J Comp Physiol B (1994) 164:299–305

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

The degree of dietary fatty acid unsaturation affects torpor patterns and lipid composition of a hibernator

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Accepted: 7 April 1994

Abstract. Diets rich in unsaturated and polyunsaturated fatty acids have a positive effect on mammalian torpor, whereas diets rich in saturated fatty acids have a negative effect. To determine whether the number of double bonds in dietary fatty acids are responsible for these alterations in torpor patterns, we investigated the effect of adding to the normal diet 5% pure fatty acids of identical chain length (C18) but a different number of double bonds (0, 1, or 2) on the pattern of hibernation of the yellow-pine chipmunk, *Eutamias amoenus*. The response of torpor bouts to a lowering of air temperature and the mean duration of torpor bouts at an air temperature of 0.5 °C (stearic acid C18:0, 4.5±0.8 days, oleic acid C18:1, 8.6±0.5 days; linoleic acid C18:2, 8.5±0.7 days) differed among animals that were maintained on the three experimental diets. The mean minimum body temperatures (C18:0, +2.3±0.3 °C; C18:1, +0.3±0.2 °C; C18:2, −0.2±0.2 °C), which torpid individuals defended by an increase in metabolic rate, and the metabolic rate of torpid animals also differed among diet groups. Moreover, diet-induced differences were observed in the composition of total lipid fatty acids from depot fat and the phospholipid fatty acids of cardiac mitochondria. For depot fat 7 of 13 and for heart mitochondria 7 of 14 of the identified fatty acids differed significantly among the three diet groups. Significant differences among diet groups were also observed for the sum of saturated, unsaturated and polyunsaturated fatty acids. These diet-induced alterations of body fatty acids were correlated with some of the diet-induced differences in variables of torpor. The results suggest that the degree of unsaturation of dietary fatty acids influences the composition of tissues and membranes which in turn may influence torpor patterns and thus survival of hibernation.

Key words: Diet – Fatty acids – Hibernation – Thermoregulation – Chipmunk, *Eutamias amoenus*

Introduction

Patterns of hibernation and daily torpor are influenced by the composition of dietary fats. Heterothermic mammals fed diets containing plant oils that are rich in UFA and PUFA have longer torpor bouts, lower T_b and lower MR during torpor than individuals fed diets containing fats that are rich in SFA and contain small amounts of PUFA (Geiser and Kenagy 1987; Geiser 1991, 1993; Geiser et al. 1992; Frank 1992; Florant et al. 1993). These diet-related differences in torpor patterns are accompanied by differences in the FA composition of body tissues and cellular membranes (Geiser 1990, 1991, 1993; Frank 1992; Florant et al. 1993). It is therefore likely that compositional differences are at least partially responsible for the physiological differences between animals on different diets. Most studies concluded that dietary essential PUFA are responsible for enhanced torpor and thus survival during the hibernation season (Geiser and Kenagy 1987; Geiser 1991; Geiser et al. 1992; Frank 1992; Florant et al. 1993; Coleman et al. 1993). However, oils and fats are complex mixtures of a large number of SFA, UFA and PUFA. It is therefore difficult to ascertain if the physiological impact of oils or fats is a reflection of overall FA saturation, the concentration of particular FAs or the position of double bonds (Hazel 1988). Furthermore, fats may contain cholesterol and oils may contain vitamins (Lehninger 1982) which also could influence the pattern of torpor.

We therefore investigated how addition of pure FAs to the normal diet of a small hibernator, the yellow-pine chipmunk *Eutamias amoenus* (50 g), affected the pattern of torpor. The FAs were identical in number of carbon atoms (C18), but differed in the number of double bonds (0, 1, or 2). An increase in the number of double bonds increases the disorder of FAs and results in a lowering of

Abbreviations: bm, body mass; T_a, air temperature; T_b, body temperature; FA, fatty acid; MR, metabolic rate; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; $\dot{V}O_2$, rate of oxygen consumption; SFA, saturated fatty acids; UFA, unsaturated fatty acids; UI, unsaturation index; SNK, Student-Newman-Keuls test

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the melting point (Hochachka and Somero 1980). Animals were maintained on three diets containing 5% addition by weight of methyl esters of either stearic acid (C18:0, 18 carbons, no double bond) oleic acid (C18:1, 18 carbons, one double bond), or linoleic acid (C18:2, 18 carbons, two double bonds). Physiological variables of torpor were measured in the three experimental groups. This experimental manipulation should show whether MUFA or essential PUFA or both are responsible for enhancing torpor in hibernating mammals. The FA composition of total lipids in the depot fat and of membrane phospholipids in cardiac mitochondria was determined at the end of the physiological experiments and correlations between body FA composition and variables of torpor were investigated.

