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The effect of unsaturated and saturated dietary lipids on the pattern of daily torpor and the fatty acid composition of tissues and membranes of the deer mouse *Peromyscus maniculatus**

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Summary. Dietary lipids strongly influence the pattern of torpor and the body lipid composition of mammalian hibernators. The object of the present study was to investigate whether these diet-induced physiological and biochemical changes also occur in species that show shallow, daily torpor. Deer mice, *Peromyscus maniculatus*, were fed with rodent chow (control diet) or rodent chow with either 10% sunflower seed oil (unsaturated diet) or 10% sheep fat (saturated diet). Animals on the unsaturated diet showed a greater occurrence of torpor (80–100% vs 26–43%), longer torpor bouts (4.5 vs 2.25 h), a lower metabolic rate during torpor (0.96 vs 2.25 ml $O_2 \cdot g^{-1} \cdot h^{-1}$), and a smaller loss of body mass during withdrawal of food (2.35 vs 3.90 g) than animals on the saturated diet; controls were intermediate. These diet-induced physiological changes were associated with significant alterations in the fatty acid composition of depot fat, leg muscle and brain total lipids, and heart mitochondrial phospholipids. Significant differences in the total unsaturated fatty acid (UFA) content between animals on saturated and unsaturated diet were observed in depot fat (55.7% vs 81.1%) and leg muscle (56.4% vs 72.1%). Major compositional differences between diet groups also occurred in the concentration of n6 and/or n3 fatty acids of brain and heart mitochondria. The study suggests that dietary lipids may play an important role in the seasonal adjustment of physiology in heterothermic mammals.

Key words: Dietary lipids – Fatty acids – Daily torpor – Thermoregulation – Metabolism

Abbreviations: EDTA ethylenediaminetetra-acetic acid; HEPES N-2 hydroxyethylpiperazine-N^1-2-ethanesulphonic acid; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; RMR resting metabolic rate; SD standard deviation; SFA saturated fatty acids; SNK Student-Newman-Keuls test; T_a air temperature; T_b body temperature; UFA unsaturated fatty acids; $\dot{V}O_2$ rate of oxygen consumption

* Dedicated to the late John K. Raison

Introduction

Mammalian torpor is expressed by a periodic reduction in T_b and other physiological functions. Torpor can be deep and prolonged, as in hibernators with a T_b of 0 °C or less, and animals may remain torpid for several weeks (Barnes 1989; Wang 1989). In contrast to deep hibernation, T_b during shallow, daily torpor falls only to about 10–25 °C and torpor bouts always last less than 24 h (Hudson 1973). However, T_bs in this range are still fatal for many homeothermic mammals, and physiological and biochemical adaptations are required for survival of shallow, daily torpor.

Ectothermic organisms acclimated to low T_bs increase concentrations of UFA in tissues and cell membranes (Cossins and Bowler 1987; Hazel 1988). This enrichment of UFA appears to maintain a suitable fluidity ["a measure of rates of molecular motion derived from correlation times and rotational diffusion coefficients of membrane probes of membranes" (Hazel 1988)] for normal physiological function at low temperatures (Cossins et al. 1977; Hazel 1984, 1988; Cossins and Bowler 1987). Hibernating endotherms which also experience low T_bs show a less pronounced seasonal shift in membrane lipid fatty acid composition or bulk membrane fluidity than ectotherms (Goldman 1975; Aloia and Pengelley 1979; Augee et al. 1984; Geiser et al. 1984; Montaudon et al. 1986; Raison et al. 1988). This may be because mammalian hibernators rewarm periodically from torpor and their cellular processes must cope with short-term fluctuations. However, as with some cold-acclimated ectotherms, hibernating mammals contain relatively large proportions of unsaturated lipids in some membranes and tissues (White 1973; Aloia 1979, 1988; Aloia and Raison 1989; Geiser 1990), and it is likely that some subtle, but physiologically important, seasonal differences in the physical properties of membrane lipids occur in hibernators (Raison 1984). Furthermore, torpid hibernators burn predominantly SFA and conserve PUFA in the depot fat (Florant et al. 1990). These ob-

servations indicate that maintenance of a high concentration of UFA in tissue and membrane lipids is a general requirement for organisms experiencing low T_bs.

The lipid composition of tissues and cell membranes is not only affected by temperature acclimation or the pattern of thermoregulation of the animal, but also by dietary fats (Fawcett and Lyman 1954; McMurchie 1988). Diet-induced changes in lipid composition in turn alter thermal properties of membranes and the activity of membrane-associated enzymes (McMurchie 1988). Moreover, diet-induced alterations of the composition of tissues and membranes are correlated with pronounced changes in thermoregulation and the pattern of torpor of hibernating animals; hibernation is deeper and longer in animals on an unsaturated diet than in individuals on saturated diet (Geiser and Kenagy 1987; Geiser 1990). Because the PUFA linoleic acid (18:2) and linolenic acid (18:3) are essential in the diet of mammals and are required for biosynthesis of most longer PUFA (Lehninger 1982), selection of appropriate food may be important for their seasonal adjustment in physiology.

The present study was undertaken to investigate the influence of unsaturated and saturated lipid diets on shallow, daily torpor in the deer mouse, *Peromyscus maniculatus*. This species shows torpor bouts of several hours and lowers its T_b to about 15 °C (Morhardt 1970; Tannenbaum and Pivorun 1984). The occurrence of torpor, duration of torpor, and reduction in metabolism during torpor were compared between groups of *P. maniculatus* maintained on diets supplemented with sunflower oil and sheep fat. The differences in the fatty acid unsaturation of the experimental diets was mainly due to different concentrations of linoleic acid (18:2). After the organismal experiments were completed, the fatty acid composition of depot fat, leg muscle, brain, and heart mitochondrial membranes of the diet groups were determined and compared with the differences in the pattern of torpor.

