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Influence of polyunsaturated and saturated dietary lipids on adipose tissue, brain and mitochondrial membrane fatty acid composition of a mammalian hibernator

Fritz Geiser

Department of Zoology, University of Washington, Seattle, WA (U.S.A.)

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Dietary lipid composition profoundly influences the hibernation pattern of the chipmunk *Eutamias amoenus*. The object of the present study was to investigate whether these physiological changes following feeding of saturated and unsaturated lipids were associated by compositional changes of fatty acids of tissues and membranes. Animals were fed with rodent chow (control diet), rodent chow with 10% sunflower seed oil (unsaturated diet) and rodent chow with 10% sheep fat (saturated diet). Diet-induced changes in the fatty acid composition of depot fat and brain total lipids and of mitochondrial phospholipids were determined. The fatty acid unsaturation index was lower in animals on saturated diet than in animals on unsaturated diet (depot fat 86.1 vs. 145.9; heart mitochondria 207.6 vs. 247.1; liver mitochondria 148.4 vs. 173.5). Pronounced differences between dietary groups were also observed in $n-3$ or $n-6$ fatty acids or their ratios of depot fat, brain and liver mitochondria. Generally, the diet-induced differences in tissue and membrane fatty acid composition in *E. amoenus* were more pronounced than those observed previously in non-hibernating species. Selective feeding and incorporation of high amounts of unsaturated fatty acids into tissues and cell membranes may be an important preparation for hibernation in *E. amoenus* which lowers its body temperature during torpor to about 0°C.

Introduction

During periods of torpor many hibernating mammals reduce their body temperature (T_b) to about 0°C or even below 0°C [1,2]. This is in contrast to homeothermic endotherms which always maintain a high T_b of about 35–40°C and generally do not survive a substantial reduction in T_b. The difference in thermoregulation between homeothermic and hibernating endotherms is reflected in the function and properties of their cell membranes [3]. Cells of hibernating mammals can maintain ionic gradients at low temperatures and their enzymes are less temperature sensitive than those from homeotherms [3]. These functional differences may be in part due to differences in cell membrane lipid composition of the two groups [3–6]. One of the major difference in the lipid composition appears to be the greater proportion of unsaturated fatty acids in some cell membranes of hibernators than in homeotherms [3,5]. A comparatively higher proportion of unsaturated fatty acids has also been observed in the membranes of cold acclimated ectothermic organisms and thus appears to be a general requirement for physiological function at low temperatures [7–11].

The pattern of thermoregulation or thermal acclimation are not the only factors that influence the lipid composition of tissues and membranes in animals. Composition of both tissues and membranes reflect dietary lipids ingested by the animal [12,13]. Compositional changes of cell membranes in turn influence their physical properties and the activity of membrane-associated enzymes [14–16]. At the organismal level, dietary lipids alter the hibernation pattern of hibernating chipmunks, *Eutamias amoenus* [17]. Chipmunks on a unsaturated diet show lower body temperatures and longer torpor duration than chipmunks on a saturated diet and it was proposed that diet-induced changes in the composition of cell membranes may be responsible for these physiological changes [17].

In the present study I investigated to what extent the diet-induced alterations in the hibernation pattern of chipmunks were accompanied by changes of fatty acid composition of tissues and membranes. Because com-

Correspondence (present address): F. Geiser, Department of Zoology, University of New England, Armidale, New South Wales 2351, Australia.

positional differences in lipid diet so profoundly affected whole animal physiology it was particularly interesting to know whether compositional changes of tissues and membranes of these hibernators are more pronounced than has been observed previously in non-hibernating mammals.

Materials and Methods

22 *Eutamias amoenus* were trapped in early September 1985, about 8 weeks before they would naturally begin to hibernate, in the Cascade Mountains near Lake Wenachee, Chelan County, Washington, at a mean mass of 42.8 g. They were transported to the University of Washington, divided into three groups of matched body mass and sex ratio and were kept individually in cages at an air temperature (T_a) of 22 ± 1°C with a 12L : 12D photoperiod (light from 0600–1800 h PST). Animals were fed ad libitum throughout the experiment with water and three diets: (i) Ralston Purina rodent laboratory chow 5001 as 'control diet', (ii) rodent chow with a 10% addition by weight of sunflower seed oil as 'unsaturated diet', and (ii) rodent chow with 10% addition sheep kidney fat as 'saturated diet'. Pellets were soaked in oil or fat overnight at 60 °C. Pellets were then transferred to a strainer at 60°C and repeatedly weighed until their fat content was 10 ± 0.5%. The fat or oil was equally distributed through the pellets and partly eaten pellets were not removed from animals to ensure that they consumed the intended amount of fat. The total lipid fatty acid composition of the three diets differed substantially (Table I). The energy content of the control diet was about 18 kJ/g and that of the fat diets about 22 kJ/g (39 kJ/g fat). Animals reached a peak body mass (mean 66.4 g; control diet 74.2 ± 7.8 g; unsaturated diet 64.7 ± 6.7 g; saturated diet 63.7 ± 4.6) after 8 weeks on their diet and were then transferred to a controlled-environment chamber at T_a 10°C.

