J Comp Physiol B (2001) 171: 189–194
DOI 10.1007/s003600000157

ORIGINAL PAPER

F. Falkenstein · G. Körtner · K. Watson · F. Geiser

Dietary fats and body lipid composition in relation to hibernation in free-ranging echidnas

Accepted: 29 October 2000 / Published online: 24 January 2001

Abstract Laboratory studies have shown that high levels of dietary unsaturated fatty acids prolong torpor and lower body temperatures in hibernating herbivorous rodents, which may in turn improve winter survival. The importance of nutritional ecology in relation to hibernation in insectivorous hibernators is unknown. We therefore studied fatty acid composition of dietary insects and the depot fat of echidnas *Tachyglossus aculeatus* (Monotremata) during the pre-hibernation season and compared depot fat fatty acid composition before and after hibernation. Echidna depot fat fatty acid composition during the pre-hibernation season was almost identical to that of the most abundant prey species, the ant *Iridomyrmex* sp. Oleic acid (C18:1) was by far the most common fatty acid in both *Iridomyrmex* sp. (60%) and echidna depot fat (62%). After about 5 months of hibernation and an 18% loss of body mass, echidna fatty acid composition had changed significantly. The percentage of the monounsaturated oleic acid (C18:1) and palmitoleic acid (C16:1) had declined, whereas that of the saturated fatty acids (C12:0, C16:0, C18:0) and the polyunsaturated linoleic acid (C18:2) had increased. Our study suggests that, unlike herbivorous rodent hibernators, echidnas rely to a large extent on monounsaturated fatty acids as fuel for hibernation, reflecting the most common fatty acid in their food. Moreover, it appears that the high concentration of monounsaturated fatty acids compensates for the moderate availability of polyunsaturates and enables them to hibernate at low body temperatures.

Communicated by I. D. Hume

F. Falkenstein (✉) · G. Körtner · F. Geiser
Zoology, School of Biological Sciences,
University of New England,
Armidale, NSW 2351, Australia
E-mail: ffalkens@metz.une.edu.au
Tel.: +61-2-67733923
Fax: +61-2-67733814

K. Watson
Human Biology, University of New England, Armidale,
NSW 2351, Australia

Abbreviations *C14:0* myristic acid · *C16:0* palmitic acid · *C16:1* palmitoleic acid · *C17:1* heptadecenoic acid · *C18:0* stearic acid · *C18:1* oleic acid · *C18:2* linoleic acid · *C18:3* linolenic acid · *MUFA* monounsaturated fatty acids · *PUFA* polyunsaturated fatty acids · *SFA* saturated fatty acids · T_b body temperature · *UFA* unsaturated fatty acids · *UI* unsaturation index

Introduction

Hibernating mammals, in contrast to their homeothermic relatives, are able to survive low body temperatures (T_b) between about 0 °C and 10 °C (Barnes 1989; Wang 1989; Arnold 1993). This thermal tolerance requires biochemical adaptations to ensure that physiological functions can be maintained (Azzam et al. 2000). In ectothermic organisms that are also able to tolerate low T_b, the most important adaptation for function at low T_b appears to be a high proportion of unsaturated fatty acids (Cossins and Lee 1985). While the role of unsaturated fatty acids for function at low T_b in hibernating mammals is less clear cut than in ectotherms (Aloia 1988; Hulbert 1993), they nevertheless appear to play an important role. Laboratory studies have shown that dietary unsaturated fatty acids result in lower T_b during torpor and lengthen torpor in rodent hibernators (Geiser and Kenagy 1987; Frank 1992; Florant 1998). This is believed to be due to the observed diet-induced increases of unsaturation in body lipids (Geiser 1990, 1993). An increase in depot fat unsaturation appears to be important for maintaining depot fat fluidity at low T_b so fatty acids can be metabolised during torpor. Moreover, membrane structure and function at low T_b appear to be improved by an increase in the proportion of unsaturated fatty acids (Aloia 1988). Thus, uptake of unsaturated fatty acids, and apparently most importantly essential fatty acids, which cannot be synthesised by mammals and must be incorporated through the diet, may form part of the preparation for hibernation

(Geiser and Kenagy 1987; Frank 1994; Schalk and Brigham 1995; Florant 1998).