Materials and methods

Twenty-one *E. amoenus* were trapped on 6 and 7 September 1986 in the Cascade Mountains near Fish Lake, Chelan County, Washington, USA, and transported to the University of Washington and kept individually in cages provided with wood shavings. They were maintained with water and Purina rodent chow 5001 and libitum at a T_a of 23 ± 1 °C ($\pm$standard deviation, SD) and a photoperiod of LD 12:12, lights on 0600–1800 hours PST. On 8 September they were divided into three groups of matched bm and sex ratio. From 18 September until the end of the experiment these groups were fed three isocaloric diets: (i) Purina rodent laboratory chow 5001 with 5% addition by weight of stearic acid (C18:0; purity 99%; Research Organics Inc. Cleveland, Ohio, USA) ($n=8$); (ii) rodent chow with 5% addition of oleic acid (C18:1, purity 95%; United States Biochemical Corporation, Cleveland, Ohio, USA) ($n=7$); and (iii) rodent chow with 5% addition of linoleic acid (C18:2; purity 99%; United States Biochemical Corporation) ($n=6$). A greater number of experimental animals on stearic acid was selected because in a previous study some *E. amoenus* on a SFA diet did not enter torpor (Geiser and Kenagy 1987). Diets were prepared as previously described (Geiser 1990). Rodent chow 5001 contains 4.5% crude fat and the total lipid FA composition (weight %) of the fat was: C10:0, 0.1%; C12:0, 0.1%; C13:0, 1.5%; C14:0, 1.8%; C15:0, 0.2%; C16:0, 20.6%; C16:1, 2.4%; C17:0, 0.3%; C18:0, 7.2%; C18:1, 26.6%; C19:0, 1.9%; C18:2, 30.4%; C18:3, 2.6%; C20:0, 0.3%; C20:1, 0.74%; C20:4, 0.2%; C20:5, 1.5%; C24:0, 0.3%; C22:5, 0.3%; C22:6, 1.1%. The C18:0 diet therefore contained about 56% of C18:0, 13% of C18:1 and 14% of C18:2, the C18:1 diet contained about 3.5% of C18:0, 65% of C18:1 and 14% of C18:2, and the C18:2 diet contained about 3.5% of C18:0, 13% of C18:1 and 67% of C18:2. FAs were extracted and methylated using the method of Lepage and Roy (1986) and analyzed as previously described (Geiser 1990).

On 27 October (after 39 days on the respective diets) animals were transferred to a walk-in temperature-controlled room at $T_a = 4 \pm 1$ °C ($\pm$SD). We held animals successively at $T_a = 4 \pm 1$ °C (SD) from 27 October to 11 December, $T_a = 2 \pm 1$ °C from 11 December to 10 January, and $T_a = 0.5 \pm 1$ °C from 10 January to 8 February. These temperatures are in the range of mid to late winter soil temperatures experienced in the field in the area from which the animals were captured (Kenagy et al. 1989). The duration of torpor bouts at these T_as was determined by observing daily at 1000 h the displacement of fine sand from the back of each animal that occurs during arousal; sand was replaced after each normothermic period. Mean duration of torpor bouts for each diet group and T_a were calculated from the mean of all undisturbed torpor bouts for each individual. The first torpor bout of each individual at $T_a = 4$ °C at the beginning of the hibernation season was excluded from calculations.

Thermoregulatory patterns in torpid individuals were examined using an experimental cooling procedure. Individuals that were torpid at $T_a = 2$ °C were transferred to a 2-l respirometer vessel (flow rate 200 ml·min^{-1}) in a small constant-temperature cabinet at $T_a = 2.0 \pm 0.5$ °C ($\pm$SD). $\dot{V}O_2$ as an estimate of MR was measured at $T_a = 2$ °C, and when $\dot{V}O_2$ had stabilized the system was slowly cooled (<0.02 °C·min^{-1}) until the animal showed a conspicuous increase in ventilation and $\dot{V}O_2$ to avoid a further decline in T_b or to initiate arousal. When this increase in ventilation and $\dot{V}O_2$ was observed the animals was immediately removed from the respirometer and its T_b measured by 3-cm rectal insertion of a fine thermocouple probe that was calibrated to the nearest 0.1 °C and read with a digital thermometer. The T_b at that point was defined as minimum T_b, the T_a measured at the time of $\dot{V}O_2$ increase was defined as minimum T_a (Geiser and Kenagy 1987). The minimum $\dot{V}O_2$ of torpid individuals was measured over 30 min before the thermoregulatory increase of $\dot{V}O_2$.

