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RESEARCH ARTICLE

Effects of unsaturated fatty acids on torpor frequency and diet selection in Djungarian hamsters (*Phodopus sungorus*)

Victoria Diedrich, Stephan Steinlechner and Frank Scherbarth*

ABSTRACT

Essential polyunsaturated fatty acids (PUFA) have been shown to play a beneficial role in hibernating mammals. High amounts of dietary PUFA led to an earlier hibernation onset, deeper and longer hibernation bouts and a higher proportion of hibernating animals in several species. In contrast, the relevance of dietary PUFA for daily heterotherms exhibiting only brief and shallow torpor bouts is less well studied. Therefore, diets differing in PUFA composition were used to examine the effects on the frequency of spontaneous daily torpor in Djungarian hamsters (*Phodopus sungorus*). In contrast to earlier studies, we were interested in whether the ratio of n-6 to n-3 PUFA affects torpor expression, and in comparison with a diet rich in monounsaturated fatty acids (MUFA). Although we found a positive effect on torpor frequency in hamsters fed a diet rich in n-6 PUFA compared with the groups fed diets either rich in n-3 PUFA or MUFA, the latter two groups did not show unusually low torpor frequencies. The results of the additional diet choice experiment indicated that hamsters in short photoperiod select food with only a slight excess of n-6 PUFA compared with n-3 PUFA (ratio of 1 to 1.5). However, there was no significant difference in torpor frequency between the diet choice group and hamsters fed on standard chow with a sevenfold excess of n-6 PUFA. In summary, the present data strongly indicate that the dietary composition of unsaturated fatty acids plays a minor role in the occurrence of spontaneous daily torpor in Djungarian hamsters.

KEY WORDS: Heterothermic, PUFA, MUFA, Thermoregulatory behaviour, Diet choice

INTRODUCTION

In heterothermic mammals, especially hibernators, the torpid state with body temperatures (T_b) far below normothermia appears to necessitate changes in body lipids. The fatty acid composition of stored fat and membrane phospholipids is assumed to play an important role for maintenance of energy supply and organ function (Abbott et al., 2012; Ruf and Arnold, 2008). For instance, proper functioning of the heart at low T_b is likely to be dependent on appropriate membrane fatty acid composition of the sarcoplasmic reticulum. In Syrian hamster (*Mesocricetus auratus*) cardiomyocytes, activity of a calcium pump in sarcoplasmic reticulum membranes (SERCA) was higher in cooling or torpid animals than during normothermic periods. Increased SERCA activity was associated with high membrane proportions of linoleic acid (LA) and low proportions of docosahexaenoic acid (DHA) (Giroud et al., 2013). In addition, the minimum T_b of torpor bouts was positively correlated with LA content and inversely correlated with proportions of DHA in sarcoplasmic reticulum membranes.

Like hibernation, seasonal daily torpor is a thermoregulatory behaviour that is employed mainly as an energy-saving mechanism throughout the winter (Heldmaier and Steinlechner, 1981). Unlike hibernators, animals that use seasonal daily torpor such as Djungarian hamsters [*Phodopus sungorus* (Pallas)] may reduce their T_b by only about 20°C. Nevertheless, acclimation of Djungarian hamsters to short photoperiod (SP) resulted in changes of tissue lipid composition in all examined tissues, i.e. heart, leg muscle and brown adipose tissue (Geiser et al., 2013). The fact that the composition of somatic lipids changed while food remained the same is in accordance with diet-independent remodelling of membranes before hibernation in alpine marmots (*Marmota marmota*) (Arnold et al., 2011). However, intake of fatty acids and proportions in triglycerides and membranes cannot be entirely uncoupled. Mammals have to ingest polyunsaturated fatty acids (PUFA), such as LA (18:2n-6) and α-linolenic acid (ALA, 18:3n-3), because desaturation enzymes for their synthesis are lacking. Thus, incorporation of PUFA into both triglycerides and membrane phospholipids is dependent on dietary content and nutritional availability to the animals. This, in turn, corresponds to the finding that the fatty acid composition of brown fat reflected that of dietary fatty acids in Djungarian hamsters (Geiser and Heldmaier, 1995), and is also in line with increased dietary PUFA intake before hibernation in golden-mantled ground squirrels (*Spermophilus lateralis*) (Frank, 1994).

