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Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator

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GEISER, FRITZ, AND G. J. KENAGY. *Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator*. Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21): R897-R901, 1987.—Membrane lipids of vertebrate animals that tolerate cold are high in polyunsaturated fatty acids. Because the lipid composition of cellular membranes in mammals can be experimentally altered by diet, we investigated whether a diet rich in polyunsaturated fatty acids would lengthen bouts of torpor and reduce the minimum body temperature in hibernating chipmunks (*Eutamias amoenus*) compared with a diet rich in saturated fatty acids. Animals on the highly unsaturated diet showed significantly longer bouts of torpor, lower minimum body temperatures, and lower metabolic rates than those on a saturated diet. Animals on a control diet were intermediate. These dietary adjustments apparently influence the control of body temperature by the central nervous system, which results in a modification of the pattern of torpor. The observations also suggest a role of nutritional ecology in hibernation.

rodent; *Eutamias amoenus*; unsaturated and saturated fats; metabolic rate; minimum body temperature; temperature regulation

HIBERNATING MAMMALS can withstand body temperatures (T_b) close to 0°C (20). Species that show this natural tolerance to hypothermia differ in the functional properties of their cell membranes from the usual mammalian pattern (11, 15, 31), which appears to result from the composition of their membrane phospholipids and fatty acids. For example, the phospholipids of cardiac muscle and mitochondrial membranes show a higher proportion of unsaturated fatty acids in hibernating (heterothermic) than in nonhibernating (homeothermic) species (1, 7, 23). Similarly, the membrane phospholipids of fish (ectotherms) that live in cold environments contain more unsaturated fatty acids than those from warm environments, and cold acclimation within a species increases the proportion of unsaturated fatty acids (3, 13, 30). These observations indicate that a high concentration of unsaturated fatty acids in membrane molecules is required for function at low T_b .

Polyunsaturated fatty acids are essential in the diet of mammals (18). Experimental manipulation of dietary lipids in hibernating and nonhibernating rodents alters the composition of body fats (5) and cell membranes, and this is further associated with changes in thermal properties of the membrane and activity of membrane-bound enzymes (21, 22, 24). Although such diet-induced

alterations of tissues and membrane lipids have been reported in a considerable number of studies (26), we are not aware of any previous report that dietary alteration of membrane lipids influences thermoregulation of an animal.

Because membrane lipids rich in unsaturated fatty acids are associated with function at low T_b and because membrane composition can be selectively altered by dietary lipids, we have tested the hypothesis that a diet enriched with polyunsaturated lipids should enhance the state of hibernation. We studied a small (50-g) hibernator, the yellow pine chipmunk, *Eutamias amoenus*, which arouses from torpor at 5- to 7-day intervals during its 4-mo hibernation season (17). Because the central nervous system maintains control of T_b during torpor (14), we were particularly interested to see whether chipmunks on an unsaturated diet would regulate T_b during torpor at a lower "set point" and have longer bouts of torpor than those on a saturated diet.

MATERIALS AND METHODS

Wild chipmunks were caught during September in the Cascade Mountains near Lake Wenatchee, Chelan County, WA, at a mean mass of 48.2 g, ~8 wk before they would naturally begin hibernating. They were transported to the University of Washington, divided into three groups of matched body mass and sex ratio, and kept at 22°C with a 12-h light-dark photoperiod. They were fed ad libitum throughout the experiment with water and 1) a control diet of Ralston Purina rodent laboratory chow 5001 containing 23% crude protein, 4.5% crude fat (mixed animal and plant fat preserved with butylated hydroxytoluene), 6% crude fiber, 8% ash, 2.5% added minerals, and vitamins ($n = 6$), 2) an "unsaturated" diet of rodent chow with a 10% addition by weight of sunflower oil ($n = 8$), or 3) a "saturated" diet of rodent chow with 10% sheep kidney fat ($n = 8$). The percent fatty acid composition of total lipids in the control diet was 10:0, 0.14%; 12:0, 0.13%; 13:0, 1.45%; 14:0, 1.84%; 15:0, 0.24%; 16:0, 20.6%; 16:1 ω 7, 2.41%; 17:0, 0.33%; 18:0, 7.15%; 18:1 ω 9, 26.6%; 19:0, 1.88%; 18:2 ω 6, 30.4%; 18:3 ω 3, 2.56%; 20:0, 0.29%; 20:1 ω 9, 0.74%; 20:4 ω 6, 0.21%; 20:5 ω 3, 1.47%; 24:0, 0.3%; 22:5 ω 3, 0.29%; and 22:6 ω 3, 1.12%. The lipids were extracted and transesterified using the method of Lepage and Roy (19); the fatty acid composition was determined with a Hewlett-Packard 5790 gas-liquid chromatograph. Sunflower oil is rich in polyunsaturated fatty acids (linoleic acid 66% of all

