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The Relationship Between Lipid Peroxidation, Hibernation, and Food Selection in Mammals[1]

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SYNOPSIS. A diet that has high levels of polyunsaturated fatty acids enhances mammalian torpor. Polyunsaturated fatty acids are not synthesized by mammals, but are incorporated into both membrane and storage lipids when they occur in the diet. Polyunsaturated fatty acids also undergo autoxidation more readily than other fatty acids, thereby producing highly toxic lipid peroxides. Lipid peroxidation increases during torpor. Natural selection in mammalian hibernators should thus have favored the evolution of dietary preferences that maximize hibernation ability while simultaneously minimizing the degree of lipid peroxidation during torpor. This hypothesis was tested in laboratory experiments and field studies involving golden-mantled ground squirrels (*Spermophilus lateralis*). We found that the intake of polyunsaturated fatty acids is restricted during the fall and autoxidation in tissues occurs mostly during the later phases of hibernation.

INTRODUCTION

Numerous mammals in the orders Carnivora, Chiroptera, Insectivora, Marsupialia, Monotremata, Primates, and Rodentia use torpor to survive seasonally cold periods and/or food shortages (Geiser, 1994; Lyman, 1982; Nicol and Andersen, 1996; Schmid, 1996). Although the cellular basis of torpor is unknown (Malan, 1996), a number of studies involving six rodent and two marsupial species have demonstrated that a diet with high levels of polyunsaturated fatty acids is required for hibernation. Animals fed diets containing relatively more polyunsaturated fatty acids 1) are more likely to hibernate, 2) hibernate earlier in the season, 3) have lower body temperatures/metabolic rates during torpor, and 4) have longer bouts of torpor than those given diets with lower levels of polyunsaturated fatty acids (see Florant, 1997, for review). Mammals can synthesize fatty acids containing either no or one carbon-carbon double bonds per molecule, but they are incapable of producing those containing two or more double bonds per molecule. A fatty acid with no carbon-carbon double bonds is called a saturated fatty acid, a fatty acid with one such bond is known as a monounsaturated fatty acid, and a polyunsaturated fatty acid (PUFA) contains two or more carbon-carbon double bonds. Most plant species produce two polyunsaturated fatty acids: linoleic acid (18 carbon atoms, 2 double bonds) and alpha-linolenic acid (18 carbon atoms, 3 double bonds). When mammals ingest plant tissues the PUFAs consumed are incorporated into membrane and storage lipids (Gunstone, 1996; Mead *et al.*, 1986).

A possible physiological constraint associated with diets containing large amounts of polyunsaturated fatty acids may be increased peroxidation of storage and membrane lipids. Under normal physiological conditions, the polyunsaturated fatty acids in mammalian cells undergo autoxidation more readily and rapidly than saturated or monounsaturated fatty acids (Gunstone, 1996; Mead *et al.*, 1986). Autoxidation (also called lipid peroxidation) is a self-sustaining chain reaction between polyunsaturated fatty acids and reactive oxygen species, and this reaction produces lipid peroxides that are highly toxic to cells. Autoxidation is initiated by the singlet ox-

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ygen ($^1O_2$), hydroxyl ($^{\cdot}OH$), and hydroperoxyl ($^{\cdot}OOH$) radicals that are found in most mammalian cells. High levels of lipid peroxidation in mammalian tissues may lead to hemolysis, muscle degeneration, nervous system damage, and eventual death (Frankel, 1995; Halliwell and Gutteridge, 1989; Southorn and Powis, 1992). Laboratory experiments with ground squirrels (*Spermophilus citellus*) revealed that autoxidation increases during torpor (Buzadzic *et al.,* 1990). When the PUFA content of the diet is increased, the rate of lipid peroxidation during hibernation is further increased (Frank and Storey, 1995). Hibernating mammals may thus be particularly susceptible to tissue damage by lipid peroxidation due to both torpor and the high PUFA contents of their tissues.

Previous experiments with golden-mantled ground squirrels (*Spermophilus lateralis*) revealed that their diet selection is based on PUFA content, and that items with intermediate levels are the most preferred (Frank, 1994). We propose that the selection of food items by mammalian hibernators prior to the onset of torpor is based on minimizing the degree of lipid peroxidation and maximizing the extent of torpor. This is accomplished by restricting the level of PUFA intake to the minimum amount required for proper torpor, thereby restricting tissue PUFA contents as well.