Materials and methods

Thirty *P. maniculatus* were trapped in early September in the Cascade Mountains near Fish Lake, Chelan County, Washington, at a mean body mass of 16.7±2.2 g (SD). They were transported to the University of Washington, divided into three groups of matched body mass and sex ratio, and maintained individually in cages at an air temperature (T_a) of 22±1 °C with a 12L:12D photoperiod (light from 06:00 to 18:00 hours PST). Animals were fed ad libitum with water and three diets: (i) Ralston Purina rodent laboratory chow 5001 as "control diet" ($n=10$) containing 30.4% of 18:2; (ii) rodent chow with a 10% addition by weight of sunflower oil as "unsaturated diet" ($n=10$) containing 60.5% of 18:2; and (iii) rodent chow with 10% addition of sheep kidney fat as "saturated diet" ($n=10$) containing 7.3% of 18:2. Diets were prepared as previously described (Geiser 1990) and the total lipid fatty acid composition of the three diets differed substantially (Table 1). Animals of each group were kept on the same diet throughout the experimental period, and physiological measurements began after the animals had been on the diets for 8 weeks.

Two methods were used to determine the frequency, duration and depth of torpor:

Table 1. Percent fatty acid composition of total lipids in the three different diets used in this study

Fatty acid	Saturated fat diet	Control diet	Unsaturated oil diet
10:0	0.36	0.14	–
12:0	0.44	0.13	–
13:0	0.58	1.45	0.44
14:0	4.9	1.84	0.1
15:0	0.8	0.24	–
16:0	22.4	20.57	10.31
16:1n7	1.44	2.41	0.55
17:0	1.56	0.33	0.15
18:0	25.0	7.15	5.35
18:1n9	27.23	26.6	17.4
19:0	–	1.88	–
18:2n6	7.3	30.4	60.5
18:3n3	1.56	2.56	1.1
20:0	0.41	0.29	0.29
20:1n9	0.19	0.74	0.26
21:0	0.22	–	–
20:2n6	–	–	0.17
20:4n6	–	0.21	–
22:0	–	–	0.57
20:5n3	0.14	1.4	0.29
24:0	–	0.3	0.22
22:5n3	0.13	0.29	–
22:6n3	0.26	1.12	0.46
SFA	56.67	34.32	17.43
UFA	38.25	65.73	80.73
PUFA	9.39	35.98	62.52
MUFA	28.86	29.75	18.21
n6	7.30	30.61	60.67
n3	2.09	5.37	1.85

Each number presents the mean of two diet preparations. Standard deviation was less than 0.7% for all fatty acids. SFA = saturated fatty acids, UFA = unsaturated fatty acids, PUFA = polyunsaturated fatty acids, MUFA = monounsaturated fatty acids

(i) The $\dot{V}O_2$ was measured continuously for 23 h between 1500 and 14:00 hours at T_a 17.0±0.5 °C. Food and water were not provided. For these measurements animals were transferred from their holding chamber to a 2-l respirometer in a small temperature-controlled cabinet. Flow rates of dry air were adjusted to 400 ml · min^{-1} with a Brooks thermal mass flow controller. The $\dot{V}O_2$ was measured with an Electrochemistry S-3A oxygen analyzer. The occurrence of torpor (the percentage of torpid individuals observed), the duration of torpor, and daily $\dot{V}O_2$ minimum, determined over an interval of at least 30 min, were derived from these measurements. Animals were considered torpid when $\dot{V}O_2$ fell below 75% of the normothermic resting $\dot{V}O_2$ (Hudson and Scott 1979), but usually $\dot{V}O_2$ fell well below this value. To assess the amout of energy saved by entering into torpor, $\dot{V}O_2$ from entry to post-arousal was integrated and expressed as a percentage of the RMR (=100%); for individuals that did not enter torpor, RMR was used for calculation of diet group means.

(ii) Animals were transferred to a small walk-in, temperature-controlled room and were exposed to L12:D12 and T_a of either 19±1 or 10±1 °C from 15:00 to 14:30 hours. Food and water were not provided. Animals were checked at 09:00 hours because it was observed during measurements of $\dot{V}O_2$ that this was the most likely time to encounter torpor in this species. Torpor was determined by observation of the animals' coordination which is substantially reduced at low T_b. If no clear assessment of torpor could be made from these observations, T_b was measured by insertion of a thermocouple probe 3 cm into the rectum. Animals with $T_b < 31$ °C were considered to be torpid (Hudson and Scott 1979). Body masses were determined at the beginning and at the end of each experiment. Measurements were repeated at $T_a = 19$ °C after animals had recovered for 3 days in their holding room at $T_a = 22$ °C

Table 2. Physiological parameters determined during measurements of $\dot{V}O_2$ in *Peromyscus maniculatus* on three different diets