Almost all published information on the effect of dietary fats on hibernation patterns and body lipid composition involved herbivorous rodent hibernators in the laboratory (Florant 1998). Hibernation is, however, not restricted to herbivorous mammals, but is common in insectivorous mammals such as bats and insectivores (Lyman et al. 1982), and also the echidna (*Tachyglossus aculeatus,* Monotremata), an egg-laying mammal (Grigg et al. 1992; Nicol and Andersen 1996). Echidnas are known to hibernate in many areas of Australia (Grigg et al. 1992; Nicol and Andersen 1996) including the New England Tablelands where their T_b falls to about 6 °C (Falkenstein et al. 1998). Nevertheless, the uptake of dietary fatty acids in relation to hibernation in echidnas has never been studied. Moreover, the utilization of depot fat fatty acids during hibernation in echidnas, as for other insectivorous hibernators, is presently unknown. To determine the possible involvement of dietary fatty acids in echidna hibernation we compared: (i) fatty acid composition of depot fat of dietary items and free-ranging echidnas during the pre-hibernation period and (ii) depot fat fatty acid composition before and after the hibernation season.

Materials and methods

Five echidnas were captured on the New England Tablelands at Oxley Wild Rivers National Park (30°40′S, 151°45′E, about 1000 m ASL) between 13 March 1998 and 6 April 1998 and six between 26 February 1999 and 5 April 1999 shortly before the onset of hibernation. Four echidnas were captured between 16 September 1999 and 4 September 1999 after their final arousal (Fig. 1). Animals were transferred to the University of New England and weighed to the nearest 1 g. Total mass loss and specific mass loss (g g^{-1} day^{-1}) were calculated from pre- and post-hibernation masses and the duration of the hibernation season of individuals was determined by temperature-telemetry. Fat samples (approx. 50 mg; $n=5$ pre-hibernation 1998, $n=6$ pre-hibernation 1999; $n=4$ post-hibernation 1999) were taken under general anaesthesia (Forthane) from peritoneal and subcutaneous depot fats. Animals were released at the point of capture after 3 days observation.

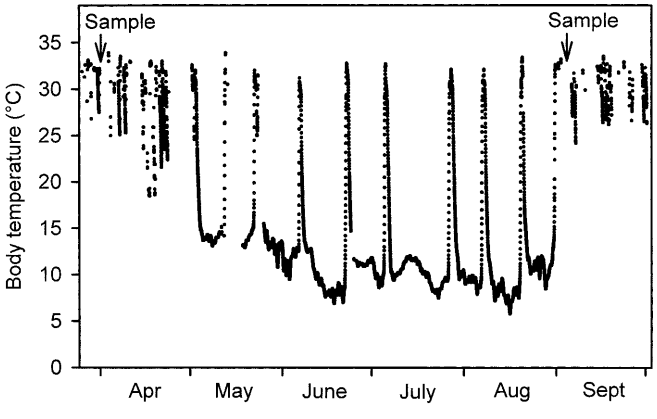

Fig. 1 Body temperature trace of a female echidna (4.1 kg) from pre-hibernation fattening to post-hibernation in 1999. *Arrows* denote when fat samples for fatty acid analysis were taken. T_b was about 32 °C during activity and fell by about 18 °C in April during brief bouts of torpor. The lowest T_b during prolonged bouts of torpor, was 6 °C and was observed between June and August

For temperature-telemetry, eight echidnas were fitted with intraperitoneal temperature-sensitive transmitters (Sirtrack: double stage, 22 g, frequency 151.220–151.780 MHz). Prior to implantation, transmitters were calibrated to the nearest 0.1 °C in a water bath using a mercury thermometer (Körtner and Geiser 1998). After release, temperature readings were taken three times per week using a tracking receiver (TR-4, Telonics) and a stopwatch. In addition, recording stations inside the animals' home ranges were erected. A recording station consisted of a modified scanner receiver and a custom-designed data logger based on a microprocessor, programmed to sample every 20 min (see Körtner and Geiser 1998 for detailed methods and Fig. 1 for body temperatures).

Major diet components were determined from faecal scats ($n=31$) collected over 2 years and from field observations. Fresh scats were collected from open ground or collected directly from echidnas which frequently defecated when lifted off the ground and left to curl up. Echidna scats found in the open are fresh as they disintegrate within a few days (Smith et al. 1989). Scats were soaked for 1 week in water, separated and washed with water through two different sized meshes (1 mm and 0.2 mm). Intact specimens were separated and classified using a stereomicroscope. From these and opportunistic observations in the field, we could establish that ants from the genera *Iridomyrmex* (ants 2–5 mm) and *Rhytidoponera* (ants 6–10 mm), and Isoptera (termites, 4–7 mm) were the major diet components during the active season. Coleoptera (beetle larvae 1.5–2.5 cm) were also often consumed.

