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The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*)

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Abstract. Golden-mantled ground squirrels (*Spermophilus lateralis*) are herbivores that hibernate during winter. Although little is known about the nutritional/physiological constraints on hibernation, numerous studies have demonstrated that increasing the amount of linoleic acid (a polyunsaturated fatty acid) in the diet enhances hibernation. This is probably because high linoleic acid diets reduce the melting points of the depot fats produced for hibernation which makes them more metabolizable at low body temperatures. This suggests that a major limitation on hibernation may be obtaining enough linoleic acid in the diet for proper hibernation. In all previous studies, however, the amount of linoleic acid in the diets of free-ranging animals was either not considered, or the range of dietary linoleic acid contents in the experiments was less than that of natural diets. It is thus not known whether the amount of linoleic acid available to hibernators under natural conditions actually limits their torpor patterns. A series of laboratory feeding and hibernation experiments were conducted with *S. lateralis* and artificial diets with different linoleic acid contents that were either below or above the linoleic acid content of the natural diet. The results demonstrated that when dietary linoleic acid contents are either below or above natural levels, hibernation ability is greatly reduced. Hibernation ability was reduced when the squirrels were maintained on a high linoleic acid diet probably by the production of toxic lipid peroxides in brown adipose tissues. The results indicate that there is an optimal level of dietary linoleic acid for proper hibernation, and this is equal to that of the natural diet. The amount of linoleic acid available in the diet thus does not limit hibernation under normal natural conditions.

Key words: Hibernation – Ground squirrel – Lipids – Diet – Polyunsaturates

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Abbreviations: BAT, brown adipose tissue; bm, body mass; FA, fatty acid; PUFA, polyunsaturated fatty acid; T_a, ambient temperature; T_b, body temperature, WAT, white adipose tissue

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Introduction

Ground squirrels are herbivorous rodents that hibernate during winter. For about 5–7 weeks prior to hibernation their feeding rate more than doubles (Loehr and Risser 1977; Kenagy 1987) and their body fat content increases to 35–40% (Morton 1975; Kenagy and Barnes 1988). These depot fats are the primary energy source utilized during hibernation (Kayser 1965). Although the nutritional and physiological constraints on prehibernatory fattening and subsequent winter survival are poorly understood, one important constraint is the melting point of depot fats. It is widely assumed that lipids must be in a fluid state to be metabolizable since the T_b of ground squirrels during torpor is often close to 0 °C (Wang 1979), which is about 25 °C below the melting points of most mammalian fats (Harwood and Geyer 1964).

Mammalian depot fats are triacylglycerols, which are esters of a single glycerol molecule and three FA molecules. The melting point of a triacylglycerol depends on the degree of unsaturation in the FA portion of the molecule, greatly decreasing as FA unsaturation increases (Lehninger 1982). Mammals can synthesize FAs containing either no (saturates) or one (monounsaturates) carbon-carbon double bond per molecule, but they are incapable of producing FAs containing two or more (polyunsaturates) double bonds (Stryer 1988). Most species of plants, in contrast, synthesize large amounts of polyunsaturates, which are incorporated into their lipids (Lehninger 1982). When mammals ingest plant lipids, the polyunsaturates consumed are incorporated into their own body fats, thereby lowering depot fat melting points (Mead et al. 1986).

Analyses of the depot fats produced for hibernation by free-ranging marmots and ground squirrels revealed that they contained large amounts of two polyunsatu-

Table 1. Fatty acid compositions of the various synthetic diets

Fatty acid type	Fatty acid notation[a]	Diet (mg · g^{-1} diet)[+]			
		Min 18:2	Med 18:2	H 18:2	H 18:2/18:3
Lauric acid	12:0	40	–	–	–
Myristic acid	14:0	16	–	1	–
Palmitic acid	16:0	9	5	9	9
Stearic acid	18:0	2	1	3	2
Oleic acid	18:1	7	22	44	26
Linoleic acid	18:2	3	15	31	45
alpha-Linolenic acid	18:3	–	–	–	6

[a] Fatty acids are represented using standard biochemical notation. The number to the left of the colon indicates the number of carbon atoms in the fatty acid molecule, and the number to the right denotes the number of double bonds (Lehninger 1982)