Animals were held at T_a 10°C from 20 November to 5 December, T_a 5°C from 6 December to 10 January, and T_a 0.5°C from 11 January. Hibernating animals were decapitated between day 2 and day 4 of a torpor bout in early February 1986. February is in the central part of the hibernation season of this species [18]. Animals used for lipid analyses were the same individuals that were used in the previous study and body mass was indistinguishable between diet-groups [17]. Depot fat (white adipose tissue) around the kidney was immediately removed and frozen. Brains were removed and divided into cerebrum and the rest of the brain without the cerebrum (i.e., olfactory bulb, diencephalon, mesencephalon, cerebellum and medulla oblongata). Both brain samples were immediately homogenized in destilled water and frozen. Hearts and livers were removed and washed in ice-cold mitochondrial isolation medium consisting of 250 mM Sucrose, 2 mM Hepes, 0.5 mM EDTA and 0.5 mg/ml bovine serum albumin adjusted to pH 7.4 with KOH. Hearts and livers of individual animals were chopped into small pieces with scissors and rinsed repeatedly with isolation medium to remove blood. The tissue was then homogenized with a glass-Teflon homogenizer. Mitochondria were isolated by differential centrifugation at 2°C as described [19]. Mitochondria were washed twice, resuspended in H_2O and frozen. All samples were frozen at −30°C.

Total lipids of fat and brain were extracted and transesterified [20] within 2 months of preparation. The total lipids of mitochondria were extracted [21], after addition of about 0.1% butylated hydroxytoluene. Phospholipids were separated from the total lipids by thin-layer chromatography on Silica-gel H plates which were developed in petroleum ether/acetone (3 : 1, v/v). The phospholipids remaining at the origin were eluted from the silica and transesterified [20]. 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

TABLE I

Percent fatty acid composition of total lipids in the three different diets

Number of carbon atoms, number of double bonds and position of double bonds of fatty acids are shown. Percentage values of different fatty acids represent the means of two diet samples. Unsaturation index (U.I.) is the sum of % unsaturated fatty acids multiplied by their number of double bonds. S.D. < 0.1 not given. Sheep fat and sunflower oil diets were considered different (*) if the ratio of their fatty acid composition differed > 20%. –, Fatty acid not present.

Fatty acid	Sheep fat diet (1) ($n = 2$)	Control diet (2) ($n = 2$)	Sunflower oil diet (3) ($n = 2$)	Ratio (1)/(3)
10:0	0.36	0.14	–	–
12:0	0.44	0.13	–	–
13:0	0.58	1.45	0.44	1.32 *
14:0	4.9	1.84	0.1	49 *
15:0	0.8	0.24	–	–
16:0	22.4 ±0.4	20.57 ± 0.46	10.31 ± 0.16	2.17 *
16:1 ($n-7$)	1.44	2.41	0.55	2.62 *
17:0	1.56	0.33	0.15	10.4 *
18:0	25.0 ±0.6	7.15 ± 0.14	5.35 ± 0.2	4.67 *
18:1	27.23 ± 0.67	26.6 ±0.14	17.4 ±0.2	1.56 *
19:0	–	1.88	–	–
18:2 ($n-6$)	7.3 ±0.4	30.4 ±0.35	60.5 ±1.2	0.12 *
18:3 ($n-3$)	–	2.56	1.1	–
20:0	0.41	0.29	0.29	1.41 *
20:1 ($n-9$)	0.19	0.74	0.26	0.73 *
21:0	0.22	–	–	–
20:2 ($n-6$)	–	–	0.17	–
20:4 ($n-6$)	–	0.21	–	–
22:0	–	–	0.57	–
20:5 ($n-3$)	0.14	1.47	0.29	0.48 *
24:0	–	0.3	0.22	–
22:5	0.13	0.29	–	–
22:6 ($n-3$)	0.26	1.12	0.46	0.57 *
U.I.	46	115	147	0.31 *

column and a flame ionization detector. The percent fatty acid concentration was determined with a Hewlett-Packard 3390A integrator.