Group (diet)	n(N)	Occurrence of torpor (%)	Duration of torpor (h)	Min $\dot{V}O_2$ (all) ($ml \cdot g^{-1} \cdot h^{-1}$)	Min $\dot{V}O_2$ ($n=5$) ($ml \cdot g^{-1} \cdot h^{-1}$)	(% RMR)	Body mass (g)
Saturated (1)	9 (14)	43±51	2.25±2.08	2.15±1.31	1.21±0.64	76.2±24.6	18.5±2.7
Control (2)	10 (14)	64±50	3.05±3.04	1.52±1.18	0.55±0.14	66.8±27.3	18.2±1.9
Unsaturated (3)	10 (10)	100± 0	4.50±2.20	0.92±0.79	0.45±0.14	46.8±18.3	17.4±1.5
ANOVA		$P<0.01$	ns	ns	$P<0.025$	$P<0.05$	ns
SNK 1vs2		ns	–	–	$P<0.05$	ns	–
SNK 1vs3		$P<0.05$	–	–	$P<0.05$	$P<0.05$	–
SNK 2vs3		$P<0.05$	–	–	ns	ns	–
t-test 1vs3		$P<0.01$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.01$	ns

Food and water were not available during measurements and T_a was 17 °C. Values represent means±SD. The mean minimum $\dot{V}O_2$ was determined for all individuals and for the five individuals from each group showing the most pronounced reduction in $\dot{V}O_2$. The % RMR is the percentage reduction of the metabolic rate of torpid animals that was integrated from entry into torpor to the end of arousal, in comparison to that of normothermic resting animals at $T_a=17$ °C (RMR=100%); if individuals did not enter torpor RMR was used for calculation of group means. Body masses refer to measurements at the beginning of experiments. *n*, number of individuals: *N*, number of determinations

with surplus food and water. Organismal experiments were carried out between 5 November and 20 January.

Experimental animals were decapitated in the first week of February and depot fat around the kidney, upper hind leg muscle, and brain immediately removed and frozen. Hearts were removed and washed in ice-cold mitochondrial isolation medium consisting of 250 m*M* sucrose, 2 m*M* HEPES, 0.5 m*M* EDTA, and 0.5 mg bovine serum albumin · ml^{-1} adjusted to pH 7.4 with KOH. Hearts were chopped into small pieces with scissors and rinsed repeatedly with isolation medium to remove blood. Heart tissue was then homogenized with a glass-Teflon homogenizer. Heart mitochondria were isolated by differential centrifugation at 2 °C as previously described (Geiser et al. 1984). Mitochondria were washed, resuspended in distilled H_2O, and frozen. All tissues and membranes were frozen at −30 °C.

Total lipids of tissues were extracted and transesterified using the method of Lepage and Roy (1986). The total lipids of heart mitochondria were extracted, after addition of 0.1% butylated hydroxytoluene, using the method of Bligh and Dyer (1959). Phospholipids were separated from the total lipids by thin-layer chromatography on Analtech Silica Gel G plates, which were developed in petroleum ether/acetone (3:1). The phospholipids remaining at the origin were eluted from the silica and transesterified (Lepage and Roy 1986). Fatty acid methyl esters were extracted in hexane, and analyzed by gas-liquid chromatography in a Hewlett-Packard 5790A gas chromatograph fitted with a Supelco SP–2330 capillary column and a flame ionization detector. The percent fatty acid concentration was determined with a Hewlett-Packard 3390A Integrator. All lipid analyses were carried out within 2 months of tissue preparation.

Numeric values are expressed as means±1 standard deviation (SD). One-way analysis of variance (ANOVA) and Student-Newman-Keuls test (SNK) were performed to detect differences in physiology and biochemistry between diet groups. Because the caloric contents of unsaturated and saturated diets were the same, but differed from the control group, a t-test was also used to compare mean physiological values of animals on saturated and unsaturated diet. Percentage values were arcsine-transformed before statistical tests were performed (Sokal and Rohlf 1981).

Results

Dietary lipids and thermal physiology

Daily torpor in *Peromyscus maniculatus* was strongly influenced by the lipid diets. The occurrence of torpor and the minimum metabolic rate during torpor differed among diet groups, and torpor was generally most pronounced in animals on the unsaturated diet (Table 2). Torpor occurred during all measurements of $\dot{V}O_2$ in animals on unsaturated diet, but only during 42% of measurements in animals on saturated diet. The minimum $\dot{V}O_2$ during the 23-h measurements in animals on unsaturated diet was less than 50% of that in animals on saturated diet (Table 2). The drop in $\dot{V}O_2$ showed a large variation among individuals; therefore the $\dot{V}O_2$ of five individuals of each group in which the reduction of $\dot{V}O_2$ was most pronounced were compared. In this instance, the $\dot{V}O_2$ of animals fed saturated fat was almost three times that in animals fed unsaturated oil ($P<0.05$; ANOVA, SNK). The duration of torpor was longer (4.50±2.20 vs 2.25±2.08 h) in animals on the unsaturated diet than in animals on the saturated diet ($P<0.05$; *t*-test). Controls showed intermediate torpor responses between the two experimental diet groups (Table 2). Normothermic T_b and RMR did not differ between diet groups. The mean T_b for normothermic,

Table 3. The occurrence of torpor and the weight loss of *Peromyscus maniculatus* on three different diets

Diet Group	*n*(*N*)	Occurrence of torpor (%)	Weight loss (g)	Body mass (g)
Saturated (1)	10 (19)	26±45	3.90±1.10	17.1±2.3
Control (2)	10 (20)	60±50	2.56±0.60	18.2±1.2
Unsaturated (3)	10 (20)	80±41	2.35±0.62	16.6±1.7
ANOVA		$P<0.01$	$P<0.01$	ns
SNK 1vs2		$P<0.05$	$P<0.05$	–
SNK 1vs3		$P<0.05$	$P<0.05$	–
SNK 2vs3		ns	ns	–
t-test 1vs3		$P<0.001$	$P<0.01$	ns