It is worth mentioning that the vast majority of feeding experiments were based on diets high in LA (for review, see Munro and Thomas, 2004), which also applies to the pioneering work in ground squirrels (*Spermophilus saturatus*) (Geiser and Kenagy, 1993). This might have been due to the assumption that a high ratio between LA and ALA should be most beneficial for membrane properties at low T_b (for review, see Ruf and Arnold, 2008). In fact, in comparison to a diet high in saturated lipids, diets rich in polyunsaturated LA have been shown to enhance the incidence of daily torpor in Djungarian hamsters kept at moderate cold ambient temperature (T_a) (Geiser and Heldmaier, 1995). However, there are also contradictory results that raise reasonable doubt as to whether LA plays a consistent role. For instance, a 4-fold dietary content of LA compared with ALA prevented hibernation at low T_a in golden-mantled ground squirrels, unlike a balanced nutritional ratio of the two essential PUFA (Frank et al., 2004). In contrast, prevention of hibernation in marmots (*Marmota flaviventris*) by a considerable dietary excess of ALA versus LA indicates a potentially inhibitory effect of ALA on torpor (Hill and Florant, 2000). Although there is convincing evidence that dietary PUFA may affect hibernation and daily torpor, further studies appear to be necessary to ascertain whether there are general effects or considerable species-dependent differences. This includes the examination of different dietary PUFA and their influence on torpor expression as previous studies compared fatty acids of different

Department of Zoology, University of Veterinary Medicine Hannover, 30559 Hannover, Germany.

*Author for correspondence (frank.scherbarth@tiho-hannover.de)

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List of symbols and abbreviations

ALA	α-linolenic acid
CET	Central European Time
DHA	docosahexaenoic acid
LA	linoleic acid
LP	long photoperiod
MUFA	monounsaturated fatty acid(s)
M_b	body mass
OA	oleic acid
PUFA	polyunsaturated fatty acid(s)
SERCA	sarcoplasmic/endoplasmic reticulum calcium ATPase
SFA	saturated fatty acids
SP	short photoperiod
T_a	ambient temperature
T_b	body temperature
T_s	surface temperature
UFA	unsaturated fatty acid(s)

saturation, i.e. PUFA, monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) (Munro and Thomas, 2004). Therefore, in this study we aimed to find out whether the dietary ratio between LA and ALA affects the occurrence of spontaneous daily torpor in Djungarian hamsters. In addition, an extensive food choice experiment was carried out to examine whether the seasonal hamsters show photoperiod-dependent preferences for one of the two essential PUFA and their ratio.

RESULTS

Surface temperature measurements

Non-invasive temperature measurements of the hamsters' body surface (from above, T_s) with a small infrared thermometer inside the nest box revealed values closely correlated to core body temperature (Fig. 1). Although short excursions from the nest box resulted in a temperature drop, the rapid decline and rise (1 min resolution) were easy to distinguish from torpor events by differences in time course (supplementary material Fig. S1). Furthermore, the hamsters entered torpor only when retreated inside the nest box. Although the method did not provide accurate temperature values of T_s, the changes in T_s allowed for reliable identification of torpor by visual inspection of the plotted raw data.

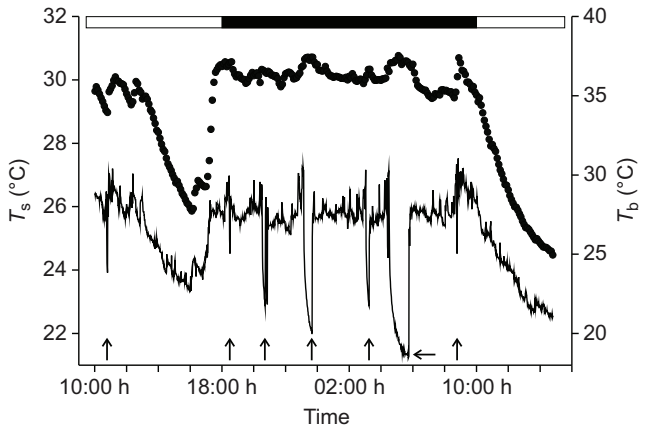

Fig. 1. Comparison between two methods for determination of torpor. Core body temperature (T_b, dots) and approximate body surface temperature (T_s, solid line) of a hamster exhibiting spontaneous daily torpor. T_b was measured with an implanted temperature-sensitive transmitter and, simultaneously, T_s was monitored with an infrared thermometer at 20±1°C. The arrows depict periods when the hamster was outside the nest box (drop in T_s) or just returning (steep rise in T_s). Black and white bars at the top indicate times of lights off and lights on, respectively.