fatty acids) and <10% of all fatty acids are saturated; sheep kidney fat mainly contains saturated fatty acids (palmitic and stearic acid 55% of total fatty acids) and is only 4% polyunsaturated fatty acids (2). The energy content of the control diet was 18 kJ/g and that of the lipid diets 20 kJ/g. When animals reached a peak body mass (mean 66.4 g), after 8 wk, they were transferred to a walk-in controlled-environment chamber held at 10°C to promote hibernation.

We held the animals successively at air temperature (T_a) = 10°C from 20 November to 5 December, 5°C from 6 December to 10 January, and at 0.5°C from 11 January to 4 February. The length of the torpor bouts was determined by daily observations at 1000 and 1700 h of the displacement of fine sand from each animal's back that occurs when it aroused.

We examined thermoregulatory patterns of torpid individuals by measuring O_2 consumption in a respirometer that was slowly cooled (<0.02°C/min) until an animal increased O_2 consumption to avoid further decline in T_b or to initiate arousal. For these experiments torpid individuals were transferred from the holding chamber at T_a = 5°C, from 7 December to 10 January to a respirometry vessel in a small temperature-controlled ($\pm 0.2^\circ\text{C}$) cabinet at the same T_a . Because the sensitivity to disturbance increases toward the end of torpor bouts, cooling experiments were performed on day 2 or 3 of a bout. After transfer to the respirometer, T_a was not decreased until O_2 consumption ($\dot{V}O_2$) stabilized. To maximize heat exchange with the air during the cooling experiments, the animal was suspended in a plastic-mesh hammock in the 2-liter respirometer. Flow rate was 70 ml/min. We define the minimum rate of O_2 consumption prior to arousal as $\dot{V}O_{2\min}$ and the corresponding air temperature as $T_{a,\min}$. We designate the T_b that we measured by interrupting the experiment to obtain a rectal measurement with a thermocouple probe as $T_{b,\min}$. Because cooling of the animals always resulted in an irreversible arousal, even when T_a was increased after the initial thermoregulatory response of the torpid animal, rectal temperatures were determined (30-mm insertion of a 36-gauge thermocouple) within 15 min of the onset of arousal and immediately after removal of each animal from the respirometer. Determination of the thermostatic increase in $\dot{V}O_2$ during torpor was further facilitated by the conspicuous increase in ventilation. We estimate that T_b at the time of measurement would have increased by <0.2°C, because the total increase in $\dot{V}O_2$ was equivalent to <1 J/g. O_2 consumption was monitored continuously with an Applied Electrochemistry S-3A O_2 analyzer. T_a was measured continuously by thermocouple and recorded along with $\dot{V}O_2$ on a Leeds and Northrup Speedomax 250. Point readings of T_a were taken using a digital thermometer. The digital thermometer was checked daily for calibration to the nearest 0.1°C with a glass Bureau of Standards thermometer.

RESULTS

Chipmunks on the unsaturated diet had longer bouts of torpor and lower body temperatures compared with animals on the saturated diet. In addition, the tendency

to enter torpor was greater in chipmunks on the unsaturated diet; of all 22 experimental animals two, both maintained on the saturated diet, never became torpid.