Food and water were withheld from 1500 to 1430 hours, T_a was 19 °C, and single observations were made at 0900 hours. Values represent means±SD. Body masses refer to measurements at beginning of experiments. Weight loss was measured over the 23.5 h period of food withdrawal

Table 4. Percent fatty acid composition of depot fat total lipids of *P. maniculatus* maintained on different lipid diets

Fatty acid	Saturated fat diet (1) $n=4$	Control diet (2) $n=4$	Unsaturated oil diet (3) $n=4$	ANOVA $P<$	SNK $P<$		
					1vs2	1vs3	2vs3
8:0	tr	tr	tr	–			
10:0	tr	tr	tr	–			
12:0	0.20±0.04	0.11±0.01	tr	0.0001	0.05	0.05	0.05
14:0	3.21±0.14	1.70±0.13	0.67±0.05	0.0001	0.05	0.05	0.05
15:0	0.70±0.04	0.42±0.03	0.21±0.01	0.0001	0.05	0.05	0.05
16:0	20.81±1.95	18.93±0.44	10.68±1.14	0.0001	ns	0.05	0.05
16:1n7	3.13±0.26	5.49±1.47	1.28±0.14	0.0001	0.05	0.05	0.05
17:0	1.36±0.11	0.52±0.04	0.36±0.07	0.0001	0.05	0.05	0.05
18:0	14.42±1.17	4.66±0.55	6.08±1.08	0.0001	0.05	0.05	0.05
18:1n9	40.31±3.36	41.42±3.04	22.28±1.07	0.0001	ns	0.05	0.05
18:2n6	10.64±0.91	23.26±3.18	56.53±2.09	0.0001	0.05	0.05	0.05
18:3n6	–	tr	–	ns			
18:3n3	1.05±0.20	1.02±0.16	0.47±0.07	0.001	ns	0.05	0.05
20:0	0.42±0.05	–	tr	0.0001	0.05	0.05	ns
20:1n9	0.14±0.10	0.38±0.04	0.21±0.03	0.05	0.05	ns	ns
21:0	–	tr	–	ns			
20:2n6	0.25±0.18	0.19±0.03	0.14±0.04	ns			
20:3n6	tr	tr	–	ns			
20:4n6	tr	tr	0.24±0.07	0.07	ns	0.05	ns
22:0	–	–	tr	ns			
20:5n3	–	0.18±0.03	tr	0.0001	0.05	ns	0.05
22:5n3	–	tr	–	ns			
22:6n3	tr	0.24±0.17	0.14±0.07	ns			
SFA	41.17±3.17	26.41±0.68	18.20±2.20	0.0001	0.05	0.05	0.05
UFA	55.70±2.56	72.40±0.45	81.10±2.23	0.0001	0.05	0.05	0.05
PUFA	12.13±1.14	25.12±3.25	57.54±2.09	0.0001	0.05	0.05	0.05
MUFA	43.57±3.58	47.28±3.64	23.76±1.13	0.0001	ns	0.05	0.05
n6	10.98±0.98	23.56±3.07	56.91±2.06	0.0001	0.05	0.05	0.05
n3	1.15±0.28	1.53±0.34	0.63±0.17	0.01	ns	0.05	0.05
n6/n3	9.96±2.26	15.90±3.83	94.67±20.1	0.0001	ns	0.05	0.05

Fatty acid concentrations are shown as the mean percentage±SD of the number of individuals investigated. Trace (tr) is shown where the fatty acid concentration was less than 0.1%. – Fatty acid not present or statistical test not performed

resting individuals from all diet groups at $T_a = 17$ °C was 35.2±0.8 °C ($n=18$), and RMR was 3.67±0.33 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($n=22$). The reduction of metabolic rate during a complete bout of torpor was more pronounced in animals on unsaturated diet than in animals on saturated diet; controls were intermediate (Table 2).

During single observations of torpor (0900 hours) at $T_a = 19$ °C, 26.3% of the animals on saturated diet showed torpor in contrast to 80% of animals on unsaturated diet (Table 3). This difference was accompanied by a lower weight loss during 23.5 h withdrawal of food in animals on unsaturated diet than in animals on saturated diet ($P<0.05$; ANOVA, SNK). The body mass of the three dietary groups was indistinguishable ($P>0.1$; ANOVA). At $T_a = 10$ °C, the differences in the occurrence of torpor between diet groups were similar to those at $T_a = 19$ °C. Torpor was observed in 70% of animals on unsaturated diet, but in only 30% of animals on saturated diet. At both T_as, control animals showed intermediate occurrence of torpor and weight loss.

Diet-induced differences in body fatty acid composition

The differences in fatty acid composition among diet groups were distinct, but not all tissues and membranes of *P. maniculatus* responded in the same way. Pronounced differences among animals on the three diets were observed in the total lipid fatty acid composition of depot fat (Table 4). Of the 21 fatty acids tested, only 7 did not show significant differences among the diet groups. One of these 21 fatty acids (20:4n6) was close to being significant ($P=0.07$; ANOVA); however, SNK revealed compositional differences ($P<0.05$) between animals on saturated and unsaturated diets (Table 4). The sum of SFA, UFA, MUFA, PUFA, n6, and n3, and the n6/n3 fatty acid ratio also differed among diet groups. The fatty acid composition of depot fat of *P. maniculatus* (Table 4) strongly reflected that of the diet (Table 1), although the differences were less pronounced than differences between the diets. Animals on the sheep-fat diet contained more UFA in their depot fat than the diet; animals on control diet and unsaturated diets contained less UFA than the diet.