[+] Min 18:2 – minimum linoleic acid diet, Med 18:2 – medium linoleic acid diet, H 18:2 – high linoleic acid diet, H 18:2/18:3 – high linoleic/α-linolenic acid diet

rates: linoleic and α-linolenic acids (Florant et al. 1990; Frank 1991, 1992) which reduced the melting points of these fats to between −6.5 and 5.0 °C (Frank 1991). These observations have led some authors to predict that large amounts of PUFAs are required in the diet for hibernation (Tait 1922; Fawcett and Lyman 1954; Geiser and Kenagy 1987; Frank 1991). Laboratory feeding/hibernation experiments with chipmunks (*Eutamias amoenus*), deer mice (*Peromyscus maniculatus*), gliders (*Acrobates pygmaeus*), ground squirrels (*Spermophilus lateralis* and *S. saturatus*), and marmots (*Marmota flaviventris*) have demonstrated that increasing the amount of linoleic acid in the diet enhances hibernation (Geiser and Kenagy 1987, 1993; Geiser 1991; Geiser et al. 1992; Frank 1992; Florant et al. 1993). It has thus been suggested that obtaining enough linoleic acid in the diet for proper hibernation may be an important limitation on hibernation under natural conditions (Geiser and Kenagy 1987; Frank 1992; Florant et al. 1993).

All of the previous studies on the effects of the amount of linoleic acid in the diet on hibernation, except those conducted by Frank (1992) and Florant et al. (1993), did not measure or consider the amount of linoleic acid in the natural diets of the species examined. The diets used by Frank (1992) and Florant et al. (1993), furthermore, had linoleic acid contents that were less than or equal to that of the natural diets as indicated by the compositions of the depot fats produced. It is thus unclear from these studies if the amount of linoleic acid available in the diet limits hibernation ability under natural conditions. Feeding and hibernation experiments with diets containing more linoleic acid than the natural diet are required to determine if this is an important limitation on hibernation by free-ranging mammals.

There are also physiological limitations associated with high linoleic acid diets which have not been considered previously. Linoleic acid is 12 times more likely to undergo autoxidation than more saturated FAs in the same chemical environment (Mead et al. 1986). Autoxidation is a self-sustaining chain reaction between FAs and oxygen radicals that produces FA peroxides which are highly toxic to cells. Mammals have several antioxidant enzymes which act as defenses against lipid peroxides. The enzymes superoxide dismutase and catalase act to prevent FA autoxidation by removing the hydroxyl and hydroperoxyl radicals that initiate autoxidation. The enzyme glutathione peroxidase uses the peptide glutathione to breakdown any lipid peroxides that may have already formed (Southorn and Powis 1992). Examination of WATs located in the gonads of marmots have also revealed that linoleic acid is not mobilized as quickly as other FA types during torpor (Florant et al. 1990). Considering these factors, we predicted that high (relative to the natural diet) linoleic acid diets may actually reduce hibernation ability. There may be an optimal level of linoleic acid in the diet/depot fats for hibernation, above or below which hibernation ability is reduced. These hypotheses were tested in laboratory feeding and hibernation experiments conducted with ground squirrels (*Spermophilus lateralis*) fed artificial diets of different linoleic acid contents.

Materials and methods

Animals. Twenty-eight adult *Spermophilus lateralis* were captured in the Crooked Creek area of the White Mountains (37°30′N, 118°10′W, elevation 3094 m) of California during July 1991. All animals were housed individually in standard rat cages at the University of California-Irvine and maintained at 22 °C on a fall (10L:14D) photoperiod for 8 weeks. During this period, the squirrels were divided into four groups ($n = 7$ each) that were maintained on different synthetic diets and water for 8 weeks. The diets differed greatly in linoleic acid content (Table 1) and each animal was given 175 g of diet every 5 days during this period.

At the end of the 8 week feeding and fattening period (September 1991) all squirrels (in their cages) were placed at 3.5 °C and on a 10L:14D photoperiod in an environmental chamber to induce hibernation. Food was withheld from all animals after 24 h. At high elevations, *S. lateralis* usually begins intensive feeding and fattening during July, with maximum bm and the onset of hibernation attained by early September (Skryja and Clark 1970; Blake 1972). This laboratory schedule thus followed the natural cycle of the squirrels.