Thermoregulation and heat production (measured indirectly as $\dot{V}O_2$) by torpid chipmunks differed significantly in relation to diet. Figure 1 demonstrates our experimental determination of $T_{a,\min}$, $T_{b,\min}$, and $\dot{V}O_{2\min}$ measured by slowly cooling torpid animals until they showed an increase in $\dot{V}O_2$. In the typical case illustrated in Fig. 1, the saturated animal increased $\dot{V}O_2$ at T_a = 1.1°C (T_b = 2.5°C), but the unsaturated animal did not decrease $\dot{V}O_2$ until T_a declined to -1.7°C (T_b = 0.1°C). For all animals measured, mean $T_{a,\min}$, $T_{b,\min}$, $\dot{V}O_{2\min}$, and $\dot{V}O_2$ at T_a = 2.0°C were significantly lower in the chipmunks on an unsaturated diet than in those on the saturated diet with controls intermediate (Table 1). The lowest measured $T_{b,\min}$ (-0.2°C) occurred in an individual on the unsaturated diet. It is remarkable that when O_2 consumption is compared at the same temperature (2°C), the metabolic rate of torpid animals on the highly saturated diet was nearly twice that of animals on the unsaturated diet (Table 1).

Duration of torpor was strongly dependent on T_a in all three groups, but at each temperature torpor was longest in the unsaturated-diet animals, shortest in the saturated animals, and intermediate in controls (Fig. 2). The decrease in T_a from 10 to 5°C resulted in a parallel increase in duration of torpor in all three groups. We further reduced T_a to 0.5°C to determine how a T_a below $T_{a,\min}$ for the saturated animals but above the $T_{a,\min}$ for the others would influence the duration of torpor bouts in the three groups. This reduction in T_a led to further increases in torpor duration of the unsaturated and control animals. However, the saturated animals showed a reversal of this pattern and a great decrease in the mean duration of torpor to ~1.5 days (34 h) compared with 7.5 days (181 h) in the unsaturated group (Fig. 1). Our choice of a final experimental T_a that was below the $T_{a,\min}$ of only the saturated animals resulted in an amplification of our findings of diet-induced changes in the interrelationship of thermoregulation and duration of torpor.

DISCUSSION

Our experiment clearly demonstrates a profound effect of dietary lipids on the duration of torpor and the minimum T_b in a hibernating rodent. These findings indicate that the previously reported diet-induced alterations in cell membrane composition (21, 22, 24, 26) are accompanied by changes in whole-animal thermoregulation. Our observation of an altered thermoregulatory response induced by dietary lipids is consistent with previous correlations of high levels of membrane lipid unsaturation with ability to function at low temperatures (1, 3, 30). However, our experiment provides the first direct conclusion, involving an intraspecific experimental manipulation, that membrane fatty acids influence regulation of T_b in a mammal at low temperatures. The shift in the set point for regulation of T_b during torpor by dietary lipids is important because it suggests that both cellular heat production and whole-animal temperature regulation are influenced by the composition and thus