Diet-induced changes in the total lipid fatty acid composition of leg muscle were also very pronounced (Table 5). Of the 20 fatty acids tested only 3 did not differ among diet groups. One of these 20 fatty acids (20:3n6) was close to being significant ($P=0.08$; ANOVA), but significant differences between animals on saturated and unsaturated diet were revealed by SNK ($P<0.05$). The sum of SFA, UFA, PUFA, and n6, and the n6/n3 fatty acid ratio also differed among diet groups.

Brain total lipid fatty acids showed much smaller differences among diet groups than those from depot fat and leg muscle (Table 6). Nevertheless, of the 23 fatty

Table 5. Percent fatty acid composition of leg muscle total lipids of *P. maniculatus* maintained on different lipid diets

Fatty acid	Saturated fat diet (1) $n=4$	Control diet (2) $n=4$	Unsaturated oil diet (3) $n=4$	ANOVA P<	SNK $P<$		
					1vs2	1vs3	2vs3
12:0	0.12±0.02	tr	tr	0.01	0.05	0.05	ns
14:0	1.82±0.26	1.07±0.33	0.56±0.14	0.001	0.05	0.05	0.05
15:0	0.46±0.07	0.28±0.09	0.20±0.07	0.005	0.05	0.05	ns
16:0	20.23±1.39	20.04±1.14	15.45±1.76	0.005	ns	0.05	0.05
16:1n7	3.67±1.29	4.68±1.73	1.84±0.34	0.05	ns	0.05	0.05
17:0	0.99±0.04	0.36±0.23	0.42±0.11	0.05	0.05	0.05	ns
18:0	12.98±1.64	9.43±2.76	10.19±2.75	ns			
18:1n9	24.00±2.71	23.19±7.34	15.32±1.64	0.05	ns	0.05	0.05
18:2n6	11.72±1.27	16.19±2.97	37.04±7.76	0.001	ns	0.05	0.05
18:3n3	0.76±0.09	0.59±0.22	tr	0.01	ns	0.05	0.05
20:0	0.23±0.12	–	–	0.0001	0.05	0.05	ns
20:1n9	–	0.23±0.06	tr	0.001	0.05	ns	0.05
20:2n6	0.15±0.17	tr	tr	ns			
20:3n6	0.20±0.16	tr	–	0.08	ns	0.05	ns
20:4n6	2.03±0.50	–	–	0.0001	0.05	0.05	ns
20:3n3	–	2.65±1.23	3.34±1.01	0.0001	0.05	0.05	ns
20:5n3	0.43±0.29	0.70±0.54	tr	0.05	ns	ns	0.05
22:4n6	–	tr	0.32±0.04	0.0001	ns	0.05	0.05
22:5n3	1.72±0.26	1.78±0.67	0.72±0.24	0.05	ns	0.05	0.05
22:6n3	15.04±2.65	16.75±7.14	13.23±4.72	ns			
SFA	36.85±1.82	31.24±3.00	26.81±4.47	0.01	0.05	0.05	ns
UFA	59.70±1.87	66.95±3.12	72.10±4.48	0.01	0.05	0.05	ns
PUFA	32.03±3.74	38.84±5.74	54.90±3.29	0.0001	ns	0.05	0.05
n6	14.09±1.11	16.36±3.15	37.45±7.94	0.0001	ns	0.05	0.05
n3	17.95±2.98	22.25±8.74	17.44±5.59	ns			
n6/n3	0.79±0.11	0.87±0.74	2.46±1.36	0.05	ns	0.05	0.05

Fatty acid concentrations are shown as the mean percentage±SD of the number of individuals investigated. Trace (tr) is shown where the fatty acid concentration was less than 0.1%. – Fatty acid not present or statistical test not performed

Table 6. Percent fatty acid composition of brain total lipids of *P. maniculatus* maintained on different lipid diets

