Comparative Biochemistry and Physiology, Part A 166 (2013) 555–563

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Physiological adaptations to prolonged fasting in the overwintering striped skunk (*Mephitis mephitis*)

Anne-Mari Mustonen [a,b,*], Jeff Bowman [c], Carrie Sadowski [c], Larissa A. Nituch [c], Laura Bruce [c], Toivo Halonen [d], Katri Puukka [e,f], Kirsti Rouvinen-Watt [g], Jari Aho [h], Petteri Nieminen [a,b]

[a] Department of Biology, Faculty of Science and Forestry, University of Eastern Finland, P.O. Box 111, FI-80101, Joensuu, Finland
[b] Institute of Biomedicine/Anatomy, School of Medicine, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211, Kuopio, Finland
[c] Wildlife Research and Development Section, Ontario Ministry of Natural Resources, 2140 East Bank Drive, Peterborough, ON K9J 7B8, Canada
[d] Eastern Finland Laboratory Centre (ISLAB), P.O. Box 1700, FI-70211, Kuopio, Finland
[e] NordLab Oulu, Oulu University Hospital, P.O. Box 500, FI-90029, OYS, Finland
[f] Department of Clinical Chemistry, Faculty of Medicine, University of Oulu, P.O. Box 5000, FI-90014, University of Oulu, Finland
[g] Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University, P.O. Box 550, Truro, NS B2N 5E3, Canada
[h] Municipal Veterinary Clinic of Joensuu, Takilatie 5, FI-80110, Joensuu, Finland

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form 15 August 2013
Accepted 15 August 2013
Available online 24 August 2013

Keywords:
Body temperature
Fatty acids
Hematology
Mephitis mephitis
Striped skunk
Torpor

ABSTRACT

Wintertime physiology of captive striped skunks (*Mephitis mephitis*) in response to cold ambient temperature (T_a) and fasting was investigated with body temperature (T_b) and activity recordings and analyses of hematology, plasma biochemistry and tissue fatty acids (FA). After 105 days of food deprivation, the skunks were in phase II of fasting indicated by the elevated plasma nonesterified FA and glycerol but no accumulation of nitrogen end products. Shorter-chain saturated and monounsaturated FA together with C18–20 n−3 polyunsaturated FA were preferentially mobilized. Individual amino acids responded to fasting in a complex manner, while essential and nonessential amino acid sums remained stable. Increases in hemoglobin and hematocrit suggested dehydration. The activity levels were lower in mid-January–early March, and the activity bouts were mostly displayed between 17:00–23:00 h. Daily torpor was observed in two females with 29 and 46 bouts. The deepest torpor (T_b < 31 °C) occurred between dawn and early afternoon and lasted for 3.3 ± 0.18 h. The average minimum T_b was 29.2 ± 0.15 °C and the lowest recorded T_b was 25.8 °C. There was significant relation between the average 24-h T_b and T_a. Increases in wintertime T_a, as predicted by climate change scenarios, could influence torpor patterns in the species.

1. Introduction

Climate change models predict that by the 2050's the greatest increases in the average wintertime ambient temperatures (T_a) in Canada will occur in the Hudson Bay and high Arctic areas (Warren and Egginton, 2008). Higher T_a can have implications for population dynamics of northern mammals displaying inactivity during the seasonal energetic bottleneck. Their successful overwintering depends on the adequacy of fat stores accumulated in autumn, rate of energy mobilization during dormancy and duration of the cold season. Climate change can lead to increased population sizes and also pronounced northward range expansion of hibernators (Humphries et al., 2002, 2004; Ozgul et al., 2010). In species displaying more flexible wintering strategies with resting days in a den interspersed by foraging bouts, global warming can eventually lead to year-round foraging effort thereby increasing intra- and interspecies interactions and the transmission risk of zoonotic diseases and parasites (Mustonen et al., 2012b). On the other hand, mammals relying on T_a as a cue for termination of seasonal inactivity can be at a risk of starvation if they emerge too early in spring when the snow cover is still deep, preventing successful foraging (Inouye et al., 2000).

The striped skunk (*Mephitis mephitis*) is an attractive carnivore model species with a wintering strategy that varies between periods of physical passivity and activity depending on the geographical area, environmental conditions, gender and age (Hamilton, 1937; Rosatte, 1999). Skunks can be active throughout the year in southern parts of their range, but in more northern latitudes they employ seasonal inactivity to overcome periods of cold T_a and food shortage (Wade-Smith and Verts, 1982; Rosatte, 1999). Their seasonal lethargy is sometimes

Abbreviations: AA, amino acid; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BM, body mass; BMI, body mass index; CBC, complete blood count; CK, creatine kinase; EDTA, ethylenediaminetetraacetic acid; FA, fatty acid; IM, intramuscular; MUFA, monounsaturated fatty acid; PCA, principal component analysis; PUFA, polyunsaturated fatty acid; SC, subcutaneous; SFA, saturated fatty acid; T_a, ambient temperature; T_b, body temperature; T_n, nest temperature; U/C, urea/creatinine.

* Corresponding author at: Department of Biology, Faculty of Science and Forestry, University of Eastern Finland, P.O. Box 111, FI-80101, Joensuu, Finland. Tel.: +358 294 45 1111; fax: +358 13 318 039.
E-mail address: Anne-Mari.Mustonen@uef.fi (A.-M. Mustonen).

1095-6433/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.cbpa.2013.08.008

intermittent and some individuals, often males, can be active during warm spells (Hamilton, 1937; Rosatte, 1999). The time period spent in the winter den is variable with observations ranging from 30 days (Storm, 1972) to 118 days (Sunquist, 1974). As fat storage specialists, striped skunks lose 14–58% of their body mass (BM) during winter (Hamilton, 1937; Allen, 1939; Storm, 1972; Sunquist, 1974; Rosatte, 1999), while body fat content can decrease from the autumnal 32–50% to the springtime 9–26% (Mutch and Aleksiuk, 1977; Wade-Smith and Verts, 1982; Hwang et al., 2007).

Average body temperatures (T_b) of captive striped skunks decreased from 37.7 °C in summer to 34.4 °C in winter (Mutch and Aleksiuk, 1977). The lowest recorded T_b was 26.0 °C during a torpor bout in captivity in MB, Canada (Hwang et al., 2007). Metabolic rate is assumed to decline during winter as well to provide animals with significant energy savings, but to our knowledge, direct metabolic rate measurements have not been conducted during dormancy. The recent study of Hwang et al. (2007) verified that captive striped skunks were capable of entering spontaneous daily torpor. In solitary animals, the torpor bouts were deeper and more frequent than in communally-housed skunks, among which females exhibited irregular and shallow torpor, while males were not torpid at all. Solitary animals lost fat reserves more rapidly than grouped skunks, which reduced their thermoregulatory costs by social thermoregulation (huddling). As T_a was suggested to function as a principal regulator of the winter rest in the species (Hamilton, 1937; Selko, 1938), it would be interesting to study the patterns of torpor, correlate these with environmental factors and assess the outcomes of seasonal passivity under climate change scenarios.

While daily torpor has been reported earlier for the striped skunk (Hwang et al., 2007), its fasting response (hematology, plasma biochemistry, fatty acid [FA] mobilization, etc.) has been investigated surprisingly little compared to more traditional models among fasting-adapted mammals. Studying overwintering will add to our understanding of the effects of global warming on carnivore populations by focusing on the physiologically most vulnerable part of the yearly cycle. A relatively small change in average monthly T_a may influence the duration of winter passivity, a variable that can be measured easily and reliably by using T_b data loggers. The first aim of the present study was to investigate if a factor prone to climate change (T_a) could have an influence on the skunk T_b and activity patterns. Secondly, we examined how prolonged wintertime fasting affects a wide array of hematological and plasma biochemical variables and if changes in these parameters can be used to determine the health and nutritional status of striped skunks. Finally, proportional changes in tissue FA profiles were determined to pinpoint the FA preferentially mobilized by fasting skunks and those retained during negative energy balance. We hypothesized that *i*) fluctuations in T_b and activity of overwintering striped skunks would follow changes in T_a, *ii*) phase of fasting could be defined from the levels of circulating metabolites and *iii*) FA mobilization would be selective in skunks similar to some other carnivores.

2. Material and methods

The experiment took place at the Ontario Ministry of Natural Resources' Codrington Wildlife Facility (Codrington, ON, Canada; 44.16°N, 77.80°W) in late fall 2010–early spring 2011. The procedures were approved by the Ontario Ministry of Natural Resources Wildlife Animal Care Committee (Protocol #10-226) and complied with the current laws of Finland and with the EU Directive 2010/63/EU for animal experiments. Three male and four female 1.5-year-old farmed striped skunks were purchased from the Ruby Fur Farm in New Sharon (IA, USA) in August 2010. The animals had been descented and tested to be rabies-free. The skunks were housed individually in cages (152 × 61 × 61 cm) with wooden nest boxes (61 × 61 × 36 cm) within a roofed (filtered skylights) and partially walled (south and north-facing ends) enclosure at the facility, but were exposed to natural T_a and photoperiod. From September to late November 2010, the skunks were fed ad libitum with Purina* 5568 Extruded Fox Breeder feed (crude protein min = 34.0%, crude fat min = 7.0%, max = 11.0%, crude fiber max = 5.0%, 3775 kcal/kg dry matter, Agribrands Purina Canada Inc., Woodstock, ON, Canada) in early to mid-morning on a daily basis to allow the natural autumnal fat storage to occur (Rosatte, 1999; Hwang, 2005). The FA composition of the diet is depicted in Supplement. Nesting material, such as dry grass and hay, was also provided, and the animals had access to water or ice at all times.