During the first 12 days of fasting at 3.5 °C, the telemetered T_b of each squirrel was recorded every 12 h to determine the onset of hibernation. The squirrels were maintained continuously at a T_a of 2–4 °C for the next 7 months, which is the length of the natural hibernation period (Blake 1972). During this period, both the T_b and torpor bout duration of each squirrel was measured every 24 h.

T_b was measured using Mini-mitter model V temperature telemeters surgically implanted into the abdominal cavity of each animal during the sixth week of feeding. During surgery, a 1-g abdominal fat biopsy was collected from each squirrel and stored at −20 °C for later FA analysis

Synthetic diets. The synthetic diets were modified versions of Purina 5803 rat diet produced by the Test Diet Division of Purina Mills (Richmond, Ind., USA). The base of these diets was sucrose, with high nitrogen casein, vitamins, minerals, fiber, and plant oils added to produce a nutritionally complete diet. To produce the FA contents listed in Table 1, the lipid fraction of each diet was a different plant oil. The minimum linoleic acid diet contained coconut oil, the medium and high linoleic acid diets both contained corn oil, and the high linoleic α-linolenic acid diet contained soybean oil. All dietary FAs were thus in a natural (triacylglycerol) form.

All four diets were 19% protein, 5% ash, 3% fiber, and 2% vitamins. The mimimun linoleic acid, high linoleic acid, and high linoleic α-linolenic acid diets were also 60% carbohydrate and 10% lipid, whereas the medium linoleic acid diet was 5% lipid and 65% carbohydrate. All diets were homogenized and pressed into 1-g feed pellets. Linoleic acid was the primary polyunsaturate utilized in this study because previous examinations of the depot fats from free-ranging *S. lateralis* revealed that this FA accounts for most of the polyunsaturates found in these lipids, with α-linolenic acid comprising the rest (Frank 1992). The high linoleic/α-linolenic acid diet was produced to examine the effects of α-linolenic acid in the diet on hibernation ability. Linoleic acid is an essential nutrient required in the diet of all mammals for proper health (Robbins 1983). The minimum linoleic acid diet thus was formulated to contain the minimum amount of linoleic acid required by rodents for survival (Pudelkewicz et al. 1968).

Analyses of body fat compositions. The FA contents of both the synthetic diets and the squirrel adipose samples were determined by gas-liquid chromatography using the techniques described previously (Frank 1991, 1992). All FA analyses were conducted at the University of California-Irvine. The total body fat content (% bm) of each squirrel was determined after 6 weeks of feeding by measuring total body electrical conductivity via the techniques of Walsburg (1988). The amount of PUFAs stored in the WATs of mammals depends directly on the amount of these FAs in the diet (Mead et al. 1986). The total amounts (grams) of linoleic and α-linolenic acids stored in the WATs of each squirrel at the end of the feeding period was thus calculated and compared to those previously obtained for the depot fats of free ranging *S. lateralis* (Frank 1992) to determine if the synthetic diets contained more or less of these FAs than the natural diet.

Measurements of lipid peroxidation and metabolism during hibernation. At the end of the hibernation period (April 1992) all ground squirrels were sacrificed by decapitation while torpid. The liver, abdominal WATs, and axillary BATs were immediately removed from each carcass and frozen in liquid N_2. These tissues were stored at −80 °C for later analysis. When mammalian tissues are exposed to increased levels of lipid peroxides the amounts of one or more of the antioxidant enzymes that protect cells from these compounds are increased (Southorn and Powis 1992). In order to determine if increasing the amount of dietary/depot fat linoleic acid increases FA autoxidation during torpor, the activities of these enzymes were measured in the liver and BATs, the major sites of lipid metabolism in hibernation ground squirrels, (Stryer 1988). Tissue extracts were prepared using the techniques of Hermes-Lima and Storey (1993), and enzyme activity levels were assayed spectrophotometrically using assays developed previously (Aebi 1984; Flohe and Gunzler 1984; Paoletti et al. 1986). The amount of the peptide glutathione was measured via the assay of Tietze (1969). Enzyme activity and glutathione levels were measured at Carleton University.