Fatty acid	Saturated fat diet (1) $n=4$	Control diet (2) $n=4$	Unsaturated oil diet (3) $n=4$	ANOVA P<	SNK $P<$		
					1vs2	1vs3	2vs3
13:0	tr	tr	–	0.0001	0.05	0.05	0.05
14:0	0.14±0.14	0.12±0.02	0.12±0.02	ns			
16:0	15.91±0.82	17.01±1.37	16.53±0.37	ns			
16:1n7	0.52±0.11	0.57±0.09	0.55±0.09	ns			
17:0	0.27±0.01	0.20±0.02	0.20±0.02	0.01	0.05	0.05	ns
18:0	17.88±1.25	18.18±0.72	18.33±0.32	ns			
18:1	18.93±1.41	18.79±0.61	18.60±0.74	ns			
18:2n6	0.93±0.09	0.95±0.14	1.70±0.21	0.0001	ns	0.05	0.05
20:0	0.53±0.11	0.48±0.11	0.45±0.07	ns			
20:1n9	2.65±0.57	2.13±0.46	2.15±0.41	ns			
21:0	0.24±0.03	0.31±0.05	0.24±0.09	ns			
20:2n6	0.19±0.03	tr	0.32±0.02	0.01	0.05	ns	0.05
20:3n6	0.42±0.04	0.37±0.06	0.39±0.05	ns			
20:4n6	8.32±0.81	8.68±0.48	9.33±0.34	ns			
22:0	0.56±0.21	0.43±0.15	0.48±0.10	ns			
22:1n9	0.28±0.06	0.20±0.11	0.21±0.06	ns			
20:5n3	–	tr	–	ns			
23:0	–	–	0.27±0.07	0.0001	ns	0.05	0.05
22:4n6	2.40±0.29	2.31±0.14	2.71±0.14	0.06	ns	0.05	ns
24:0	1.10±0.19	0.62±0.29	0.39±0.13	0.005	0.05	0.05	ns
24:1n9	2.19±0.70	1.61±0.72	1.72±0.45	ns			
22:5n3	0.38±0.08	0.45±0.07	0.21±0.06	0.005	ns	0.05	0.05
22:6n3	16.41±1.15	17.50±1.29	16.10±0.83	ns			
SFA	36.72±1.61	37.52±1.38	37.06±0.43	ns			
UFA	53.51±0.72	53.71±0.79	53.95±0.48	ns			
PUFA	29.02±2.11	30.43±1.38	30.72±1.36	ns			
n6	12.24±1.01	12.42±0.33	14.46±0.59	0.005	ns	0.05	0.05
n3	16.78±1.14	17.87±1.41	16.28±0.82	ns			
n6/n3	0.73±0.02	0.70±0.05	0.88±0.04	0.01	ns	0.05	0.05

Fatty acid concentrations are shown as the mean percentage±SD of the number of individuals investigated. Trace (tr) is shown where the fatty acid concentration was less than 0.1%. – Fatty acid not present or statistical test not performed

Table 7. Percent fatty acid composition of heart mitochondrial phospholipids of *P. maniculatus* maintained on different lipid diets

Fatty acid	Saturated fat diet (1) $n=4$	Control diet (2) $n=4$	Unsaturated oil diet (3) $n=4$	ANOVA $P<$	SNK $P<$		
					1vs2	1vs3	2vs3
10:0	tr	tr	–	ns			
12:0	tr	–	–	ns			
15:0	0.08±0.02	0.08±0.05	tr	0.07	ns	0.05	ns
15:1n9	0.77±0.17	1.20±0.25	0.81±0.23	0.05	0.05	ns	0.05
16:0	11.75±0.17	13.24±0.30	12.64±1.47	ns	0.05	ns	ns
16:1n7	0.23±0.09	0.35±0.07	0.13±0.15	0.08	ns	ns	0.05
17:0	0.80±0.02	0.49±0.03	0.52±0.10	0.0001	0.05	0.05	ns
17:1n9	1.20±0.25	0.95±0.14	1.04±0.15	ns			
18:0	21.42±0.80	18.40±0.57	22.07±2.25	0.01	0.05	ns	0.05
18:1	7.18±1.01	6.95±0.07	4.63±0.51	0.005	ns	0.05	0.05
18:2n6	13.11±2.46	16.32±1.01	20.96±1.83	0.001	0.05	0.05	0.05
18:3n3	0.45±0.19	0.33±0.11	tr	0.0001	ns	0.05	0.05
20:2n6	tr	0.19±0.22	0.19±0.23	ns			
20:3n6	0.13±0.15	0.14±0.13	tr	ns			
20:4n6	2.83±0.47	2.94±0.97	4.36±1.14	ns			
20:5n3	0.48±0.07	0.59±0.21	tr	0.0001	ns	0.05	0.05
22:4n6	tr	tr	0.95±0.37	0.0001	ns	0.05	0.05
24:0	0.52±0.14	0.49±0.09	1.19±0.81	ns			
22:5n3	2.79±0.09	2.27±0.52	1.34±0.16	0.001	ns	0.05	0.05
22:6n3	35.50±2.00	34.87±1.65	29.01±4.39	0.05	ns	0.05	0.05
SFA	34.63±0.67	32.60±0.85	36.43±3.57	ns			
UFA	64.26±1.28	67.09±0.81	63.42±3.41	ns			
PUFA	55.31±2.48	57.66±1.14	56.85±2.62	ns			
MUFA	9.38±1.36	9.45±1.03	6.60±0.99	0.01	ns	0.05	0.05
n6	16.09±2.73	19.59±1.32	26.45±3.15	0.001	ns	0.05	0.05
n3	39.21±2.14	38.06±0.99	30.34±4.48	0.01	ns	0.05	0.05
n6/n3	0.41±0.08	0.52±0.05	1.17±0.27	0.001	ns	0.05	0.05

Fatty acid concentrations are shown as the mean percentage±SD of the number of individuals investigated. Trace (tr) is shown where the fatty acid concentration was less than 0.1%. – Fatty acid not present or statistical test not performed

acids tested, 8 showed significant differences among diet groups. The fatty acid 22:4n6 differed only between animals on saturated and unsaturated diets ($P<0.05$; SNK). The sum of SFA, UFA, and PUFA did not differ among diet groups; however, the n6 fatty acids and the n6/n3 fatty acid ratio showed distinct differences.

The phospholipid fatty acids of heart mitochondria also differed substantially among diet groups (Table 7). Of 20 fatty acids tested, 12 showed significant differences; 2 of these 12 (15:0 and 16:1n7) were only distinguishable by SNK. Furthermore, the sum of MUFA, n6, n3, and the ratio of n6/n3 fatty acids differed significantly among diets. The experimental diets did not induce a difference in the sum of the SFA, UFA, and PUFA of heart mitochondrial membranes, but rather induced in a shift from n6 to n3 fatty acids. Animals on the unsaturated diet showed an increase in n6 fatty acid content, whereas animals on the saturated diet contained more n3 fatty acids than animals on the unsaturated diet. A high concentration (>29%) of docosahexaenoic acid (22:6n3) was observed in all diet groups.