On November 17, 2010, the skunks were anesthetized with intramuscular (IM) injection of ketamine hydrochloride (5 mg/kg) and medetomidine hydrochloride (50 μg/kg). BM, body lengths and chest circumferences were measured and body mass indices (BMI) calculated as described for other carnivores (Mustonen et al., 2007c). A sterile thermosensitive data logger (iButton Thermochron DS1922L, Maxim Integrated, San Jose, CA, USA) registering the T_b at 60-min intervals was inserted surgically into the abdominal cavity. The accuracy and precision of the loggers had been tested previously (van Marken Lichtenbelt et al., 2006). A small patch of abdominal skin was shaved along the ventral midline and a 3-cm incision was made caudally from the umbilicus. 1–2 g of subcutaneous (SC) white adipose tissue was removed, frozen in liquid nitrogen and stored at −70 °C. The peritoneum was opened along the *linea alba* to insert the logger. The peritoneum and skin were sutured with resorbable thread. During the same anesthesia, a blood sample was collected from the subclavian vein using a needle and Vacutainer with ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged at 1500 *g* for 15 min, after which plasma was removed and stored at −70 °C. Atipamezole hydrochloride (0.2 mg/kg) was administered to each animal after surgery and blood sampling to reverse the sedative effects of medetomidine. IM benzylpenicillin (30,000 IU/kg) was administered for antibiotic prophylaxis and IM meloxicam (0.2 mg/kg) as an analgesic. The animals were allowed to recover in private nest boxes. The surgical procedure was performed by Dr. Graham Crawshaw of the Toronto Zoo (Toronto, ON, Canada).

As communal denning is known to decrease the occurrence and depth of daily torpor in striped skunks (Hwang et al., 2007), the animals of the present experiment were housed singly in conditions described above. They were fed with Purina* 5568 feed as follows: ad libitum until November 23, ca. 16.8 g (63 kcal) between November 24–30, 8.4 g between December 1–7 and 4.2 g between December 8–14 to simulate the natural decrease in food availability. Regular feeding ceased on December 15, emulating the lack of food sources experienced by wild skunks in northern part of their range (Rosatte, 1999). During the fasting period, there were no other experiments conducted in the same enclosure and the skunks were able to rest in a relatively disturbance-free environment. Eliminating food sources and providing suitable nesting areas have been previously shown to induce a winter sleep-like state of reduced activity and T_b in captive skunks (Mutch and Aleksiuk, 1977). Each skunk was given half a can of sardines (ca. 95 kcal) on milder days on January 18, 25, February 17 and March 23, 2011, to mimic natural conditions, as wild skunks can become more active and seek food when T_a rises (Hamilton, 1937; Rosatte, 1999). The FA composition of the sardines is represented in Supplement. Water was provided daily or as needed.

Four infrared cameras were set up to monitor the activity of the skunks between December 15, 2010–March 29, 2011. The cameras were triggered to record by motion (e.g., a skunk coming out of the nest box) and, when triggered, captured 3 still images every 20 s. In order to preserve battery life, there was a 10 min delay before the camera could detect another event of activity. Each occurrence of activity producing 3 images was coded as a single event and the sum of these events within each 24-h period per animal was used to indicate activity level. The value = 0 depicted that the skunk did not show any observable activity on that date. Thermosensitive loggers (iButton Thermochron model DS1922L) registered the T_a and nest temperature (T_n) at 60-min intervals synchronized with the T_b recordings.

At the end of the winter rest on March 29, 2011, the animals were anesthetized and sampled for blood as described above. BM, body lengths and chest circumferences were measured. The skunks were subsequently euthanized with T-61 (embutramide 60 mg/kg, mebezonium iodide 15 mg/kg, tetracaine hydrochloride 1.5 mg/kg) followed by dissection of tissue samples including ventral SC fat, retroperitoneal fat, omental fat, mesenteric fat, liver and muscle (*m. quadriceps femoris dx.*) and their storage at −70 °C.

Complete blood count (CBC) was determined with the IDEXX LaserCyte® hematology analyzer (IDEXX Laboratories Inc., Westbrook, ME, USA) adjusted to the canine hematologic profile. Variables of plasma clinical chemistry were determined with the Technicon RA-XT™ analyzer (Technicon Ltd., Swords, Ireland) as outlined previously (Mustonen et al., 2005a, 2007c, 2009a). Activities of plasma alanine (ALT) and aspartate (AST) aminotransferases were measured using Reflotron® GPT (ALT) and GOT (AST) test strips with the Reflotron Plus analyzer (Roche Diagnostics Ltd., Basel, Switzerland). The aminotransferase analyses were originally validated for whole blood, serum, heparinized blood and heparinized plasma and as we used EDTA-plasma, the obtained results cannot be taken as reference values for future studies. Amino acid (AA) concentrations in plasma were measured with ion-exchange chromatography (Biochrom 30 Amino Acid Analyzer, Biochrom Ltd., Cambridge, UK). To our knowledge, essential AA have not been defined for the studied species. For this reason, we calculated the total essential AA as the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine as performed previously for some other carnivores (Wright et al., 1999).

For the determination of FA profiles, tissue samples were transmethylated by heating with 1% H_2SO_4 in methanol under nitrogen atmosphere. The formed FA methyl esters were extracted with hexane and analyzed by an Agilent 6850 gas–liquid chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA; Mustonen et al., 2012a). They were identified based on the retention time, mass spectrum and comparison with authentic standards of a known composition (Sigma-Aldrich, St. Louis, MO, USA). The results represent the FA composition (mol-%) of total lipids. Relative changes in the proportions of FA during wintering were calculated by the formula: [(mol-% in spring) − (mol-% in autumn)] / [mol-% in autumn]. Fractionation coefficients between the dietary and tissue levels of each FA were calculated as follows: (mol-% in tissue) / (average mol-% in Purina* 5568 diet).

The T_b recordings were divided into the periods of feeding (November 17–December 14, 2010) and fasting (December 15, 2010–March 29, 2011). T_b at < 31 °C was selected as the limit below which the skunks were considered to be torpid, as the same threshold was used also in a previous study on striped skunks (Hwang et al., 2007). Torpor bout duration and the time of day for each bout were determined based on the time points at which T_b decreased < 31 °C and increased above it. Theoretical percentages of energy preservation caused by reductions in T_b during torpor bouts were calculated according to Nieminen et al. (2013) with the formula: $(T_{b:fed} - T_{b:fasted}) \times 100\% / (T_{b:fed} - T_a)$.

Differences in the general parameters such as the BM, BMI and biochemical variables were analyzed with the Student's *t*-test or Mann–Whitney *U*-test for parametric and nonparametric data, respectively (SPSS *v*19 software package, IBM, Armonk, NY, USA). An activity score sum and average 24-h T_b were calculated for each date of each individual. Activity score sums during early, mid- and late winter were tested for statistical difference with the Kruskal–Wallis analysis of variance (ANOVA). Individual amplitude spectra were calculated with the Fast Fourier Transform. To analyze the relationships between the average 24-h T_b and the covariates T_a, T_n and day length, the linear mixed model was performed. Bivariate correlations were calculated with the Spearman correlation coefficient (r_s). To analyze the relationships in hematology, clinical chemistry and AA profiles between the seasons, the data were also subjected to the multivariate principal component analysis (PCA) using the Sirius *v*6.5 software package (Pattern Recognition Systems AS, Bergen, Norway; Kvalheim and Karstang, 1987). $P < 0.05$ was considered statistically significant. The results are presented as the mean ± SE.

3. Results

The average autumnal BM (November 17, 2010) of the skunks was 5.2 ± 0.35 kg and the vernal BM (March 29, 2011) 2.0 ± 0.15 kg, the average decrease being 62 ± 1.6%. The BMI decreased by 50 ± 1.5% and the chest circumference by 31 ± 2.2%. The autumnal BM or BMI did not correlate significantly with the number of torpor bouts, although the two animals with the highest occurrence of torpor had also the lowest initial BM. The vernal BM correlated inversely with the number of bouts ($r_s = -0.807$, $P < 0.028$), and there was a positive association between the average 24-h min T_b and the absolute BM loss during winter ($r_s = 0.829$, $P < 0.021$).

The average wintertime T_a (December 15, 2010–March 29, 2011) was −4.9 ± 0.1 °C (min = −29.6 °C, max = 22.5 °C) and the average T_n −3.5 ± 0.1 °C (min = −26.4 °C, max = 22.0 °C). The average T_b during feeding (November 17–December 14, 2010) was 36.1 ± 0.01 °C. Torpor bouts were documented to occur between December 27 and March 28, and there was large interindividual variation in the number and frequency of bouts (Fig. 1). No incidences of torpor (i.e., $T_b < 31$ °C) were recorded for 2 animals (a male and a female), 3 individuals (2 males and a female) showed only 1–2 torpor bouts and 2 female skunks exhibited more frequent torpor (29 and 46 bouts in 105 days). The average bout duration ($T_b < 31$ °C) was 3.3 ± 0.18 h, the average min T_b during the bouts was 29.2 ± 0.15 °C and the lowest recorded T_b was 25.8 °C. The majority of torpor bouts occurred between 07:00 and 14:00 h, while the highest T_b were usually recorded between 18:00 and 22:00 h (Fig. 2).