The FA compositions of the WATs collected after hibernation were also measured to determine if linoleic acid was retained in these tissues during torpor, as suggested by Florant et al. (1990). All multiple comparisons were conducted with the general linear models ANOVA procedure (SAS Institute 1985). Antioxidant enzyme activities and glutathione levels were analyzed using a student's *t*-test, whereas the fatty acid compositions of depot lipids before and after hibernation were compared with a paired *t*-test (Snedecor and Cochran 1980).

Table 2. Mean (±SE) body masses and fat contents of *Spermophilus lateralis* after fattening

Diet group	Mean body mass (g)*	Mean body fat (%)*
Minimum linoleic acid	277±7	61±1
Medium linoleic acid	321±24	57±4
High linoleic acid	290±24	51±6
High linoleic α-linolenic acid	329±15	61±2

* All means within a category are statistically equivalent at the $P=0.05$ level. $n=5$ for each mean

Table 3. Mean (±SE) linoleic acid contents of white adipose tissues

Diet group	Mean linoleic acid content (g per animal)*
Minimum linoleic acid	7.3±1.8
Medium linoleic acid	23.3±0.3
High linoleic acid	28.1±1.7
High linoleic /α-linoleic acid	33.6±2.9
Natural diet[a]	18.1±0.4

* Means with the same superscript are not statistically different at the $P=0.05$ level, $n=5$ for each mean
[a] Data from Frank (1992)

Results

Body fat compositions

After 6 weeks of feeding all body fat contents (Table 2) were statistically equivalent ($F=1.78$, $P=0.21$). After 8 weeks of feeding all ground squirrel groups had equal bm ($F=1.13$, $P=0.36$; Table 2). the WATs produced during this period differed greatly in linoleic acid content among the diet groups. The total linoleic acid content (g per animal) of the depot fats from the squirrels fed the minimum linoleic acid diet was significantly lower than that of lipids from free-ranging *S. lateralis* (Table 3), whereas the linoleic acid contents of the other diet groups were significantly greater ($F=38.00$, $P=0.01$). This indicates that the linoleic acid content of the minimum linoleic acid diet was less than that of the diets of free-ranging *S. lateralis*, whereas the linoleic acid contents of the other experimental diets were greater. The mean (±SE) α-linolenic acid contents of the depot fats from the high linoleic/α-linolenic acid group and from free-ranging *S. lateralis* (Frank 1992) were 4.1±0.6 and 5.2±0.1 g per animal, respectively, and wee not significantly different ($t=-1.7$,

Table 4. Number of squirrels from each diet group that hibernated during 12 days of fasting at $T_a = 3.5$ °C

Diet group	Number fasted[a]	Number hibernated
Minimum linoleic acid	4	4
Medium linoleic acid	6	2
High linoleic acid	7	2
High linoleic α-linoleic acid	5	0

[a] Although each group originally contained seven squirrels, some animals refused to ingest the synthetic diet and were removed from the study

$P = 0.23$). α-Linolenic acid was not present in the depot fats from the other experimental diet groups

Hibernation ability

After 12 days of fasting at $T_a = 3.5$ °C none of the squirrels in the high linoleic/α-linolenic acid group entered hibernation, and only a third of the animals in the medium and high linoleic acid groups became torpid during this period (Table 4). All of the squirrels from the minimum linoleic acid group hibernated, however. The mean bm of the non-hibernating squirrels decreased by 110–120 g by the end of the 12-day fasting period. All non-hibernating squirrels were thus removed from the study at this point, since they appeared to have depleted their fat stores. Because only two squirrels each from the medium and high linoleic acid groups hibernated, the hibernation data of these animals were pooled into a single group ($n = 4$) for comparison to the minimum linoleic acid group. This pooled group will henceforth be referred to as the medium/high linoleic acid group.