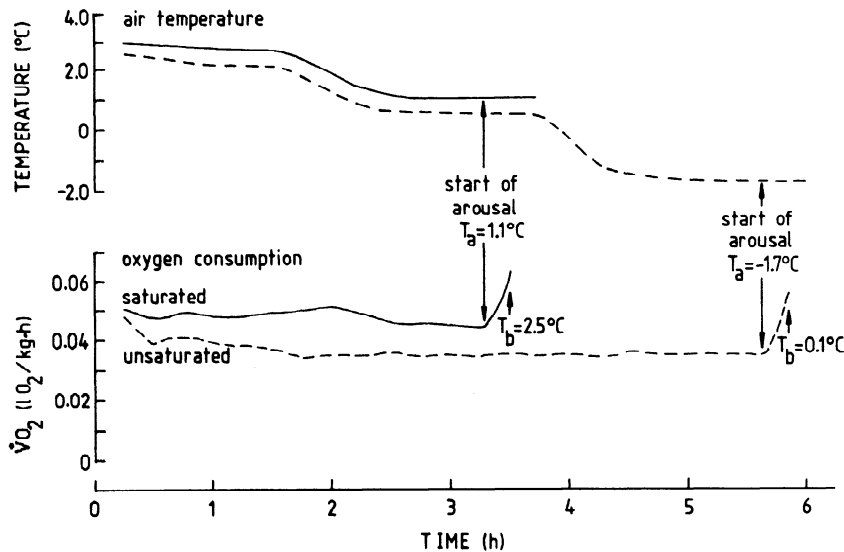


FIG. 1. Simultaneous records of air temperature (T_a) and O_2 consumption ($\dot{V}O_2$) in 2 torpid chipmunks that were slowly cooled by experimental decrease of T_a . Cooling was continued until each animal initiated arousal, as indicated by abrupt increase in O_2 consumption and ventilation; on initial increase animals were removed and rectal temperature was measured. Animal on saturated diet (solid lines) initiated arousal at $T_a = 1.1^\circ\text{C}$ and had body temperature (T_b) of 2.5°C on removal. Animal on unsaturated diet (broken lines) did not initiate arousal until T_a had dropped to -1.7°C and on removal it had T_b of only 0.1°C .

TABLE 1. Body and air temperatures, O_2 consumption, and body mass of torpid chipmunks maintained on 3 different diets

Diet	$T_{b,\min}$, $^\circ\text{C}$	$T_{a,\min}$, $^\circ\text{C}$	$l\ O_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$		Body Mass, g
			$\dot{V}O_{2\min}$	$\dot{V}O_2$ at 2.0°C	
Unsaturated	0.58 ± 0.62 (5)	-0.96 ± 0.37 (6)	0.029 ± 0.010 (6)	0.034 ± 0.009 (6)	55.9 ± 5.8
Control	1.21 ± 0.83 (5)	0.02 ± 0.86 (6)	0.042 ± 0.011 (5)	0.047 ± 0.013 (5)	59.8 ± 8.4
Saturated	2.22 ± 0.65 (5)	1.03 ± 0.25 (6)	0.052 ± 0.011 (6)	0.064 ± 0.018 (6)	55.7 ± 1.9

Values are means \pm SD for no. of individuals in parentheses. Each individual was measured once or twice. Values of body mass correspond to O_2 consumption ($\dot{V}O_2$) measurements. Statistical differences were tested using one-way analysis of variance. Minimum body temperature ($T_{b,\min}$): $F = 6.80$, $df = 2, 12$, $P < 0.025$. Air temperature when $\dot{V}O_2$ increased indicating onset of arousal ($T_{a,\min}$): $F = 19.16$, $df = 2, 15$, $P < 0.001$. Minimum rate of O_2 consumption ($\dot{V}O_{2\min}$): $F = 6.77$, $df = 2, 14$, $P < 0.01$. $\dot{V}O_2$ at $T_a 2.0^\circ\text{C}$: $F = 7.09$, $df = 2, 14$, $P < 0.01$. Body mass, $F = 0.83$, $df = 2, 14$, $P > 0.25$. All pairs of all 4 variables other than body mass differed significantly (Student-Newman-Keuls test; $P < 0.05$) except for $T_{b,\min}$ of control and unsaturated animals and $\dot{V}O_2$ of control and saturated animals and control and unsaturated animals.

functional properties of cellular membranes. Although there is presently no basic understanding of a molecular mechanism for thermoregulation (4, 27), correlations between the minimum T_b during torpor and the thermal response of membrane function or permeability suggest that thermoregulation is physiologically linked to membrane properties (7, 10, 25).

When animals were transferred from $T_a 5.0$ to 0.5°C , the duration of torpor decreased in animals on the saturated diet, whereas it increased in controls and saturated animals. This distinctive reversal of torpor duration can be explained by the $T_{a,\min}$ of the saturated group (Table 1), which, unlike that of the two other groups, was greater than the experimental T_a of 0.5°C . The intraspecific differences we report in thermal sensitivity of torpor duration in relation to diet represent the first demonstration of such variation to our knowledge. An increase in torpor duration with decrease in T_a is expected for hibernators in general (6). A reversal of this relationship has also been documented for species in situations where T_a falls below $T_{b,\min}$, in which case the animal increases its metabolic rate for thermostatic heat production (9, 28). Our choice of a particular experimental T_a that was below $T_{a,\min}$ for the saturated animals but above that of

the others for determination of torpor duration thus further accentuates results of our thermoregulatory measurements.

