The Role of α-Linolenic Acid (18:3) in Mammalian Torpor

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Abstract. Field studies were conducted with free-ranging golden-mantled ground squirrels (*Spermophilus lateralis*) to determine the relationship between fall diet polyunsaturated fatty acid (PUFA) content, subsequent torpor patterns, and over-winter survival over a two-year period. Blood plasma PUFA levels increase with diet PUFA content. The PUFA contents of fall diets were thus estimated by measuring blood plasma PUFA levels. Torpor patterns and over-winter survival were measured by radio telemetry using temperature-sensitive collars. Mean plasma PUFA levels for juvenile *S. lateralis* were nearly twice those of adults, due to elevated linoleic acid (18:2) contents. One third of the juvenile *S. lateralis* did not survive the winter, whereas all of the adults survived this period. These findings indicate that the food plant selection of juvenile ground squirrels differs substantially from those of adults and produces a relatively higher PUFA intake. These results suggest that one consequence of juvenile dietary preferences may be a lowered over-winter survival rate through the inhibition of hibernation by high diet 18:2 contents, rather than due to differences in diet 18:3 levels.

Introduction

Laboratory experiments with chipmunks (*Tamias amoenus*), two species of ground squirrels (*Spermophilus lateralis* and *S. saturatus*), and marmots (*Marmota flaviventris*) have revealed that their torpor is enhanced when they ingest diets with moderately high levels of linoleic acid (18:2). Sciurids fed a moderately high linoleic acid diet were more likely to enter torpor, spent less time fasting

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Life in the Cold: Evolution, Mechanisms, Adaptation, and Application. Twelfth International Hibernation Symposium. Biological Papers of the University of Alaska, number 27. Institute of Arctic Biology, University of Alaska Fairbanks, Alaska, USA.

prior to torpor, had lower metabolic rates, and had longer torpor bouts than those given diets containing relatively less linoleic acid (Florant et al., 1993; Frank, 1992; Geiser and Kenagy, 1987, 1993; Thorp et al., 1994). Similar experiments conducted with mice and 2 marsupials produced identical results (Geiser, 1991; Geiser et al., 1992; Withers et al., 1996). Torpor by golden-mantled ground squirrels (*Spermophilus lateralis*) is actually inhibited, however, when diet linoleic acid contents are above 62 mg/g (Frank and Storey, 1996), possibly due to enhanced lipid peroxidation (Frank and Storey, 1995), and is thus most likely when linoleic acid levels are 33–62 mg/g diet (Frank et al., 1998). Linoleic acid is a polyunsaturated fatty acid (PUFA) having more than one carbon-carbon double bond, as opposed to either a saturated fatty acid containing no carbon-carbon double bonds or a monounsaturated fatty acid containing only one such bond. Mammals can synthesize saturated and monounsaturated fatty acids, but they are incapable of producing PUFAs. Most plant species, however, produce two PUFAs: linoleic acid (18 carbon atoms, two double bonds) and α-linolenic acid (18 carbon atoms, three double bonds). When mammals consume PUFAs these are incorporated into their cell membranes and storage lipids (Gunstone, 1996).

Feeding by ground squirrels dramatically increases for two months prior to onset of hibernation, and a body fat content of 35–40% is attained (Kenagy, 1987; Kenagy and Barnes, 1988). The fall diet of ground squirrels consists mostly of plant tissues (Eshelmann and Jenkins, 1989; Kenagy et al., 1989; Tevis, 1953). The PUFA content of plants varies with species, season, and between different parts of the same plant (Florant et al., 1990; Gunstone, 1996). Alpha-linolenic acid (18:3) comprises ⅓ to ¼ of all PUFAs found in the fall diets of herbivorous hibernators (Florant et al., 1990; Frank 1994), but little is known about the influence of it on torpor. Laboratory experiments using a 4.5% linseed oil diet as a source of α-linolenic acid suggested that this PUFA might actually inhibit torpor (Hill and Florant, 2000), although the authors indicated that this inhibition may have actually been due some compound(s) present in the linseed oil diet other than α-linolenic acid. We predict that: (1) a moderate level of α-linolenic acid in the diet actually enhances torpor, (2) a moderate intake of α-linolenic acid is maintained by mammalian hibernators through the selection of food items based on fatty acid composition, and (3) hibernator over-winter mortality is associated with exceedingly high diet PUFA contents during the previous fall. The first two hypotheses were tested in laboratory experiments involving *S. lateralis*, and the third was examined by a field study on this species.