In spectral analysis, the T_b displayed 24-, 12-, 8- and 6-h oscillations without statistically significant increases in amplitude between feeding and food deprivation (data not shown). The duration of torpor bouts correlated positively ($r_s = 0.508$, $P < 0.001$) and the min T_b during the bouts negatively with the date of fasting ($r_s = -0.435$, $P < 0.001$). There was also an inverse correlation between the min T_b and the duration of torpor bouts ($r_s = -0.834$, $P < 0.001$). In linear mixed model, the average 24-h T_b showed significant relation with the average 24-h T_a ($F_{1,6.012} = 8.941$, $P < 0.05$) and T_n ($F_{1,6.006} = 7.678$, $P < 0.05$), but not with day length.

There was relatively large variation in the levels of physical activity between the individuals. In general, the activity scores were higher in early and late winter than in mid-January–early March (Kruskal–Wallis ANOVA, $P < 0.001$; Fig. 3A). However, there were several occurrences of increased activity also during this period, often in association with elevated T_a (Fig. 3B). February 12, 2011 was the only date during which none of the skunks showed any activity (average $T_a = -4.1$ °C). Most of the activity bouts were recorded between 17:00 and 23:00 h, but they could be detected also at other points of the 24-h cycle. There was no significant relation between the 24-h activity score sums and T_a, T_n or day length in linear mixed model.

The erythrocyte count, hematocrit, hemoglobin concentration, lymphocyte-%, lymphocyte, monocyte and platelet counts and plateletcrit increased from autumn to spring, while the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and neutrophil-% decreased (Table 1). According to PCA, the variable separating the fed and fasted skunks the most clearly was platelet count. The plasma glucose and high-density lipoprotein cholesterol concentrations, urea–creatinine (U/C) ratio, creatine kinase (CK), ALT and AST activities were higher in autumn than in spring (Table 2). In contrast, the nonesterified FA, glycerol, low-density lipoprotein cholesterol and creatinine levels increased from autumn to spring. In PCA, CK, glycerol, ALT and AST were the parameters distinguishing the fed from the fasted animals. Regarding the variables of nitrogen metabolism, the plasma concentrations of alanine, cystine, histidine, lysine, methionine,

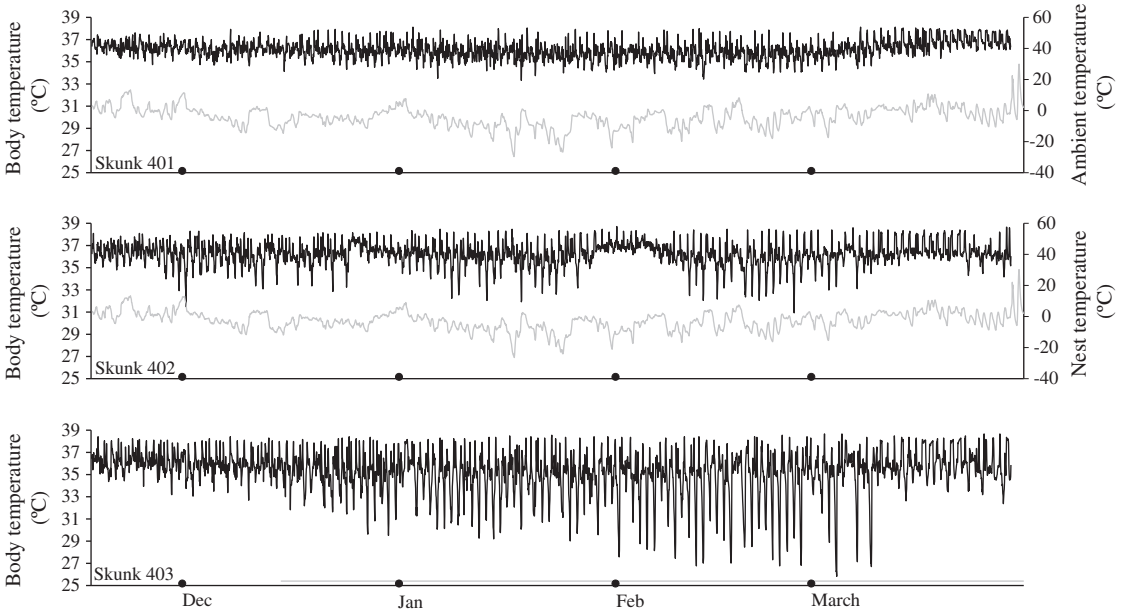

Fig. 1. Body temperatures of 3 female striped skunks with different torpor patterns (black lines) and ambient and nest temperatures (gray lines) measured at 60-min intervals. The bullets on the X-axes depict the first day of each month and the horizontal gray line in the lowest panel represents the fasting period between December 15, 2010–March 29, 2011.

phosphoserine and taurine decreased during wintering (Table 3). The levels of α-aminobutyrate, glutamate, glycine, 3-methylhistidine and valine increased, while the sums of essential and nonessential AA remained stable. According to PCA, alanine, glutamine, taurine, lysine and glutamate were the AA separating the fed and fasted skunks the most.

The total lipids of the ventral SC fat contained lower proportions of FA such as 15:0, 16:0, 14:1n−5, 16:1n−7, 17:1n−8, 18:2*c*9*t*11, 18:3n−6, 18:3n−3, 20:4n−6, 20:5n−3, 21:5n−3 and n−3 polyunsaturated FA (PUFA) sum together with a lower n−3/n−6 PUFA ratio in spring than in autumn (Table 4). In contrast, the FA with higher proportions in spring included 18:0, 19:0, 20:0, 22:0, 16:1n−9, 18:1n−9, 20:1n−11, 20:1n−9, 20:1n−7, 22:1n−11, 22:1n−7, 20:2n−6, 20:3n−6, 22:4n−6, 22:5n−6, 22:5n−3, 22:6n−3, n−6 PUFA sum and total PUFA sum.

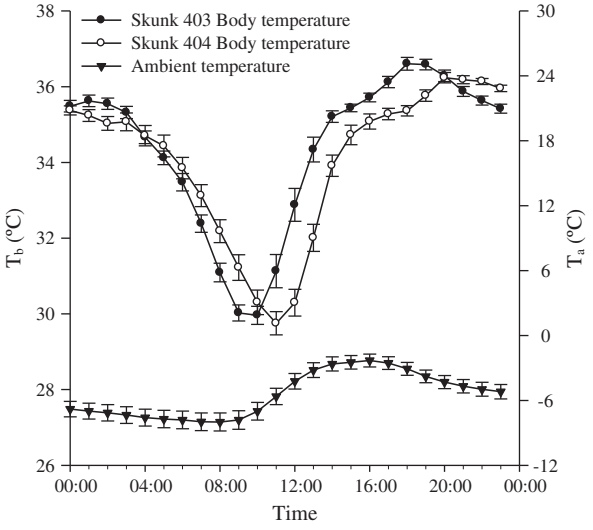

Fig. 2. 24-h fluctuations in body temperature (T_b) of two female striped skunks during the days with torpor and diurnal variations in ambient temperature (T_a) during the same days (mean ± SE).

The preferentially mobilized and preserved FA in the SC fat are depicted in Fig. 4. In general, C14–17 saturated FA (SFA), most C14–16 monounsaturated FA (MUFA), 18:1n−7, 20:4n−6 and C18–20 n−3 PUFA had decreased proportions during fasting, while C18–24 SFA, 16:1n−9, C20–24 MUFA, most C20–22 n−6 PUFA and C22 n−3 PUFA were conserved. In plasma, the proportions of 20:3n−6, 20:4n−6, 20:5n−3, 22:6n−3 and the sums of n−3, n−6 and total PUFA increased during winter, while the percentages of, e.g., 15:0, 17:0, 18:0, 24:0, total SFA, 16:1n−9, 16:1n−7, 18:1n−7, 22:1n−11, 22:1n−9, 18:3n−6, 18:4n−3, 20:3n−3, 20:4n−3, 21:5n−3, 22:4n−6, 22:4n−3 and 22:5n−6 decreased (Table 4).

FA with efficient net incorporation from the diet into the SC fat often included C12–20 SFA, C16 MUFA, 18:1n−7, C20 MUFA and most longer-chain PUFA. In contrast, the dietary proportions of C22–24 SFA and MUFA, C18 PUFA and C20 n−3 PUFA were usually higher than in the tissues. In several occasions, the liver and muscle FA profiles differed from the analyzed adipose tissues, e.g., by containing more 18:0, 24:1n−9 and long-chain PUFA but less 18:1n−9 and 18:3n−3 (data not shown). In muscle, the vernal PUFA sum ($r_s = 0.800$, $P < 0.031$) and n−3/n−6 PUFA ratio ($r_s = 0.891$, $P < 0.007$) correlated with the number of torpor bouts.