Diet selection as a defense against tissue lipid peroxidation

The enzymes superoxide dismutase and catalase inhibit lipid peroxidation in mammalian tissues by removing the hydroxyl and hydroperoxyl radicals that initiate this process. The enzyme glutathione peroxidase uses the peptide glutathione to breakdown any fatty acid peroxides that may have already formed. When lipid peroxidation increases, the amount of one or more of these enzymes also increases (Southorn and Powis, 1992). Mammals also use Beta-carotene, ascorbic acid, and Vitamin E as biochemical defenses against lipid peroxidation; of these, Vitamin E is by far the most important (Halliwell and Gutteridge, 1989). Vitamin E is not synthesized by animals but is commonly produced by most plants; it is required in the diets of all animals for proper health and survival. Eight different isomers of Vitamin E (four tocopherols and four tocotrienols) are found in plants (Kasparek, 1980; Sies, 1993). The amount of Vitamin E required in the diet for proper health depends mostly on the amount of polyunsaturated fatty acids present in the diet. Increasing the amount of polyunsaturated fatty acids in the diet without also sufficiently raising Vitamin E levels leads to increased tissue damage by lipid peroxidation (Kaasgard *et al.,* 1992).

Our analyses of the food plants ingested by free-ranging ground squirrels revealed that they usually have low Vitamin E contents, and their PUFA levels vary greatly with species (see results). Previous laboratory experiments with *S. lateralis* revealed that the hibernation ability of this species is maximal when the diet has a PUFA level of at least 33 mg/g during the fall fattening period. Increasing the PUFA content of the diet to as much as 59 mg/g does not enhance hibernation further, and raising it to 62 mg/g diet actually reduces hibernation ability. In addition to the required amount of polyunsaturated fatty acids, the diet must also contain sufficient amounts of oleic acid (18 carbon atoms, 1 double bond) to bring the total (monounsaturated + polyunsaturated) amount of unsaturated fatty acids in the diet to at least 100 mg/g (Frank, 1992; Frank and Storey, 1996). Thus to both maximize hibernation ability and minimize tissue lipid peroxidation, *S. lateralis* should maintain a dietary PUFA level as close to 33 mg/g as possible during the fall. This hypothesis was tested in laboratory experiments and field studies. Experiments were also conducted to detail further the influence of dietary fatty acid content on the degree of tissue lipid peroxidation during both fattening and hibernation.

METHODS

Ground squirrels are herbivorous/granivorous rodents that hibernate for periods of up to 8 months during the fall/winter. Feeding dramatically increases for 5–7 weeks prior to the onset of hibernation, and body fat reaches 35–40% by the end of this period (Kenagy, 1987; Kenagy and Barnes,

TABLE 1. *Fatty acid compositions of the semisynthetic diets.*

Fatty acid	Fatty acid notation*	Diet (mg/g)		
		Low PUFA	Med. PUFA	High PUFA
Lauric	12:0	59	2	—
Myristic	14:0	22	2	2
Palmitic	16:0	16	22	19
Stearic	18:0	4	—	8
Oleic	18:1	14	84	39
Linoleic	18:2	10	23	62
Alpha-Linolenic	18:3	—	—	1

* The number to the left of the colon indicates the number of carbon atoms in the molecule; the number to the right denotes the number of double bonds (Mead *et al.,* 1986).

1988). These body fats are the primary source of energy during hibernation (Kayser, 1965). At high elevations, *S. lateralis* usually begins intensive feeding in early July, with maximal body fat content and the onset of hibernation attained by early September (Blake, 1972). Twenty-one adult *S. lateralis* were thus captured for laboratory experiments during the last week of June in the Crooked Creek area of the White Mountains of California (37°30′N, 118°10′W, elevation = 3,094 m). All animals were individually housed in standard plastic rat cages at the Animal Care Facility of the University of California-Irvine. They were maintained on a fall (10L:14D) photoperiod and a diet of Purina 5001 Rodent Chow.

Semisynthetic diets

The laboratory experiments used diets that varied only in fatty acid composition. These diets were produced by the Test Diets Division of Purina Mills (Richmond, Indiana), and each consisted of 9 parts Purina 5001 Rodent Chow added to one part plant oil. The fatty acid contents of these diets are listed in Table 1. To produce these fatty acid contents, the lipid fraction of each diet contained a different plant oil. All fatty acids were thus in a natural (triacylglycerol) form. The only PUFA in these diets was linoleic acid because this fatty acid accounts for more than two thirds of all PUFAs found in natural diets (Frank, 1991, 1992).