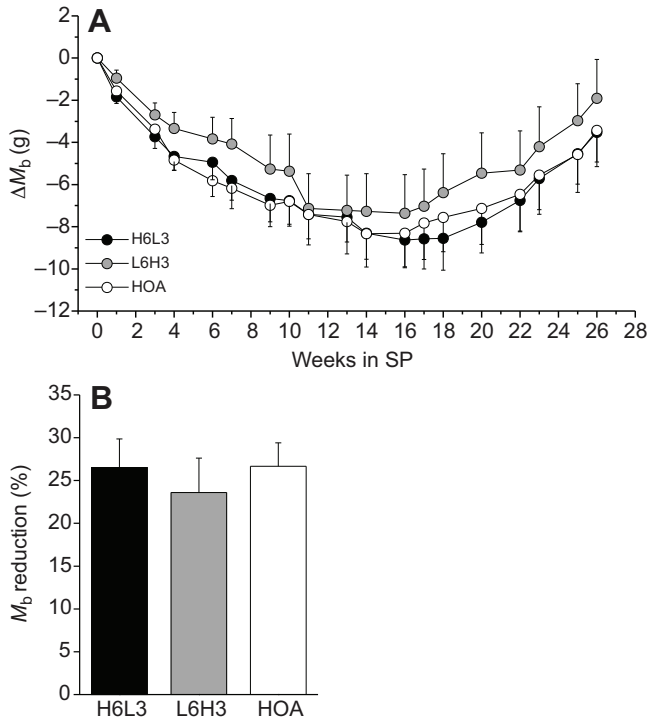

Fig. 2. Changes in body mass. (A) Body mass change (ΔM_b; mean ± s.e.m.) of the three treatment groups since the beginning of short photoperiod (SP) exposure. Groups received diets with high n-6:n-3 PUFA ratio (H6L3), low n-6:n-3 PUFA ratio (L6H3) and a low proportion of n-6 and n-3 PUFA (HOA). (B) Maximum mean (±s.e.m.) relative M_b reduction of the three treatment groups during SP exposure.

Experiment 1

Body mass and food intake

Exposure to SP resulted in a loss of body mass (M_b) in all treatment groups (Fig. 2A). Changes in M_b over the whole time course of the experiment did not differ between the groups at any time point [repeated measures (RM) ANOVA: $F_{32,432}$=0.32, P=1.0]. This was also the case for the maximum relative reduction in M_b. In total, animals of the H6L3 (high n-6 to n-3 PUFA ratio), L6H3 (low n-6 to n-3 PUFA ratio) and HOA [high proportion of MUFA (oleic acid, OA)] groups reduced their M_b by 26.5±3.4%, 23.6±4.0% and 26.7±2.7%, respectively (ANOVA: $F_{2,27}$=0.04, P=0.96; Fig. 2B).

The maximum reductions in relative daily food intake were 35.5±3.2% (H6L3), 33.9±3.0% (L6H3) and 29.8±1.8% (HOA) (ANOVA: $F_{2,27}$=1.43, P=0.256). Relative daily food intake did not differ significantly between treatment groups at any time point of the experiment, although animals of the H6L3 group tended to show a higher reduction (RM ANOVA: $F_{34,442}$=1.31, P=0.119; Fig. 3).

Fur index

All animals exhibited changes in fur colour in response to SP exposure (see Materials and methods). Between the groups, the highest fur index did not differ significantly (H6L3: 3.3±0.3; L6H3: 3.3±0.3; HOA: 3.4±0.2; ANOVA: $F_{2,27}$=0.17, P=0.845).

Torpor expression

The different groups showed a total number of 437 (H6L3), 283 (L6H3) and 300 (HOA) torpor bouts. Animals of the H6L3 group contributed 43% to the overall number of detected torpor bouts, which was significantly higher than the percentage of both the L6H3

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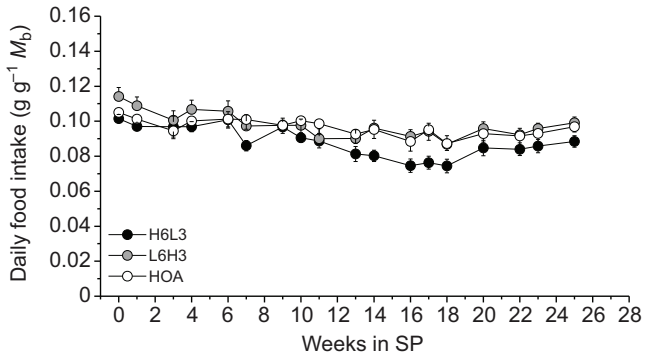

Fig. 3. Food intake per day per gram of M_b. Mean (±s.e.m.) relative daily food intake of the three treatment groups described in Fig. 2.