Numeric values are expressed as mean ± 1 S.D. Statistical tests were performed on arcsine-transformed percentage values [22]. Unsaturation index (U.I.) and ratios were not transformed. Statistical tests used were one-way analysis of variance (ANOVA) and the Scheffé method range test [22]. Statistical differences were assumed significant at the 95% level ($P < 0.05$). Only two samples were collected for lipid diet preparations and brain parts from each diet group and therefore no statistical test was performed. However, it was assumed that saturated and unsaturated diets and brains of animals on these diets differed if the difference in fatty acid composition was > 20%.

Results

Dietary lipids had a pronounced effect on the appearance of *E. amoenus* depot fat. When tissues were prepared the depot fat of unsaturated fat fed animals had the appearance of oil whereas that from the saturated fat fed animals looked more like their dietary animal fat. Compositional differences of depot fat total lipids were also very pronounced (Table II). Of the 14 fatty acids detected, 10 differed significantly ($P < 0.01$; ANOVA). Only linolenic acid (18:3($n-3$)) was not significantly different. Because 15:0, 19:0 and 20:0 showed no overlap between dietary groups (in some dietary groups these fatty acids were found only in traces or were not detected at all, whereas in others they represented a substantial proportion), it was assumed that they differ, although no test was performed. The pronounced differences in the composition of the various fatty acids also was reflected in the unsaturation index (U.I.), total saturated and unsaturated fatty acids, $n-6$ and $n-3$ fatty acids as well as their ratios. The unsaturation index was considerably greater in the animals on the unsaturated diet than in the other groups. The fatty acid composition of *E. amoenus* depot fat was similar to that of the diets although the unsaturation index of the saturated diet group was substantially greater than that of the diet.

Brain total lipid fatty acid composition also differed between the diet groups (Tables III and IV). For the cerebrum 21 fatty acids were detected and 6 of these differed by more than 20% between animals on unsaturated and saturated diets (Table III). Furthermore, the $n-6$ fatty acids differed distinctly between animals on saturated and unsaturated diets. The rest of the brain without the cortex showed greater diet-induced

TABLE II

Depot fat total lipid fatty acid composition of hibernating Eutamias amoenus on the three different lipid diets

The fatty acids (FA) shown as the mean percentage ± S.D. The unsaturation index (U.I.) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; trace (tr) represents fatty acids that were present at < 0.1%. –, Fatty acid not present or statistical test not performed. n.s., not significant.

Fatty acid	Sheep fat diet (1) ($n = 4$)	Control diet (2) ($n = 4$)	Sunflower oil diet (3) ($n = 4$)	$P <$			
				ANOVA	Range test		
					1–2	1–3	2–3
14:0	1.18 ± 0.23	0.82 ± 0.17	0.52 ± 0.06	0.001	n.s.	0.05	n.s.
15:0	0.23 ± 0.03	–	–	–			
16:0	11.74 ± 1.15	9.50 ± 2.0	6.76 ± 0.28	0.005	n.s.	0.05	n.s.
16:1 ($n-7$)	6.50 ± 0.51	7.30 ± 1.7	3.80 ± 1.2	0.01	n.s.	0.05	0.05
17:0	1.33 ± 0.10	0.21 ± 0.03	0.16 ± 0.02	0.00001	0.05	0.05	n.s.
18:0	8.23 ± 1.08	2.80 ± 0.3	3.72 ± 0.45	0.00001	0.05	0.05	n.s.
18:1	52.40 ± 2.4	56.10 ± 5.8	25.10 ± 2.5	0.00001	n.s.	0.05	n.s.
19:0	tr	2.30 ± 0.1	tr	–			
18:2 ($n-6$)	12.60 ± 1.06	16.64 ± 2.1	56.40 ± 2.5	0.00001	0.05	0.05	0.05
18:3 ($n-3$)	0.48 ± 0.12	0.72 ± 0.26	0.43 ± 0.03	n.s.			
20:0	0.16 ± 0.03	tr	0.10 ± 0.09	–			
20:1 ($n-9$)	0.64 ± 0.14	1.32 ± 0.67	0.26 ± 0.14	0.00001	0.05	0.05	0.05
22:5 ($n-3$)	0	0.26 ± 0.06	0.14 ± 0.05	0.00001	0.05	0.05	0.05
22:6 ($n-3$)	0	0.24 ± 0.01	0.19 ± 0.09	0.00001	0.05	0.05	n.s.
U.I.	86.1 ± 3.3	103.2 ± 0.6	145.9 ± 2.6	0.00001	0.05	0.05	0.05
Sat. FA's	22.85 ± 2.31	15.55 ± 2.64	11.24 ± 0.73	0.0001	0.05	0.05	n.s.
Unsat. FA's	72.54 ± 2.58	82.75 ± 2.65	86.53 ± 0.80	0.00001	0.05	0.05	n.s.
Sat./Unsat.	0.31 ± 0.04	0.19 ± 0.04	0.13 ± 0.01	0.0001	0.05	0.05	n.s.
n–6 FA's	12.61 ± 1.06	16.79 ± 2.11	56.72 ± 2.58	0.00001	0.05	0.05	0.05
n–3 FA's	0.48 ± 0.12	1.22 ± 0.29	0.76 ± 0.16	0.0025	0.05	n.s.	n.s.
(n–6)/(n–3)	27.45 ± 5.33	14.26 ± 2.36	76.83 ± 13.53	0.00001	n.s.	0.05	0.05