4. Discussion

An earlier study on striped skunks used T_b at < 31 °C as the criterion for torpor (Hwang et al., 2007). Although the same threshold was employed in the present study, it is important to recognize that smaller decreases in T_b can also be energetically relevant (Barclay et al., 2001). The theoretical energy savings by our skunks were calculated to reach 17% during the deepest torpor. Even though some individuals did not exhibit T_b at < 31 °C, they did have less pronounced reductions in T_b that could have saved energy. The first torpor bout was documented on December 27 and, later in winter, the bouts lasted longer and reached a lower min T_b. In comparison to previous data of Hwang et al. (2007) on solitary skunks, the torpor bouts of our animals were more shallow and most of the skunks did not use daily torpor regularly. The skunks of the earlier experiment were kept north of our station (Delta Marsh, MB [50°N] vs. Codrington, ON [44°N]), and there are fairly large differences in climate between these areas, our study region being more temperate in winter (Environment Canada, 2012). Our skunks

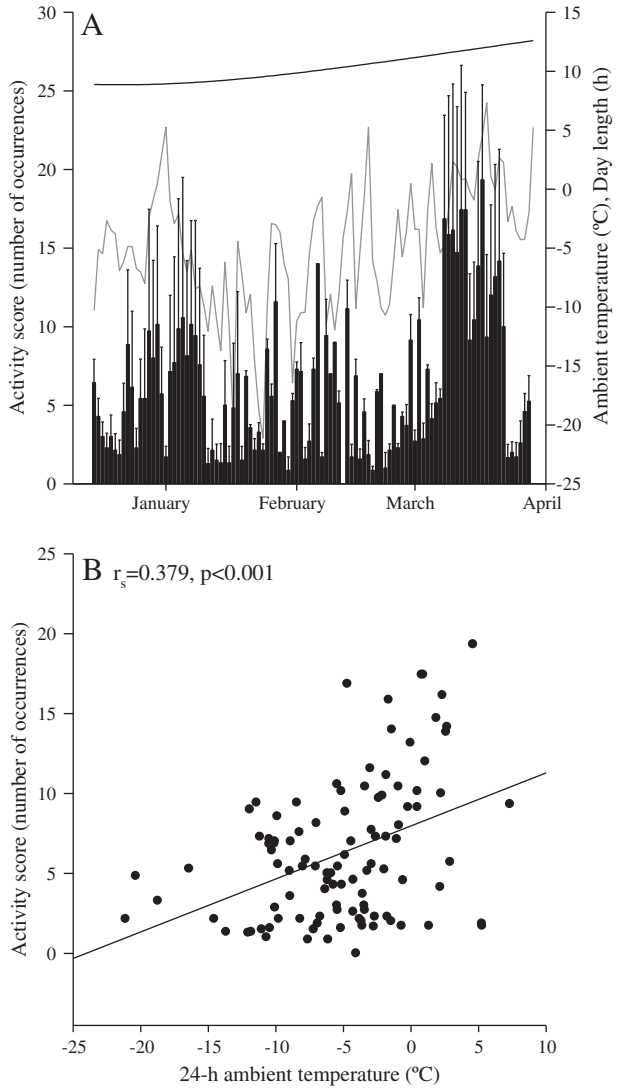

Fig. 3. A) Average activity scores (black bars) of the fasted striped skunks (n = 7, number of occurrences per day + SE), day length (black line) and average 24-h ambient temperatures (gray line) measured in December 15, 2010–March 28, 2011. B) Correlation between the average activity scores (n = 7) and average 24-h ambient temperatures.

had been obtained from a commercial fur farm in IA, USA and they originated from a more southern stock (IA and MN, USA) than the wild animals and their captive-born offspring of the earlier study in MB. As the skunk population that our animals descended from had been bred on the farm for ca. 70 years, domestication could have had effects on their seasonality. Furthermore, even with the precautions, disturbances by humans or by the skunks themselves could have affected the expression of torpor. In addition, previous reports have discussed possible trade-offs in the use of torpor (benefits of energy conservation vs. physiological costs of metabolic depression) in hibernators (Humphries et al., 2003), and it remains uninvestigated if trade-offs could affect the entry into torpor by skunks, as well.

The solitary skunks of the earlier experiment (Hwang et al., 2007) displayed torpor from midnight to dawn (nocturnal activity period of skunks) and aroused from dawn to midday, whereas the present study demonstrated the lowest T_b values (< 31 °C) at 07:00–14:00 h, the highest between 18:00 and 22:00 h and the activity bouts occurring mostly between 17:00 and 23:00 h. The timing of the T_b peaks and nadirs showed close resemblance to the wild raccoon dog (*Nyctereutes procyonoides*) studied previously with similar methods (Mustonen et al., 2007a). The period of the lowest T_b coincided relatively well

Table 1
Hematological values of the skunks (n = 7) according to season, mean ± SE.

	Autumn	Spring
Erythrocytes (10^{12}/L)	7.33 ± 0.20	10.22 ± 0.49*
Hematocrit (%)	38.8 ± 0.9	53.0 ± 2.3*
Hemoglobin (g/L)	122 ± 16	158 ± 8*
MCV (fL)	53.0 ± 0.9	52.0 ± 0.8
MCH (pg)	16.9 ± 0.5	15.5 ± 0.2*
MCHC (g/dL)	31.9 ± 0.5	29.8 ± 0.4*
RDW (%)	18.5 ± 0.2	17.7 ± 0.3
Reticulocytes (10^3/μL)	30.3 ± 6.2	32.4 ± 4.5
Reticulocytes (%)	0.4 ± 0.1	0.3 ± 0.1
Leukocytes (10^9/L)	7.67 ± 0.74	9.21 ± 0.66
Neutrophils (10^9/L)	5.17 ± 0.72	4.74 ± 0.54
Lymphocytes (10^9/L)	1.75 ± 0.18	3.07 ± 0.23*
Monocytes (10^9/L)	0.57 ± 0.08	0.87 ± 0.10*
Eosinophils (10^9/L)	0.15 ± 0.03	0.25 ± 0.09
Basophils (10^9/L)	0.03 ± 0.01	0.02 ± <0.01
Neutrophils (%)	66.3 ± 3.1	52.3 ± 2.2*
Lymphocytes (%)	24.0 ± 3.3	34.6 ± 1.8*
Monocytes (%)	7.4 ± 0.6	9.9 ± 1.2
Eosinophils (%)	2.0 ± 0.4	2.9 ± 1.0
Basophils (%)	0.4 ± 0.1	0.2 ± <0.1
Platelets (10^3/μL)	270 ± 22	450 ± 58*
Plateletcrit (%)	0.2 ± <0.1	0.4 ± 0.1*
MPV (fL)	8.8 ± 0.3	8.3 ± 0.5
PDW (%)	21.6 ± 0.3	21.4 ± 0.3

MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, MPV = mean platelet volume, PDW = platelet distribution width.
* P < 0.05 between seasons, *t*-test, Mann–Whitney *U*-test.

with the normal diurnal resting period of skunks and may, thus, represent an extension of sleep. The lowest 24-h T_a values were often recorded during the morning hours preceding the T_b nadirs. The occurrence of torpor in the sleeping phase of the daily sleep–wake cycle partly confirms the data of Hwang et al. (2007) on group-housed skunks but disagrees with their results on solitary animals that displayed torpor bouts during the nocturnal activity phase. The lowest recorded T_b were comparable in these two experiments (26.0 °C in Hwang et al., 2007, 25.8 °C in the present study) and, to our knowledge, these values are the lowest documented for carnivores (Hock, 1957; Harlow, 1981; Fowler and Racey, 1988; Hissa et al., 1994; Mustonen et al., 2007a).

When compared to other species with torpor, the hypothermia observed in the skunks shared some resemblance to the American badger (*Taxidea taxus*). One badger studied by Harlow (1981) displayed 30 torpor bouts when overwintering in an outdoor enclosure. Similar to

Table 2
Plasma clinical chemistry of the skunks (n = 7) according to season, mean ± SE.

	Autumn	Spring
Glucose (mmol/L)	13.28 ± 1.30	6.32 ± 0.28*
Triacylglycerols (mmol/L)	1.22 ± 0.39	0.81 ± 0.13
Free fatty acids (mmol/L)	0.30 ± 0.07	0.67 ± 0.15*
Glycerol (μmol/L)	110 ± 19	215 ± 43*
Total cholesterol (mmol/L)	4.2 ± 0.4	3.9 ± 0.1
LDL-cholesterol (mmol/L)	0.31 ± 0.06	0.64 ± 0.07*
HDL-cholesterol (mmol/L)	2.81 ± 0.17	2.28 ± 0.13*
Total protein (g/L)	68 ± 2	67 ± 1
Ammonia (μmol/L)	570 ± 3	570 ± 2
Urea (mmol/L)	10.5 ± 1.0	10.1 ± 0.7
Creatinine (μmol/L)	43 ± 4	76 ± 5*
Urea–creatinine ratio	276 ± 73	137 ± 12*
Uric acid (μmol/L)	43.0 ± 8.1	36.3 ± 3.3
Creatine kinase (U/L)	586 ± 66	259 ± 21*
Bilirubin (μmol/L)	4.5 ± 2.4	4.5 ± 0.3
AST (U/L)	141 ± 14	94 ± 10*
ALT (U/L)	143 ± 22	42 ± 15*
TAS (mmol/L)	1.56 ± 0.17	1.61 ± 0.05

LDL = low-density lipoprotein, HDL = high-density lipoprotein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, TAS = total antioxidant status.
* P < 0.05 between seasons, *t*-test, Mann–Whitney *U*-test.