Each diet was pressed into 1 g cylindrical pellets, and the pellets were color coded by diet type using food coloring. Previous feeding trials demonstrated that color alone does not influence the food selection of *S. lateralis* (Frank, 1994). The mean (±SE) Vitamin E content of these diets was 26.07 ± 3.63 IU/kg diet (IU = International Unit of Vitamin E activity), and was not significantly different from that of the diets of free-ranging *S. lateralis* (Student's *t*: $t = 1.45$, df = 11, $P = 0.1$).

Laboratory food selection experiments

Each of the preference experiments involved 5 different squirrels selected randomly from the pool of 21. Both experiments were conducted during early part of the fall (*i.e.,* in the second week of July). The medium and high PUFA diets were offered in the first experiment, whereas the second experiment involved the low and high PUFA diets. Every squirrel was familiarized with both diets for two days prior to each experiment. On the third day, all cages were cleaned and every squirrel was presented with 25 g of each diet simultaneously and allowed to feed for 24 hr. All remaining food pellets and fragments were then recovered, sort by color (diet), dried, and weighed. The amount of each diet type consumed was calculated as the difference between the amount of dry matter initially presented, and the amount remaining. Total PUFA intake was calculated as the fraction of the combined amounts of each diet consumed during an experiment that was linoleic acid.

Laboratory feeding/hibernation experiments

After the diet selection experiments were completed (mid July), all captive ground squirrels were divided into three different groups of n = 7. Each group was main-

tained on a different semisynthetic diet for the next 8 weeks. At the end of this feeding/fattening period (September), 3 squirrels were randomly selected from each group and sacrificed. Samples of the liver and brown adipose tissues were immediately taken from each carcass, frozen in liquid N_2, and then stored at −80°C for later analysis. The remaining 4 squirrels from each group were induced to hibernate by placing them (in their cages) at Ta = 4°C and on fall photoperiod in an environmental chamber. After the first 24 hr, all food was withheld. Hibernating squirrels were maintained under these conditions for the next month (30 days), during which body temperature and the lengths of torpor bouts were recorded every 24 hr. Body temperatures were measured using Mini-mitter model V temperature telemeters that were surgically implanted into the abdomen of each animal after four weeks of feeding. After hibernating for one month (October), all squirrels were sacrificed while torpid. Samples of their liver and brown adipose tissues were taken, frozen in liquid N_2, and stored at −80°C.

To determine the influence of diet PUFA content on lipid peroxidation, the activities of superoxide dismutase, catalase, and glutathione peroxidase were measured in the liver and brown adipose tissues. The activities of these enzymes indicate the level of lipid peroxidation *in vivo* because as lipid peroxidation increases, so does the activity of one or more of these enzymes (Southorn and Powis, 1992). This method was used to measure lipid peroxidation rather than attempting to measure the amounts of lipid peroxides in the tissues directly because none of the 14 lipid peroxide assays already developed has proven to be highly accurate (Halliwell and Chirico, 1993). Liver and brown adipose tissues were chosen for analysis because previous studies with ground squirrels revealed that these tissues are major sites of lipid peroxidation (Buzadzic *et al.,* 1990; Frank and Storey, 1995). All enzyme activities were assayed spectrophotometrically using the techniques of Aebi (1984), Flohe and Gunzler (1984), and Paoletti *et al.* (1986). For a given tissue type and thermal state (euthermic or hibernating), the enzyme activities for animals maintained on the same diets were compared using a one-way analysis of variance (ANOVA). The power of each ANOVA was calculated using the techniques of Zar (1996).

Field diet composition studies

The average amounts of monounsaturated and polyunsaturated fatty acids in natural *S. lateralis* diets during the entire fall feeding/fattening period were estimated by measuring the concentrations of these fatty acids in the white adipose tissues collected just prior to hibernation. This method was developed because rodents cannot be induced to vomit, and therefore diet composition during the entire 5–7 week fall feeding period cannot be determined by repeatedly collecting stomach contents from the same animals (see Frank, 1994). The concentrations of both monounsaturated and polyunsaturated fatty acids in the white adipose tissues of mammals depend on the amounts of these fatty acids in the diet during fattening (Mead *et al.,* 1986), however. A total of 19 adult *S. lateralis* was collected at the study site during late August. Immediately upon capture, each squirrel was sacrificed and a sample of white adipose tissue was taken from inside the abdominal cavity. These fat samples were then stored at −20°C for later analyses of fatty acids. The fatty acid compositions of 10 of these samples are summarized elsewhere (Frank, 1992).