From each of these diet components, samples were collected at the study site prior to echidna hibernation on 8–10 March 1999. The insect samples were obtained using an aspirator (Shattuck 1999) with a 6 mm glass tube connected to a sampling vial from mostly under rocks (<10 kg and >20 cm in diameter) and decomposing wood (area covered $>10\times30$ cm). Sampling was conducted for three afternoons for 5 h each when most echidnas are active and foraging. During the pre-hibernation season, about 100 rocks and 30 logs were turned and we found four nests of *Rhytidoponera* sp. (four larval and four adult samples), five nests of *Iridomyrmex* sp. (five larval samples, adult numbers were too low to comprise a significant dietary item), five Isoptera nests (five adult samples, larval numbers were too low to comprise a significant dietary item) and four Coleoptera (four larval samples). We assume that our sampling closely matched available prey because echidnas usually feed only briefly on insects close to the surface (Abensperg-Traun 1988). Samples used for fatty acid analysis weighed between 0.05 g and 0.2 g and the individual beetle larvae weighed between 1 g and 2 g. The insects were frozen at –30 °C, and were separated into adults and juvenile forms in the laboratory shortly after collection.

Echidna depot fats and insect samples were transesterified using a direct transesterification procedure (Rule 1997). This one-step procedure allowed us to extract all methylated fatty acids in a single tube minimising contamination and loss of lipid. Before methylation, insect samples were homogenised with a glass rod in their sampling tubes. For the methylation of total lipid fatty acids 2 ml 14% boron-trifluoride in methanol and 2 ml methanol were added to tubes, capped under a stream of N_2 to prevent oxidation and placed on a heating block set at 80 °C for 3 h (vortexed every 10 min). The methyl esters were dissolved in hexane, bubbled with N_2, capped under a stream of N_2, and stored in a freezer at –30 °C for up to 1 month or at –70 °C if they had to be stored for longer than 1 month.

A gas chromatograph (HP 5890 Series II, flame ionisation detection) with a silica capillary column (Alltech, EC-1000) was used for analysis of fatty acid methyl esters. Samples were run isothermically at 200 °C for 60 min using Helium as carrier gas. To

identify the lipids, two standards were used: Sigma 189–19 (37 fatty acid methyl esters, ranging from C4:0 to C24:1) and Sigma 189–1 (C16:0, C18:0, C18:1, C18:2, C18:3 in equal parts). Standards were run after every ten samples. The chromatograms were integrated with DELTA Junior V.4 software (Digital Solutions) and the fatty acid composition expressed as percentages.

For statistical analysis, all percentage values were converted to $x = arcsine[square - root(y/100)]$. Fatty acids below 0.1% (using pre-hibernation 1999 echidna samples as reference) were excluded from further analysis – these were C8:0, C20:1 and one unidentified fatty acid between C13:0 and C14:0. Sums of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA, two double bonds or more) total unsaturated fatty acids (UFA) and the unsaturation index (UI, the sum of the percentage of unsaturated fatty acids multiplied by their number of double bonds) were also tested. As percentages for peritoneal and subcutaneous depot fat fatty acid types in all individuals investigated ($n = 6$, pre-hibernation 1999) did not differ (t-test $P > 0.05$) they were subsequently combined into one mean. Depot fat fatty acid composition in other hibernating species (*Marmota flaviventris*) have been found previously to be uniform between different white adipose depots in the same animal (Florant et al. 1990). Likewise, the fatty acids of *Rhytidoponera* sp. larva did not differ significantly from the adults and were also pooled.

To test if echidna depot fat fatty acids differed from those of insects sampled before the hibernation season, a one-way ANOVA was applied. This was followed by Dunnett's one-way multiple comparison test (MINITAB) to determine which fatty acid of which insect differed or matched the echidna depot fat. To test whether echidna depot fat fatty acid composition changed during hibernation and between years a t-test was used. Numeric values are expressed as means ± 1SD for the number of individuals sampled (n).

Results

Body mass of echidnas decreased significantly from the pre-hibernation season to the post-hibernation season. Average mass during the pre-hibernation season was 4,673 ± 849 g ($n = 7$) and decreased to 3,934 ± 986 g ($n = 5$) after the final arousal. Body mass loss over the 149 ± 32 day hibernation season was 839 ± 250 g and 0.044 ± 0.027 g $g^{-1}day^{-1}$ ($n = 5$). For those individuals for whom fat samples were available for both the pre-hibernation (body mass 4288 ± 800 g, $n = 3$) and post-hibernation (body mass 3470 ± 601 g, $n = 3$) season, body mass loss was similar (818 ± 300 g, $n = 3$).