Discussion

The present study shows that the composition of dietary lipids has a pronounced effect on the pattern of torpor in *P. maniculatus*. Some of these changes in torpor patterns may be explained by the compositional changes of fatty acids in tissues and membranes that were induced by the lipid diets.

Torpor was most pronounced in animals on the unsaturated diet. Torpor was more frequent, torpor bouts lasted about twice as long, metabolic rate was lower, and the loss of body mass in animals without food and water was lower in animals on unsaturated diet than in those on saturated diet. The more pronounced torpor in animals on unsaturated diet would probably increase their chance of survival during cold exposure and food shortage in the wild. Therefore, selection of diets with high amounts of UFA (e.g., oily seeds) may be important for winter preparation in this species. It is known that seed intake increases in autumn in *P. maniculatus* (Howard 1961), and a seasonal change in diet may play an important role in the seasonal adjustment of physiology that occurs in this and many other heterothermic species (Lynch et al. 1978; Heldmaier and Steinlechner 1981; Geiser and Baudinette 1987; Fowler and Racey 1990).

The consistent differences in the physiological variables of torpor among the diet groups suggest that they are interrelated. A more frequent occurrence of torpor in animals receiving an unsaturated diet perhaps reflects the greater reduction of T_b and $\dot{V}O_2$ which enhance energy savings and make the use of torpor more "profitable" to the individual. Energy savings by deeper, longer, and more frequent torpor in animals on unsaturated diet are substantially greater than in animals on the saturated diet (Table 3). The lower minimum $\dot{V}O_2$ of torpid animals on the unsaturated diet is most probably due to a greater drop of T_b than in animals on saturated diet. The longer torpor bouts in animals fed unsaturated oil probably result from low T_b and $\dot{V}O_2$ since these are inversely

related to the duration of torpor bouts (Geiser et al. 1990).

Diet-induced changes in torpor patterns observed here are in agreement with previous observations on hibernating chipmunks, *Eutamias amoenus* (Geiser and Kenagy 1987). *E. amoenus* showed longer torpor bouts, lower T_bs, and lower $\dot{V}O_2$ on unsaturated than on saturated diet (Geiser and Kenagy 1987). The similar diet-induced physiological response in the daily heterotherm *P. maniculatus* and the deep hibernator *E. amoenus* suggest that at least some of the physiological adaptations underlying daily torpor and hibernation are similar. Although T_b during daily torpor in *P. maniculatus* falls by only about 20 °C, such a drop would be fatal for many homeothermic species, and adaptations for normal physiological function at these T_bs are required. Since increases in PUFA in *P. manicultus* and *E. amoenus* were correlated with enhanced torpor, it is likely that the lipid composition of tissues and membranes plays an important part in surviving daily torpor and hibernation.

Diet-induced changes in fatty acid composition were observed in all tissue and membranes; however, the extent of these changes differed substantially among tissues and membranes. Changes of tissue fatty acid composition were most pronounced in the depot fat and muscle tissue, were intermediate in heart mitochondrial membranes, and the smallest changes were observed in brain tissue. The lipid composition of membranes and tissue is genetically controlled (Lehninger 1982). The different changes in various tissues suggests that some tissues (e.g., brain) are more conservative in maintaining their composition than others (e.g., depot fat). Furthermore, analysis of total brain tissue may not reveal compositional changes in different membrane fractions (Hazel 1988). Some tissues may require less compositional changes than others for normal function at low T_b. For example, the heart must maintain its physical activity during hibernation, and heart mitochondria may therefore require substantial compositional changes. Despite the differences between tissues in their response to dietary lipid treatment, it appears that lipid diet had a much stronger effect on the composition of tissues and membranes of both the daily heterotherm *P. maniculatus* and the hibernator *E. amoenus* than on non-hibernators (White 1973; McMurchie 1988; Geiser 1990; present study). This difference in diet-induced changes in fatty acid composition of heterotherms and non-hibernators appears to reflect their mode of thermoregulation. Non-hibernators, with their constant high T_b, do not require adaptations for function at low T_b and diet-induced changes in body lipid composition may be undesirable. In contrast, hibernators and daily heterotherms must survive pronounced drops in T_b and may profit from incorporating large amounts of UFA from their diet into tissues and membranes.

The correlation between the pattern of torpor and the fatty acid composition of tissues and membranes raises the question of functional linkage. Diet-induced changes in membrane composition affect the activity of membrane-associated enzymes (McMurchie 1988), and it seems reasonable to assume that cellular metabolism could be directly affected by compositional changes. This may explain differences in the metabolic rate of the diet groups. However, it is likely that membrane compostion is not the only factor that contributes to the physiological differences among the diet groups. Since the fatty acids of depot fat changed so substantially, it is possible that the composition of the depot fat directly affects metabolism. It has been shown that unsaturated fatty acids are potent inhibitors of thyroid hormone binding to isolated rat liver cells, and it is possible that this lowers cellular metabolism and the metabolic rate of the animal (Wiersinga et al. 1988).

This study shows that the composition of dietary fatty acids influences the pattern of daily torpor. Hence dietary lipids may have an important role in nutritional ecology and the seasonal adjustment of thermal physiology in heterothermic mammals.

