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The Effect of Dietary Fatty Acids on the Pattern of Torpor in a Marsupial

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Abstract

Experimental evidence suggests that unsaturated dietary fatty acids enhance daily torpor and hibernation in rodents. We were interested in whether such dietary effects on torpor patterns are restricted to rodents or are a more general feature of mammalian torpor. We investigated the effect of an "unsaturated" diet (containing 10% sunflower seed oil) and a "saturated" diet (containing 10% sheep fat) on torpor of the feathertail glider, Acrobates pygmaeus *(Marsupialia). Torpid gliders on the unsaturated diet showed lower minimum body temperatures (T_b) (2.4° ± 0.3° C vs. 3.9° ± 0.6° C) and longer torpor bouts (5.0 ± 1.1 d vs. 2.1 ± 1.0 d) than animals on the saturated diet. The results indicate that dietary, unsaturated fatty acids are important in the seasonal acclimatization of heterothermic mammals.*

Introduction

Ectothermic vertebrates that are acclimated to cold temperatures generally show an increase in the proportion of unsaturated fatty acids in tissues and cellular membranes. This appears to be important for the maintenance of a suitable membrane fluidity, which is required for normal function at low body temperature (T_b) (Cossins, Friedlander, and Prosser 1977; Hazel 1988). Seasonal hibernation is also accompanied by compositional changes of tissues and cell membranes (Goldman 1975; Platner et al. 1976; Raison et al. 1981; Aloia and Raison 1989), but it appears that seasonal changes of membrane fluidity in hibernators are less pronounced than in ectothermic organisms (Aloia et al. 1986; Aloia and Raison 1989). However, measurements of membrane fluidity are unlikely to provide a complete molecular inter-

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pretation of membrane processes (Hazel 1988), and it is possible that some subtle but physiologically important seasonal differences in the physical properties of membrane lipids occur in hibernators (Raison 1984). This notion is supported by the observation that a diet containing high proportions of polyunsaturated fatty acids, which profoundly affected the lipid composition of tissues and mitochondrial membranes, enhanced daily torpor and hibernation in heterothermic rodents (Geiser and Kenagy 1987; Geiser 1990, 1991). It is therefore likely that the uptake of essential fatty acids, which are required for synthesis of most longer-chain polyunsaturated fatty acids, form part of the preparation for hibernation (Florant et al. 1990).

We were interested in whether the diet-induced changes in torpor patterns are restricted to rodents or are a more general feature of heterothermic mammals. We investigated the effect of unsaturated (10% sunflower seed oil) and saturated (10% sheep fat) lipid diets on the pattern of torpor in the marsupial feathertail glider, *Acrobates pygmaeus* (Acrobatidae) (10–14 g), which enters daily and prolonged torpor in the laboratory (Jones and Geiser 1992). The fatty acid composition of the two diets differed particularly in the content of polyunsaturated fatty acids, which were 2.8-fold higher in the unsaturated diet than in the saturated diet (table 1).

Material and Methods

Ten feathertail gliders (six males and four females) of mean body mass 11.6 ± 1.6 g (SD) were caught in winter 1989 on the New England Plateau of New South Wales at altitudes between 1,000 and 1,300 m. Although many attempts were made to obtain more animals from this area, no more individuals could be caught. The animals were transported to the University of New England in Armidale, New South Wales, and held in a controlled temperature cabinet at an air temperature (T_a) of 18.0° ± 0.5°C and a photoperiod of 12L:12D (light from 0600 to 1800 hours AEST). Animals were housed individually in cages provided with bedding and nesting material. They were fed daily with a mixture of Heinz high-protein cereal, honey, and water with a small addition of calcium and Pentavite. Apples and canned baby food (fruits) were also offered. Water was always provided. On September 25 animals were divided into two groups of matched body mass and sex ratio and were fed a mixture of 12.5% Heinz high-protein cereal, 12.5% honey, 75% water, and either (1) a 10% addition by weight of sunflower seed oil (unsaturated diet; polyunsaturated fatty acids 46.7%; body mass 11.7 ± 1.2 g) or (2) a 10% addition by weight of sheep kidney fat (saturated diet; polyunsaturated fatty acids 16.8%; body mass 12.3 ± 0.8 g). The fatty

TABLE 1

Percentage fatty acid composition of the two diets fed to the two groups of Acrobates pygmaeus