TABLE III

Cerebrum total lipid fatty acid composition of hibernating Eutamias amoenus on the three different lipid diets

The fatty acids (FA) shown as the mean percentage ± S.D. The unsaturation index (U.I.) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; trace (tr) represents fatty acids that were present at < 0.1%. Statistical test were not performed because of the small *n*. Animals on sheep fat and animals on sunflower oil were considered different (*) when the ratio of their fatty acid composition showed a change of > 20%. –, Fatty acid not present.

Fatty acid	Sheep fat diet (1) ($n = 2$)	Control diet (2) ($n = 2$)	Sunflower oil diet (3) ($n = 2$)	Ratio (1)/(3)
14:0	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.02	1.07
15:0	tr	–	–	–
15:1 (*n*–9)	0.83 ± 0.16	0.61 ± 0.06	0.66 ± 0.06	1.26 *
16:0	20.26 ± 0.36	19.68 ± 0.45	20.88 ± 0.38	0.97
16:1 (*n*–9)	0.33 ± 0.09	0.32 ± 0.01	0.30 ± 0.05	1.10
16:1 (*n*–7)	1.37 ± 0.46	2.01 ± 0.10	1.38 ± 0.22	0.99
17:0	0.42 ± 0.04	0.23 ± 0.02	0.22 ± 0.01	1.91 *
17:1 (*n*–9)	2.41 ± 0.02	2.55 ± 0.08	2.58 ± 0.66	0.93
18:0	18.20 ± 0.61	16.49 ± 0.11	17.71 ± 0.24	1.03
18:1	20.00 ± 0.15	22.44 ± 0.57	17.90 ± 0.56	1.12
18:2 (*n*–6)	1.43 ± 0.60	3.23 ± 0.18	6.11 ± 2.26	0.23 *
20:0	0.55 ± 0.03	0.62 ± 0.03	0.53 ± 0.04	1.04
20:1 (*n*–9)	0.50 ± 0.07	0.41 ± 0.26	0.49 ± 0.01	1.02
20:2 (*n*–6)	0.14 ± 0.06	0.15 ± 0.07	0.22 ± 0.02	0.64 *
20:3 (*n*–6)	0.46 ± 0.22	0.77 ± 0.02	0.76 ± 0.02	0.61 *
20:4 (*n*–6)	13.22 ± 0.60	11.13 ± 0.16	13.36 ± 0.63	0.99
22:0	–	0.22 ± 0.09	0.14 ± 0.05	–
22:4 (*n*–6)	2.88 ± 0.18	2.32 ± 0.06	2.84 ± 1.01	1.01
24:1 (*n*–9)	0.62 ± 0.02	0.99 ± 0.05	0.44 ± 0.04	1.41 *
22:5 (*n*–3)	–	0.26 ± 0.06	0.14 ± 0.06	–
22:6 (*n*–3)	12.42 ± 1.37	11.26 ± 0.76	10.60 ± 0.10	1.17
U.I.	169.5	159.8	167.8	1.01
Sat. FA's	39.59	37.39	39.63	1.0
Unsat. FA's	56.61	58.45	57.64	0.98
Sat./Unsat.	0.70	0.64	0.68	1.03
n–6 FA's	18.13	17.60	23.29	0.78 *
n–3 FA's	12.42	11.52	10.74	1.15
(*n*–6)/(*n*–3)	1.46	1.52	2.17	0.67 *

changes than the cortex. 24 fatty acids were detected and 10 of these differed by more than 20% between animals on saturated and unsaturated diets (Table IV). The $n-6$, $n-3$ fatty acids and the $n-6/n-3$ ratio also showed distinct differences between the two experimental diets.