Methods

A total of 28 adult *S. lateralis* were collected from the Crooked Creek (37° 30' N, 118° 10' W, elevation = 3094 m) area of the White Mountains in California during August 2000/2001. All were individually housed in rat cages located in the Wildlife Vivarium of Fordham University and maintained at 22–24° C on a natural fall (10L:14D) photoperiod. Three different semisynthetic diets varying only in fatty acid composition (Table 1) were produced: each used ground flax seed for an α-linolenic acid source. The low linoleic acid/low α-linolenic acid (L 18:2/L 18:3) diet was a mixture of 90% Purina 5001 Rodent Chow, 5% flax seed, and 5% coconut oil, the high linoleic acid/low α-linolenic acid (H 18:2/L 18:3) diet was composed of 90% Purina 5001 Rodent Chow, 5% flax seed, and 5% sunflower oil, and the low linoleic acid/high α-linolenic acid (L 18:2/H 18:3) diet consisted of 90% Purina 5001 rodent chow and 10% ground flax seed. The Test Diets Division of Purina Mills, Inc. produced the diets pressed into 1g cylindrical pellets. All diets were 26% protein, 12% lipid, 51% carbohydrate, and 7.3% ash. Commercial food colorings were used to code for diet type. Color does not influence the diet selection of *S. lateralis* (Frank, 1994). Diet and blood plasma fatty acid compositions were determined by gas-liquid chromatography following the procedures summarized by Frank (2002).

Table 1. Fatty acid compositions of the semisynthetic diets.

Fatty acid type	Notation*	Diet type (mg/g) L18:2/L18:3	L18:2/H18:3	H18:2/L18:3
Lauric acid	12:0	22.4	–	–
Myristic acid	14:0	13.1	0.4	–
Palmitic acid	16:0	19.1	16.2	17.9
Stearic acid	18:0	5.8	5.3	5.3
Oleic acid	18:1	22.4	23.8	23.3
Linoleic acid	18:2	23.4	27.8	45.6
α-Linolenic acid	*18:3*	13.0	26.5	11.9
Total PUFA	–	36.4	54.3	57.5

* The number to the left of the colon indicates the number of carbon atoms, the number to the right denotes the number of carbon-carbon double bonds (Gunstone, 1996).

Laboratory Hibernation Experiment

Two *S. lateralis* groups (N = 6 each) were maintained on different semisynthetic diets for 3 months; one was fed the H 18:2/L 18:3 diet while the other was given the L 18:2/H 18:3 diet. Both groups were then placed at 5° C in an incubator during October 2000 to induce hibernation. All food was withheld after a two-day adjustment period. All squirrels not displaying torpor during 10 days of fasting were removed from the incubator and fed their regular semisynthetic diets for three more weeks. These squirrels were then fasted inside the incubator again for another 10 days to induce hibernation, and any squirrel not displaying torpor was removed from the study. All hibernating squirrels were maintained at 5° C for four months, and body temperatures were continuously recorded using "Mini-Mitter" transmitters surgically implanted into the abdomen of each squirrel.

Diet Preference Trials

Two consecutive diet selection experiments were conducted during October 2001 with 16 captive adult *S. lateralis;* both involved the L 18:2/L 18:3 and L18:2/H 18:3 diets. Each squirrel was given 120 g of each diet simultaneously at the onset of the first experiment. All remaining pellets were collected after six days. All squirrels were then placed in the second experiment by presenting each with 80 g of both diet types. All remaining pellets were collected after four days. The remaining pellets recovered at the end of each experiment were sorted by diet type (color), dried, and weighed. The amount of each diet consumed was calculated as the difference between the amount of dry matter initially presented and that remaining.

Overwinter Survival Study

Fifteen free-ranging *S. lateralis* were examined in the Barcroft area (37° 35.013' N, 118° 14.208' W, elevation = 3776 m) of the White Mountains of California. Torpor patterns and over-winter survival were monitored during the September–June periods of 2000–2001 and 2001–2002 using radio collars placed on each individual that transmitted pulse rates corresponding to skin temperatures. Blood plasma PUFA concentrations depend mostly on diet PUFA content during feeding (Gunstone, 1996). Blood samples were thus collected from each radio collared squirrel just prior to the onset of torpor (between 1 September and 5 October) and analyzed for plasma fatty acid content to provide a relative index of individual diet PUFA level.

Results

Laboratory Hibernation Experiment

Only three of the six squirrels fed the H 18:2/L 18:3 diet eventually displayed torpor, whereas a significantly greater proportion, six out of six, of those given the L 18:2/H 18:3 diet did (FI = 41.3, P < 0.05). All squirrels in the L 18:2/H 18:3 diet group continued to display regular torpor bouts throughout the four-month period at T_a = 5° C, but two of the three squirrels from the H 18:2/L 18: 3 group that initially entered torpor spontaneously stopped displaying regular torpor bouts during this same period. Hibernating members of both groups displayed statistically equivalent minimum body temperatures (Fig. 1A) and torpor bout lengths (Fig. 1B), however.