Table 3
Plasma concentrations of amino acids and nitrogen metabolites (μmol/L) of the skunks (n = 7) according to season, mean ± SE.

	Autumn	Spring
Alanine	451.89 ± 55.46	309.80 ± 30.29*
α-Aminoadipic acid	7.86 ± 1.58	5.62 ± 0.63
α-Aminobutyrate	12.06 ± 1.60	40.94 ± 4.80*
Arginine	67.70 ± 3.83	56.89 ± 8.44
Asparagine	33.37 ± 2.89	28.37 ± 4.77
Citrulline	9.48 ± 0.61	8.44 ± 0.69
Cystine	29.78 ± 3.29	11.88 ± 2.32*
Glutamate	35.98 ± 8.28	131.33 ± 14.35*
Glutamine	469.69 ± 40.01	482.47 ± 64.76
Glycine	168.33 ± 13.48	217.72 ± 14.11*
Histidine	69.61 ± 3.65	49.39 ± 4.88*
Isoleucine	39.41 ± 2.10	52.01 ± 4.54
Leucine	89.19 ± 6.01	117.06 ± 9.28
Lysine	174.85 ± 16.78	106.89 ± 14.55*
Methionine	29.68 ± 1.25	21.75 ± 2.61*
1-Methylhistidine	5.71 ± 0.56	7.90 ± 0.93
3-Methylhistidine	5.26 ± 0.97	24.05 ± 4.05*
Ornithine	21.95 ± 8.58	12.10 ± 5.00
Phenylalanine	74.63 ± 4.60	58.54 ± 5.48
Phosphoserine	11.18 ± 0.79	8.57 ± 0.30*
Serine	97.23 ± 11.43	106.59 ± 21.86
Taurine	319.33 ± 29.47	188.12 ± 11.20*
Threonine	113.99 ± 6.82	104.07 ± 7.47
Tryptophan	85.00 ± 9.08	67.96 ± 8.09
Tyrosine	37.93 ± 2.36	33.40 ± 4.80
Valine	120.13 ± 9.05	180.36 ± 12.18*
Nonessential amino acids	1818.45 ± 126.57	1706.88 ± 140.40
Essential amino acids	796.49 ± 41.00	758.03 ± 52.34
Total amino acids	2614.93 ± 161.32	2464.91 ± 175.82

* $P < 0.05$ between seasons, *t*-test, Mann–Whitney *U*-test.

the skunks, the bouts did not occur daily. The torpor cycle of the badger lasted altogether for 29 h and the periods of the lowest T_b for 6–18 h. During the bouts, T_b typically reduced by 9 °C to approximately 28 °C and heart rate decreased by 50%. The average min T_b during the skunk torpor bouts (29.2 °C in the present study) was close to that of the badger but the periods with the lowest T_b were shorter (range 1–7 h).

The distribution areas and seasonal activity levels of many species are strongly influenced by climatic factors. In the case of the striped skunk, T_a was suggested to function as an important external factor affecting its winter activity (Hamilton, 1937; Selko, 1938; Aleksiuk and Stewart, 1977) and in the present study, the average 24-h T_a showed significant relation with the average 24-h T_b. The loose association between the T_b and activity levels may be related to the method of activity recording that did not distinguish isolated activity bouts from more enduring activity but, instead, every occurrence of skunks outside the den from the duration of a few s to < 10 min was coded as a single event. It is also possible that the limited cage size could have constrained the activity of the skunks.

Expected increases in wintertime T_a due to climate change (Plummer et al., 2006) may affect the duration and number of torpor bouts in northern skunks. A phenomenon with some similarities has been observed in Colorado yellow-bellied marmots (*Marmota flaviventris*) which displayed advanced arousal from hibernation than was the case a couple of decades earlier (Inouye et al., 2000). Global warming may lead to year-round foraging of skunks north of the present distribution area of active wintering and subsequently promote intra- and interspecies interactions and enhanced transmission of zoonoses (see Mustonen et al., 2012b for the raccoon dog), as the striped skunk is an important vector and reservoir of diseases and parasites (Dragoo, 2009; Gehrt et al., 2010). A phylogeographic study by Barton and Wisely (2012) illustrated how striped skunks responded to the Holocene climatic warming and expanded their North American distribution area northwards. Future range expansion may be possible and might occur at the expense of year-round active mammals (see also Humphries et al., 2004). In fact, there are quite recent observations of skunks in Kuujjuarapik, Quebec ([55°N]; George, 2006).

Although the skunks of the present study lost an average of 62% BM, they were in good body condition after the fasting period. The total weight loss was in concert with the results of Hwang et al. (2007), who also documented that striped skunks could reproduce successfully after the pronounced wintertime loss of approximately half of their BM. The animals of our study had sufficient fat reserves to survive winter and their elevated plasma levels of nonesterified FA and glycerol indicated extended phase II of fasting with stimulated fat utilization (Castellini and Rea, 1992; Mustonen et al., 2005a). The mobilization of FA from adipose tissue of mammals is selective according to the molecular structure of FA (Raclot, 2003). Generally, FA are mobilized more efficiently when they are short, unsaturated and when their double bonds are located closer to the terminal methyl group of the chain. In the ventral SC fat of the skunks, the preferentially mobilized FA included, e.g., C14–17 SFA, most C14–16 MUFA and C18–20 n−3 PUFA, while the proportions of C18–24 SFA, C20–24 MUFA and C22 n−6 and n−3 PUFA increased. These data mostly conform to previous results on raccoon dogs (Mustonen et al., 2007b, c). The most polar triacylglycerols rich in highly-mobilized FA (16–20 carbon atoms and 4–5 double bonds) could be more accessible to hydrolysis by hormone-sensitive lipase at the lipid–water interface resulting in the preservation of long-chain SFA and MUFA (Raclot, 1997, 2003). The net increase of 16:1n−9 deviated from the general pattern of mobilization and could have resulted from chain-shortening of 18:1n−9. It cannot be ruled out that the feeding of the skunks with sardines on four occasions could have affected the retention of, e.g., long-chain n−3 PUFA. This is, however, unlikely, as 20:5n−3 abundant in fish oil was among the highly-mobilized FA during food deprivation as usual (Raclot, 2003).

Previous literature is fairly inconsistent regarding responses of plasma FA profiles to negative energy balance in carnivores (Nieminen et al., 2006, 2009; Mustonen et al., 2007b). Some of the changes documented in the fasted skunks, such as the decreases in 18:0 and 16:1n−7 and increases in 20:4n−6, 22:6n−3 and unsaturated FA/SFA ratio, were similar to previous observations in denning European brown bears (*Ursus arctos arctos*; Hissa et al., 1998a), although several differences were also noted between these studies. The elevated proportions of 20:4n−6 and 22:6n−3 also fit earlier data of fasted European polecats (*Mustela putorius*; Nieminen et al., 2009). However, changes observed in plasma nonesterified FA of denning American black bears (*Ursus americanus*; LeBlanc et al., 2001) diverged clearly from those detected in the plasma total lipids of our skunks. The efficient net incorporation of short-chain SFA, C16 MUFA and 18:1n−7 from the diet into the fat tissue mostly conformed to previous studies on other mammals as did the inefficient incorporation of longer-chain SFA and essential PUFA precursors (Mustonen et al., 2007d, 2009b, 2012a; Paakkonen et al., 2011). The divergence of the FA profiles of liver and muscle from adipose tissues also followed the previously documented pattern (Mustonen et al., 2009b, 2012a). Earlier studies have suggested that dietary PUFA (especially 18:2n−6) could participate in the regulation of hibernation in rodents, for instance, by reducing the melting point of membrane lipids and depot fats (Geiser and Kenagy, 1987; Frank and Storey, 1995). The skunks do not display T_b at a level that could be comparable to true hibernators (close to 0 °C) and, perhaps for this reason, a clear connection between the tissue FA signatures and the use of torpor was not observed.

Similar to many fasting mammals (Harlow and Seal, 1981; Mustonen et al., 2004; McCue, 2010), the circulating glucose levels decreased in the skunks but the animals were not hypoglycemic at the time of sampling. Normoglycemia has been a common finding also in other fasting musteloids (Mustonen et al., 2005a; Nieminen et al., 2007). The reason for the high autumnal plasma glucose concentrations compared to previous serum data from island spotted skunks (*Spilogale gracilis amphiala*; Crooks et al., 2003) remains obscure and warrants further investigation, as the animals had been fasted overnight prior to the surgery and

Table 4
Proportions (mol-%) of the most abundant fatty acids in subcutaneous white adipose tissue and plasma of the skunks (n = 7) according to season, mean ± SE.