The mean ($\pm$ SE) times spent fasting before hibernation by the minimum and medium/high linoleic acid groups were 138 ± 6 and 96 ± 24 h, respectively, which were significantly different ($t = -2.68$, $P = 0.04$; Student's t-test). During the first 4 months of hibernation, the minimum and medium/high linoleic acid groups maintained statistically equivalent T_bs during torpor (Fig. 1 A). The T_b of the minimum linoleic acid group was significantly lower during both the fifth ($t = 4.60$, $P = 0.01$) and sixth ($t = 4.65$, $P = 0.01$) months of hibernation (Fig. 1 A), but not during the last month of hibernation. Although during the first week of hibernation the medium/high linoleic acid group had significantly longer torpor bouts ($t = 3.00$, $P = 0.04$) than the minimum linoleic acid group, torpor bout duration did not significantly differ between these two groups during any subsequent period (Fig. 1 B).

Lipid peroxidation and metabolism during hibernation

Liver tissue collected from the minimum and medium/high linoleic acid groups had no significant differences in the activities of superoxide dismutase ($t = 2.12$, $P = 0.10$), catalase ($t = 1.07$, $P = 0.33$), or glutathione peroxidase

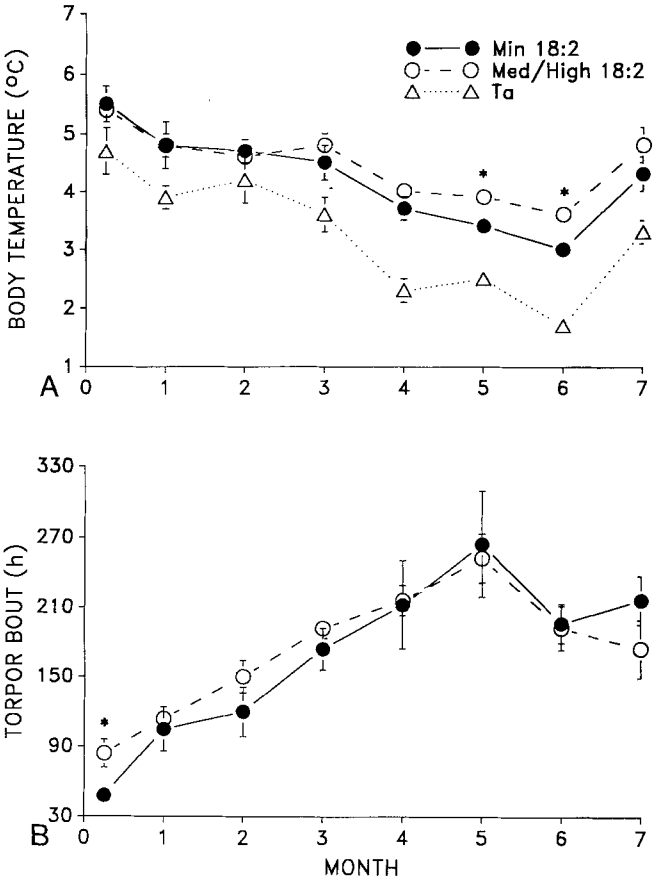

Fig. 1. Mean ($\pm$ SE) body temperatures (**A**) and (**B**) torpor bout durations (h) during hibernation by *Spermophilus lateralis* fed the minimum (*closed circles*) and medium/high (*open circles*) linoleic acid diets. The mean ambient temperature (T_a) for a particular month is indicated by an *open triangle*. * Significant differences between the two squirrel groups during a particular month at the $P = 0.05$ level

($t = 1.37$, $P = 0.23$; Table 5). The liver tissues also contained equivalent amounts of both the reduced ($t = 0.08$, $P = 0.53$) and oxidized ($t = 0.50$, $P = 0.60$) forms of glutathione (Table 5). In the BATs from the medium/high linoleic acid group, however, the activities of both superoxide dismutase ($t = 3.76$, $P = 0.03$) and catalase ($t = 3.07$, $P = 0.01$) were significantly greater than the corresponding activities in the BATs from the minimum linoleic acid group (Table 5). The two BAT groups did not significantly differ in glutathione peroxidase activity ($t = 1.77$, $P = 0.21$), reduced glutathione ($t = -0.47$, $P = 0.68$), or oxidized glutathione content ($t = 0.1$, $P = 0.9$; Table 5).