(28%) and HOA group (29%) (χ^2-test, 3×2 table: χ^2=70.74, P<0.001; H6L3 versus L6H3 χ^2=63.59, P<0.001 and H6L3 versus HOA χ^2=35.02, P<0.001).

The following analysis of torpor expression included only those animals in each group that showed torpor more than once (H6L3 N=10, L6H3 N=10 and HOA N=10). Animals of the H6L3 group showed a significantly higher torpor incidence (9.2±1.2) compared with those in the L6H3 group (5.4±1.2) and the HOA group (5.7±1.5) only during weeks 16 and 17 of SP exposure (RM ANOVA: $F_{14,189}$=1.79, P=0.043; Tukey *post hoc* test: H6L3 versus L6H3 P=0.013 and H6L3 versus HOA P=0.042) (Fig. 4A). Accordingly, comparison of the total number of torpor bouts did not reveal any significant difference (H6L3: 43.7±8.0, L6H3: 28.3±7.3, HOA: 30.0±7.9; ANOVA: $F_{2,27}$=1.2, P=0.318) (Fig. 4B). The latency between the beginning of SP exposure and first torpor expression (between weeks 9 and 10) as well as the duration of torpor episodes did not differ between the experimental groups (data not shown).

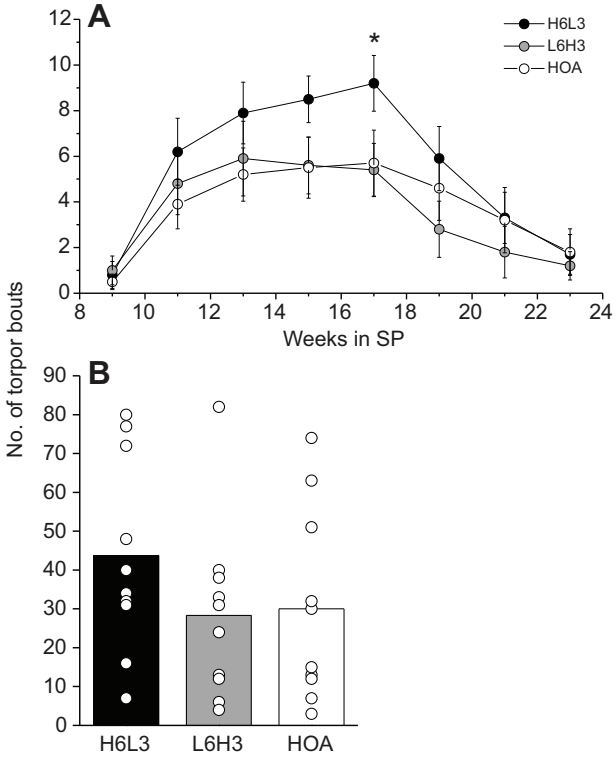

Fig. 4. Torpor occurrence. (A) Mean (±s.e.m.) number of torpor bouts per 2 weeks of observation of the three treatment groups described in Fig. 2. The asterisk denotes a significantly higher number of torpor bouts in H6L3 compared with both other groups (RM ANOVA: F=1.79, P=0.043; Tukey *post hoc* test: H6L3 versus L6H3 P=0.013 and H6L3 versus HOA P=0.042). (B) Mean number of torpor bouts for the whole observation period. Circles represent individual values (for s.e.m., see Results).

Experiment 2

M_b and food choice

Comparison of photoperiod-induced changes in M_b revealed no significant differences between controls and the food choice group (RM ANOVA, $F_{26,468}$=0.99, P=0.48) (Fig. 5).