Depot fat samples were collected from *S. lateralis* maintained on the semisynthetic diets during the laboratory experiments, and analyzed for fatty acid composition. White adipose tissue biopsies were taken from the abdomens of 7 squirrels during the surgical implantation of the temperature telemeters after 4 weeks of feeding. Abdominal white adipose tissue samples were also collected from the carcasses of the 9 squirrels sacrificed after 8 weeks of feeding on the diets. The fatty acid compositions of the white adipose tissues were examined by least squares regression to quantify the relationship between dietary oleic acid content (mg/g) and the amount of oleic acid (%) in white adipose tissues, as well as that

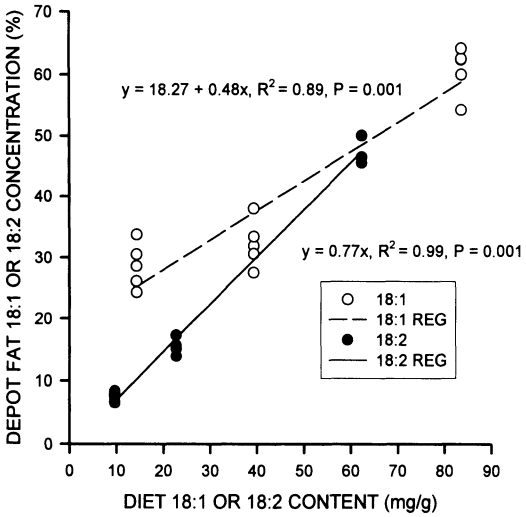

FIG. 1. The relationship between dietary and depot fat levels of oleic acid (open circles) and that for linoleic acid (closed circles). The line drawn through each set of points represents the least squares linear regression.

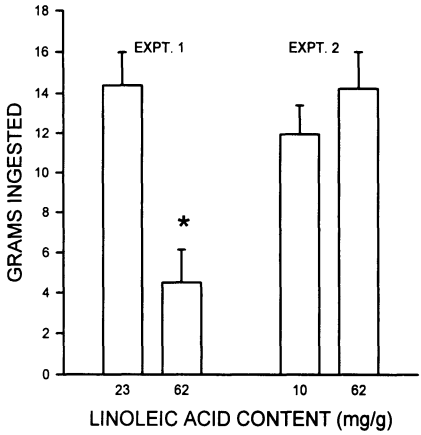

FIG. 2. Histograms indicating the mean (±SE) intake of each semisynthetic diet per subject during the laboratory food selection experiments. *Significant difference between means in the same experiments at $P = 0.05$.

between dietary linoleic acid content (mg/g) and the level of linoleic acid (%) in white adipose tissues (Fig. 1). Separate comparisons were made for oleic and linoleic acids because mammals can synthesize monounsaturated fatty acids (MUFA) but not polyunsaturated fatty acids. The equation describing the relationship between dietary and adipose levels of oleic acid ($y = 18.27 + 0.48x$) was used to estimate the oleic acid contents of natural *S. lateralis* diets from the levels of this fatty acid found in their depot fats. Oleic acid was the only monounsaturated fatty acid considered because this is the only MUFA produced by most plant species (Gunstone, 1996) and the only MUFA found in the diets of free-ranging *S. lateralis* (Frank, 1994).

The equation summarizing the relationship between levels of linoleic acid in the diet and depot fat ($y = 0.77x$) was used to estimate dietary linoleic acid content from the concentration of linoleic acid found in the depot fats of free-ranging *S. lateralis*. Because mammals absorb and store linoleic and alpha-linolenic acids equally well (Mead *et al.*, 1986), the linoleic acid equation was also used to estimate dietary alpha-linolenic acid content from the amount of this fatty acid present in the depot fat.

In order to estimate the average Vitamin E content of natural *S. lateralis* diets during the fall fattening period, stomach contents from 9 of the squirrels collected and sacrificed at the study site were analyzed for Vitamin E using the H. P. L. C. techniques of Tramontano *et al.* (1993). This provided an accurate estimate of dietary Vitamin E because this nutrient is neither digested nor absorbed in the stomach (Gallo-Torres, 1980). Behavioral observations were also made of free-ranging *S. lateralis* foraging at the Crooked Creek site during August to determine the plant species and parts ingested during the fattening period. Samples of these species and parts were collected, frozen at −20°C, and later analyzed for fatty acid composition. The semisynthetic diets, plant parts, and depot fat samples all were analyzed for fatty acid composition by gas chromatography using the techniques described previously (Frank 1991).