Diet Preference Trials

The mean (± SE) amount of the L 18:2/L 18:3 diet consumed during the first preference trial was 55.8 ± 4.9g whereas that for the L 18:2/H 18:3 was 33.9 ± 4.3 g and significantly less (paired t = –3.45, P = 0.004). The mean (± SE) amount of the L 18:2/L 18:3 diet ingested during the second experiment was 43.9 ± 4.9 g and significantly more (t = –2.86, P = 0.01) than the amount of the L 18:2/H 18:3 diet consumed (26.5 ± 3.5 g) during the same period. The L 18:2/L 18:3 acid diet constituted a mean (± SE) proportion of the total food

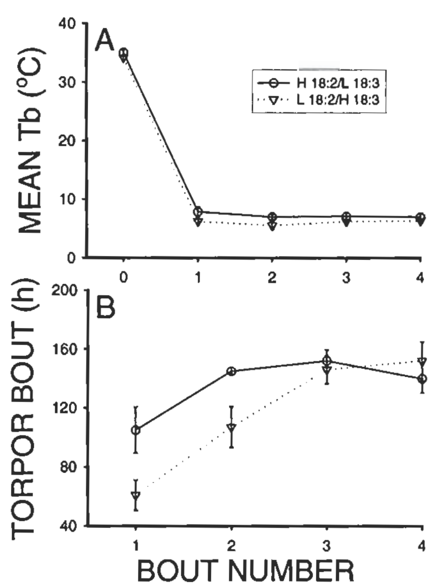

Fig. 1 (A). Minimum body temperatures recorded for each animal during torpor by S. lateralis. *(B) Duration of torpor bouts (h) by* S. lateralis *in different diet groups. Symbols indicate means, vertical lines represent + SE.*

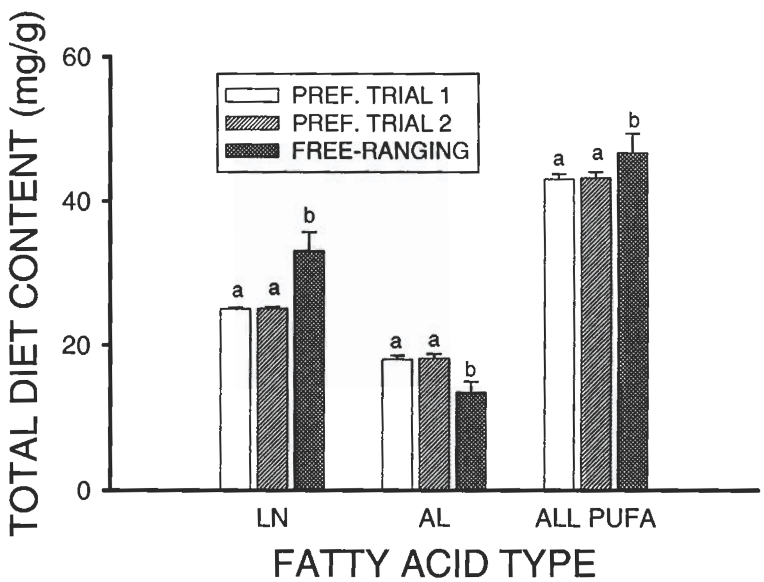

Fig. 2. Histograms indicating the mean (+ SE) total intakes of linoleic (LN) and α-linolenic (AL) acids during the selection experiments. Data for free-ranging S. lateralis *are from Frank et al. (1998). Means sharing a common lowercase letter are not significantly different at the P < 0.05 level.*

ingested that was 62.8 ± 3.8% during the first experiment, whereas this fraction was 61.8 ± 4.8% during the second experiment and did not significantly differ between the two experiments (t = 0.25, P = 0.81). Total 18:2 intakes were calculated as the fraction of the combined amounts of each diet consumed during an experiment that was 18:2, and total 18:3 intakes were calculated in a likewise fashion. The two preference experiments did not significantly differ in total 18:2 (t = 0.25, P = 0.41), total 18:3 (t = –0.25, P = 0.81), or overall PUFA (t = –0.26, P = 0.81) intakes (Fig. 2). The total 18:2 intakes observed during both laboratory experiments were significantly lower (Fig. 2) than those observed for the natural diets of free-ranging *S. lateralis* by Frank et al. (1998) (Student's t = 47.8, P < 0.001, and, t = 38.3, P = 0.002, respectively), whereas both laboratory total 18:3 intakes observed (Fig. 2) were significantly greater (t = 8.7, P < 0.001, and t = 7.3, P < 0.001, respectively). The natural diets of free-ranging *S. lateralis* thus had significantly greater overall PUFA intake (Fig. 2) than those of the first (t = –5.3, P < 0.001) and second (t = – 4.1, P = 0.002) laboratory selection experiments.