Because the sensitivity to disturbance increases toward the end of torpor bouts, cooling experiments were performed between day 2 and 4 of a torpor bout. Cooling experiments were conducted between 17 and 25 December. Flow rates of dry air were adjusted and measured with a Brooks thermal mass flow controller. $\dot{V}O_2$ was measured with an Applied Electrochemistry S-3A oxygen analyzer and recorded together with T_a on a Leeds and Northrup Speedomax 250.

In early March four hibernating individuals of each group were decapitated and depot fat around the kidney immediately removed and frozen at −30 °C. Hearts were removed and washed in ice-cold mitochondrial isolation medium consisting of 250 mmol·l^{-1} sucrose, 2 mmol·l^{-1} HEPES, 0.5 mmol·l^{-1} EDTA, and 0.5 mg·ml^{-1} bovine serum albumin adjusted to pH 7.4 with KOH. Hearts were chopped into small pieces with scissors and rinsed repeatedly to remove blood. Heart tissue was then homogenized with a glass-Teflon homogenizer and 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 at −30 °C. FA composition of depot fat was determined because fat is the major source of energy during hibernation (Florant et al. 1990). The FA composition of heart mitochondrial membranes was determined because the heart remains active during hibernation and the mitochondria have to provide ATP even at very low T_b (Raison et al. 1988).

Total lipid FAs of depot fat were extracted and methylated (Lepage and Roy 1986). The FA methyl esters of heart mitochondrial phospholipids were prepared and FA methyl esters were extracted in hexane and analysed by gas-liquid chromatography as previously described (Geiser 1990). All lipid analyses were carried out within 2 months of tissue or membrane preparation.

One-way analysis of variance (ANOVA) followed by a pairwise Student-Newman-Keuls test (SNK; significance level 5%) were performed to detect differences among the diet groups. Percentage values were arcsine-transformed before testing (Sokal and Rohlf 1981). Linear regressions were performed using the method of least squares. Results are expressed as mean $\pm$ standard error (SE).

Results

Dietary fatty acids, body mass and torpor patterns

The bm of the three diet groups increased in a similar manner. At capture bm was 48.0 ± 0.7 g (C18:0 diet), 49.5 ± 0.7 g (C18:1 diet) and 50.4 ± 1.3 g (C18:2 diet); bm had increased to 65.5 ± 2.4 g (C18:0 diet), 65.2 ± 3.0 (C18:1 diet) and 70.7 ± 5.6 g (C18:2 diet) by 25 October when animals were transferred to T_a of 4 °C. This represents an increase in bm of 36% (C18:0 diet), 32% (C18:1 diet) and 40% (C18:2 diet).

The thermal response of the duration of torpor bouts of *E. amoenus* was dependent on the number of double

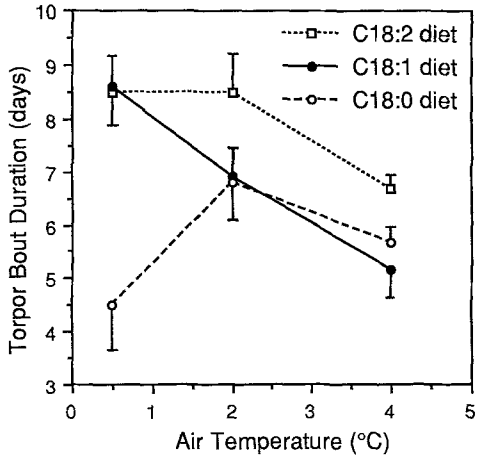

Fig. 1. The duration of torpor bouts (means with SE) of hibernating *Eutamias amoenus* on diets containing 5% of C18:0 ($n=8$), C18:1 ($n=7$), or C18:2 ($n=6$). Torpor bouts differed among diet groups at T_a 0.5 °C (ANOVA, $P<0.001$; SNK $P<0.01$ for C18:2 versus C18:0 diets and for C18:1 versus C18:0 diets)

