization is continuously adjacent to the study area, with vegetation types varying between wet sedge/tussock tundra to heath and open shrub meadows. This suggests that *S. parryii* feeding on a high-PUFA diet during the fall are much more likely to subsequently disperse to another location than those that were able to obtain a moderate-PUFA diet in the same population. The PUFA contents of natural seed oils increase with latitude (Linder 2000). It is therefore likely that food plant species/parts with moderate (33–74 mg/g) PUFA content are less common in the Arctic than in more southern areas. The PUFA content of plants varies both with species (Frank et al. 1998) and between different parts of the same plant (Florant et al. 1990). Laboratory experiments with golden-mantled ground squirrels (*S. lateralis*) revealed that their diet selection is based on PUFA content (Frank 1994) and that they maintain a total (18:2 and 18:3) dietary PUFA intake of 33 mg/g through their food item choice. The average PUFA content of the diets consumed by free-ranging *S. lateralis* during the fall is 33–47 mg/g, with about 25% of the adults ingesting fall diets with PUFA contents that were outside the moderate (33–74 mg/g) range (Frank et al. 1998, 2004). Interpreting these findings in conjunction with the diet compositions observed for free-ranging *S. parryii* suggests that populations of arctic ground squirrels may frequently face shortages of food items (plants and plant parts) that enable them to maintain an overall diet with a moderate PUFA content, forcing some individuals to consume a high-PUFA (>74 mg/g) diet during the fall. This, in turn, may lead to greater propensity to disperse to new habitats in an effort to obtain a diet more conducive to hibernation.

The T_b data for all 24 arctic ground squirrels revealed that each had long (multiday) torpor bouts during which supercooling occurred. The minimum T_b measured for each individual varied from -2.2° to -1.0°C . Arctic ground squirrels are the only species of hibernating mammal known to supercool

naturally during torpor, and this phenomenon has been demonstrated in outdoor-enclosure (Barnes 1989; Barnes and Ritter 1993) and laboratory (Buck and Barnes 2000) experiments involving captive *S. parryii* maintained on a diet consisting mostly of a commercial rodent feed. A preliminary study involving just one free-ranging *S. parryii* indicated that supercooling occurs regularly during natural torpor (Boyer and Barnes 1999). Our study confirms this prediction, revealing that free-ranging *S. parryii* consuming their natural diets normally supercool during torpor. Laboratory experiments conducted with Cascade ground squirrels (*Spermophilus saturatus*) by Geiser and Kenagy (1993) suggested that the minimum T_b maintained during torpor by squirrels that ingested a moderate-PUFA (33–74 mg/g) diet is not different from the minimum T_b defended during torpor by those that consumed a high-PUFA (>74 mg/g) diet. Free-ranging *S. parryii* in our study that ingested a moderate-PUFA diet during the fall maintained a mean minimum T_b during torpor that was not significantly different from that of *S. parryii* that consumed a high-PUFA diet (Table 2). The findings of our study are thus consistent with those of Geiser and Kenagy (1993).

It would be of interest to determine whether the greater rate of disappearance for squirrels ingesting a high-PUFA diet is due to a high rate of overwinter mortality, increased dispersal, or a combination of both. Further investigations into both the etiology of natural variations in dietary PUFA levels and the relationships between tissue lipid peroxidation, diet, and torpor will provide new insights into both the ecology and evolution of mammalian torpor.

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Literature Cited

- Barnes B.M. 1989. Freeze avoidance in a mammal: body temperatures below 0°C in an arctic hibernator. *Science* 244: 1593–1595.
- Barnes B.M. and D. Ritter. 1993. Patterns of body temperature change in hibernating arctic ground squirrels. Pp. 119–130 in C. Carey, G.L. Florant, B.A. Wunder, and B. Horwitz, eds. *Life in the Cold: Ecological, Physiological, and Molecular Mechanisms*. Westview, Boulder, CO.
- Boyer B.B. and B.M. Barnes. 1999. Molecular and metabolic aspects of mammalian hibernation. *BioScience* 49:713–724.
- Bruns U., F. Frey-Roos, S. Pudritz, F. Tataruch, T. Ruf, and W. Arnold. 2000. Essential fatty acids: their impact on free-living alpine marmots (*Marmota marmota*). Pp. 215–222 in G. Heldmaier and M. Klingenspor, eds. *Life in the Cold*. Springer, New York.

