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The effect of total unsaturate content on hibernation

Craig L. Frank and Kenneth B. Storey
Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6,
Present address of C.L.F.: The Louis Calder Center, P.O. Box K, Armonk, N.Y. 10504, U.S.A.

Golden-mantled ground squirrels (*Spermophilus lateralis*) are herbivorous rodents that hibernate during winter. For 7 weeks prior to hibernation, their feeding rate more than doubles (Kenagy 1987), and body fat content increases to 35-40% (Kenagy & Barnes 1988). These stored fats are the primary energy source utilised during hibernation (Kayser 1965). One important constraint on hibernation appears to be the melting point of depot fats, since it is widely assumed that lipids must be in a fluid state to be metabolisable (Mead *et al.* 1986). The body temperatures of ground squirrels during torpor are often close to 0°C (Wang 1979), which is about 25°C below the melting points of most mammalian fats (Harwood & Geyer 1964).

The melting points of mammalian triacylglycerols depend mostly on the degree of unsaturation in the fatty acid portion of these molecules, decreasing as unsaturation increases. Mammals can synthesise fatty acids containing either no (saturates) or one (monounsaturates) carbon-carbon double bonds per molecule, but they are incapable of producing most fatty acids containing two or more (polyunsaturates) double bonds. Most plants, in contrast, synthesise large amounts of polyunsaturates incorporated into their lipids. 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).

These observations have led some authors to predict that large amounts of polyunsaturated fatty acids are required in the diet for hibernation (Fawcett & Lyman 1954; Frank 1991; Geiser & Kenagy 1987). Laboratory experiments with chipmunks, deer mice, gliders, ground squirrels and marmots have demonstrated that increasing the amount of the polyunsaturate linoleic acid in the diet enhances hibernation ability (Florant *et al.* 1993; Frank 1992; Geiser 1991; Geiser & Kenagy 1987; Geiser *et al.* 1992). In contrast to the effect of polyunsaturated fatty acids, little is known about the effect of dietary monounsaturates on hibernation ability. Triacylglycerols containing two or three monounsaturated fatty acids per molecule have melting points near 0°C (O'Connor 1960). Monounsaturates are major components of most plant lipids, and when ingested by mammals, they are also incorporated into their body fats, thereby lowering adipose tissue melting points (Mead *et al.* 1986). Ground squirrels may not be able to synthesise the highly unsaturated depot fats required for proper hibernation without high levels of monounsaturates or polyunsaturates in the diet. We therefore predicted that a diet with a high total unsaturate content should also enhance hibernation ability, with total unsaturate content defined as the sum of the monounsaturate and polyunsaturate contents. We tested this hypothesis in laboratory feeding and hibernation experiments conducted with *Spermophilus lateralis* fed diets of different fatty acid contents.

Twelve adult *Spermophilus lateralis* were captured in the Crooked Creek area of the White Mountains in California during June 1992. All animals were individually housed in rat cages and maintained at 22°C on a 10L:14D photoperiod. The squirrels were divided into three groups of n=4, each of which was maintained on a different semisynthetic diet and water for 8 weeks. Each animal was given 150g of diet every 5 days. At the end of the 8 week feeding period (September 1992), the squirrels were placed at 4.0°C and on a constant fall photoperiod (10L:14D) in an environmental chamber to induce hibernation. After a 24h period, all food was withheld. During the first 9 days of fasting, the body temperature of each squirrel was measured every 12h to determine the onset of hibernation. All squirrels from each group that did not hibernate during this first 9-day fasting period were removed from the environmental chamber and placed at Ta=22°C. These euthermic animals were fed their usual semisynthetic diets for 8 days. These squirrels were then placed back in the environmental chamber and fasted at Ta=4.0°C for another 9-day period to induce hibernation.

Hibernating squirrels were maintained continuously at Ta=4.0°C for a month, during which both the body temperature (Tb) and torpor bout duration of each animal was measured every 24h. Body temperatures were measured using Mini-mitter model V temperature telemeters that were surgically implanted into the abdominal cavity of each squirrel after 4 weeks of feeding. During surgery, a 1g biopsy of abdominal fat was collected from each animal and stored at -20°C for later analysis.

Table 1. Fatty acid compositions of the various semisynthetic diets

Fatty Acid Type	Fatty Acid Notation	Diet (mg/g)		
		Saturate	Monounsaturate	Polyunsaturate
Lauric acid	12:0	59.1	2.4	—
Myristic acid	14:0	22.0	1.8	1.6
Palmitic acid	16:0	16.3	22.3	19.4
Stearic acid	18:0	4.3	—	8.0
Oleic acid	18:1	14.3	83.6	39.4
Linoleic acid	18:2	9.7	22.8	62.4
Linolenic acid	18:3	—	—	0.8
Total Unsaturate (TU)		24.0	106.4	102.6