Food choice resulted in significantly different percentage choice of the three diets, but only during exposure to SP (RM ANOVA, $F_{56,588}$=2.83, P<0.001) (Fig. 6A). Selection of the diet rich in monounsaturated oleic acid (OA; diet HOA) was almost constantly higher compared with selection of the diet rich in polyunsaturated LA (H6L3). In comparison with the diet rich in polyunsaturated ALA (L6H3), the percentage of HOA was significantly increased only during weeks 6, 7, 10 and 17. There was no significant difference between the percentage of H6L3 and L6H3. For long photoperiod (LP) exposure, the mean percentages were almost identical if compared between the two LP periods (H6L3: 28.0±3.2% versus 28.4±1.8%; L6H3: 35.6±2.9% versus 35.9±3.4%; HOA: 36.4±0.8% versus 35.7±2.6%; Fig. 6B). However, proportions of HOA were significantly lower in LP compared with the two periods of cold exposure in SP (RM ANOVA, $F_{12,162}$=7.3, P<0.001; followed by Tukey test, P<0.02, each).

As a result of diet choice, fatty acid intake relative to M_b showed a significantly increased uptake of OA compared with LA and/or ALA almost exclusively during SP exposure (Fig. 7A). The possible range for the ratio between dietary LA and ALA was 0.3–11.8 (control chow: 7.3), where the extreme values would represent exclusive feeding on either L6H3 or H6L3. The range for the ratio between PUFA and MUFA ([LA+ALA]:OA) was 0.4–3.6 (2.6 for the standard chow). Calculation of the mean ratios revealed only a slight tendency towards reduced PUFA:MUFA ratios in selected food during SP exposure (0.9–1.4) compared with LP conditions (1.4–1.8) (Fig. 7B). The mean ratio between the two PUFA, LA (n-6) and ALA (n-3), ranged from 0.9 to 2.0 during exposure to LP, and between 0.8 and 1.5 in SP.

Torpor

Beginning in week 11 of SP exposure, the control group (N=10) showed a total number of 182 torpor bouts compared with 199 in

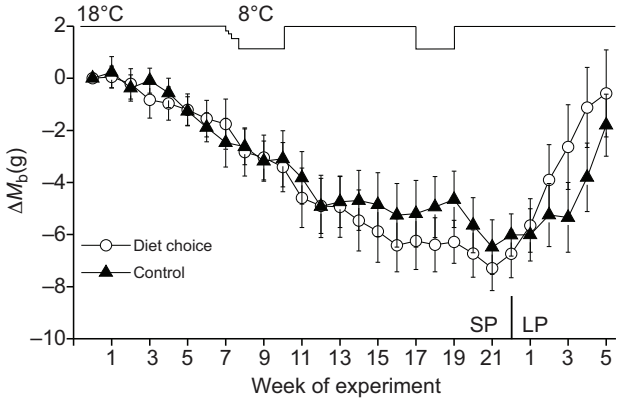

Fig. 5. Changes in M_b. M_b (mean ± s.e.m.) following the switch from long photoperiod (LP) to SP and back to LP after 22 weeks of SP exposure. Ambient temperature (T_a) is schematically depicted as a black line in the upper part of the graph.

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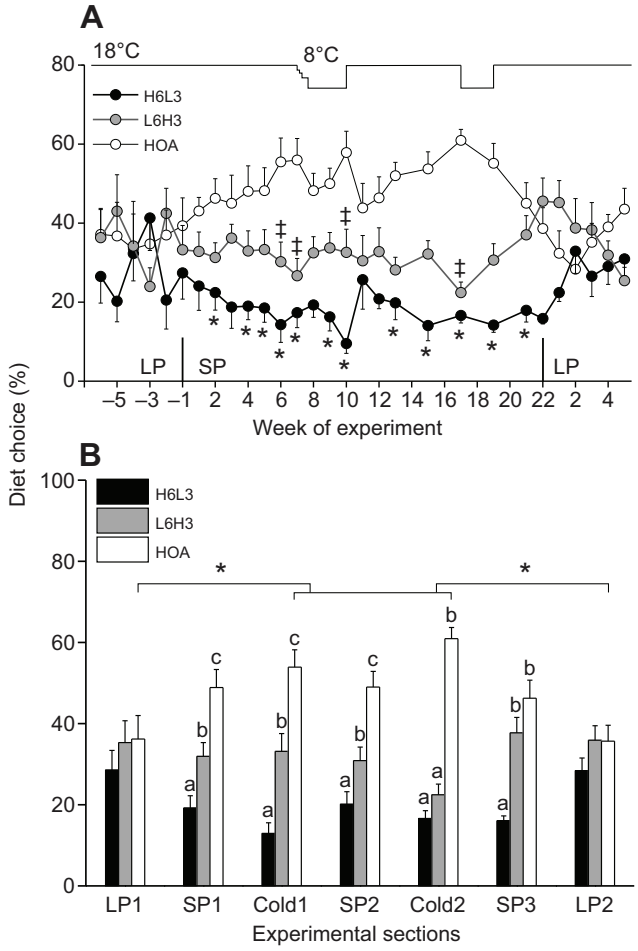