Oleic acid (C18:1) was the most abundant fatty acid in both insects (40–60%) and echidnas (50–62%; Table 1). Second most abundant was the saturate palmitic acid (C16:0) in the echidna (17–21%), *Iridomyrmex* sp. (20%) and beetle larvae (26%), but ranked third in *Rhytidoponera* sp. (15%) and the Isoptera (11%). Palmitoleic acid (C16:1) was the third most

Table 1 Percent total lipid fatty acid composition of echidna depot fat and major dietary items during the pre-hibernation season and in echidnas after hibernation. The various fatty acids are quantified as the mean percent ± SD. Significant differences are marked and the sums of the number of differences are shown. [*MUFA* sum of monounsaturated fatty acids, *PUFA* sum of polyunsaturated fatty acids, *SFA* sum of saturated fatty acids, *Tr* fatty acids that were present at < 0.1% (trace), *UFA* sum of unsaturated fatty acids (MUFA + PUFA), *UI* the unsaturation index is the sum of percentage of unsaturated fatty acids multiplied by their number of double bonds]

Fatty acid type	1998 pre-hibernation Echidna $n = 5$	1999 pre-hibernation					1999 post-hibernation Echidna $n = 4$
		Echidna $n = 6$	*Iridomyrmex* $n = 5$ (Larvae)	*Rhytidoponera* $n = 8$ (4 larvae, 4 adult)	Coleoptera $n = 4$ (Larvae)	Isoptera $n = 5$ (Adult)	
C10:0	0.95 ± 0.19	0.52 ± 0.09[a]	0.47 ± 0.22	1.06 ± 0.88	Tr[b]	0.11 ± 0.17[b]	0.45 ± 0.6
C12:0	3.46 ± 0.61	2.58 ± 0.73	1.86 ± 0.88	2.57 ± 1.01	0.92 ± 0.78[b]	1.79 ± 1.89	3.82 ± 0.73[c]
C13:0	0.45 ± 0.28	0.17 ± 0.06	0	0.16 ± 0.32	1.18 ± 0.85	1.57 ± 0.35 [b]	0.26 ± 0.31
C14:0	0.17 ± 0.27	0.10 ± 0.11	0	0.11 ± 0.15	1.36 ± 1.57	0.55 ± 0.32	0.13 ± 0.25
C16:0	19.47 ± 1.85	17.25 ± 0.92	20.18 ± 1.96	14.84 ± 3.03	26.18 ± 3.78[b]	10.69 ± 2.01[b]	20.80 ± 1.24[c]
C16:1	7.27 ± 0.47	6.76 ± 0.86	4.62 ± 1.02	4.69 ± 1.18 [b]	4.98 ± 2.61	3.13 ± 0.18[b]	4.90 ± 0.35[c]
C17:0	0.28 ± 0.23	0.32 ± 0.14	Tr	0.23 ± 0.20	0.73 ± 0.85	0.64 ± 0.38	0.15 ± 0.29
C17:1	Tr	0.46 ± 0.03[a]	0.17 ± 0.16[b]	0.62 ± 0.05	1.27 ± 0.63[b]	1.46 ± 0.41[b]	Tr [c]
C18:0	3.98 ± 0.59	3.13 ± 0.17[a]	2.68 ± 1.23	4.71 ± 0.95	2.76 ± 2.18	7.29 ± 1.68[b]	7.78 ± 1.0 [c]
C18:1	56.81 ± 0.98	61.67 ± 1.50[a]	60.13 ± 6.30	46.81 ± 5.03[b]	39.68 ± 15.15[b]	55.60 ± 2.46	50.29 ± 4.96[c]
C18:2	5.72 ± 1.90	5.38 ± 1.27	5.28 ± 2.56	18.30 ± 3.45[b]	18.02 ± 9.39[b]	12.23 ± 1.27[b]	7.55 ± 1.28[c]
C18:3	0.44 ± 0.24	0.63 ± 0.38	0.88 ± 0.33	1.56 ± 0.37[b]	1.92 ± 1.22[b]	0 [b]	0.23 ± 0.46
C20:0	0.25 ± 0.19	0.58 ± 0.11	0.72 ± 0.51	1.02 ± 0.24	0.40 ± 0.32	1.52 ± 0.39[b]	0.26 ± 0.36
C20:4	0.11 ± 0.11	0.25 ± 0.09	0.32 ± 0.25	1.56 ± 0.97[b]	0.13 ± 0.27	1.61 ± 0.98[b]	1.94 ± 3.27
C22:1	0.10 ± 0.10	0.12 ± 0.31	0.13 ± 0.12	0.97 ± 0.80[b]	Tr	0.50 ± 0.32	Tr
No. of significant differences		4	1	6	7	10	7
SFA	29.19 ± 2.47	24.67 ± 1.25[a]	28.45 ± 6.26	25.44 ± 3.35	33.53 ± 7.46[b]	24.17 ± 2.16	34.90 ± 1.75[c]
MUFA	64.38 ± 0.90	69.05 ± 1.31[a]	65.06 ± 5.66	53.11 ± 4.74[b]	45.92 ± 17.13[b]	60.78 ± 2.33	55.19 ± 5.09[c]
PUFA	6.27 ± 2.10	6.26 ± 1.37	6.49 ± 2.73	21.43 ± 4.59[b]	20.08 ± 10.67[b]	13.84 ± 2.24[b]	9.72 ± 3.87
UFA	70.65 ± 2.38	75.32 ± 1.23[a]	71.55 ± 6.26	74.54 ± 3.34	66.00 ± 7.83[b]	74.62 ± 3.2	64.91 ± 1.56[c]
UI	77.59 ± 4.59	82.70 ± 2.45	79.56 ± 8.5	100.67 ± 8.52[b]	88.27 ± 8.22	91.68 ± 6.89	78.75 ± 9.96