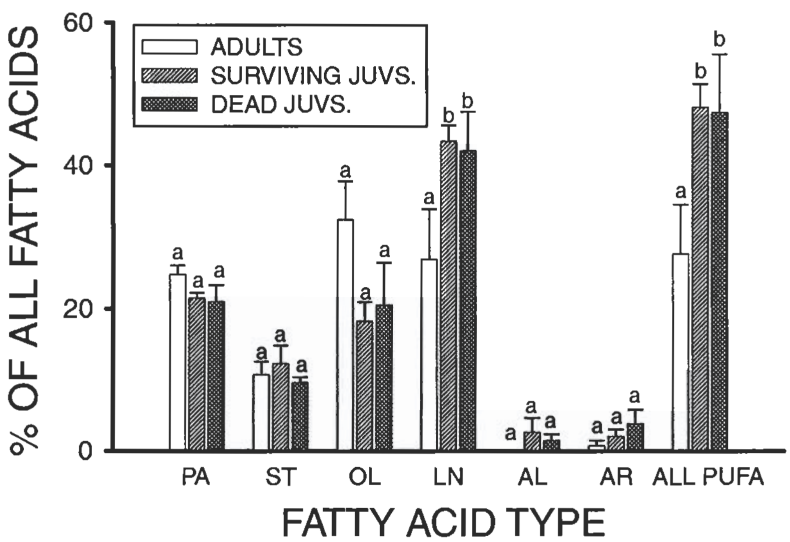

Fig. 3. Histograms indicating the mean (+ SE) levels of palmitic (PA), stearic (ST), oleic (OL), linoleic (LN), α-linolenic (AL), and arachidonic (AR) acids found in the blood plasma of free-ranging adult and juvenile S. lateralis. *Means sharing a common lowercase letter are not significantly different at the P <0.05 level.*

Over-Winter Survival Study

All five of the radio-collared adult *S. lateralis* survived the winter, whereas only 7 of the 10 radio-collared juvenile *S. lateralis* survived during this same period. The fall blood plasma of adults, juvenile survivors, and juveniles that subsequently died did not significantly differ in palmitic (GLM ANOVA F = 2.80, P = 0.10) stearic (F = 0.30, P = 0.75), oleic (F = 3.40, P =.0.07), α-linolenic (F = 3.77, P = 0.51) or arachidonic (F = 1.42, P = 0.28) acid contents (Fig. 3). Both juvenile groups had significantly greater overall plasma PUFA levels, however (F = 4.53, P = 0.03) than the adults (Fig. 3), due to correspondingly greater linoleic acid (F = 3.77, P = 0.05) levels (Fig. 3).

Discussion

Squirrels fed the L 18:2/H 18:3 diet had a greater propensity to both enter and remain in torpor than those given the H 18:2/L 18:3 diet, even though these two diets had virtually identical total PUFA contents (Table 1). These findings support our hypothesis that moderate diet α-linolenic acid levels enhance mammalian torpor. They are also in contrast to those of Hill and Florant (2000) in which their 4.5% linseed oil diet actually inhibited torpor, even though the α-linolenic acid content was only 20.6 mg/g. Hill and Florant (2000) were surprised by their finding and speculated that their results were due to compounds in the linseed oil diet other than α-linolenic acid content. Our study supports their supposition. Diet α-linolenic acid content was not maximized by *S. lateralis* during the laboratory selection experiments but was instead maintained between 18.0 and 18.2 mg/g, on average. The susceptibility of α-linolenic acid to peroxidation is far greater than that of linoleic acid (Gunstone, 1996). Interpreting the diet selection experiments in conjunction with this fact suggests that *S. lateralis* maintains a diet α-linolenic acid content of 18.0–18.2 mg/g to both maximize torpor propensity while minimizing the rate of tissue lipid peroxidation during torpor. The blood plasma of juvenile *S. lateralis* contained nearly twice the level of linoleic acid as that of adults collected during same period, indicating that they have a correspondingly higher diet linoleic acid content during the fall. Exceedingly high levels of linoleic acid in the fall diet inhibits torpor (Frank, 2002), thus the greater over-winter mortality of juveniles may be due in part to their fall diet selection. Further investigation of this system may therefore provide valuable insights into the ecological/nutritional constraints on mammalian torpor.

Acknowledgments

We thank S. J. Wickler, D. F. Hoyt, and K. Stanton for their generous assistance. This study was supported by a grant from the National Science Foundation (IBN-9986620) awarded to CLF.

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