Dietary lipids also had a distinct effect on heart mitochondrial phospholipid fatty acids of *E. amoenus* (Table V). Of the 16 fatty acids detected, 6 showed significant differences ($P < 0.05$; ANOVA). A further 6 fatty acids for which no statistical test was performed showed no overlap in values. Only 4 fatty acids were not significantly different. There was a notably high proportion (> 20%) of docosahexaenoic acid (22 : 6($n-3$)) in all dietary groups. Significant differences between the dietary groups were also observed for the unsaturation index and the total proportion of all unsaturated fatty acids. The unsaturation index of heart mitochondria was substantially greater than that observed for other membranes and tissues.

Liver mitochondrial phospholipid fatty acids composition also differed between the three diets (Table VI). Of the 17 fatty acids detected four differed significantly and a further seven fatty acids did not show overlap in values. Only six fatty acids were indistinguishable between the diet groups. The sum of the

TABLE IV

Brain (without cerebrum) total lipid fatty acid composition of hibernating Eutamias amoenus on the three different lipid diets

The fatty acids (FA) shown as the mean percentage ± S.D. The unsaturation index (U.I.) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; trace (tr) represents fatty acids that were present at < 0.1%. Statistical tests were not performed because of the small *n*. Animals on sheep fat and animals on sunflower oil were considered different (*) when the ratio of their fatty acid composition showed a change of > 20%. –, Fatty acid not present.

Fatty acid	Sheep fat diet (1) ($n = 2$)	Control diet (2) ($n = 2$)	Sunflower oil diet (3) ($n = 2$)	Ratio (1)/(3)
14:0	0.13 ± 0.02	0.13 ± 0	0.14 ± 0.02	0.93
15:0	tr	–	–	–
15:1 (*n*–9)	0.55 ± 0.04	0.52 ± 0.01	0.53 ± 0.05	1.04
16:0	15.84 ± 0.91	15.16 ± 0.02	15.51 ± 0.81	1.02
16:1 (*n*–9)	0.35 ± 0.03	0.28 ± 0.01	0.26 ± 0.01	1.35 *
16:1 (*n*–7)	1.70 ± 0.49	2.15 ± 0.06	1.72 ± 0.04	0.99
17:0	0.41 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	1.64 *
17:1 (*n*–9)	2.32 ± 0.12	2.42 ± 0.04	2.46 ± 0.05	0.94
18:0	14.08 ± 0.97	13.02 ± 0.54	13.18 ± 0.25	1.07
18:1	28.03 ± 1.31	30.04 ± 0.26	26.58 ± 0.42	1.05
18:2 (*n*–6)	1.59 ± 0.71	3.05 ± 0.16	5.59 ± 1.00	0.28 *
20:0	0.73 ± 0.02	0.85 ± 0.03	0.84 ± 0.08	0.87
20:1 (*n*–9)	1.57 ± 0.18	1.68 ± 0.01	1.58 ± 0.06	0.99
20:2 (*n*–6)	0.17 ± 0.10	0.23 ± 0.06	0.45 ± 0.11	0.38 *
20:3 (*n*–6)	0.66 ± 0.11	0.91 ± 0.08	0.96 ± 0.11	0.69 *
20:4 (*n*–6)	8.30 ± 0.62	7.13 ± 0.09	7.96 ± 0.02	1.04
22:0	0.68 ± 0.23	0.84 ± 0.12	0.86 ± 0.19	0.79 *
22:1 (*n*–9)	0.23 ± 0.02	0.21 ± 0.01	0.23 ± 0.01	1.00
20:5 (*n*–3)	–	tr	–	–
22:4 (*n*–6)	2.17 ± 0.57	1.75 ± 0.02	1.99 ± 0.13	1.09
24:0	0.74 ± 0.29	0.95 ± 0.09	0.97 ± 0.22	0.76 *
24:1 (*n*–9)	2.61 ± 0.59	3.36 ± 0.27	3.39 ± 0.18	0.77 *
22:5 (*n*–3)	0.04 ± 0.01	0.20 ± 0.16	0.34 ± 0.36	0.12 *
22:6 (*n*–3)	9.31 ± 1.02	7.51 ± 0.67	6.67 ± 0.13	1.40 *
U.I.	140.8	131.6	133.2	1.06
Sat. FA's	32.62	31.19	31.75	1.03
Unsat. FA's	59.60	61.55	60.70	0.98
Sat./Unsat.	0.55	0.51	0.52	1.06
n–6 FA's	12.89	13.07	16.95	0.76 *
n–3 FA's	9.35	7.71	7.01	1.33 *
(*n*–6)/(*n*–3)	1.38	1.70	2.42	0.57 *

TABLE V

Heart mitochondrial phospholipid fatty acid composition of hibernating Eutamias amoenus on the three different lipid diets

The fatty acids (FA) are shown as the mean percentage ± S.D. of the number of individuals investigated. The Unsaturation Index (U.I.) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; trace (tr) represents fatty acids that were present at < 0.1%. –, Fatty acid not present or statistical test not performed. n.s., not significant.