	WAT, autumn	WAT, spring	Plasma, autumn	Plasma, spring
14:0	2.831 ± 0.137	2.662 ± 0.098	0.472 ± 0.028	0.455 ± 0.026
15:0	0.165 ± 0.006	0.143 ± 0.005*	0.140 ± 0.006	0.114 ± 0.006*
16:0	25.472 ± 0.818	22.939 ± 0.821*	21.071 ± 0.219	21.474 ± 0.187
17:0	0.201 ± 0.009	0.197 ± 0.004	0.306 ± 0.006	0.203 ± 0.011*
18:0	6.283 ± 0.309	7.798 ± 0.513*	14.318 ± 0.694	9.632 ± 0.263*
19:0	0.038 ± 0.003	0.067 ± 0.006*	0.126 ± 0.009	0.116 ± 0.011
20:0	0.087 ± 0.006	0.210 ± 0.026*	0.314 ± 0.033	0.337 ± 0.035
22:0	0.007 ± 0.001	0.021 ± 0.004*	0.209 ± 0.018	0.190 ± 0.019
24:0	0.017 ± 0.005	0.017 ± 0.002	0.364 ± 0.034	0.179 ± 0.018*
Σ SFA	35.605 ± 1.176	34.526 ± 0.752	37.960 ± 0.867	33.222 ± 0.402*
14:1n−5	0.317 ± 0.030	0.216 ± 0.018*	0.070 ± 0.006	0.062 ± 0.006
16:1n−9	0.434 ± 0.009	0.557 ± 0.015*	0.456 ± 0.014	0.265 ± 0.012*
16:1n−7	7.629 ± 0.616	4.872 ± 0.352*	2.260 ± 0.123	1.792 ± 0.102*
18:1n−9 + 11	30.626 ± 0.489	32.399 ± 0.288*	8.542 ± 0.375	9.119 ± 0.385
18:1n−7	2.658 ± 0.086	2.502 ± 0.091	3.171 ± 0.083	2.718 ± 0.070*
20:1n−11	0.165 ± 0.005	0.432 ± 0.077*	0.100 ± 0.006	0.091 ± 0.007
20:1n−9	0.524 ± 0.027	1.201 ± 0.214*	0.245 ± 0.009	0.296 ± 0.031
22:1n−11	0.113 ± 0.009	0.343 ± 0.133*	0.313 ± 0.069	0.120 ± 0.015*
Σ MUFA	43.111 ± 1.010	43.158 ± 0.485	18.342 ± 0.337	17.246 ± 0.419
18:3n−3	1.018 ± 0.019	0.657 ± 0.027*	0.265 ± 0.019	0.230 ± 0.010
20:4n−3	0.030 ± 0.001	0.029 ± 0.002	0.175 ± 0.055	0.046 ± 0.009*
20:5n−3	0.067 ± 0.004	0.046 ± 0.006*	0.390 ± 0.047	0.567 ± 0.062*
22:4n−3	0.007 ± <0.001	0.008 ± 0.001	1.052 ± 0.123	0.033 ± 0.004*
22:5n−3	0.164 ± 0.008	0.362 ± 0.034*	1.013 ± 0.097	0.915 ± 0.062
22:6n−3	0.213 ± 0.021	0.317 ± 0.033*	1.372 ± 0.325	3.813 ± 0.176*
Σ n−3 PUFA	1.583 ± 0.019	1.479 ± 0.052*	4.767 ± 0.357	5.734 ± 0.241*
18:2n−6	18.197 ± 0.311	19.017 ± 0.298	22.076 ± 1.880	24.028 ± 0.533
20:4n−6	0.488 ± 0.009	0.431 ± 0.024*	11.709 ± 0.859	17.164 ± 0.612*
22:4n−6	0.235 ± 0.015	0.530 ± 0.045*	1.167 ± 0.146	0.550 ± 0.039*
22:5n−6	0.085 ± 0.004	0.147 ± 0.015*	2.325 ± 0.234	0.521 ± 0.054*
Σ n−6 PUFA	19.453 ± 0.342	20.668 ± 0.305*	38.315 ± 1.068	43.409 ± 0.519*
Σ PUFA	21.284 ± 0.351	22.316 ± 0.310*	43.698 ± 0.801	49.533 ± 0.410*
n−3/n−6 PUFA ratio	0.082 ± 0.001	0.072 ± 0.003*	0.126 ± 0.013	0.132 ± 0.007
UFA/SFA ratio	1.828 ± 0.097	1.905 ± 0.064	1.641 ± 0.060	2.013 ± 0.037*

WAT = white adipose tissue, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids.
* $P < 0.05$ between seasons within a tissue, *t*-test, Mann–Whitney *U*-test.

sampling. However, even higher serum concentrations were presented as the upper limit of reference range for striped skunks (Dragoo, 2009). The anesthetic agents utilized could have affected the plasma glucose levels of our animals (Ambrisko et al., 2005) but their potential effects should have been noticeable also in spring. However, the vernal glycogen levels were quite low (2.4 μg/mg) and this could have limited the ability of hepatic glycogenolysis to increase the circulating glucose levels in the fasted skunks.

No accumulation of nitrogen end products was observed in the food-deprived skunks, which displayed stable concentrations of plasma urea and ammonia and markedly decreased U/C ratios. Reduced kidney filtration was probably responsible for the elevated creatinine levels (Adlercreutz et al., 1983), which are commonly observed in overwintering mammals during negative energy balance (Hissa et al., 1994; Mustonen et al., 2004). Although our data suggest that the skunks were not in phase III of fasting with stimulated proteolysis, muscle AA

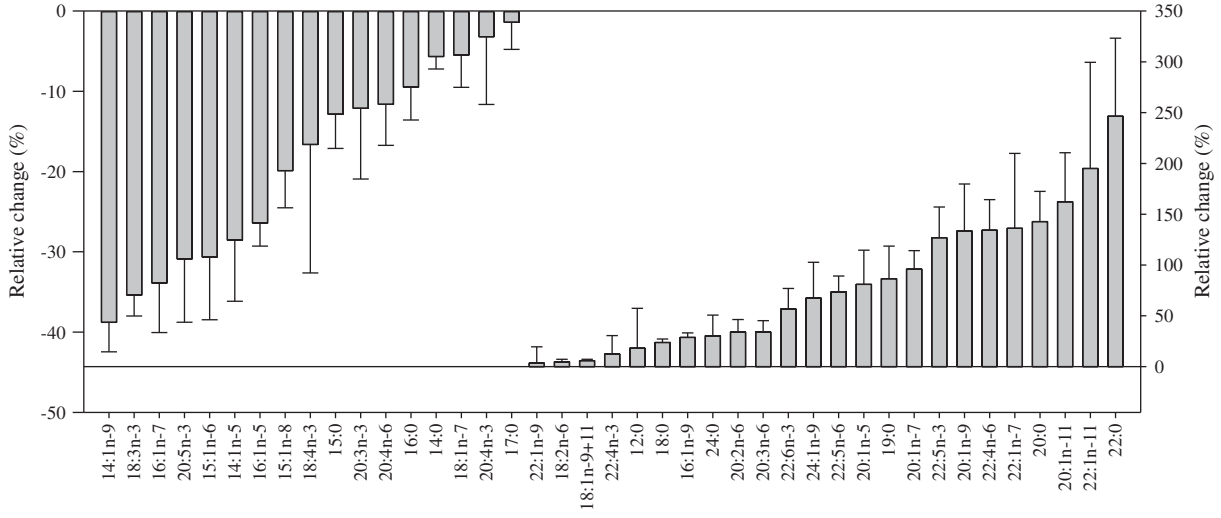

Fig. 4. Relative changes in the proportions of selected fatty acids (FA) in subcutaneous adipose tissue of the fasted striped skunks (n = 7) during winter. + values indicate that a FA increased in proportion and − values signify its decrease in proportion during overwintering (mean + SE).

are mobilized as substrates for the de novo synthesis of glucose also during phase II, which is generally characterized with protein conservation (Castellini and Rea, 1992). The observed hypoalaninemia occurs commonly during prolonged fasting (Mustonen et al., 2004, 2005b, 2006b) and results from the decreased release of alanine from muscle reducing the availability of this major precursor for gluconeogenesis (Felig, 1975).

Similar to American black bears (Wright et al., 1999), the skunks maintained the circulating concentrations of total nonessential and essential AA during fasting. In addition to alanine and taurine, some essential AA, such as histidine, lysine and methionine, were noted to decrease in concentration while the valine level increased. Branched-chain AA, such as valine, can be used as indicators of protein catabolism (Ruderman, 1975; Harlow and Buskirk, 1996). Another sign of muscle protein breakdown could be the elevated plasma 3-methylhistidine concentration (Hissa et al., 1998b; Mustonen et al., 2005b) as displayed in the skunks. Moreover, glutamine is an important carrier of carbon from breakdown of muscle proteins (Ruderman, 1975) and fasting-induced hyperglutaminemia has been documented in some carnivores (Wright et al., 1999; Mustonen et al., 2004, 2006b). This did not occur in the skunks (see also Mustonen et al., 2006a, 2009a) that, on the other hand, experienced increases in their glutamate levels as observed previously in fasted blue foxes (*Vulpes lagopus*; Mustonen et al., 2006b). Glycine concentrations could have been elevated due to increased breakdown of collagen during fasting (Lohuis et al., 2005).

Elevated blood hemoglobin, hematocrit and red blood cell counts were documented in the skunks at the end of the food deprivation period supporting earlier data on fasted gray wolves (*Canis lupus*; DelGiudice et al., 1987) and denning European brown bears (Hissa et al., 1994). Our results were at the upper end of striped skunk reference values reported by Dragoo (2009). Negative energy balance caused dehydration in experimental animals and men leading to increased hemoglobin, hematocrit and erythrocyte counts (Matsuzawa and Sakazume, 1994; Bouhlel et al., 2006). Although not measured in the present study, decreased wintertime water intake in the form of ice could have led to hemoconcentration in the fasted skunks. This may also explain the increase in the platelet count at the end of fasting (Matsuzawa and Sakazume, 1994), and dehydration could have theoretically masked some other hematological changes associated with food deprivation and/or seasonality.