RESULTS

Laboratory food selection experiments

The squirrels ingested over three times more of the medium PUFA diet (Fig. 2) than the high PUFA diet during the first diet selection experiment (paired $t = 3.89$, df = 4, $P < 0.025$). All 5 squirrels ingested more

TABLE 2. *Total intakes of polyunsaturated fatty acids.*

Experiment number	Mean (±SE) PUFA intake (mg/g diet)*
1	31.8 ± 2.6[a]
2	38.2 ± 2.9[a]
Field	46.7 ± 2.7[b]

* Means with the same lower case letter are not different at $P = 0.05$.

of the medium PUFA diet. The resulting total PUFA intake (Table 2) was not significantly different from the predicted level of 33 mg/g diet (two-tailed $t = -0.46$, df = 4, $P > 0.5$). The squirrels ingested statistically equivalent amounts of the low and high PUFA diets (Fig. 2) in the second experiment ($t = 1.83$, df = 4, $0.1 < P < 0.2$), with only 3 of them ingesting more of the high PUFA diet. The total PUFA intake produced (Table 2) was not significantly different from 33 mg/g diet (two-tailed $t = 1.76$, df = 4, $0.1 < P < 0.20$).

Laboratory fattening/hibernation experiments

The three groups had equivalent superoxide dismutase activities in their liver tissues (Fig. 3) during the feeding period (F = 4.26, df = 2,6, $P = 0.08$, Power = 0.25) and after one month of hibernation (F = 2.55, df = 2,9, $P = 0.14$, Power = 0.50). The superoxide dismutase activities of the brown adipose tissues (Fig. 3) from the three groups also did not vary significantly during either feeding (F = 2.02, df = 2,6, P = 0.21, Power = 0.25) or the first month of hibernation (F = 3.41, df = 2,9, $P = 0.10$, Power = 0.35). The liver tissues of the three groups did not significantly differ in catalase activity (Fig. 4) during feeding (F = 1.04, df = 2,6, $P = 0.42$, Power = 0.25) or the first month of hibernation (F = 3.17, df = 2,9, $P = 0.10$, Power = 0.30). The catalase activities of the brown adipose tissues did not vary statistically with diet type (Fig. 4) during feeding (F = 2.17, df = 2,6, $P = 0.20$, Power = 0.25) and one month of hibernation (F = 1.75, $P = 0.24$, Power = 0.25). The level of glutathione peroxidase activity found in the liver tissues (Fig. 5) also did not significantly vary during both feeding (F = 0.78, df = 2,6, P = 0.49, Power = 0.40) and the first month of hibernation (F = 3.76, df = 2,9, $P = 0.09$, Power = 0.40). The brown adipose tissues from the medium PUFA group (Fig. 5), however, had significantly lower glutathione peroxidase levels than the other groups during both feeding (F = 96.0, df = 2,6, P

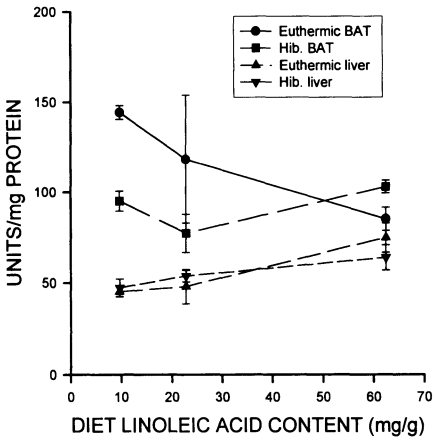

FIG. 3. Superoxide dismutase activities (Means ± SE) in the liver and brown adipose tissues during feeding and hibernation. One unit of activity is the amount of enzyme inhibiting the oxidation of NADH by 50% under reaction conditions. n = 3 for each mean involving euthermic squirrels; n = 4 for each mean involving hibernating squirrels.

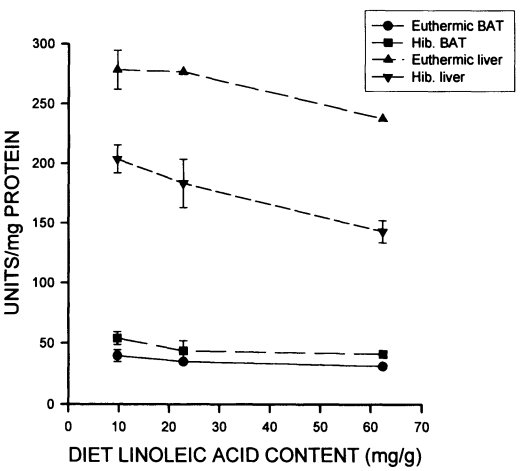

FIG. 4. Mean (±SE) catalase activities of brown adipose and liver tissues during feeding and hibernation. One unit of activity is the amount of enzyme that reduces 1 μmol of H_2O_2 per minute. n = 3 for each mean involving euthermic squirrels; n = 4 for each mean involving hibernation squirrels.