- Buck C.L. and B.M. Barnes. 1999a. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. *J Mammal* 80:430–442.
- . 1999b. Temperatures of hibernacula and changes in body composition of arctic ground squirrel over winter. *J Mammal* 80:1264–1276.
- . 2000. Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *Am J Physiol* 279:R255–R262.
- Carey H.V., C.L. Frank, and T.Y. Aw. 2000. Cellular responses to metabolic stress in hibernating mammals. Pp. 339–346 in G. Heldmaier and M. Klingenspor, eds. *Life in the Cold*. Springer, New York.
- Christie W.W. 1989. Gas chromatography and lipids. *Oily, Ayr*.
- Feldhamer G.A., L.C. Drickhamer, S.H. Vessey, and J.F. Merritt. 1999. *Mammalogy: Adaptation, Diversity, and Ecology*. WCB McGraw-Hill, New York.
- Florant G.L., H.S. Ameenuddin, and D.A. Rintoul. 1993. The effect of a low essential fatty acid diet on hibernation in marmots. *Am J Physiol* 264:R747–R753.
- Florant G.L., L.C. Nuttle, D.E. Mullinex, and D.A. Rintoul. 1990. Plasma and white adipose tissue lipid composition in marmots. *Am J Physiol* 258:R1123–R1131.
- Folch J., M. Lees, and G. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–507.
- Frank C.L. 1991. Adaptations for hibernation in the depot fats of a ground squirrel (*Spermophilus beldingi*). *Can J Zool* 69:2707–2711.
- . 1992. The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiol Zool* 65:906–920.
- . 1994. Polyunsaturate content and diet selection by ground squirrels (*Spermophilus lateralis*). *Ecology* 75:458–463.
- . 2002. The effects of short-term variations in diet fatty acid composition on mammalian torpor. *J Mammal* 83:1013–1019.
- Frank C.L., E.S. Dierenfeld, and K.B. Storey. 1998. The relationship between lipid peroxidation, hibernation, and food selection in mammals. *Am Zool* 38:341–349.
- Frank C.L., W.R. Hood, and M.C. Donnelly. 2004. The role of α -linolenic acid (18:3) in mammalian torpor. Pp. 71–80 in B.M. Barnes and H.V. Carey, eds. *Life in the Cold: Evolution, Mechanisms, Adaptation and Application*. Institute of Arctic Biology, Fairbanks, AK.
- Frank C.L. and K.B. Storey. 1995. The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). *J Comp Physiol B* 164:536–542.
- . 1996. The effect of total unsaturate content on hibernation. Pp. 211–216 in F. Geiser, A.J. Hulbert, and S.J. Nicol, eds. *Adaptations to the Cold*. University of New England Press, Armidale.
- Frankel E.N. 1995. Oxidation of polyunsaturated lipids and its nutritional consequences. Pp. 265–270 in W.A.M. Castenmiller, ed. *Oils-Fats-Lipids: Proceedings of the 21st World Congress of the International Society for Fat Research (ISF)*. Vol. 2. P.J. Barnes, Bridgewater.
- Freeman G.H. and J.H. Halton. 1951. Note on an exact treatment of contingency, goodness of fit and other problems of significance. *Biometrika* 38:141–149.
- Geiser F. 1991. The effect of unsaturated and saturated dietary lipids on the patterns of daily torpor and the fatty acid composition of tissues and membranes of the deer mouse *Peromyscus maniculatus*. *J Comp Physiol B* 161:590–597.
- . 1994. Hibernation and daily torpor in marsupials: a review. *Aust J Zool* 42:1–16.
- . 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annu Rev Physiol* 66:239–274.
- Geiser F. and G.J. Kenagy. 1987. Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *Am J Physiol* 252:R897–R901.
- . 1993. Dietary fats and torpor patterns in hibernating ground squirrels. *Can J Zool* 74:1182–1186.
- Geiser F. and T. Ruf. 1995. Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol Zool* 68:935–966.
- Geiser F., B. Stahl, and R.P. Learmonth. 1992. The effect of dietary fatty acids on the pattern of torpor in a marsupial. *Physiol Zool* 65:1236–1245.
- Gunstone F.D. 1996. *Fatty Acid and Lipid Chemistry*. Blackie Academic & Professional, Glasgow.
- Harlow H.J. and C.L. Frank 2001. The role of dietary fatty acids in the evolution of spontaneous and facultative hibernation patterns in prairie dogs. *J Comp Physiol B* 171:77–84.
- Karels T.J., A.E. Byrom, R. Boonstra, and C.J. Krebs. 2000. The interactive effects of food and predators on reproduction and overwinter survival of arctic ground squirrels. *J Anim Ecol* 69:235–247.
- Kayser C. 1965. Hibernation. Pp. 180–296 in W. Mayer and R. VanGelder, eds. *Physiological Mammalogy*. Vol. 2. Academic Press, New York.
- Linder C.R. 2000. Adaptive evolution of seed oils in plants: accounting for biogeographic distribution of saturated and unsaturated fatty acids in seed oils. *Am Nat* 156:442–458.
- Long R.A., R.A. Hut, and B.M. Barnes. 2007. Simultaneous collection of body temperature and activity data in burrowing mammals: a new technique. *J Wildl Manag* 71:1375–1379.
- Long R.A., T.J. Martin, and B.M. Barnes. 2005. Body temperature and activity patterns in free-living arctic ground squirrels. *J Mammal* 86:314–322.
- Mead J., D. Alfin-Slater, D. Howton, and G. Popjak. 1986. *Lipids: Chemistry, Biochemistry, and Nutrition*. Plenum, New York.
- Michener G.R. 1992. Sexual differences in over-winter torpor

- patterns of Richardson's ground squirrels in natural hibernacula. *Oecologia* 89:397–406.
- Munro D. and D.W. Thomas. 2004. The role of polyunsaturated fatty acids in the expression of torpor by mammals: a review. *Zoology* 107:29–48.
- Mzilikazi N., B.G. Lovegrove, and D.O. Ribble. 2002. Exogenous passive heating during torpor arousal in free-ranging rock elephant shrews, *Elephantus myurus*. *Oecologia* 133: 307–314.
- Thorp C.R., R.P. Kodanda, and G.L. Florant. 1994. Diet selection alters metabolic rate in the yellow-bellied marmot (*Marmota flaviventris*) during hibernation. *Physiol Zool* 67:1213–1229.
- Willmer P., G. Stone, and I. Johnson. 2000. *Environmental Physiology of Animals*. Blackwell Science, Boston.
- Withers K., J. Billingsley, D. Hirning, A. Young, P. McConnell, and S. Carlin. 1996. Torpor in *Smithopsis macroura*: effects of dietary fatty acids. Pp. 217–222 in F. Geiser, A.J. Hulbert, and S.J. Nicol, eds. *Adaptations to the Cold*. University of New England Press, Armidale.
- Young P.J. 1990. Hibernating patterns of free-ranging Columbian ground squirrels. *Oecologia* 83:504–511.