The semisynthetic diets were modified versions of Purina 5001 rodent diet produced by the Test Diet Division of Purina Mills, Inc. (Richmond, Indiana). To produce the fatty acid contents listed in Table 1, a different plant oil was added to each diet. The high saturate diet contained coconut oil, the high monounsaturate diet contained olive oil, and sunflower oil was added to the high polyunsaturate diet. In each case, a mixture of 1 part plant oil to 9 parts Purina 5001 rodent diet was produced. The diets were then homogenised and pressed into 1g feed pellets using a laboratory pellet mill. The overall composition of the diets were 21% protein, 49% carbohydrate, 15% lipid, 7% ash, 5% fiber, and 3%

minerals. The high saturate diet was produced to test the ability of *S. lateralis* to synthesise unsaturated body fats when maintained on a low monounsaturate/polyunsaturate diet, and subsequently hibernate. The high monounsaturate diet was used to determine the effects of dietary oleic acid on hibernation ability, whereas the high polyunsaturate diet provided a comparison to any effects observed in the high monounsaturate group. The high monounsaturate and the high polyunsaturate diets were also designed to have roughly equivalent total unsaturate (monounsaturate + polyunsaturate) contents.

The white adipose tissues collected after 4 weeks of feeding, as well as the semisynthetic diets, were analysed for fatty acid content using the gas chromatography techniques described previously (Frank 1991). These data were compared to the fatty acid compositions of depot fats collected from free-ranging *S. lateralis* listed previously (Frank 1992). The total body fat content (% body mass) of each squirrel was determined by measuring total body electrical conductivity after 4 weeks of feeding via the techniques of Walsberg (1988).

After 4 weeks of feeding, the mean (± SE) body fat contents of the high saturate, monounsaturate, and polyunsaturate diet groups were 49.2±1.8%, 45.9±3.0%, and 51.3 ±1.6%, respectively, and were statistically equivalent ($F=2.62$, $p=0.12$). The mean (± SE) body masses of these groups after 8 weeks of feeding were 309±20g, 299±8g, and 286 ±12g, respectively, and were also equivalent ($F=0.58$, $p=0.60$). The body fat contents could not be measured during this period due to electrical interference from the temperature telemeters. The white adipose tissues produced during feeding differed greatly in fatty acid composition (Fig.1) between the diet groups. Only the high saturate group contained the saturated fatty acids lauric (12:0) and myristic (14:0) acids, which were probably derived from the diet. The mean (± SE) proportions of these fatty acids were 32.7±0.3 and 12.0±0.4%, respectively.

The four diet groups had equivalent amounts of palmitic (16:0) acid (Fig. 1). The high monounsaturate group contained about twice as much palmitoleic acid (16:1) in the depot fat (Fig. 1) than the other groups ($F=8.04$, $p=0.002$), and this group had the highest oleic acid (18:1) content ($F=19.31$, $p=0.0001$).

Table 2. Mean (± SE) body temperatures (Tb) and torpor bout durations during hibernation

Diet Group	Mean Tb (°C)	Mean Torpor Bout (h)
Saturate	4.6 ± 0.4a*	96 ± 21a*
Monounsaturate	5.1 ± 0.3a	188 ± 7b
Polyunsaturate	6.2 + 0.5b	164 + 18b

* Means with the same letter are not significantly different at the $p=0.05$ level.

The high polyunsaturate group had significantly more linoleic acid (18:2) than the other three diet groups ($F=8.04$, $p=0.002$; Fig. 1). The total unsaturate (TU) contents of the lipids from the high monounsaturate and polyunsaturate groups were twice that of the lipids from the high saturate group ($F=143.9$, $p=0.0001$), but both were equivalent to that of free-ranging animals (Fig. 1). During the first 9-day fasting period, only 1 of the 4 squirrels in the high saturate group became torpid, whereas 3 animals from the high monounsaturate group and 2 from the high polyunsaturate group hibernated during this period. During the second 9-day fasting period, only 2 of the 3 remaining members of high saturate group hibernated, while all of the remaining members of both the high monounsaturate (1) and high polyunsaturate (2) groups became torpid.

During hibernation, the high polyunsaturate diet group maintained a slightly higher body temperature ($F=7.8$, $p=0.01$; Table 2) than the other diet groups. Both the high monounsaturate and high polyunsaturate groups had torpor bouts that were about twice the duration ($F=25.1$, $p=0.01$) of the high saturate group (Table 2).

The results of this study clearly demonstrate that a high monounsaturate (oleic acid) diet enhances hibernation by *S. lateralis* supporting observations on chipmunks, *Tamias amoenus* (Geiser *et al.* 1994). Squirrels fed the high monounsaturate diet were more likely to hibernate and had longer torpor bout durations than those maintained on the high saturate diet. As in previous studies, the hibernation ability of squirrels fed a high polyunsaturate (linoleic acid) diet was also greater than the high saturate group. The high monounsaturate diet enhanced hibernation ability to a slightly greater extent than the high polyunsaturate diet. It is important to note that the depot fats of both these groups had total unsaturate contents equal to that found under natural conditions. This indicates that the total unsaturate contents of these diets were roughly equivalent to that of the natural diet, which appears to be 103-106mg/g diet.