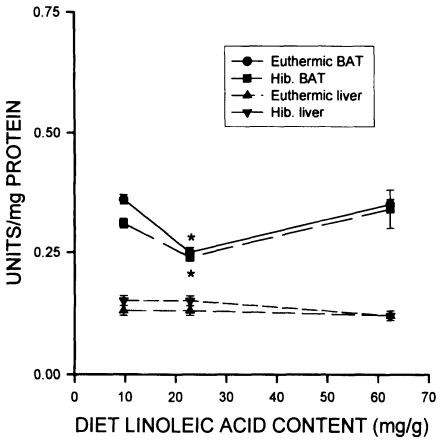

FIG. 5. Mean (±SE) glutathione peroxidase activities of brown adipose and liver tissues during feeding and hibernation. One unit of activity is the amount of enzyme required to oxidize 1 μmol of NADPH per minute. *Significantly different mean with a category at $P = 0.05$. n = 3 for each mean involving euthermic squirrels; n = 4 for each mean involving hibernating squirrels.

= 0.001) and the first month of hibernation (F = 10.58, df = 2,9, $P = 0.006$).

Field diet studies

The estimated total (linoleic + alpha-linolenic acid) PUFA intake (Table 3) was significantly greater than 33 mg/g diet (two-tailed $t = 5.02$, df = 18, $P < 0.001$) and those observed in the laboratory diet (Table 2) preference tests (One-way ANOVA: F = 4.38, df = 2,25, $P = 0.02$). The total PUFA content of the natural diet was significantly less than 59 mg/g diet ($t = 11.62$, df = 18, $P < 0.001$), however. The stomach content analyses revealed that the natural diets also had a mean (±SE) Vitamin E content of 14.11 ± 5.21 IU/kg diet. At Crooked Creek, *S. lateralis* diets consisted entirely of two

TABLE 3. *Mean (±SE) fatty acid compositions of white adipose tissues from free-ranging* S. lateralis *and the dietary fatty acid compositions indicated.*

Fatty acid	Depot fat concentration (%)	Dietary content (mg/g)
Oleic	40.6 ± 1.6	45.9 ± 2.9
Linoleic	25.5 ± 2.0	33.1 ± 2.6
Alpha-linolenic	10.4 ± 1.2	13.5 ± 1.5

n = 19 at each level.

types of plant parts during the fall: 1) the herbaceous (leaves and flowers) parts of grasses and forbs, and 2) pine seeds (Table 4). The herbaceous parts ingested had significantly lower oleic (F = 23.96, df = 4,10, $P = 0.001$) and linoleic (F = 29.12, df = 4,10, $P = 0.001$) acid contents than the seeds and they were the only items that contained alpha-linolenic acid (Table 4).

DISCUSSION

Results of the laboratory food selection experiments support the hypothesis that PUFA intake is restricted to the minimal level required for proper hibernation (33 mg/g) during the fall feeding/fattening period prior to hibernation. In both experiments, the total dietary PUFA intakes produced by food selection were not significantly different from 33 mg/g. The Vitamin E contents of the semisynthetic diets were equivalent to those of natural diets, so the *S. lateralis* in the laboratory experiments were subjected to roughly the same degree of oxidative stress as free-ranging animals. The results of the field studies also support our hypothesis. Although the mean for the total PUFA content of natural diets was greater than 33 mg/g, it was 12 mg/g below the maximum dietary PUFA level content shown to produce proper hibernation pat-

TABLE 4. *Mean (±SE) fatty acid compositions of items ingested by free-ranging ground squirrels.*

Plant species and part ingested	mg/g*		
	18:1	18:2	18:3
Agrostis exarata leaves	3 ± 1[a]	3 ± 1[a]	8 ± 1
Alopecurus aequalis leaves	1 ± 1[a]	4 ± 1[a]	12 ± 3
Taraxacum officinale flowers	2 ± 1[a]	11 ± 1[a]	10 ± 1
Pinus edulis seeds	169 ± 32[b]	208 ± 39[b]	—
Pinus flexilis seeds	78 ± 11[c]	274 ± 37[b]	—

* Means within a fatty acid category with the same superscript are not significantly different at $P = 0.05$, n = 3 for each mean.

terns (59 mg/g). This is quite remarkable considering that the food items ingested by free-ranging *S. lateralis* had total PUFA contents varying from 11 to 274 mg/g with species, and reveals that the intake of high PUFA items is restricted.