Fig. 6. Proportions of the different diets as a result of diet self-selection. (A) Percentages for the different diets rich in linoleic acid (H6L3), α-linolenic acid (L6H3) or oleic acid (HOA). The values represent means (and s.e.m.) from determinations of food intake every week or every other week (weeks 13–21 in SP). Significant differences between diets are indicated: ‡L6H3 versus HOA; *H6L3 versus HOA (RM ANOVA followed by Tukey test). T_a is schematically depicted as a black line in the upper part of the graph. (B) Percentages (mean and s.e.m.) of diets averaged for the consecutive experimental periods: LP1 (weeks –6 to –1), SP1 (weeks 1–7), Cold1 (weeks 9–10), SP2 (weeks 11–17), Cold2 (weeks 18–19), SP3 (weeks 20–22), LP2 (weeks 1–5). Significant differences within each period are denoted by different lowercase letters (one-way ANOVA followed by Student–Newman–Keuls test). Significant differences between experimental periods are indicated by asterisks (RM ANOVA followed by Tukey test).

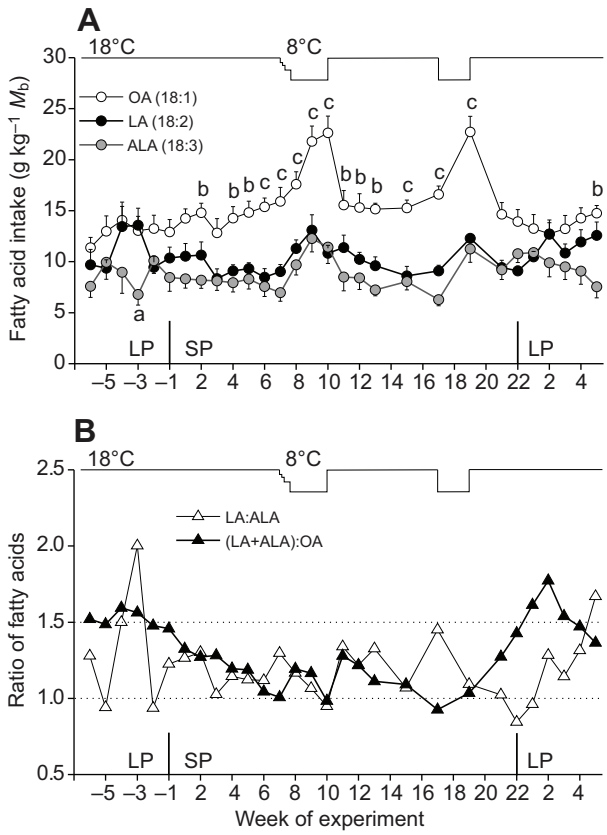

Fig. 7. Fatty acid composition of self-selected diets. (A) Weekly amounts of ingested fatty acids (mean and s.e.m.) relative to M_b. Significant differences between linoleic acid (LA), α-linolenic acid (ALA) and oleic acid (OA) are indicated by a (ALA versus LA and OA), b (OA versus ALA) or c (OA versus LA and ALA) (RM ANOVA followed by Tukey test). (B) Mean ratios between the amounts of ingested fatty acids (related to M_b). For better clarity, error bars have been omitted and dotted lines added. T_a is schematically depicted as a thin black line in the upper part of each graph.

the food choice group (N=10). However, three control hamsters did not use torpor at all compared with only one animal in the food choice group. These hamsters were excluded from the calculation of torpor frequencies. RM ANOVA revealed no significant difference between weekly numbers of torpor bouts ($F_{14,196}$=0.92, P=0.54; Fig. 8), and there was no effect of lowered T_a on torpor expression. Similarly, the total number of torpor bouts per animal for the whole observation period (including LP exposure) was not significantly different between controls (26.0±8.8; N=7) and the food choice group (22.1±6.2; N=9).