Chipmunks on the unsaturated diet should conserve more energy during hibernation than those on the saturated diet because of their significantly lower O_2 consumption (Table 1). This greater reduction of O_2 consumption in torpor is associated with a greater rate change of a process with 10°C increase (Q_{10}) ($Q_{10} = 2.77$) on the unsaturated diet than on the saturated diet ($Q_{10} = 2.46$), assuming a normothermic T_b of 37°C and calculating the standard metabolic rate from an equation of Hayssen and Lacy (12) for placental mammals. The increased Q_{10} of $\dot{V}O_2$ in the unsaturated animals may be due to an increase of the thermal coefficient of mitochondrial respiration induced by unsaturated dietary lipids (21).

The minimum T_b of -0.2°C measured in one individual is the lowest value of a regulated T_b for a hibernating rodent of which we are aware (8, 20). This differs from the argument of Hudson (16) that chipmunks generally do not reduce T_b below 5 – 7°C and therefore that torpor in chipmunks represents an intermediate physiological state between deep hibernation and shallow, daily torpor.

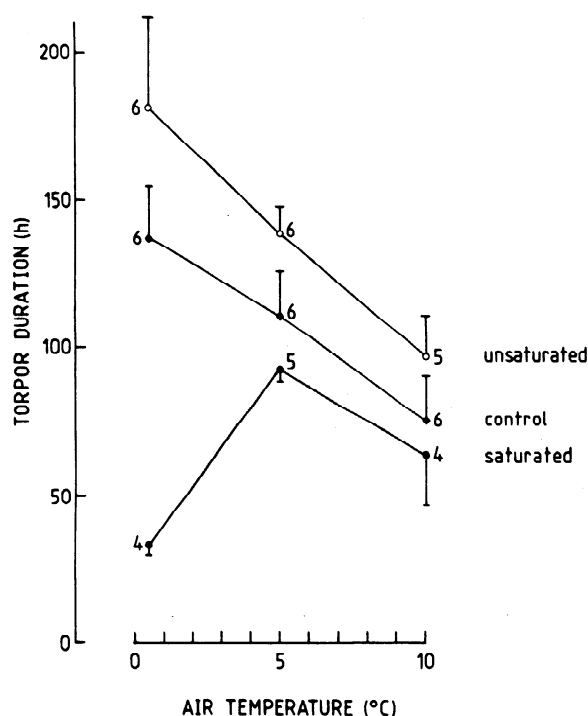


FIG. 2. Duration of bouts of torpor as function of air temperature (T_a) in chipmunks on 3 different lipid diets. Values are means \pm SD of no. of animals indicated in graph where value for each individual is means of 1–12 bouts of torpor at each T_a . Statistical differences were determined by one-way analysis of variance (0.5°C: $F = 55.3$, $df = 2,13$, $P < 0.001$; 5°C: $F = 25.8$, $df = 2,14$, $P < 0.001$; 10°C: $F = 7.7$, $df = 2,12$, $P < 0.01$). All pairwise comparisons were significantly different (Student-Newman-Keuls test; $P < 0.05$) except control and saturated animals at $T_a = 10^\circ\text{C}$.

Our observation of torpor bouts as long as 12 days in *E. amoenus* indicates a similarity to the torpor duration of ground squirrels and other "classical hibernators."

The possible ecological relevance of our observations is apparent from the fact that *E. amoenus* increases its intake of seeds from spring and summer to autumn and that more than half of the diet is seeds during the prehibernatory season, whereas the intake of animal matter decreases prior to hibernation (29). Plants seeds are rich in polyunsaturated oils (2). Selective feeding on foods that are high in unsaturated lipids would be advantageous because it would allow the animals to minimize metabolic rate during torpor, to remain torpid for longer periods, and to hibernate at lower T_a 's without the need of thermostatic heat production. These factors should increase the probability of survival during the winter hibernation period.

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