The increased lymphocyte and monocyte counts and/or percentages were similar to the patterns observed in bears (Hissa et al., 1994). Maintaining the immune response has metabolic costs and, thus, its downregulation during the seasonal rest could provide potential energetic benefits. Neutrophils are involved in the defense against bacterial pathogens (Weksler and Moore, 1990) and, during denning, the probability of encountering environmental or food-derived pathogens is presumably lower than during active foraging. This could offer one hypothetical explanation to the decreased neutrophil-% of the fasted skunks. Also the relative change in lymphocytes may be considered beneficial, as increased lymphocyte counts can reflect immunological investment and better body condition (Beldomenico et al., 2008). While the results of CBC are not totally straightforward to interpret, there were no indications that the observed changes would have been hazardous for skunk health. Regarding hematological and clinical chemistry data, PCA indicated that CK, platelet count, glycerol, aminotransferases and U/C ratio could be potentially useful indices of body condition and nutritional status in the species. Overall, the hematologic and plasma biochemical responses of the striped skunk showed many similar characteristics to fasting-adapted ursids (Hissa et al., 1994).

In conclusion, the expression of winter dormancy was flexible in the farm-bred striped skunks: torpor bouts were frequent in some individuals but rare or totally absent in others. The deepest torpor often occurred between dawn and early afternoon and could be followed by an activity phase between late afternoon and midnight. *i*) Our data suggest T_a as a strong abiotic factor influencing the T_b of skunks. Increases in wintertime T_a predicted by climate change scenarios could limit the duration and number of torpor bouts. This may promote active wintertime foraging, animal–animal and human–animal interactions and possibly the spread of zoonoses. *ii*) The striped skunk appears to be resistant to starvation (phase III of fasting) and its responses in hematology and clinical chemistry show similarities to the patterns observed in other fasting-adapted mammals. Biochemical and hematological data can be used as tools in the assessment of body condition in the species. *iii*) FA mobilization is selective in the skunk and the FA preferentially utilized (shorter-chain SFA and MUFA and C18–20 n−3 PUFA) and preserved (long-chain FA) during fasting conform to previously studied carnivores.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2013.08.008.

Acknowledgments

The technical help of Graham Branscombe, Dr. Graham Crawshaw, Marja-Leena Hannila, Anna-Liisa Karttunen, Scott Taylor and Aaron Walpole is greatly acknowledged. Financial support for A-MM was provided by the Kone Foundation and the Emil Aaltonen Foundation. Financial support for the work in Canada was provided by the Ontario Ministry of Natural Resources. The funding sources had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the article for publication. The authors declare no conflict of interest.

References

Adlercreutz, H., Saris, N.-E., Vihko, R., 1983. Analyses of Clinical Chemistry, 3rd ed. Lääketieteenkandidaattiseura ry/Kandidaattikustannus Oy, Helsinki, Finland (In Finnish).
Aleksiuk, M., Stewart, A.P., 1977. Food intake, weight changes and activity of confined striped skunks (*Mephitis mephitis*) in winter. Am. Midl. Nat. 98, 331–342.
Allen, D.L., 1939. Winter habits of Michigan skunks. J. Wildl. Manage. 3, 212–228.
Ambrisko, T.D., Hikasa, Y., Sato, K., 2005. Influence of medetomidine on stress-related neurohormonal and metabolic effects caused by butorphanol, fentanyl, and ketamine administration in dogs. Am. J. Vet. Res. 66, 406–412.
Barclay, R.M.R., Lausen, C.L., Hollis, L., 2001. What's hot and what's not: defining torpor in free-ranging birds and mammals. Can. J. Zool. 79, 1885–1890.
Barton, H.D., Wisely, S.M., 2012. Phylogeography of striped skunks (*Mephitis mephitis*) in North America: Pleistocene dispersal and contemporary population structure. J. Mammal. 93, 38–51.
Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., Begon, M., 2008. The dynamics of health in wild field vole populations: a haematological perspective. J. Anim. Ecol. 77, 984–997.
Bouhlel, E., Salhi, Z., Bouhlel, H., Mdella, S., Amamou, A., Zaouali, M., Mercier, J., Bigard, X., Tabka, Z., Zbidi, A., Shephard, R.J., 2006. Effect of Ramadan fasting on fuel oxidation during exercise in trained male rugby players. Diabetes Metab. 32, 617–624.
Castellini, M.A., Rea, L.D., 1992. The biochemistry of natural fasting at its limits. Experientia 48, 575–582.
Crooks, K.R., Garcelon, D.K., Scott, C.A., Wilcox, J.T., Timm, S.F., Van Vuren, D.H., 2003. Hematology and serum chemistry of the island spotted skunk on Santa Cruz Island. J. Wildl. Dis. 39, 460–466.
DelGiudice, G.D., Seal, U.S., Mech, L.D., 1987. Effects of feeding and fasting on wolf blood and urine characteristics. J. Wildl. Manage. 51, 1–10.
Dragoo, J.W., 2009. Nutrition and behavior of striped skunks. Vet. Clin. Exot. Anim. 12, 313–326.
Environment Canada, 2012. Canadian climate normals or averages 1961–1990. http://climate.weatheroffice.gc.ca/climate_normals/index_1961_1990_e.html (Accessed 5 July 2012).
Felig, P., 1975. Amino acid metabolism in man. Annu. Rev. Biochem. 44, 933–955.
Fowler, P.A., Racey, P.A., 1988. Overwintering strategies of the badger, *Meles meles*, at 57 °N. J. Zool. 214, 635–651.
Frank, C.L., Storey, K.B., 1995. The optimal depot fat composition for hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). J. Comp. Physiol. B 164, 536–542.
Gehrt, S.D., Kinsel, M.J., Anchor, C., 2010. Pathogen dynamics and morbidity of striped skunks in the absence of rabies. J. Wildl. Dis. 46, 335–347.
Geiser, F., Kenagy, G.J., 1987. Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. Am. J. Physiol. 252, R897–R901.
George, J., 2006. Climate change lures skunks, moose to the Arctic. Nunatsiaq News, September 29. http://www.nunatsiaqonline.ca/archives/60929/news/climate/60929_01.html (Accessed 17 October 2012).
Hamilton Jr., W.J., 1937. Winter activity of the skunk. Ecology 18, 326–327.
Harlow, H.J., 1981. Torpor and other physiological adaptations of the badger (*Taxidea taxus*) to cold environments. Physiol. Zool. 54, 267–275.