The abdominal WATs collected from the minimum linoleic acid group before and after hibernation contained statistically equivalent fractions of palmitoleic (16:1) acid ($t = 4.58$, $P > 0.05$; paired t-test), oleic (18:1) acid ($t = 3.76$, $P > 0.50$), and linoleic (18:2) acid ($t = 3.37$, $P > 0.05$; Fig. 2). The proportion of palmitic acid (16:0), however, was significantly greater prior to hibernation ($t = 15.78$, $P < 0.005$). The depot fats collected from the medium/high linoleic acid group before and after hibernation contained equivalent fractions of linoleic acid ($t = 3.37$, $P < 0.05$; Fig. 2). The WAT collected from this group prior to hibernation had significantly greater pro-

Table 5. Mean (±SE) activities of various antioxidant enzymes and glutathione levels in ground squirrel tissues

Enzyme	Tissue	Units · mg protein^{-1} [a]	
		Min 18:2	Med/High 18:2
Superoxide dismutase	Liver	103±6	139±16
	Brown adipose	95±7	140±9*
Catalase	Liver	117±24	142±11
	Brown adipose	35±4	73±12*
Glutathine peroxidase	Liver	0.16±0.01	0.20±0.02
	Brown adipose	0.18±0.01	0.23±0.03
Reduced Glutathione[b]	Liver	6989±1119	8794±2129
	Brown adipose	3273±715	2897±339
Oxidized Glutathione[b]	Liver	1106±317	1380±356
	Brown adipose	527±76	527±26

[a] One unit of superoxide dismutase is the amount of enzyme inhibiting the oxidation of NADH by 50% under the reaction conditions. One unit of catalase activity is the amount of enzyme that reduces 1 μmol H_2O_2 per min. One unit of glutathione peroxidase activity is the amount of enzyme that oxidizes 1 μmol NADPH per min
* Significant difference between diet groups at the $P=0.05$ level
[b] nmol · g tissue^{-1}

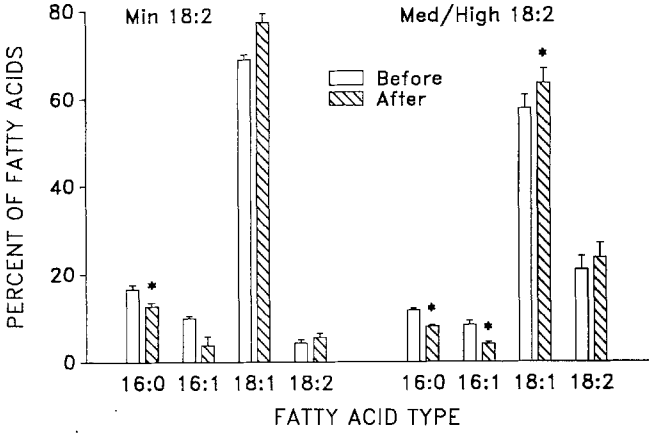

Fig. 2. Histograms indicating the mean (±SE) fatty acid contents (%) of the white adipose tissues from *Spermophilus lateralis* before and after hibernation. The abscissa indicates fatty acid type: palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1), and linoleic acid (18:2). * Significant difference within a fatty acid type between the lipids collected before and after hibernation at the $P=0.05$ level

portions of palmitic ($t=9.03$, $P<0.005$) and palmitoleic ($t=6.14$, $P<0.01$) acids than those collected after hibernation (Fig. 2), whereas the medium/high linoleic acid group had a grater fraction of oleic acid ($t=8.58$, $P<0.005$) after hibernation (Fig. 2).

Discussion

The results of this study clearly demonstrate that high levels (relative to the natural diet) of linoleic acid in the diet reduces the hibernation ability of *Spermophilus lateralis*. Ground squirrels with linoleic acid levels in their depot fats that were greater than those of free-ranging *S. lateralis* were much less likely to hibernate during fasting than squirrels maintained on a low linoleic acid diet. When hibernating members of both of these groups were compared, there were few significant differences in torpor patterns. These results indicate that in terms of hibernation performance, there are no advantages to increasing the amount of linoleic acid in the diet/depot fats above natural levels.