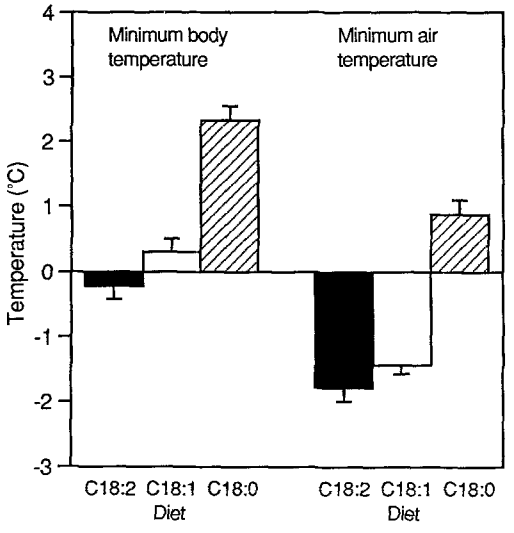

Fig. 2. The minimum T_b and the minimum T_a (means with SE for six individuals of each group) of hibernating *Eutamias amoenus* on diets containing 5% of C18:0, C18:1 or C18:2. Both the minimum T_b and the minimum T_a differed among diet groups (ANOVA $P<0.0001$; SNK $P<0.05$ for C18:2 versus C18:0 diets and for C18:1 versus C18:0 diets)

bonds in the dietary FA supplementation (Fig. 1). Torpor bout duration in all experimental groups lengthened when T_a was lowered from 4 to 2 °C. When T_a was further reduced to 0.5 °C, animals on C18:1 and C18:2 diets lengthened or maintained duration of torpor bouts, whereas animals on C18:0 diet shortened duration of torpor bouts by nearly half. Significant differences among diet groups in the duration of torpor bouts were observed at $T_a=0.5$ °C (ANOVA, $P<0.001$; SNK $P<0.01$ for C18:2 versus C18:0 diets and for C18:1 versus C18:0 diets).

Differences among diet groups were also observed in the minimum T_b and minimum T_a (Fig. 2). The minimum T_b (C18:0, $T_b+2.3\pm0.3$ °C; C18:1, $T_b+0.3\pm0.2$ °C;

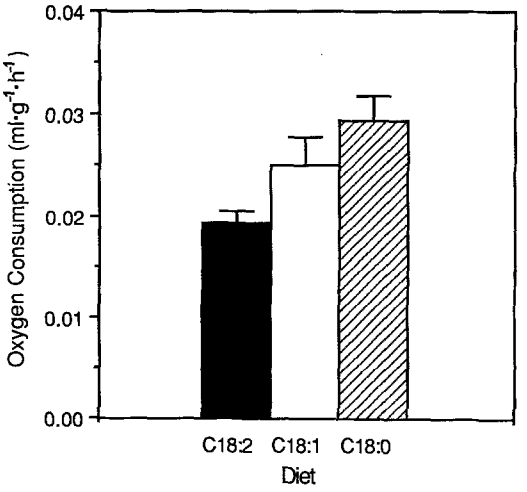

Fig. 3. The minimum oxygen consumption ($\dot{V}O_2$) (means with SE for six individuals of each group) of hibernating *Eutamias amoenus* on the three diets. The $\dot{V}O_2$ was determined over 30 min before the thermoregulatory increase was observed and differed among diet groups (ANOVA $P<0.01$; SNK $P<0.05$ for C18:2 versus C18:0 and for C18:2 versus C18:1)

C18:2, $T_b-0.2\pm0.2$ °C) and minimum T_a (C18:0, $T_a+0.9\pm0.2$ °C; C18:1, $T_a-1.4\pm0.1$ °C; C18:2, $T_a-1.8\pm0.2$ °C) differed between animals on C18:2 and C18:0 diets and between C18:1 and C18:0 diets (ANOVA, SNK, $P<0.05$). The lowest individual minimum T_b of -1.0 °C was observed in an animal on C18:2 diet.

The $\dot{V}O_2$ of torpid animals also differed among diet groups (Fig. 3). The lowest values were measured in animals on the C18:2 diet, the highest values in animals on the C18:0 diet and intermediate values in animals on the C18:1 diet (ANOVA $P<0.01$; SNK $P<0.05$ for C18:2 versus C18:0 and for C18:2 versus C18:1). The $\dot{V}O_2$ of animals on the C18:0 diet was about 50% higher than that of animals on the C18:2 diet (Fig. 3). Body mass, determined immediately after each of the $\dot{V}O_2$ measurements, did not differ among diet groups (C18:0, 54.4 ± 3.0 g; C18:1, 56.9 ± 4.9 g; C18:2, 54.0 ± 1.4 g; $P>0.5$, ANOVA).

Depot fat and heart mitochondrial fatty acid composition

The dietary FAs resulted in some differences in the body FA composition (Tables 1, 2). The total lipid FAs of depot fat showed the most pronounced differences among the dietary groups (Table 1). Of the 13 FAs identified, 7 differed significantly among diet groups. Significant differences among diet groups were also observed for the unsaturation index (UI; the sum of the percent unsaturated FAs multiplied their number of double bonds), and the sum of SFA, UFA and PUFA (Table 1). Animals on the linoleic acid (C18:2) diet contained more UFA than animals on the stearic acid (C18:0) diet. In each diet group the FA provided in the respective diet was found in the depot fat in a relatively large proportion.