Fatty acid	Sheep fat diet (1) ($n = 3$)	Control diet (2) ($n = 3$)	Sunflower oil diet (3) ($n = 5$)	$P <$			
				Anova	Range test		
					1–2	1–3	2–3
14:0	0.10 ± 0.05	–	–	–			
16:0	15.40 ± 3.00	14.83 ± 1.84	13.41 ± 3.02	ns			
16:1 (*n*–7)	0.57 ± 0.04	0.84 ± 0.16	0.27 ± 0.10	0.001	n.s.	0.05	0.05
17:0	0.86 ± 0.49	0.62 ± 0.08	0.44 ± 0.03	0.0001	0.05	0.05	n.s.
18:0	27.91 ± 3.23	22.02 ± 2.54	24.37 ± 0.77	0.05	0.05	n.s.	n.s.
18:1	7.49 ± 1.89	9.67 ± 1.18	5.14 ± 0.70	0.005	n.s.	n.s.	0.05
18:2 (*n*–6)	14.55 ± 1.95	12.04 ± 2.54	16.73 ± 3.30	n.s.			
20:1 (*n*–9)	0.11 ± 0.01	0.37 ± 0.08	tr	–			
20:2 (*n*–6)	0.12 ± 0.02	0.26 ± 0.04	0.56 ± 0.13	0.01	n.s.	0.05	0.05
20:3 (*n*–6)	0.31 ± 0.01	0.30 ± 0.04	tr	–			
20:4 (*n*–6)	10.31 ± 1.29	8.55 ± 0.89	9.92 ± 1.72	n.s.			
20:5 (*n*–3)	0.57 ± 0.05	0.45 ± 0.04	tr	–			
22:4 (*n*–6)	0.12 ± 0.28	tr	tr	–			
24:0	0.67 ± 0.09	tr	0.41 ± 0.27	–			
22:5 (*n*–3)	0.53 ± 0.91	1.29 ± 0.24	0.98 ± 0.19	0.005	0.05	0.05	n.s.
22:6 (*n*–3)	20.93 ± 0.51	27.30 ± 3.96	27.10 ± 0.19	n.s.			
U.I.	207.6 ± 15.3	242.7 ± 3.96	247.1 ± 12.5	0.05			
Sat. FA's	45.1 ± 5.8	37.4 ± 4.0	38.6 ± 2.2	n.s.			
Unsat. FA's	55.0 ± 3.6	61.0 ± 3.8	60.7 ± 1.7	0.05			
Sat./Unsat.	0.97 ± 0.19	0.76 ± 0.15	0.70 ± 0.05	n.s.			
n–6 FA's	25.20 ± 2.40	21.10 ± 2.30	27.90 ± 3.90	n.s.			
n–3 FA's	21.80 ± 1.60	29.0 ± 4.2	28.10 ± 4.10	n.s.			
(n–6)/(n–3)	1.15 ± 0.03	0.74 ± 0.13	1.02 ± 0.27	n.s.			

$n - 6$ fatty acids also differed significantly between the diet groups ($P < 0.0025$; ANOVA).

Discussion

The present study shows that physiological changes of hibernation following dietary lipid treatment previously observed in *E. amoenus* [17] are associated with significant changes in the fatty acid composition of depot fat, brain and mitochondrial membranes. These diet-induced changes in the fatty acid saturation of both tissues and membranes were more pronounced than those observed previously in non-hibernating homeothermic mammals (Table VII). Furthermore, the composition of depot fat and mitochondrial membranes in *E. amoenus* reflected the composition of the diet, i.e., the animals on the unsaturated diet contained a greater proportion of unsaturated fatty acids than those on the saturated diet, which was not always the case in non-hibernators (Table VII).

However, not all tissues and membranes of *E. amoenus* responded in the same way to the dietary lipid manipulations. The changes in depot fat were most pronounced, more or less reflecting the diets of the different groups, although the unsaturation index of the saturated animals was much higher than that of the diets. This indicates that the saturated fat fed animals selectively incorporated unsaturated fatty acids from their relatively saturated diet to maintain high proportions of unsaturated fatty acids in their body fat.