DISCUSSION

The aim of the present study was to examine the relevance of the essential fatty acids LA (n-6) and ALA (n-3), on the occurrence of spontaneous daily torpor. The non-invasive, indirect monitoring of torpor by infrared thermometers that registered the surface temperature of the hamsters during their stay in the nest box proved to be a reliable method for the determination of torpor frequency (Geiser and Heldmaier, 1995).

Djungarian hamsters show this energy-saving behaviour after a certain period of exposure to SP despite food abundance (Heldmaier and Steinlechner, 1981). The typical loss of M_b during acclimation to SP is commonly assumed to be a prerequisite for the expression of spontaneous daily torpor (Ruf et al., 1993). In the present study, the different experimental groups showed a similar reduction in M_b, regardless of dietary composition of unsaturated fatty acids (UFA). Also, the decrease in food intake, which is linked to the loss of M_b, remained unaffected by dietary fat composition. Thus, SP-mediated downregulation of M_b in Djungarian hamsters appears to be largely independent of dietary composition of UFA. This conclusion is supported by a previous finding in the same species; a diet supplemented with PUFA had no effect on the SP-induced decrease in M_b compared with food rich in MUFA and SFA (Gutowski et al., 2011). In contrast, feeding a PUFA-enriched diet resulted in a diminished loss of M_b over the whole hibernation period in alpine marmots (*M. marmota*) (Bruns et al., 2000), whereas a diet deficient in essential fatty acids increased the loss of M_b in yellow-bellied marmots (*M. flaviventris*) (Florant et al., 1993). In a daily heterotherm, the deer mouse (*Peromyscus maniculatus*), torpor-enhancing effects of increased PUFA availability were also

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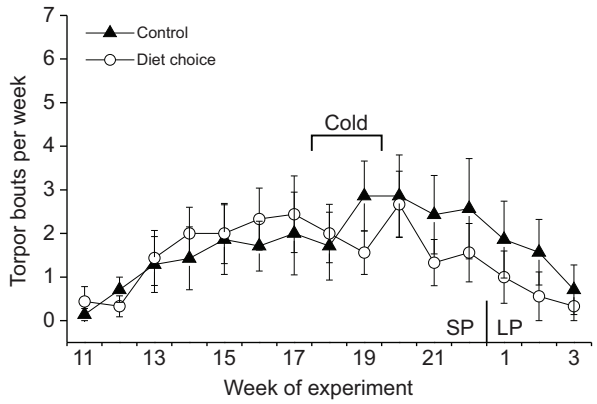

Fig. 8. Torpor frequency. Number (mean ± s.e.m.) of torpor bouts per week per animal during exposure to SP followed by LP. Sample sizes are limited to the hamsters that displayed torpor. During weeks 18 and 19 in SP, T_a was reduced from 18 to 8°C (Cold).

accompanied by a decreased loss of M_b (Geiser, 1991). However, torpor was induced by cold exposure and withdrawal of food and water.

Although M_b change of the Djungarian hamsters was not affected by different compositions of dietary fat, we found a stimulating effect on daily torpor in animals fed the diet with a high LA:ALA ratio (H6L3). This is in accordance with previous findings (Geiser and Heldmaier, 1995) and the hypothesis of beneficial effects for torpor being highest when membrane phospholipids contain a high n-6:n-3 PUFA ratio (LA:ALA) (Ruf and Arnold, 2008). However, in experiment 2 (the food choice experiment), the LA:ALA ratio was much higher in the control chow compared with the results of diet self-selection, but there was no difference in torpor frequency. The fact that a slight surplus of dietary LA versus ALA did not diminish torpor frequency compared with a diet with a considerable LA excess suggests that Djungarian hamsters would not benefit from maximizing LA intake with regard to displaying torpor. However, diet self-selection and the lower LA:ALA ratio did not increase torpor expression, which would have been in line with the hypothesized strategy of optimized PUFA intake (Frank, 1994; Frank et al., 1998; Hill and Florant, 2000; Munro and Thomas, 2004).

While we expected PUFA intake to be higher in SP, selection of MUFA showed a tendency towards an increase in SP, although diet choice reached significance only for the two periods of cold exposure. The latter might be indicative of the T_a being more important than photoperiod for changes in diet self-selection. In accordance with this, Djungarian hamsters that were offered two diets differing in lipid composition showed similar temperature-dependent changes in diet choice independent of being kept in LP or acclimated to SP (Hiebert et al., 2000; Hiebert et al., 2003). The present finding of a tendency towards decreased PUFA:MUFA ratios in SP appears to support the view that a high proportion of MUFA in body tissue might be sufficient to maintain membrane fluidity and function within the temperature range of torpor bouts in daily heterotherms (Falkenstein et al., 2001; Schalk and Brigham, 1995). This argument is strengthened by studies providing evidence for a torpor-facilitating effect of MUFA in daily heterotherms and even hibernators (Frank and Storey, 1996; Geiser and Heldmaier, 1995).