Fatty Acid	Sheep Fat Diet (Saturated)	Sunflower Seed Oil Diet (Unsaturated)
12:0	.5	2.1
14:0	3.2	3.4
14:1	.6	1.6
16:0	17.9	13.9
16:1	1.0	1.5
18:0	16.4	5.0
18:1	42.3	21.9
18:2	15.6	45.8
18:3	1.1	.6
20:0	.6	.9
21:0	...	.7
22:0	...	1.2
20:5	.1	.3
UI	78.9	119.9
PUFA	16.8	46.7

Note. UI, the unsaturation index (the sum of the percentage of unsaturated fatty acids multiplied by their number of double bonds); PUFA, the sum of polyunsaturated fatty acids.

acid composition of the two experimental diets differed substantially especially in the content of the essential fatty acid linoleic acid (18:2) (table 1). One gram of calcium powder and 10 drops of Pentavite were added to 100 g of the food mixture. Heinz high-protein cereal contains 19% protein, 5.5% fat, 58.1% carbohydrates, 12.6% moisture, 1.5% fiber, and minerals and vitamins. Individuals were offered daily 16 g of the diet mixture. To obtain a better understanding of how long it takes for dietary lipids to affect torpor patterns, we fed animals for only 15 d prior to physiological measurements, rather than 8 wk as in the previous experiments on rodents (Geiser and Kenagy 1987; Geiser 1991). The body mass after 15 d on the diets was 11.6 ± 0.4 g (unsaturated diet) and 12.5 ± 0.9 g (saturated diet).

After 15–26 d on the diet, animals were transferred to a 0.5-L respirometer vessel at a T_a of 8.0 ± 0.5°C, 12L:12D. Oxygen consumption ($\dot{V}O_2$) was measured in individual animals from 1700 hours until the following afternoon. Food and water were not available during these measurements. Animals entered torpor during the night after a short (usually 2–4-h) activity period. Animals were considered torpid when $\dot{V}O_2$ fell below 75% of the $\dot{V}O_2$ of normothermic, resting animals (RMR) at the same T_a (Hudson and Scott 1979), but $\dot{V}O_2$ always fell below 10% of RMR within 1 h of entering torpor. Flow rates were kept high (350 mL/min) at the beginning of the experiment to measure RMR of normothermic individuals. In the morning (about 1000 hours), after the animals had been in torpor for at least 4 h, the flow rate was reduced to 70 mL/min to measure the $\dot{V}O_2$ of torpid individuals. The T_a was then slowly reduced from 8°C (<0.02°C/min) until the animal increased its metabolic rate to prevent a further drop in T_b (Heller and Hammel 1972). Animals that showed this increase in $\dot{V}O_2$ were removed from the respirometer vessel and T_b was immediately measured by 2-cm rectal insertion of a calibrated (to the nearest 0.1°C) 38-gauge thermocouple probe that was read with an Omega HH-71 T electronic thermometer. The T_a at which an increase in $\dot{V}O_2$ was observed was called the minimum T_a, the T_b measured when the increase in $\dot{V}O_2$ occurred was called the minimum T_b, and the $\dot{V}O_2$ measured over 30 min before the increase in $\dot{V}O_2$ was called the minimum $\dot{V}O_2$ (Geiser and Kenagy 1987). Determination of the minimum T_b using this cooling procedure reveals values that are statistically indistinguishable from steady-state measurements of torpid hibernators that are maintained at a T_a below their minimum T_b throughout a torpor bout (Geiser and Kenagy 1988; Geiser, Hiebert, and Kenagy 1990). Oxygen consumption was measured with an Applied Electrochemistry S-3A oxygen analyzer and calculated according to the method of Withers (1977). Cooling experiments were conducted once on each individual, and, because each measurement lasted 1 d, the collection of data took from October 9 to October 20 (i.e., after 15–26 d on the diets). To exclude possible effects of the time on the two diets, measurements were alternated between the two dietary groups (i.e., saturated diet on day 1, unsaturated diet on day 2, etc.)

On October 21, after the animals had been on their diets for 27 d, the T_a in the temperature-controlled cabinet was reduced from 18° to 8.0° ± 0.5°C, which is close to the average environmental temperature on the New England Plateau in winter. The duration of torpor bouts was measured over a 10-d period at a T_a of 8°C by observing daily (0900–1000 hours) the displacement of sawdust from the back of torpid animals, which they clean off when they are aroused. Sawdust was placed on the back of the animals when they first were observed in torpor and was replaced after each normothermic period. If animals entered only daily torpor it was assumed that torpor bouts lasted 0.5 d. Food and water were available during these measurements.