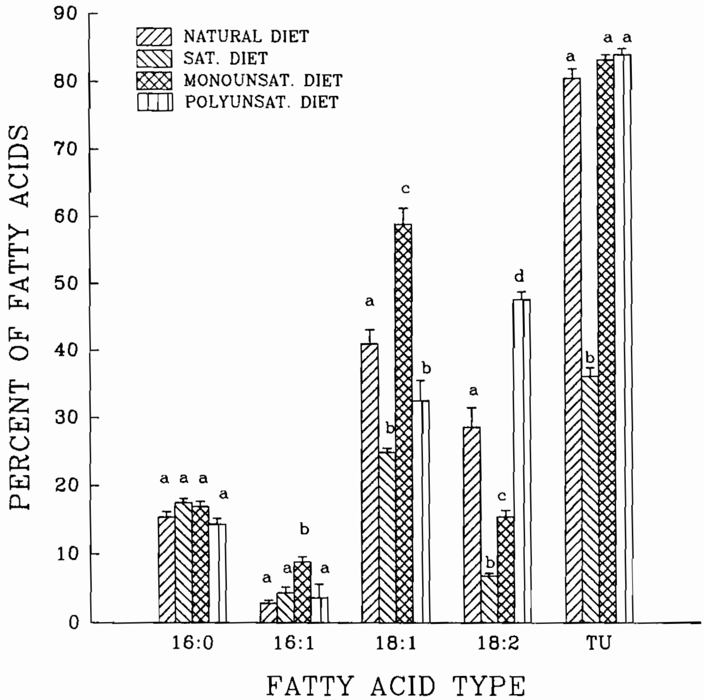

Fig. 1. Histograms indicating the mean (± SE) fatty acid contents (%) of abdominal depot fats. Bars with the same letter are not significantly different at the p=0.05 level. Data on the depot fat composition of free-ranging *S. lateralis* are from Frank (1992), n=10 for each mean. For each mean of the experimental diet groups, n=4.

Since diet composition determines depot fat composition and hibernation ability, this study, when interpreted in conjunction with the results of previous hibernation studies, reveals the optimal diet for proper hibernation. The optimal diet should contain enough unsaturated fatty acids to produce depot fats with a total unsaturate content of 80-85% during fattening. Previous studies have shown that when the polyunsaturate content of the depot fats is greater than natural levels, hibernation ability is reduced, probably due to increased lipid peroxidation (Frank & Storey 1995). The diet should thus also have a monounsaturate/polyunsaturate ratio of about 2:1.

The depot fat analyses in this study also reveal important features of the ability of *S. lateralis* to synthesise monounsaturated fatty acids. In all cases, only small amounts of palmitoleic acid were produced in the depot fats; virtually all of the monounsaturates present in the depot fats were oleic acid. Both the high saturate and high polyunsaturate diet groups had body fats that were about only 40% monounsaturate. Since a depot fat content of about 80-85% unsaturated fatty acids is required for hibernation, this indicates that *S. lateralis* cannot prepare their depot fats for hibernation through monounsaturate synthesis alone. The total unsaturate content of the diet must be considered when examining the influence of dietary composition on hibernation ability.

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References

Fawcett D.W. & Lyman C.P. (1954) The effects of low environmental temperature on the composition of depot fat in relation to hibernation. *J. Physiol. Lond.* **126**, 235-247.

Florant G.L., Hester L., Ameenuddin S. & Rintoul D.A. (1993) The effect of a low essential fatty acid diet on hibernation in marmots. *Am. J. Physiol.* **264**, R747-753.

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. & Storey K.B. (1995) The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). *J. Comp. Physiol. B*, **164**, 536-542.

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.

Geiser F. & Kenagy G.J. (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *Am. J. Physiol.* **252**, R897-R901.

Geiser F., Stahl B. & Learmonth R.P. (1992) The effect of dietary fatty acids on the pattern of torpor in a marsupial. *Physiol. Zool.* **65**, 1236-1245.

Geiser F., McAllan B.M. & Kenagy G.J. (1994) The degree of dietary fatty acid unsaturation affects torpor patterns and lipid composition of a hibernator. *J. Comp. Physiol. B,* **164**, 299-305.

Harwood H.J. & Geyer R.P. (1964) *Biology Data Book.* Federation of American Societies for Experimental Biology, Washington.

Kayser C. (1965) Hibernation. In *Physiological Mammalogy* (Edited by Mayer W. & VanGelder R.), 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. & Barnes B.M. (1988) Seasonal reproductive patterns in four coexisting rodent species from the Cascade Mountains, Washington. *J. Mammal.* **69**, 274-292.

Mead J., Alfin-Slater D., Howton D. & Popjak G. (1986) *Lipids: Chemistry, Biochemistry, and Nutrition.* Plenum Press, New York.

O'Connor R. (1960) X-ray diffraction and polymorphism. In *Fatty Acids: Their Chemistry, Properties, Production, and Uses* (Edited by Markley K.S.), pp. 285-379. Interscience, New York.

Walsberg G.E. (1988) Evaluation of a nondestructive method for determining fat stores in small birds and mammals. *Physiol. Zool.* **61**, 153-159.

Wang L.C H. (1979) Time patterns and metabolic rates of natural torpor in Richardson's ground squirrel. *Can. J. Zool.* **57**, 149-155.