Table 1. Total lipid fatty acid composition of depot fat of hibernating *Eutamias amoenus* on the three fatty acid diets

Fatty Acid	C18:0 diet (1)	C18:1 diet (2)	C18:2 diet (3)	ANOVA $P<$	SNK		
					1–2 $P<$	1–3	2–3
	$n=4$	$n=4$	$n=4$				
C14:0	0.53±0.02	0.51±0.04	0.63±0.07	ns	ns	ns	ns
C16:0	8.13±0.51	6.60±0.36	6.92±0.72	ns	ns	ns	ns
C16:1n7	6.10±0.75	4.43±0.03	4.76±0.44	ns	ns	ns	ns
C17:0	0.28±0.02	0.18±0.01	0.14±0.02	0.0001	0.05	0.05	0.05
C18:0	9.73±0.82	2.41±0.22	2.25±0.21	0.0001	0.05	0.05	ns
C18:1n9	58.30±1.05	70.11±1.20	36.40±2.81	0.0001	0.05	0.05	0.05
C18:2n6	13.28±0.05	12.57±0.64	45.15±2.59	0.0001	ns	0.05	0.05
C18:3n3	0.59±0.04	0.43±0.02	0.54±0.08	ns	0.05	ns	ns
C20:0	0.12±0.02	tr	tr	0.0001	0.05	0.05	0.05
C20:1n9	1.21±0.21	1.04±0.09	0.92±0.13	ns	ns	ns	ns
C20:2n6	0.21±0.02	0.18±0.01	0.27±0.03	0.01	ns	0.05	0.05
C22:5n3	tr	tr	0.17±0.04	ns	ns	ns	ns
C22:6n3	tr	tr	0.21±0.07	0.05	ns	0.05	ns
UI	95.33±0.68	103.22±0.44	135.93±3.48	0.0001	0.05	0.05	0.05
SFA	18.79±1.19	9.76±0.61	9.93±0.95	0.0001	0.05	0.05	ns
UFA	79.90±1.18	88.92±0.65	88.49±1.01	0.0002	0.05	0.05	ns
PUFA	14.28±0.20	13.36±0.64	46.41±2.72	0.0001	ns	0.05	0.05

Number of carbon atoms (C14–C22), double bounds (:0–:6) and position of double bonds (n3, n6, n7, n9) of fatty acids are shown. The various fatty acids are quantified as the mean %±SE of the number of individuals investigated. The Unsaturation Index (UI) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; SFA, sum of saturated fatty acids; UFA, sum of unsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; trace (tr) represents fatty acids that were present at <0.1%

Table 2. Phospholipid fatty acid composition of cardiac mitochondria from hibernating *Eutamias amoenus* on the three fatty acid diets

Fatty Acid	C18:0 diet (1)	C18:1 diet (2)	C18:2 diet (3)	ANOVA $P<$	SNK		
					1–2 $P<$	1–3	2–3
	$n=4$	$n=4$	$n=4$				
C16:0	12.19±0.39	12.26±0.61	13.02±0.61	ns	ns	ns	ns
C16:1n7	0.52±0.08	0.50±0.03	0.37±0.02	ns	ns	ns	ns
C17:0	0.44±0.02	0.44±0.02	0.51±0.02	0.025	ns	0.05	0.05
C17:1n9	0.71±0.02	0.35±0.03	0.42±0.03	0.0001	0.05	0.05	ns
C18:0	23.70±0.44	20.74±0.68	21.40±0.58	0.01	0.05	0.05	ns
C18:1	7.89±0.24	9.59±0.03	6.34±0.19	0.0001	0.05	0.05	0.05
C18:2	14.56±1.34	14.47±0.22	17.70±0.64	0.05	ns	0.05	0.05
C20:2n6	–	–	0.48±0.04	0.0001	ns	0.05	0.05
C20:3n6	0.32±0.04	0.43±0.03	0.24±0.02	0.003	0.05	ns	0.05
C20:4n6	11.61±0.67	11.45±0.92	10.70±0.58	ns	ns	ns	ns
C20:5n3	0.14±0.08	0.11±0.06	0.18±0.05	ns	ns	ns	ns
C22:4n6	tr	0.13±0.08	0.18±0.08	ns	ns	ns	ns
C22:5n3	2.04±0.08	1.77±0.08	1.76±0.15	ns	ns	ns	ns
C22:6n3	24.59±1.94	26.40±1.18	25.74±0.73	ns	ns	ns	ns
UI	244.02±6.75	254.66±3.59	251.62±3.36	ns	ns	ns	ns
SFA	36.33±0.10	33.45±0.30	34.93±0.23	0.0001	0.05	0.05	0.05
UFA	62.55±0.12	65.22±0.39	64.28±0.22	0.0001	0.05	0.05	0.05
PUFA	53.29±0.24	54.78±0.41	56.96±0.25	0.0001	0.05	0.05	0.05