The squirrels maintained on a low (relative to the natural diet) linoleic acid diet had the greatest hibernation ability in this present study. When this group is compared to the results for *S. lateralis* fed a diet containing a natural (greater) level linoleic acid in a previous study (Frank 1992), however, the minimum linoleic acid group fasted longer prior to hibernation, maintained higher T_b during torpor, and had shorter torpor bouts, although they were maintained under the same laboratory conditions. The results of both this and the previous hibernation study of *S. lateralis*, when interpreted together, thus demonstrate that the optimal amount of linoleic acid in the diet for proper hibernation is equal to the linoleic acid content of the natural diet. The optimal (natural) diet for hibernation enables *S. lateralis* to produce 80–90 g WAT with a linoleic acid content of 25% during a 7-week fattening period. A diet that results in a different body fat composition reduces hibernation ability. Previous studies reported that increasing the amount dietary linoleic acid enhances torpor, but they did not consider the linoleic acid contents of natural diets (Geiser and Kenagy 1987, 1993; Geiser 1991; Geiser et al. 1992) and probably tested a range of dietary linoleic acid contents that was below the optimal level for proper hibernation.

The linoleic acid content of mammalian depot fats is directly correlated with that of the diet (Mead et al. 1986). Free-ranging *S. lateralis* thus probably maintain the optimal depot fat composition for hibernation through active diet selection. Ground squirrels have a dietary preference for items of an intermediate linoleic acid content over those containing relatively more or less linoleic acid (Frank 1994). Such a diet selection strategy is very important because the linoleic acid content of plants varies greatly with species (Harwood and Geyer 1964), season/temperature (Quinn 1988), and among different parts of the same plant (Florant et al. 1990). The results of two field studies on *S. lateralis* in the White Mountains of California (Frank 1992, 1994) revealed that these squirrels normally maintain the linoleic acid contents of their diets at the optimal level for hibernation. This demonstrates that at this study site the amount of linoleic acid available does not limit the hibernation ability of *S. lateralis*.

The results of this study also indicate that the inhibi-

tion of hibernation by high linoleic acid diets may be due, at least in part, to increased FA peroxidation in BAT. Most of the white adipose lipids (energy) consumed during hibernation is used to support the metabolism of BAT (McKee and Andrews 1990), since these tissues are primarily responsible for thermoregulation during both torpor and arousal bouts (Trayhurn and Milner 1989). Due to their large numbers of mitochondria, BATs are particularly prone to the production of oxygen free radicals which are capable of initiating FA autoxidation (Barja 1992). These tissues are thus highly sensitive to the FA composition of the lipids used as an energy source. The elevated activities of both superoxide dismutase and catalase seen in the BATs from the medium/high linoleic acid group suggests that increasing the amount of linoleic acid in the diet above natural levels may increase the production of lipid peroxides in these tissues. The increased levels of superoxide dismutase and catalase may not be sufficient to compensate for this dietary effect. Hibernation ability may thus have been reduced in these groups by damage to the BATs.

The abdominal WATs contained the same proportion of linoleic acid before and after the 7-month hibernation period. This indicates that there was no selective retention of this FA in these tissues. These results are in contrast to the linoleic acid retention observed in the gonadal adipose tissues of marmots by Florant et al. (1990). It appears that the retention of linoleic acid only occurs in gonadal fats. Since most of the lipids produced for hibernation are located in the abdominal cavity (Kayser 1965), it is unlikely that the selective retention of linoleic acid in depot fats is a major factor affecting hibernations. The results of the abdominal depot fat analyses do suggest, however, that saturated and monounsaturated FA are utilized at different rates during hibernation. The squirrels maintained on the high linoleic/alpha-linolenic acid diet were totally incapable of hibernating. Although the depot fats of this groups were the only lipids to contain α-linolenic acid, these fats also contained the greatest amount of linoleic acid. It is thus unclear if this dramatic reduction in hibernation ability was due to the presence of α-linolenic acid in the diet, or increased linoleic acid levels. Additional experiments are necessary to determine the effects of α-linolenic acid on hibernation. The effects of dietary FA on the degree of lipid peroxidation in other tissues must also be determined in order to better understand the relationship between dietary lipids, FA autoxidation, and hibernation. We are presently conducting studies in these areas. Further investigation of this system will undoubtedly provide new insights into mammalian hibernation and herbivory.

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