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References

Aloia RC (1979) Membrane structure and function during hibernation. Chem Zool 11:49–75

Aloia RC, Pengelley ET (1979) Lipid composition of cellular membranes of hibernating mammals. Chem Zool 11:1–48

Aloia RC (1988) Lipid, fluidity and functional studies of membranes of hibernating mammals. In: Aloia RC, Curtain CC, Gordon LM (eds) Physiological regulation of membrane fluidity. Liss, New York, pp 1–39

Aloia RC, Raison JK (1989) Membrane function in mammalian hibernation. Biochim Biophys Acta 988:123–146

Augee ML, Pehowich DJ, Raison JK, Wang LCH (1984) Seasonal and temperature-related changes in mitochondrial membranes associated with torpor in the mammalian hibernator *Spermophilus richardsonii*. Biochim Biophys Acta 776:27–36

Barnes BM (1989) Freeze-avoidance in a mammal: body temperatures below 0 °C in an Arctic hibernator. Science 244:1593–1595

Bligh EC, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Cossins AR, Friedlander MJ, Prosser CL (1977) Correlations between behavioral temperature adaptations of goldfish and the viscosity and fatty acid composition of their synaptic membranes. J Comp Physiol 120:109–121

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman and Hall, London

Fawcett DW, Lyman CP (1954) The effect of low environmental temperature on the composition of depot fat in relation to hibernation. J Physiol (Lond) 126:235–247

Florant GL, Nuttle LC, Mullinex DE, Rintoul DA (1990) Plasma and white adipose tissue lipid composition in marmots. Am J Physiol 258:R1123–R1131

Fowler PA, Racey PA (1990) Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog *Erinaceus europaeus*. J Comp Physiol B 160:299–307

Geiser F (1990) Influence of polyunsaturated and saturated dietary lipids on adipose tissue, brain, and mitochondrial membrane

fatty acid composition of a mammalian hibernator. Biochim Biophys Acta 1046:159–166
Geiser F, Baudinette RV (1987) Seasonality of daily torpor and thermoregulation in three dasyurid marsupials. J Comp Physiol B 157:335–344
Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. Am J Physiol 252:R897–R901
Geiser F, Augee ML, Raison JK (1984) Thermal response of liver mitochondrial membranes of the heterothermic bat *Miniopterus schreibersii* in summer and winter. J Thermal Biol 9:183–188
Geiser F, Hiebert S, Kenagy GJ (1990) Torpor bout duration during the hibernation season of two sciurid rodents: Interrelations with temperature and metabolism. Physiol Zool 63:489–503
Goldman SS (1975) Cold resistance of the brain during hibernation. III Evidence for a lipid adaptation. Am J Physiol 228:834–839
Hazel JR (1984) Effects of temperature on the structure and metabolism of cell membranes in fish. Am J Physiol 246:R460–R470
Hazel JR (1988) Homeoviscous adaptation in animal cell membranes. In: Aloia RC, Curtain CC, Gordon LM (eds) Physiological regulation of membrane fluidity. Liss, New York, pp 149–188
Heldmaier G, Steinlechner S (1981) Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus*. Oecologia 48:265–270
Howard WE (1961) Seeds stored by prairie deer mice. J Mammal 42:260–263
Hudson JW (1973) Torpidity in mammals. In: Whitow GC (ed) Comparative physiology of thermoregulation. Academic Press, New York, pp 97–165
Hudson JW, Scott JM (1979) Daily torpor in the laboratory mouse *Mus musculus* var. albino. Physiol Zool 52:205–218
Lehninger AL (1982) Principles of biochemistry. Worth, New York
Lepage G, Roy CC (1986) Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114–120
Lynch GR, Vogt FD, Smith HR (1978) Seasonal study of spontaneous daily torpor in the white-footed mouse *Peromyscus leucopus*. Physiol Zool 51:289–297
McMurchie EJ (1988) Dietary lipids and the regulation of membrane fluidity and function. In: Aloia RC, Curtain CC, Gordon LM (eds) Physiological regulation of membrane fluidity. Liss, New York, pp 189–237
Montaudon D, Robert J, Canguilhem B (1986) Fluorescence anisotropy of kidney lipids and membranes of a hibernating mammal. Cryobiology 23:177–183
Morhardt JE (1970) Body temperatures of white-footed mice (*Peromyscus* spp.) during daily torpor. Comp Biochem Physiol 33:423–439
Raison JK (1984) On the need to distinguish between the essential and consequential changes in membrane lipids during hibernation. In: Hales JRS (ed) Thermal physiology. Raven Press, New York, pp 447–452
Raison JK, Augee ML, Aloia RC (1988) Mitochondrial membrane transitions in heart and other organs of a hibernator. Am J Physiol 254:E378–E383
Sokal RR, Rohlf FJ (1981) Biometry. Freeman, New York
Tannenbaum MG, Pivorun EM (1984) Differences in daily torpor patterns among three southeastern species of *Peromyscus*. J Comp Physiol B 154:233–236
Wang LCH (1989) Ecological, physiological, and biochemical aspects of torpor in mammals and birds. In: Wang LCH (ed) Animal adaptation to cold. Springer, Berlin, Heidelberg, pp 361–401
White DA (1973) The phospholipid composition of mammalian tissues. Biochim Biophys Acta Library 3:441–483
Wiersinga WM, Chopra IJ, Chua Teco GN (1988) Inhibition of nuclear T3 binding by fatty acids. Metabolism 37:996–1002