[a,c] Significant differences between echidna fatty acids in the two years between 1999 pre-hibernation and post-hibernation, respectively, (determined using a t-test)

[b] Significant differences between diet groups and echidnas in 1999, determined using ANOVA followed by Dunnett's one-way multiple comparison

abundant fatty acid in the echidna (5–7%) and ranked fourth in the insects (3–5%). The essential fatty acid linoleic acid (C18:2) ranked fourth in the echidna (5–8%) and second or third in the insects (5–18%). During pre-hibernation, total UFA ranged between 70.7% and 75.3% in most cases, but was slightly lower (66%) in the coleoptera.

During the pre-hibernation season of 1999, of the 15 fatty acids identified, the fatty acids in depot fats of echidnas best matched those of *Iridomyrmex* sp. (Table 1). Importantly, the essential linoleic acid (C18:2), which cannot be synthesised by echidnas, was found in almost identical proportions in *Iridomyrmex* sp. and echidna fat. Of the 15 fatty acids identified 14 matched and only heptadecenoic acid (C17:1), one of the minor fatty acids ($<0.5\%$), differed between *Iridomyrmex* sp. and echidna fat. Groups of fatty acids and the UI also best matched between echidnas and *Iridomyrmex* sp.

For *Rhytidoponera* sp. and beetle larvae, six and seven fatty acids, respectively, differed from echidna fat and substantial differences were observed for oleic acid (C18:1) and linoleic acid (C18:2; Table 1). Differences were also observed for PUFA in *Rhytidoponera* sp. and beetle larvae, and MUFA and the UI in *Rhytidoponera* sp. The Isoptera showed the greatest differences with two thirds of all fatty acids (10 of 15) and all the fatty acid sums differing from those of the echidnas.

Pre-hibernation depot fat fatty acid composition of echidnas between the years 1998 and 1999 was similar. Differences were only observed in the saturates C10:0 and C18:0 and in the monounsaturates C17:1 and C18:1. The latter was reflected in the significant differences of SFA, MFA and UFA. Amounts of polyunsaturates and their sum were almost identical between years.

The post-hibernation depot fat fatty acid composition of echidnas in 1999 differed significantly from the pre-hibernation sample (Table 1). After hibernation, the saturated fatty acids (C12:0, C16:0 and C18:0) as well as the essential polyunsaturate linoleic acid (C18:2) were present in significantly greater percentages than before hibernation. In contrast, the monounsaturates (C16:1 and C18:1) showed a significant decrease (Table 1; Fig. 2A). The sum of SFA (Fig. 2B) increased significantly after hibernation whereas the UFA showed a significant decline, which was due to the 14% reduction in MUFA. The PUFA showed a slight increase, which was due to the significant increase in linoleic acid (C18:2), but overall the seasonal change in PUFA was not significant.

Because of seasonal changes in fatty acid composition, the ranking of abundance of the different fatty acid types also changed (Table 1). Stearic acid (C18:0) and linoleic acid (C18:2) were now, unlike during the pre-hibernation season, at higher concentrations than the monounsaturate palmitoleic acid (C16:1). Oleic acid (C18:1) remained the dominant fat in echidnas and palmitic acid (C16:0) remained the second most abundant.