Compositional changes in brain tissues were relatively small when compared with depot fat. However, when the two brain samples were compared, the diet-induced changes in the fatty acid composition of the cerebrum were less pronounced than those of the rest of the brain without the cerebrum. This supports previous findings that composition of lipids in the cerebrum during hibernation is less pronounced than those in the brain stem [23], which is the brain part that remains most active during hibernation [24].

Heart mitochondria of *E. amoenus* showed much more pronounced diet-induced differences in fatty acid composition than in non-hibernators (Tables V and VII). The heart remains very active during hibernation and a high proportion of unsaturated fatty acids in heart mitochondria may be important for maintenance of cardiac function at low body temperatures [25–27]. In contrast, heart mitochondria of non-hibernators only slightly change in response to dietary lipid treatment and it appears that this composition is designed for

TABLE VI

Liver mitochondrial phospholipid fatty acid composition of hibernating Eutamias amoenus on the three different lipid diets

The fatty acids (FA) are shown as the mean percentage ± S.D. of the number of individuals investigated. The Unsaturation Index (U.I.) is the sum of the % unsaturated fatty acids multiplied by their number of double bonds; trace (tr) represents fatty acids that were present at < 0.1%. -, Statistics not performed. n.s., not significant.

Fatty acid	Sheep fat diet (1) ($n = 3$)	Control diet (2) ($n = 4$)	Sunflower oil diet (3) ($n = 4$)	$P <$			
				Anova	Range test		
					1–2	1–3	2–3
14:0	0.26 ± 0.06	0.17 ± 0.06	tr	-			
15:0	0.15 ± 0.07	tr	tr	-			
16:0	10.83 ± 1.95	12.73 ± 3.03	11.69 ± 2.69	n.s.			
16:1 ($n-7$)	1.00 ± 0.46	2.00 ± 0.59	0.63 ± 0.16	0.025	n.s.	n.s.	0.05
17:0	1.19 ± 0.11	0.40 ± 0.11	0.39 ± 0.06	0.0001	0.05	0.05	n.s.
18:0	20.54 ± 2.91	20.53 ± 3.49	23.90 ± 5.17	n.s.			
18:1	27.07 ± 2.25	25.26 ± 2.36	12.97 ± 1.89	0.00001	n.s.	0.05	0.05
18:2 ($n-6$)	16.39 ± 0.94	16.95 ± 1.35	24.41 ± 3.26	0.0025	n.s.	0.05	0.05
21:0	0.84 ± 0.34	tr	tr	-			
20:2 ($n-6$)	tr	tr	1.87 ± 0.65	-			
20:3 ($n-6$)	0.92 ± 0.31	0.87 ± 0.42	0.75 ± 0.47	n.s.			
20:4 ($n-6$)	12.99 ± 2.01	14.94 ± 3.12	17.55 ± 2.18	n.s.			
20:5 ($n-3$)	0.73 ± 0.29	0.45 ± 0.18	tr	-			
23:0	0.63 ± 0.13	0.72 ± 0.39	tr	-			
22:4 ($n-6$)	tr	tr	0.55 ± 0.18	-			
22:5 ($n-3$)	0.63 ± 0.11	0.57 ± 0.08	0.50 ± 0.12	n.s.			
22:6 ($n-3$)	4.10 ± 1.49	6.47 ± 1.10	5.65 ± 2.30	n.s.			
U.I.	148.4 ± 11.5	166.7 ± 19.8	173.5 ± 23.0	n.s.			
Sat. FA's	34.1 ± 3.1	35.6 ± 6.2	35.9 ± 7.7	n.s.			
Unsat. FA's	64.6 ± 1.5	67.5 ± 5.2	64.6 ± 7.0	n.s.			
Sat./Unsat.	0.53 ± 0.06	0.52 ± 0.12	0.57 ± 0.17	n.s.			
n–6 FA's	31.1 ± 1.0	32.8 ± 3.3	45.0 ± 5.0	0.0025	n.s.	0.05	0.05
n–3 FA's	5.5 ± 1.9	7.5 ± 1.3	6.4 ± 2.5	n.s.			
(n–6)/(n–3)	6.07 ± 1.71	4.43 ± 0.52	8.10 ± 3.60	n.s.			

TABLE VII

Fatty acid unsaturation of tissues and membranes in non-hibernators (rat, marmoset, human) and a hibernator (chipmunk) on a saturated and unsaturated diet

U.I., unsaturation index is the sum of the % unsaturated fatty acids multiplied by their double bonds. P/S ratio, polyunsaturated fatty acids/saturated fatty acids ratio. (Species are: rat, *Rattus norvegicus;* marmoset, *Callithrix jacchus;* human, *Homo sapiens;* chipmunk *Eutamias amoenus*).