Number of carbon atoms (C14–C22), double bounds (:0–:6) and position of double bonds (n3, n6, n7, n9) of fatty acids are shown. The various fatty acids are quantified as the mean %±SE of the number of individuals investigated. The Unsaturation Index (UI) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; SFA, sum of saturated fatty acids; UFA, sum of unsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; trace (tr) represents fatty acids that were present at <0.1%; – fatty acid not present or statistics were not performed

Phospholipid FA composition of heart mitochondria also differed among diet groups (Table 2). Of the 14 FAs that were identified, 7 differed significantly. Furthermore, the sum of SFA, UFA, and PUFA differed among diet groups. The amount of docosahexaenoic acid (C22:6) was very high in all three diet groups. The FA provided in each diet was present in relatively high concentrations in the heart mitochondrial phospholipid FAs of the respective diet group.

Table 3. The relationships between the Unsaturation Index (UI), the sum of saturated fatty acids SFA; the sum of unsaturated fatty acids UFA, and the sum of polyunsaturated fatty acids PUFA and variables of torpor of individual *Eutamias amoenus*

		a	*b*	r^2	*P*<
Depot fat:					
Torpor bouts vs	UI				ns
	SFA	13.10	−0.339	0.76	0.0001
	UFA	−20.95	0.346	0.78	0.0001
	PUFA				ns
Minimum T_b vs	UI	6.42	−0.050	0.49	0.01
	SFA	−2.44	0.253	0.79	0.0001
	UFA	22.44	−0.252	0.77	0.0001
	PUFA	1.96	−0.047	0.33	0.05
Torpor $\dot{V}O_2$ vs	UI	0.04	−0.002	0.48	0.01
	SFA				ns
	UFA				ns
	PUFA	0.03	−0.0002	0.38	0.05
Heart mitochondria:					
Torpor bouts vs	UI				ns
	SFA	43.15	−0.986	0.48	0.01
	UFA	−57.17	1.03	0.48	0.01
	PUFA				ns
Minimum T_b vs	UI				ns
	SFA	−22.59	0.670	0.42	0.05
	UFA	51.04	−0.785	0.52	0.01
	PUFA	35.66	−0.633	0.62	0.001
Torpor $\dot{V}O_2$ vs	UI				ns
	SFA				ns
	UFA				ns
	PUFA	0.12	−0.002	0.34	0.05

Regression analyses were performed on the 12 individuals for which data on both FA composition and variables of torpor were available. Linear regressions are described by the equations: $y=a+bx$. The torpor $\dot{V}O_2$ represents the minimum $\dot{V}O_2$ of torpid individuals. Torpor bouts were measured at $T_a=0.5$ °C

Discussion

The experimental observations reported here support the view that the degree of unsaturation of dietary FAs influences mammalian torpor. The difference of only a single double bond in a C18 FA sufficed to alter the minimum T_b, the MR during torpor and the duration of torpor bouts. As most variables of torpor were correlated with body FA composition of the chipmunks it is possible that differences in torpor patterns were caused by differences in the FA composition.

Diet-induced differences in both torpor patterns and body lipid composition invite discussion on how these phenomena might be related. Compositional differences of depot fat and mitochondrial membrane FAs as well as differences in torpor patterns were most pronounced between animals on C18:0 and C18:2 diets. Since animals on the C18:1 diet were intermediate in some variables of torpor it appears that the C18:2 diet enhanced torpor and the C18:0 diet inhibited torpor. Differences in the patterns of torpor were more pronounced between animals on C18:0 and C18:1 diets than between animals on C18:1 and C18:2 diets. These observations suggest that adding a single double bond to C18:0 caused the greatest change in the pattern of torpor. However, the MR differed more between animals on C18:1 and C18:2 diets than between C18:0 and C18:1 diets, suggesting that for this variable the second double bond in the C18 FA had the greater effect. This is further demonstrated by the Q_{10} for the minimum $\dot{V}O_2$ between the diet groups. The Q_{10} for the minimum $\dot{V}O_2$ and T_b between the C18:0 and C18:1 diets was about 2.2, which is close to the prediction for biological reactions. In contrast, the Q_{10} for the minimum $\dot{V}O_2$ and T_b between the C18:0 and C18:2 diets and between the C18:1 and the C18:2 diets was >5 and therefore well above the prediction for normal biological reactions. This observation suggests that differences among diet groups in the minimum $\dot{V}O_2$ can only partially be explained by Q_{10} effects (i.e. differences in the minimum T_b). It also suggests that the C18:2 diet resulted in an inhibition of the MR in comparison to the C18:0 and C18:1 diets.