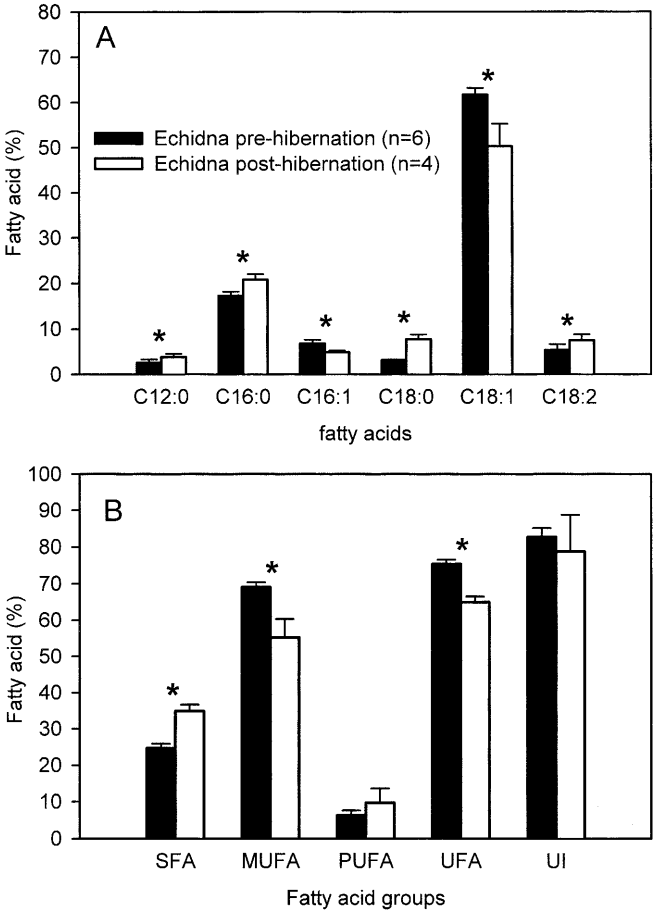

Fig. 2 **A** Major depot fat fatty acids ($>1\%$) in echidnas before and after hibernation (mean ± SD) in 1999. **B** Fatty acid groups in echidnas before and after hibernation (mean ± SD) in 1999. [*MUFA* sum of monounsaturated fatty acids, *PUFA* sum of polyunsaturated fatty acids, *SFA* sum of saturated fatty acids, *UFA* sum of unsaturated fatty acids (MUFA + PUFA), *UI* the unsaturation index is the sum of percentage of unsaturated fatty acids multiplied by their number of double bonds]. *Bars* denoted with an asterisk (*) are significantly different at $P<0.05$

Discussion

Our study provides the first evidence on dietary fatty acid uptake and fatty acid use during hibernation in free-ranging echidnas. It shows that, as in herbivorous hibernators, the fatty acid composition of depot fat of insectivorous echidnas reflects that of their diet. Pre-hibernation depot fat fatty acid compositions between years varied only little.

During the average hibernation season of 5 months, body mass of echidnas declined by about 18% and the proportion of roughly half of the depot fat fatty acids changed significantly. As the monounsaturated fatty acids C16:1 and more importantly C18:1 declined from 6.8% to 4.9% and 61.7% to 50.3%, respectively, during hibernation, it appears that they are predominately used as fuel.

Abundance in the field, scat analysis, and matching fatty acid composition strongly suggest that the ant

Iridomyrmex sp. is the most important food source of echidnas on the New England Tablelands. It has been shown previously, that depot fat of free-ranging ground squirrels *Spermophilus beldingi* closely reflects their diet (Frank 1991). As in echidnas, the high proportion of unsaturates in white adipose tissue in *S. beldingi* was apparently due to the accumulation of fatty acids derived from their food (Frank 1991). Our conclusion that depot fat fatty acid composition of echidnas reflects that of their diet is also supported by previous laboratory investigations on hibernating herbivorous, sciurid rodents, in which depot fat fatty acid composition closely resembled their diet (Geiser 1990; Frank 1992; Geiser et al. 1994). Close similarities between dietary and depot fat fatty acids also have been shown for deer mice (*Peromyscus maniculatus*), which do not hibernate, but enter daily torpor instead (Geiser 1991). Furthermore, the depot fat fatty acid composition of a captive echidna, with unusually high levels of myristic acid (C14:0), closely resembled that of their diet consisting of milk, sheep's blood and ground liver (Bolliger and Shorland 1963). Thus depot fat fatty acid composition may be a useful tool in determining major dietary items in the field.

Iridomyrmex spp. are virtually ubiquitous on the Australian continent (Shattuck 1999). They are classed as extremely abundant, active and aggressive and the presence of other ant species often depends on the adaptations employed in avoiding interaction with *Iridomyrmex* spp. (Andersen 1995). *Iridomyrmex* spp. are also the dominant ants on the New England Tablelands, as determined in a forest 30 km from our study site (Lobry de Bruyn et al. 1997). In Western Australia, *Iridomyrmex* spp. in the size range 2–4 mm account for $>75\%$ of the formicid numbers in pitfall traps, corresponding with the predominant formicid size group preyed on by echidnas (Abensperg-Traun 1988). Furthermore, Griffiths and Simpson (1966) reported that echidnas on the Southern Tablelands near Canberra penetrated mound nests of *Iridomyrmex* spp. From the above it appears that echidnas on the New England Tablelands and in Western Australia (Abensperg-Traun and De Boer 1992) are opportunistic foragers, with echidnas in the New England Tablelands often feeding on the small *Iridomyrmex* because they are abundant. However, although we found no evidence of selective foraging for less abundant food sources we cannot entirely exclude it.