The fatty acid compositions listed in Table 4 reveal a possible major limitation on hibernation and over-winter survival not considered previously. Herbaceous plant parts have PUFA contents that are far below 33 mg/g, whereas the pine seeds have PUFA contents that are 6 to 10 time greater. To maintain dietary PUFA levels at or near 33 mg/g, *S. lateralis* should ingest only enough pine seeds to bring the PUFA content of the entire diet to 33 mg/g, with most of the diet consisting of herbaceous grass and forb parts. Due to strong seasonal changes in the amounts of palatable plant species/parts available, however, the fraction of herbaceous plant parts in the diets of free-ranging ground squirrels in alpine areas decreases from 84–90% in June to 22–26% by September. During this same period, the proportion of seeds in the diet increases from 5–8% to 56% (Morton, 1975). Thus the *S. lateralis* in our field study apparently had dietary PUFA contents that were slightly greater than predicted due to limitations in the amount of low PUFA plants that are available during the fall. This may impose an important limitation on the hibernation ability and over-winter survival of ground squirrels in general.

Linoleic acid is a nutrient required in the diet of mammals for proper health, and the linoleic acid requirement of rodents is 3–5 mg/g diet. When feeding on diets low in linoleic acid (3–5 mg/g), the minimum Vitamin E requirement of rodents varies from 3–50 IU/kg food with species. Increasing dietary linoleic acid above the minimum requirement increases the amount of Vitamin E needed to about 100 IU/kg diet (Robbins, 1993). The PUFA contents of free-ranging *S. lateralis* diets are relatively higher, thus their low Vitamin E contents (14 IU/kg) are probably inadequate to prevent lipid peroxidation during torpor. Obtaining sufficient amounts of Vitamin E in the diet during fattening may thus also be an important limitation on hibernation and over-winter survival.

Increasing the polyunsaturate content of the diet by 52 mg/g did not produced any increase in the levels of superoxide dismutase, catalase, or glutathione peroxidase in liver or brown adipose tissues. This indicates that increasing dietary polyunsaturate content by 52 mg/g does not increase the level of lipid peroxidation in these tissues during either prehibernatory fattening or the first month of hibernation. In contrast, increasing the amount of polyunsaturates in the diet by only 12–28 mg/g during fattening doubled the superoxide and catalase activities in the brown adipose tissues of *S. lateralis* after 7 months of hibernation (Frank and Storey, 1995), which indicates that lipid peroxidation also increased.

The major function of restricting dietary PUFA levels to 33 mg/g during fattening appears to be to reduce the amount of lipid peroxidation that occurs in the later phases of hibernation. Our lipid peroxidation experiments are not entirely conclusive, however, because the powers of the ANOVA comparisons made were low. The possibility that increasing the amount of polyunsaturates in the diet increases the amount of lipid peroxidation during fattening and the early phases of hibernation cannot be entirely ruled out. The diet selection of free-ranging *S. lateralis* is not only based on their immediate nutritional needs, but also the physiological challenges that they will face during the next 7–8 mo. Further investigation of this system will provide new insights not only into the physiological ecology of mammalian hibernation but also the biochemistry of lipid peroxidation.

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REFERENCES

Aebi, H. 1984. Catalase in vitro. Meth. Enzymol. 105: 121–126.

Buzadzic, B., M. Spasic, Z. S. Saicic, R. Radojicic, V. M. Petrovic, and B. Halliwell. 1990. Antioxidant defenses in the ground squirrel *Citellus citellus* 2. The effect of hibernation. J. Free Radic. Biol. Med. 9:407–413.

Blake, B. H. 1972. The annual cycle and fat storage in two populations of golden-mantled ground squirrels. J. Mammal. 53:157–167.

Flohe, L. and W. A. Gunzler. 1984. Glutathione peroxidase. Meth. Enzymol. 105:115–121.

Florant, G. L. 1997. Lipid composition in hibernators: The importance of essential fatty acids. Amer. Zool. 38:331–340.

Frank, C. L. 1991. Adaptations for hibernation in the depot fats of a ground squirrel (*Spermophilus beldingi*). Can. J. Zool. 69:2707–2711.

Frank, C. L. 1992. The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). Physiol. Zool. 65: 906–920.

Frank, C. L. 1994. Polyunsaturate content and diet selection by ground squirrel (*Spermophilus lateralis*). Ecology 75:458–463.

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.

Frank, C. L. and K. B. Storey. 1996. The effect of total unsaturate content on hibernation. *In* F. Geiser, A. J. Hulbert, and S. C. Nicol (eds.), *Adaptations to the cold,* pp. 211–216. University of New England Press, Armidale, Australia.