Fatty acids of total lipids of the two diets were extracted and methylated according to the method of Lepage and Roy (1986). Methyl esters were extracted in hexane and analyses were performed in a computer-controlled Hewlett Packard 5890 Series II gas chromatograph with a 30-m capillary FFAP column (Alltech, Deerfield, Ill.). Data were analyzed with the Delta chromatography data system (Digital Systems, Brisbane).

Results are presented as means ± 1 SE; *n* is the number of individuals.

Results

All individuals entered torpor in the respirometer when food and water were withheld and T_a was 8°C. The mean time interval from the beginning of these experiments (1700 hours) until onset of torpor was 181 ± 32 min ($n = 5$) for animals on the unsaturated diet and 287 ± 129 min ($n = 5$) for animals on the saturated diet.

Significant differences between the diet groups were observed in variables derived from the cooling experiments (fig. 1). The minimum T_b was lower

Fig. 1. The minimum T_b *and the minimum* T_a *of torpid* Acrobates pygmaeus *on a saturated (*$n = 5$*) and an unsaturated (*$n = 5$*) diet. Values are means with SE, and both the minimum* T_b *and the minimum* T_a *were lower in the animals on the unsaturated diet (*$P < 0.05$*; t-test).*

in animals on the unsaturated diet (2.4° ± 0.3°C, $n = 5$) than in animals on the saturated diet (3.9° ± 0.6°C, $n = 5$; $P < 0.05$; $t = 2.34$). The lowest individual T_b measured was 1.6°C in an animal on the unsaturated diet and 2.4°C in an animal on the saturated diet. The minimum T_a also differed between diet groups (1.0° ± 0.2°C, $n = 5$, unsaturated diet; 2.8° ± 0.4°C, $n = 5$, saturated diet; $P < 0.05$; $t = 4.09$).

The minimum $\dot{V}O_2$ was indistinguishable between diet groups and was 0.056 ± 0.006 mL/g h (unsaturated diet, body mass 10.9 ± 0.4 g, $n = 5$) and 0.062 ± 0.005 mL/g h (saturated diet, body mass 11.9 ± 0.6 g, $n = 5$). The RMR at a T_a of 8°C was also similar in both diet groups (7.0 ± 0.3 mL/g h, unsaturated diet, $n = 4$; 6.9 ± 0.3 mL/g h, saturated diet, $n = 4$).

The mean duration of the longest torpor bout observed for each individual at a T_a of 8°C was 5.0 ± 1.1 d (unsaturated diet; $n = 5$) and 2.1 ± 1.0 d (saturated diet; $n = 5$), and the mean duration in the animals on the saturated diet was significantly shorter than that of the animals on the unsaturated diet (fig. 2; $P < 0.05$; $t = 1.95$).

Discussion

The present study supports previous observations that the composition of dietary lipids affects the pattern of torpor in mammals. Torpor was more pronounced in gliders on the unsaturated diet than in individuals on the saturated diets. In particular, the minimum T_b and T_a were lower and the duration of torpor was longer in animals on a diet enriched with polyunsaturated fatty acids than in animals on a saturated diet.

While most of the diet-induced physiological differences in the present study were similar to those observed previously in chipmunks, *Eutamias amoenus,* or deer mice, *Peromyscus maniculatus* (Geiser and Kenagy 1987; Geiser 1991), the metabolic rate of gliders did not seem to be affected by dietary lipids. It is possible that the gliders were not maintained on the experimental diets for long enough to elicit changes in metabolic rates. In contrast to earlier studies, in which animals were fed for more than 56 d, gliders had been maintained on the experimental diets for only 15–26 d before measurements of metabolism were made. Another likely reason for the lack of diet-induced differences in metabolic rates is the composition of experimental diets. In previous studies on chipmunks and deer mice, concentrations of polyunsaturated fatty acids, which appear to be responsible for the enhanced torpor, were more than sixfold greater in the unsaturated diets than in the saturated diets (Geiser 1990, 1991). In the present study the difference in the concentration of polyunsaturated fatty acids of the two