Species, Sample	Saturated diet (1)	Unsaturated diet (2)	Ratio (2)/(1)	Source
Depot fat (U.I.)				
Rat	88.2	142.8	1.62	[34]
Chipmunk	86.1	145.9	1.69	present study
Depot fat (P/S ratio)				
Human	0.44	0.62	1.41	[35]
Chipmunk	0.60	5.09	8.48	present study
Heart mitochondria (U.I.)				
Rat	205	207	1.01	[28]
Marmoset	162	153	0.94	[28]
Chipmunk	208	247	1.19	present study
Liver mitochondria (U.I.)				
Rat	183	197	1.08	[28]
Marmoset	164	161	0.98	[28]
Chipmunk	148	174	1.18	present study

optimal function at a constant high T_b. The compositional change of liver mitochondrial fatty acids in *E. amoenus* was less pronounced than that of heart mitochondria but clearly greater than that of non-hibernating species (Ref. 28; Table VII).

Geiser and Kenagy [17] suggested that the changes in thermoregulation, torpor duration and metabolic rate may be caused by diet-induced compositional changes of membrane lipid composition. The pronounced compositional changes of mitochondrial membranes of *E. amoenus* would support this notion. However, the present study shows that not only the membranes of *E. amoenus* are altered by diet and that the most pronounced changes occur in depot fat. It is, therefore, likely that changes of lipid composition of tissues and membranes affect the physiology of animals in a variety of ways.

Temperature acclimation is associated with a shift in thermal optimum of ectothermic organisms. The shift of the thermal optimum in turn is largely achieved by changes in membrane lipid composition which creates an appropriate physical environment for cellular functions [10]. Cold acclimation in particular is associated by increase in unsaturated fatty acids and increase in membrane fluidity [10,11]. Similar physical changes may occur in the unsaturated-fat-fed *E. amoenus*. Like in cold-acclimated ectotherms, the highly unsaturated membranes of the *E. amoenus* on unsaturated diet may allow the animal to lower their body temperature further than the animals on saturated diet because they can maintain membrane structure and function at lower T_b [3,6,10]. The set point for the regulation of body temperature could be directly influenced by the fatty acid composition of neural and other cellular membranes, or indirectly influenced by prostaglandins that are formed from polyunsaturated fatty acids and are involved in thermoregulation [29].

The diet-induced change in depot fat offers an alternative explanation. It has been suggested previously that the depot fat of hibernators must be fluid at low T_b because it supplies most fuel during the hibernation period [12,30]. Saturated depot fat would solidify at the low T_b values during hibernation and the supply of fuel would be interrupted. Hibernating animals with saturated lipid stores would have two options to avoid nutrient depletion, they either could regulate T_b at relatively high levels to prevent solidification of depot fat, or rewarm in relatively short intervals and restore supply of lipids in their blood stream. Both short torpor bouts and increase in body temperature have been observed in torpid animals on saturated diet [17,31]. The associated metabolic costs would most likely prevent successful completion of the hibernation season [17].

These interpretations may explain the differences in minimum T_b and duration of torpor of the experimental groups to a certain extent. However, they do not explain why at the same T_b (above the minimum T_b) animals on unsaturated diet have lower metabolic rates than those on saturated diet [17]. If the supply of lipid fuel were directly responsible one would predict the opposite effect because access to to fuel at low T_b should be easier and the metabolic rate higher in the unsaturated animals than the saturated ones. Recent observations may provide an explanation for the different metabolic rates in animals on different lipid diets. Unsaturated fatty acids are potent inhibitors of the binding of thyroid hormone T3 to isolated rat livers [32]. Thyroid hormones increase the metabolic rate of all body cells, therefore, a reduced binding of T3 should result in a metabolic depression. The depot fat of the unsaturated *E. amoenus* consisted of almost 60% linoleic acid, which proved to be a very potent inhibitor of T3 binding in the rat [32]. There are other observations on the differential effect of fatty acids of different chain length and saturation on cellular activity [33]. Proton conductance of brown fat mitochondria of hamsters (*Phodopus sungorus*) [33] differs substantially between various fatty acids and it is possible that the metabolic rate of animals is affected by the composition of their lipid fuel.

The results of the present study support the view that unsaturated lipids in tissues and membranes of mammals are important for successful hibernation. They suggest that the thermoregulation and metabolism of the hibernating animal are affected by the lipid composition of their tissues and membranes. However, there are a number of possibilities how the physiology of an animal could be affected by the lipid composition of their body.

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