Frankel, E. N. 1995. Oxidation of polyunsaturated lipids and its nutritional consequences. *In* W. A. M. Castenmiller (ed.), *Proceedings of the 21st world congress of the International Society for fat research (ISF),* Vol. 2., pp. 265–269. P. J. Barnes and Associates, Bridgewater, England.

Gallo-Torres, H. E. 1980. Biochemistry, absorption, transport, and metabolism. *In* L. J. Machlin (ed.), *Vitamin E: A comprehensive treatise,* pp. 169–267. Marcel Dekker, Inc., New York.

Geiser, F. 1994. Hibernation and daily torpor in marsupials: A review. Aust. J. Zool. 42:1–16.

Gunstone, F. D. 1996. *Fatty acid and lipid chemistry.* Blackie Academic and Professional, Glasgow, Scotland.

Halliwell, B. and J. M. Gutteridge. 1989. *Free radicals in biology and medicine.* Claredon Press, Oxford.

Halliwell, B. and S. Chirico. 1993. Lipid peroxidation: Its mechanism, measurement, and significance. Amer. J. Clin. Nutr., 57:715S–725S.

Kaasgard, S. G., G. Holmer, C. Hoy, W. A. Behrens, and J. L. Beare-Rogers. 1992. Effects of dietary linseed oil and marine oil on lipid peroxidation in monkey liver *in vivo* and *in vitro.* Lipids 27:740–745.

Kasparek, S. 1980. Chemistry of tocopherols and tocotrienols. *In* L. J. Machlin (ed.), *Vitamin E: a comprehensive treatise,* pp. 7–65. Marcel Dekker, Inc., New York.

Kayser, C. 1965. Hibernation. *In* W. Mayer and R. VanGelder (eds.), *Physiological mammalogy,* Vol. II, pp. 180–296. Academic Press, New York.

Kenagy, G. J. 1987. Energy allocation for reproduction in the golden-mantled ground squirrel. Symp. Zool. Soc. Lond. 57:259–273.

Kenagy, G. J. and B. M. Barnes. 1988. Seasonal reproductive patterns in four coexisting rodent species from the Cascade Mountains, Washington. J. Mammal. 69:274–292.

Lyman, C. P. 1982. Who is who among the hibernators. *In* C. P. Lyman, J. S. Willis, A. Malan, and L. C. H. Wang (eds.), *Hibernation and torpor in mammals and birds.* pp. 12–36. Academic Press, New York.

Malan, A. 1996. The origins of hibernation: A reappraisal. *In* F. Geiser, A. J. Hulbert, and S. C. Nicol (eds.), *Adaptations to the cold,* pp. 1–6. University of New England Press, Armidale, Australia.

Mead, J., D. Alfin-Slater, D. Howton, and G. Popjak. 1986. *Lipids: Chemistry, biochemistry, and nutrition.* Plenum Press, New York.

Morton, M. L. 1975. Seasonal cycles of body weights and lipid in Belding ground squirrels. Bull. S. C. Acad. Sci. 74:128–143.

Nicol, S. and N. A. Andersen. 1996. Hibernation in the echidna: Not an adaptation to the cold? *In* F. Geiser, A. J. Hulbert, and S. C. Nicol (eds.), *Adaptations to the cold,* pp. 7–12. University of New England Press, Armidale, Australia.

Paoletti, F., D. Aldinucci, and A. Caparrini. 1986. A sensitive spectrophotometric method for the determination of superoxide dismutase in tissue extracts. Anal. Biochem. 154:536–541.

Robbins, C. T. 1993. *Wildlife feeding and nutrition.* Academic Press, New York.

Schmid, J. 1996. Oxygen consumption and torpor in mouse lemurs (*Microcebus murinus* and *M. myoxinus*): Preliminary results of a study in western Madagascar. *In* F. Geiser, A. J. Hulbert, and S. C. Nicol (eds.), *Adaptations to the cold,* pp. 47–54. University of New England Press, Armidale, Australia.

Sies, H. 1993. Strategies of antioxidant defense. Eur. J. Biochem. 215:213–219.

Southorn, P. A. and G. Powis. 1992. Free radicals in cell biology. Fund. Med. Cell Biol. 3B:529–558.

Tramontano, W. A., D. Ganci, M. Pennino, and E. S. Dierenfeld. 1993. Distribution of alpha-tocopherol in early foliage samples in several forage crops. Phytochemistry 34:389–390.

Zar, J. H. 1996. *Biostatistical analysis.* Prentice Hall, Upper Saddle River, New Jersey.

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