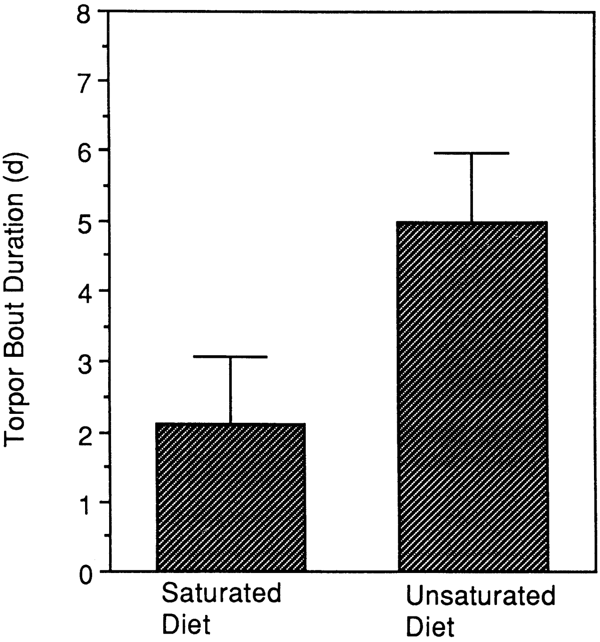

Fig. 2. The mean (with SE) of the longest torpor bout observed at a T_a *of 8° C in* Acrobates pygmaeus *on a saturated (*n = *5) and an unsaturated (*n = *5) diet. Torpor bouts were significantly longer in the animals on the unsaturated diet (*$P < 0.05$*;* t*-test).*

diets was only about threefold. In a similar study on hamsters (*Mesocricetus brandti*), in which no effects of dietary fatty acids on torpor patterns were observed, the difference between polyunsaturated fatty acid content of the two experimental diets was much less pronounced than in the previous and the present studies (Bartness et al. 1991), which lends some support to the second interpretation.

The diet-induced changes in torpor patterns of gliders observed here are most likely due to diet-induced differences in body lipid composition that are known to occur in homeothermic and heterothermic mammals (McMurchie 1988; Geiser 1990, 1991). The lipid composition of tissues and cell membranes could influence the physiology of the animals in a number of ways. For example, an increase of unsaturated fatty acids in cellular membranes may improve function of membrane-associated enzymes at low temperatures (McMurchie 1988) and therefore may allow a further drop in T_b. Alternatively, Hulbert and Else (1989) have suggested that the concentration of polyunsaturated fatty acids in cellular membranes may affect cellular metabolism via altered membrane permeability. Furthermore, compositional changes of neural membranes may directly affect thermoregulation, and the set point for T_b may be lowered when the concentration of unsaturated fatty acids is increased. A low T_b, no matter how it is caused, may in turn affect the duration of torpor bouts, because the minimum T_b is inversely related to the duration of torpor bouts in hibernators (Geiser et al. 1990).

Enhanced torpor may be important for the survival of gliders in the wild. On the New England Plateau, where the animals were collected, T_a during winter nights falls regularly below 0°C and may not rise above 10°C during daytime for a number of days. A low minimum T_b would ensure that the animals would less frequently encounter the T_a at which they need to increase their metabolism for defense of T_b during torpor. Furthermore, long torpor bouts may enable them to survive cold spells and to await improved conditions for foraging.

It is of particular interest that the thermal physiology is influenced by diet in a species that appears to be an opportunistic feeder that consumes insects, pollen, and nectar (Huang, Ward, and Lee 1987). Selection of unsaturated diets for winter preparation does not seem to occur in *Acrobates pygmaeus,* because seasonal changes in diet are coincidental with the seasonal change in abundance of food items (Huang et al. 1987). However, seed intake was particularly high in autumn, which suggests that the animals consume a diet rich in unsaturated fatty acids. Furthermore, insects, which form a major part (up to 80%) of the diet of this species, increase their content of unsaturated fatty acids during cold exposure (Fast 1970). As pollen intake also increases in winter (Huang et al. 1987), and pollen grains have a lipid cover that is rich in polyunsaturated fatty acids (Simpson and Neff 1983), it is possible that *A. pygmaeus* ingests more unsaturated and polyunsaturated fatty acids during autumn and winter without diet selection.

Marsupial and placental mammals have been separated for at least 100 million yr (Dawson 1983). The similarities in the diet-induced changes in torpor patterns of the unrelated marsupial *A. pygmaeus* and rodents suggest that torpor is affected by diet in at least some species of both mammalian subclasses. Thus, dietary lipids may play a role in seasonal acclimatization of many heterothermic mammals.

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