Diet-induced changes in both variables of torpor and as well as body FA composition suggest that they may be interrelated. Therefore, linear regressions were performed with the UI, SFA, UFA and PUFA of depot fat and heart mitochondrial membranes as independent variables and the duration of torpor bouts at $T_a=0.5$ °C, the minimum T_b and the MR of torpid individuals as dependent variables for the 12 individuals for which data on both were available (Table 3). For the depot fat 8 of 12 regression analyses revealed significant correlations between FA composition and variables of torpor (Table 3). The best correlations were observed between the duration of torpor bouts and the SFA and UFA and between the minimum T_b and the SFA and UFA (Table 3; Fig. 4). For heart mitochondrial phospholipids 6 of 12 regression analyses revealed significant correlations (Table 3). The best correlation was observed between the minimum T_b and the PUFA (Table 3; Fig. 5).

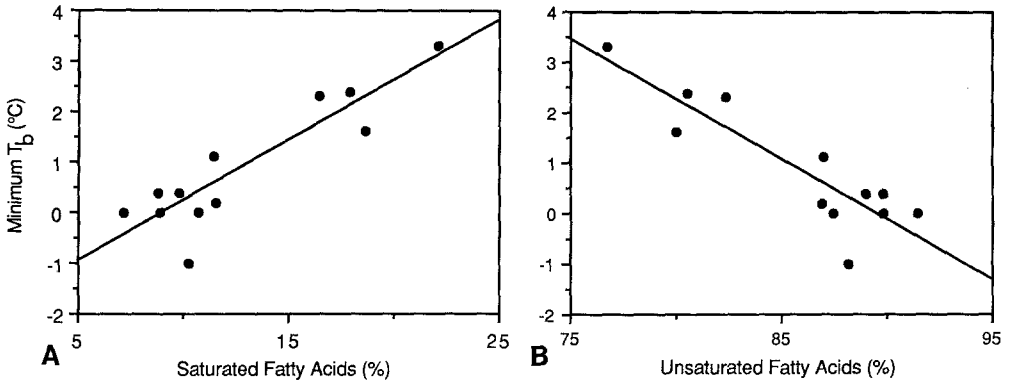

Fig. 4. The relationship between the minimum T_b of *Eutamias amoenus* and (**A**) the sum of depot fat saturated fatty acids (SFA) and (**B**) the sum of depot fat unsaturated fatty acids (UFA). Equations are given in Table 3

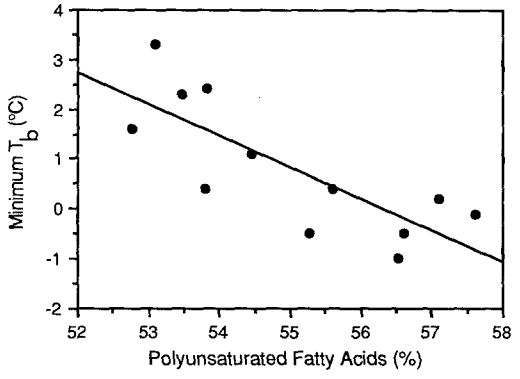

Fig. 5. The relationship between the minimum T_b of *Eutamias amoenus* and the sum of heart mitochondrial polyunsaturated fatty acids (PUFA). Equations are given in Table 3

The extend of diet-induced compositional changes differed among depot fat and heart mitochondrial membranes. The most pronounced compositional differences were observed in depot fat suggesting that the FA composition of different tissues is not uniformally controlled by endogenous processes and is strongly influenced externally by diets. A diet of inappropriate composition therefore would have a very strong impact on the depot fat composition. We do not know how the composition of stored fat affects hibernation, but it is possible that the melting point of stored fat influences regulation of T_b to maintain fluid fat stores. Regression analyses of fatty acid composition and variables of torpor suggest that the minimum T_b is strongly affected by the composition of depot fat which would support this notion. However, duration of torpor bouts and MR of torpid individuals were also correlated with FA composition of depot fat. It is therefore possible that the FAs provided from the depot fat as metabolizable substrate may influence MR which in turn may affect duration of torpor bouts (Geiser et al. 1990).