The results of the present study indicate that echidnas metabolise MUFA, mainly C16:1 and C18:1, during hibernation in preference over SFA and PUFA. In particular, linoleic acid (C18:2) was present at a higher percentage after hibernation than before. It is possible that linoleic acid is important for reproduction after the hibernation season, as it is a precursor for prostaglandins (Gurr and James 1980). The saturated fatty acids C12:0, C16:0 and C18:0 were also spared, and linolenic acid (C18:3) did not change significantly. White adipose tissue fatty acid composition changes during hibernation in ground squirrels *Spermophilus lateralis* showed some similarities and some differences to our study. As in echidnas, C16:1 decreased and C18:2 increased in ground squirrels, however, C16:0 decreased in ground squirrels whereas it increased in echidnas. Moreover, oleic acid (C18:1) apparently most important for fuel in hibernating echidnas, increased proportionally in hibernating ground squirrels (Frank and Storey 1995). In yellow-bellied marmots, *Marmota flaviventris,* SFA were predominantly metabolised, dienes were spared and MUFA showed inconsistent changes among individuals (Florant et al. 1990). Thus, it appears that the use of fatty acids for fuel during the hibernation season differs among hibernators, likely reflecting abundance in their diet, physiological needs, and perhaps phylogenetic differences.

In comparison to free-ranging Belding's ground squirrels (*Spermophilus beldingi*) during autumn (Frank 1991), the essential fatty acid content in echidna depot fat was very low. Whereas in the abdominal fats of *S. beldingi* the sum of essential fatty acids (C18:2 and C18:3) was about equal to levels of C18:1 during prehibernation (approx. 35%), this was not the case in echidnas. In depot fat of echidnas C18:1 was about ten-times more abundant than the essential fatty acids (C18:2, C18:3). Nevertheless, echidnas are obviously capable of metabolising C18:1 at T_bs below 10 °C. As discussed above, low levels of polyunsaturate content in depot fats are likely a reflection of availability of fatty acids in the natural diet. However, as echidnas in New England hibernate under much milder conditions than for example arctic ground squirrels (Barnes 1989), oleic acid, which will be fluid at the T_b of torpid echidnas, appears suitable to maintain fuel supply during hibernation.

It appears that low levels of essential fatty acids in depot fat of hibernators can be compensated by high levels of MUFA. Two laboratory studies support this interpretation. Geiser et al. (1994) and Frank and Storey (1996) have provided evidence that support the argument that hibernators may not necessarily need high levels of polyunsaturates to function at low T_b. The yellow-pine chipmunk *Tamias amoenus* fed on three different diets supplemented with 5% of a saturated, monounsaturated and a diunsaturated C18 fatty acid showed big differences in pattern of hibernation and minimum T_b between the saturated and the unsaturated group (Geiser et al. 1994). Only minor differences were observed between the monounsaturated and the diunsaturated group (Geiser et al. 1994). A similar response was observed in *Spermophilus lateralis* (Frank and Storey 1996). Torpor bout duration in *S. lateralis* on the high SFA diet was significantly shorter than those on the high MUFA diet and the high PUFA diet, but no differences were observed between high MUFA and PUFA (Frank and Storey 1996). These observations may explain why echidnas survive hibernation with a low level of essential fatty acids (linoleic C18:2 and linolenic C18:3 acid about 10%). It appears the high concentra-

tion of oleic acid (C18:1, 62%) can compensate for the low PUFA. It has been established that the addition of one double bond has the most profound effect on fatty acid fluidity, whereas the addition of a further double bond only causes a minor physical change (Cossins and Lee 1985).

Our study shows that unlike in rodent hibernators, the insectivorous echidna relies to a large extent on monounsaturated fatty acids as a fuel source and perhaps to maintain physiological function during hibernation. It would be interesting to know whether this is a general feature of insectivorous hibernators or whether nutritional requirements differ between hibernators from different climatic regions.

Acknowledgements We would like to thank Kerryn Carter, Mark Deutschlander, Bert Jenkins, Florian Lüders, Martyn Prior, Annette Templeton and Wendy Westman for help with field work, Tracy Swan for help with the gas chromatograph, and Stuart Cairns for statistical advice. This work was supported by a grant from the Australian Research Council to FG. Experiments were approved by the University of New England Animal Ethics Committee.