Harlow, H.J., Buskirk, S.W., 1996. Amino acids in plasma of fasting fat prairie dogs and lean martens. J. Mammal. 77, 407–411.
Harlow, H.J., Seal, U.S., 1981. Changes in hematology and metabolites in the serum and urine of the badger, *Taxidea taxus*, during food deprivation. Can. J. Zool. 59, 2123–2128.
Hissa, R., Siekkinen, J., Hohtola, E., Saarela, S., Hakala, A., Pudas, J., 1994. Seasonal patterns in the physiology of the European brown bear (*Ursus arctos arctos*) in Finland. Comp. Biochem. Physiol. A 109, 781–791.
Hissa, R., Hohtola, E., Tuomala-Saramäki, T., Laine, T., Kallio, H., 1998a. Seasonal changes in fatty acids and leptin contents in the plasma of the European brown bear (*Ursus arctos arctos*). Ann. Zool. Fenn. 35, 215–224.
Hissa, R., Puukka, M., Hohtola, E., Sassi, M.-L., Risteli, J., 1998b. Seasonal changes in plasma nitrogenous compounds of the European brown bear (*Ursus arctos arctos*). Ann. Zool. Fenn. 35, 205–213.
Hock, R.J., 1957. Metabolic rates and rectal temperatures of active and "hibernating" black bears. Fed. Proc. 16, 440.
Humphries, M.M., Thomas, D.W., Speakman, J.R., 2002. Climate-mediated energetic constraints on the distribution of hibernating mammals. Nature 418, 313–316.
Humphries, M.M., Thomas, D.W., Kramer, D.L., 2003. The role of energy availability in mammalian hibernation: a cost-benefit approach. Physiol. Biochem. Zool. 76, 165–179.
Humphries, M.M., Umbanhowar, J., McCann, K.S., 2004. Bioenergetic prediction of climate change impacts on northern mammals. Integr. Comp. Biol. 44, 152–162.
Hwang, Y.T., 2005. Physiological and ecological aspects of winter torpor in captive and free-ranging striped skunks. (PhD thesis) University of Saskatchewan, Saskatoon.
Hwang, Y.T., Larivière, S., Messier, F., 2007. Energetic consequences and ecological significance of heterothermy and social thermoregulation in striped skunks (*Mephitis mephitis*). Physiol. Biochem. Zool. 80, 138–145.
Inouye, D.W., Barr, B., Armitage, K.B., Inouye, B.D., 2000. Climate change is affecting altitudinal migrants and hibernating species. Proc. Natl. Acad. Sci. U S A 97, 1630–1633.
Kvalheim, O.M., Karstang, T.V., 1987. A general-purpose program for multivariate data analysis. Chemom. Intell. Lab. 2, 235–237.
LeBlanc, P.J., Obbard, M., Battersby, B.J., Felskie, A.K., Brown, L., Wright, P.A., Ballantyne, J.S., 2001. Correlations of plasma lipid metabolites with hibernation and lactation in wild black bears *Ursus americanus*. J. Comp. Physiol. B 171, 327–334.
Lohuis, T.D., Beck, T.D.I., Harlow, H.J., 2005. Hibernating black bears have blood chemistry and plasma amino acid profiles that are indicative of long-term adaptive fasting. Can. J. Zool. 83, 1257–1263.
Matsuzawa, T., Sakazume, M., 1994. Effects of fasting on haematology and clinical chemistry values in the rat and dog. Comp. Haematol. Int. 4, 152–156.
McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A 156, 1–18.
Mustonen, A.-M., Nieminen, P., Puukka, M., Asikainen, J., Saarela, S., Karonen, S.-L., Kukkonen, J.V.K., Hyvärinen, H., 2004. Physiological adaptations of the raccoon dog (*Nyctereutes procyonoides*) to seasonal fasting-fat and nitrogen metabolism and influence of continuous melatonin treatment. J. Comp. Physiol. B 174, 1–12.
Mustonen, A.-M., Pyykönen, T., Paakkonen, T., Ryökkynen, A., Asikainen, J., Aho, J., Mononen, J., Nieminen, P., 2005a. Adaptations to fasting in the American mink (*Mustela vison*): carbohydrate and lipid metabolism. Comp. Biochem. Physiol. A 140, 195–202.
Mustonen, A.-M., Puukka, M., Pyykönen, T., Nieminen, P., 2005b. Adaptations to fasting in the American mink (*Mustela vison*): nitrogen metabolism. J. Comp. Physiol. B 175, 357–363.
Mustonen, A.-M., Puukka, M., Saarela, S., Paakkonen, T., Aho, J., Nieminen, P., 2006a. Adaptations to fasting in a terrestrial mustelid, the sable (*Martes zibellina*). Comp. Biochem. Physiol. A 144, 444–450.
Mustonen, A.-M., Pyykönen, T., Puukka, M., Asikainen, J., Hänninen, S., Mononen, J., Nieminen, P., 2006b. Physiological adaptations to fasting in an actively wintering canid, the arctic blue fox (*Alopex lagopus*). J. Exp. Zool. 305A, 32–46.
Mustonen, A.-M., Asikainen, J., Kauhala, K., Paakkonen, T., Nieminen, P., 2007a. Seasonal rhythms of body temperature in the free-ranging raccoon dog (*Nyctereutes procyonoides*) with special emphasis on winter sleep. Chronobiol. Int. 24, 1095–1107.
Mustonen, A.-M., Asikainen, J., Aho, J., Nieminen, P., 2007b. Selective seasonal fatty acid accumulation and mobilization in the wild raccoon dog (*Nyctereutes procyonoides*). Lipids 42, 1155–1167.
Mustonen, A.-M., Käkelä, R., Käkelä, A., Pyykönen, T., Aho, J., Nieminen, P., 2007c. Lipid metabolism in the adipose tissues of a carnivore, the raccoon dog, during prolonged fasting. Exp. Biol. Med. 232, 58–69.
Mustonen, A.-M., Käkelä, R., Nieminen, P., 2007d. Different fatty acid composition in central and peripheral adipose tissues of the American mink (*Mustela vison*). Comp. Biochem. Physiol. A 147, 903–910.
Mustonen, A.-M., Puukka, M., Rouvinen-Watt, K., Aho, J., Asikainen, J., Nieminen, P., 2009a. Response to fasting in an unnaturally obese carnivore, the captive European polecat *Mustela putorius*. Exp. Biol. Med. 234, 1287–1295.
Mustonen, A.-M., Käkelä, R., Asikainen, J., Nieminen, P., 2009b. Selective fatty acid mobilization from adipose tissues of the pheasant (*Phasianus colchicus mongolicus*) during food deprivation. Physiol. Biochem. Zool. 82, 531–540.
Mustonen, A.-M., Käkelä, R., Halonen, T., Kärjä, V., Vartiainen, E., Nieminen, P., 2012a. Fatty acid mobilization in voles—model species for rapid fasting response and fatty liver. Comp. Biochem. Physiol. A 163, 152–160.
Mustonen, A.-M., Lempiäinen, T., Aspelund, M., Hellstedt, P., Ikonen, K., Itämies, J., Vähä, V., Erkinaro, J., Asikainen, J., Kunnasranta, M., Niemelä, P., Aho, J., Nieminen, P., 2012b. Application of change-point analysis to determine winter sleep patterns of the raccoon dog (*Nyctereutes procyonoides*) from body temperature recordings and a multi-faceted dietary and behavioral study of wintering. BMC Ecol. 12, 27.
Mutch, G.R.P., Aleksiuk, M., 1977. Ecological aspects of winter dormancy in the striped skunk (*Mephitis mephitis*). Can. J. Zool. 55, 607–615.
Nieminen, P., Käkelä, R., Pyykönen, T., Mustonen, A.-M., 2006. Selective fatty acid mobilization in the American mink (*Mustela vison*) during food deprivation. Comp. Biochem. Physiol. B 145, 81–93.
Nieminen, P., Rouvinen-Watt, K., Saarela, S., Mustonen, A.-M., 2007. Fasting in the American marten (*Martes americana*): a physiological model of the adaptations of a lean-bodied animal. J. Comp. Physiol. B 177, 787–795.
Nieminen, P., Mustonen, A.-M., Kärjä, V., Asikainen, J., Rouvinen-Watt, K., 2009. Fatty acid composition and development of hepatic lipidosis during food deprivation—mustelids as a potential animal model for liver steatosis. Exp. Biol. Med. 234, 278–286.
Nieminen, P., Hohtola, E., Mustonen, A.-M., 2013. Body temperature rhythms in *Microtus* voles during feeding, food deprivation and winter acclimatization. J. Mammal. 94, 591–600.
Ozgul, A., Childs, D.Z., Oli, M.K., Armitage, K.B., Blumstein, D.T., Olson, L.E., Tuljapurkar, S., Coulson, T., 2010. Coupled dynamics of body mass and population growth in response to environmental change. Nature 466, 482–485.
Paakkonen, T., Mustonen, A.-M., Käkelä, R., Kiljander, T., Kynkäänniemi, S.-M., Laaksonen, S., Solismaa, M., Aho, J., Kortet, R., Puukka, K., Saarela, S., Härkönen, L., Kaitala, A., Ylönen, H., Nieminen, P., 2011. Experimental infection of the deer ked (*Lipoptena cervi*) has no negative effects on the physiology of the captive reindeer (*Rangifer tarandus tarandus*). Vet. Parasitol. 179, 180–188.
Plummer, D.A., Caya, D., Frigon, A., Côté, H., Giguère, M., Paquin, D., Biner, S., Harvey, R., de Elia, R., 2006. Climate and climate change over North America as simulated by the Canadian RCM. J. Clim. 19, 3112–3132.
Raclot, T., 1997. Selective mobilization of fatty acids from white fat cells: evidence for a relationship to the polarity of triacylglycerols. Biochem. J. 322, 483–489.
Raclot, T., 2003. Selective mobilization of fatty acids from adipose tissue triacylglycerols. Prog. Lipid Res. 42, 257–288.
Rosatte, R.C., 1999. Striped, spotted, hooded, and hog-nosed skunk. In: Novak, M. (Ed.), Wild Furbearer Management and Conservation in North America, Section IV: Species Biology, Management, and Conservation. Queen's Printer for Ontario, Toronto, pp. 598–613.
Ruderman, N.B., 1975. Muscle amino acid metabolism and gluconeogenesis. Annu. Rev. Med. 26, 245–258.
Selko, L.F., 1938. Hibernation of the striped skunk in Iowa. J. Mammal. 19, 320–324.
Storm, G.L., 1972. Daytime retreats and movements of skunks on farmlands in Illinois. J. Wildl. Manage. 36, 31–45.
Sunquist, M.E., 1974. Winter activity of striped skunks (*Mephitis mephitis*) in east-central Minnesota. Am. Midl. Nat. 92, 434–446.
van Marken Lichtenbelt, W.D., Daanen, H.A.M., Wouters, L., Fronczek, R., Raymann, R.J.E.M., Severens, N.M.W., Van Someren, E.J.W., 2006. Evaluation of wireless determination of skin temperature using iButtons. Physiol. Behav. 88, 489–497.
Wade-Smith, J., Verts, B.J., 1982. *Mephitis mephitis*. Mamm. Species 173, 1–7.
Warren, F.J., Egginton, P., 2008. Background information: concepts, overviews and approaches. In: Lemmen, D.S., Warren, F.J., Lacroix, J., Bush, E. (Eds.), From Impacts to Adaptation: Canada in a Changing Climate 2007. Government of Canada, Ottawa, pp. 27–56.
Weksler, B.B., Moore, A., 1990. Leukocyte disorders. In: Andreoli, T.E., Carpenter, C.C.J., Plum, F., Smith Jr., L.H. (Eds.), Cecil Essentials of Medicine, 2nd ed. WB Saunders, Philadelphia, pp. 366–369.
Wright, P.A., Obbard, M.E., Battersby, B.J., Felskie, A.K., LeBlanc, P.J., Ballantyne, J.S., 1999. Lactation during hibernation in wild black bears: effects on plasma amino acids and nitrogen metabolites. Physiol. Biochem. Zool. 72, 597–604.