The diet-induced compositional differences of heart mitochondrial FAs were less distinct than those of depot fat. However, *E. amoenus* showed about a 7% difference of PUFA in heart mitochondrial membranes between the C18:0 and C18:2 diets. This compositional difference was paralleled by a 2.5 °C difference in minimum T_b. Correlations between the low minimum T_b, the long bouts of torpor and high concentrations of heart mitochondrial UFA and PUFA suggest that the minimum T_b and the duration of torpor bouts may be somehow linked with the composition of mitochondrial FAs. It is possible that the animals defend their T_b during torpor at a T_b that allows unimpaired function of heart mitochondria (Geiser et al. 1986) and that this critical temperature is lowered by an increase of UFA and PUFA in the heart mitochondrial membrane.

The effect of dietary C18:2 (linoleic acid) on hibernation observed here is important because mammals require these essential FAs for synthesis of most longer chain PUFA (Lehninger 1982). PUFA in turn appear to be a general requirement for physiological function at low temperatures in animals (Hazel 1988). Since *E. amoenus* on the C18:2 diet showed particularly deep torpor, with T_b falling as low as -1 °C, and very low MR, the view that selective feeding as well as selective uptake of essential FAs may be important for preparation for hibernation (Florant et al. 1990; Coleman et al. 1993) is supported by our study. The diet-induced physiological and biochemical changes observed here further emphasize the importance of compositional, physical, and functional seasonal changes of tissues and cell membranes that have been previously observed in hibernators (Aloia and Pengelley 1979; Raison et al. 1988; Aloia and Raison 1989).

It has long been recognized that many heterothermic rodents increase the intake of oily seeds in autumn (Tevis 1953; Howard 1961). Furthermore, it has been demonstrated experimentally that chipmunks (*Tamias striatus*) collect oily seeds for food caches irrespective from the distance from their burrow, whereas other food items were only cached if they were found close to their burrow (Wood 1993). As oily seeds are rich in energy, this appears to be an appropriate strategy for winter preparation. Many hibernators rely largely on stored body fats during the hibernation season and an increase of fat stores is essential for survival (Florant et al. 1990). However, oily seed are not only rich in energy generally they also are rich in essential PUFA. Thus, it is possible that hibernators select oily seeds before the hibernation season because of both energy content and the content of MUFA and PUFA.

While all diet-induced alterations of physiological variables measured here are important for hibernation, the most significant effect is that on torpor bout duration.

Torpor bout length differed markedly at T_a of 0.5 °C, which is below the minimum T_a of animals on the C18:0 diet, but above the minimum T_a of animals on C18:1 and C18:2 diets. This is further evidence that the duration of torpor bouts is not always inversely related with T_a, but decreases when the torpid animal metabolically defends its T_b (Geiser and Kenagy 1987). The animals on the C18:0 diet therefore did not only increase their MR to maintain their T_b during torpor, they also reduced the duration of torpor bouts and had to arouse more frequently than animals on the other diets. Thus, a relatively small difference in the minimum T_b during torpor had a very marked effect on the duration of torpor bouts at low T_a.

Differences in the thermal response of torpor bout duration are an important observation because soil temperatures at a hibernaculum site of the species are not constant throughout winter (Kenagy et al. 1989). Because arousals consume most of the energy used during hibernation, energy expenditure in hibernators with longer torpor bouts and less frequent arousals should be reduced. Under field conditions, *E. amoenus* hibernates for about 4–5 months (Kenagy and Barnes 1988). If a hibernation period of 135 days and a T_a regime as in the present study are assumed, animals on the C18:0 diet would arouse about 25 times, those on C18:1 diet about 21 times and those on C18:2 diet only about 17 times. These more frequent arousals in animals on C18:0 diet would increase their energy expenditure in comparison to animals on C18:2 diet by about 47%. If other factors such as lower MR during torpor were also considered, energy savings in animals on the C18:2 diet in comparison to the C18:0 diet would be even greater. These considerable diet-induced energy savings may have an impact on survival of individuals in the wild. As the hibernation season can be a time of high mortality in mammals (Arnold 1990; Broome and Mansergh 1990) a diet rich in UFA and PUFA may increase the proportion of animals surviving the winter.

Acknowledgements. This work was supported by a grant of the Australian Research Council and a Feodor Lynen Fellowship of the Alexander von Humboldt-Stiftung to F. G., and grants of the University of Washington Graduate School Research Fund and the NSF (BSR 8401248) to G.J.K. We would like to thank M. T. Childs and I. B. King for advice and research facilities and two anonymous referees for constructive comments on the manuscript.

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