References

Abensperg-Traun MA (1988) Food preference of the echidna, *Tachyglossus aculeatus* (Monotremata: Tachyglossidae), in the wheatbelt of Western Australia. Aust Mammal 11:117–123

Abensperg-Traun MA, De Boer ES (1992) The foraging ecology of a termite- and ant-eating specialist, the echidna *Tachyglossus aculeatus* (Monotremata: Tachyglossidae). J Zool (Lond) 226:243–257

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

Andersen AN (1995) A classification of Australian ant communities, based on functional groups which parallel plant life-forms in relation to stress and disturbance. J Biogeogr 22:15–29

Arnold W (1993) Energetics of social hibernation. In: Carey C, Florant GL, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological and molecular mechanisms. Westview, Boulder, pp 65–80

Azzam NA, Hallenbeck JM, Kachar B (2000) Membrane changes during hibernation. Nature 407:317–318

Barnes BM (1989) Freeze avoidance in a mammal: body temperatures below 0 °C in an arctic hibernator. Science 244:1521–1616

Bolliger A, Shorland FB (1963) Investigation on fats of Australian mammals. Aust J Sci 25:453–456

Cossins AR, Lee JAC (1985) The adaptation of membrane structure and lipid composition to cold. In: Gilles R (ed) Circulation, respiration, and metabolism. Springer, Berlin Heidelberg New York, pp 543–552

Falkenstein F, Körtner G, Geiser F (1998) Hibernation of echidnas on the New England Tablelands (abstract). Scientific Meeting Australian Mammal Society, Perth, Western Australia, p 9

Florant GL (1998) Lipid metabolism in hibernators: the importance of essential fatty acids. Am J Zool 38:331–340

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

Frank CL (1991) Adaptations for hibernation in the depot fats of a ground squirrel (*Spermophilus beldingi*). Can J Zool 69: 2707–2711

Frank CL (1992) The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). Physiol Zool 65:906–920

Frank CL (1994) Polyunsaturate content and diet selection by ground squirrels (*Spermophilus lateralis*). Ecology 75:458–463

Frank CL, Storey KB (1995) The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). J Comp Physiol B 164:536–542

Frank CL, Storey KB (1996) The effect of total unsaturate content on hibernation. In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold. Tenth International Hibernation Symposium, University of New England Press, Armidale, pp 211–216

Geiser F (1990) The effect of polyunsaturated and saturated dietary lipids on adipose tissue, brain and mitochondrial membrane fatty acid composition of a mammalian hibernator. Biochim Biophys Acta 1046:159–166

Geiser F (1991) The effect of unsaturated and saturated dietary lipids on the pattern of daily torpor and the fatty acid composition of tissues and membranes of the deer mouse *Peromyscus maniculatus*. J Comp Physiol B 161:590–597

Geiser F (1993) Dietary lipids and thermal physiology. In: Carey C, Florant BA, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological and molecular mechanisms. Westview, Boulder, pp 141–153

Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. Am J Physiol 252:R897–R901

Geiser F, McAllan BM, Kenagy GJ (1994) The degree of dietary fatty acid unsaturation affects torpor patterns and lipid composition of a hibernator. J Comp Physiol B 164:299–305

Griffiths M, Simpson KG (1966) A seasonal feeding habit of spiny ant-eaters. CSIRO Wildl Res 11:137–143

Grigg GC, Augee ML, Beard LA (1992) Thermal relations of free-living echidnas during activity and in hibernation in a cold climate. In: Augee ML (ed) Platypus and echidnas. R Zool Soc NSW, Sydney, pp 160–173

Gurr MI, James AT (1980) Lipid biochemistry: an introduction, 3rd edn. Chapman and Hall, London

Hulbert AJ (1993) Membrane adaptations in ectotherms and endotherms. In: Carey C, Florant BA, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological and molecular mechanisms. Westview, Boulder, pp 417–428

Körtner G, Geiser F (1998) Ecology of natural hibernation in the marsupial mountain pygmy-possum (*Burramys parvus*). Oecologia 113:170–178

Lobry de Bruyn LA, Jenkins BA, Sutrisno (1997) Soil invertebrate biodiversity in stringybark forest in the New England Tablelands before clearing. Mem Mus Victoria 56:295–303

Lyman CP, Willis JS, Malan A, Wang LCH (eds) (1982) Hibernation and torpor in mammals and birds. Academic Press, New York

Nicol S, Andersen NA (1996) Hibernation in the echidna: not an adaptation to the cold? In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold. Tenth International Hibernation Symposium, University of New England Press, Armidale, pp 7–12

Rule DC (1997) Direct transesterification of total fatty acids of adipose tissue, and of freeze-dried muscle and liver with boron-trifluoride in methanol. Meat Sci 46:23–32

Schalk G, Brigham RM (1995) Prey selection by insectivorous bats: are essential fatty acids important? Can J Zool 73:1855–1859

Shattuck SO (1999) Australian ants: their biology and identification, CSIRO, Collingwood

Smith AP, Wellham GS, Green SW (1989) Seasonal foraging activity and microhabitat selection by echidnas (*Tachyglossus aculeatus*) on the New England Tablelands. Aust J Ecol 14:457–466

Wang LCH (1989) Ecological, physiological, and biochemical aspects of torpor in mammals and birds. In: Wang LCH (ed) Animal adaptation to cold. Springer, Berlin Heidelberg, New York, pp 361–401