Observations in hibernators such as ground squirrels (*S. lateralis*) provided evidence for PUFA-selective feeding in preparation for hibernation (Frank, 1994); marmots (*M. flaviventris*) even extended their home range to specifically include PUFA-rich food items in their diet prior to hibernation (Florant et al., 1990). Interestingly, field studies also indicated seasonal food preferences in Djungarian hamsters (Flint, 1966). Winter burrows contained predominantly composite seeds, which are rich in PUFA, predominantly LA (Hitchcock and Nichols, 1971). However, it is possible that the stored seeds simply reflected the availability of this food item rather than food preferences (cf. Fine and Bartness, 1996). In fact, high calorie supplementary feeding of LA-rich sunflower seeds did not enhance torpor expression in Djungarian hamsters (Ruf et al., 1991). At this point, it should be mentioned that besides diet composition and selective food uptake, factors such as preferential intestinal absorption, storage, incorporation into membranes, retention or oxidation might also affect membrane FA composition (Florant, 1998; Giroud et al., 2009; Price et al., 2013; Ruf and Arnold, 2008; Zhou and Nilsson, 2001). Although we cannot provide data on tissue composition from our experiments, Geiser and Heldmaier (Geiser and Heldmaier, 1995) found a close correlation between fatty acid composition of diet and brown adipose tissue in Djungarian hamsters.

A diet high in LA (n-6 PUFA) only slightly increased the number of torpor bouts compared with diets rich in either ALA (n-3 PUFA) or OA (MUFA), indicating that the dietary composition of UFA is of minor relevance for the occurrence of daily torpor.

The conclusion that the dietary composition of UFA plays a minor role in affecting torpor is supported by diet self-selection, which revealed LA:ALA (n-6:n-3 PUFA) ratios considerably lower than in the standard hamster chow, but without affecting torpor frequency. Therefore, the present data support the conjecture that the torpor-enhancing effect of PUFA should be more important for hibernators (Munro and Thomas, 2004), which show much longer and deeper reductions in T_b and rely completely on accessible fat stores for energy supply (Geiser and Ruf, 1995), whereas daily heterotherms spent a great proportion of time in a normothermic state.

MATERIALS AND METHODS

Animal husbandry and all experiments were in accordance with the German Animal Welfare Act and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (11/0372, 13/1175).

To investigate the potential influence of different nutritional ratios of n-6 and n-3 PUFA on the expression of spontaneous daily torpor in Djungarian hamsters (*P. sungorus*), two experiments were conducted, as described below.

Animals

The female Djungarian hamsters used in the following experiments originated from the breeding colony of the Institute of Zoology (University of Veterinary Medicine Hannover, Germany) and were born and raised in an outdoor enclosure under natural photoperiod (Hannover, ~52° N latitude) and ambient temperatures. The hamsters were housed in Type II Makrolon cages (16.5×22.0×14.0 cm) with wood shavings and soft tissue as bedding material. In addition to tap water and standard chow (7014, hamster breeding diet, Altromin Spezialfutter, Lage, Germany) which were provided *ad libitum*, the animals received oat flakes, sunflower seeds and apple until 4 weeks before the beginning of the experiments.

Table 1. Composition of experimental diets according to manufacturer's specifications

Macronutrients (% of diet)	H6L3	L6H3	HOA	Control
Protein	22	22	22	23
Fat	5	5	5	5
Carbohydrates	46	46	46	50

H6L3, L6H3 and HOA diets were from ssniff; control diet was from Altromin (see Materials and methods).

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Table 2. Dietary composition of fatty acids (%) according to the manufacturer's specifications

Fatty acids					
C:D	Name	H6L3	L6H3	HOA	Control
C14:0	Myristic acid	0.4	0.4	0.4	–
C16:0	Palmitic acid	9.5	6.9	10.8	11.7
C18:0	Stearic acid	3.2	4.1	3.1	3.4
C20:0	Arachidic acid	0.4	–	0.4	0.4
C16:1	Palmitoleic acid	–	–	1.0	0.2
C18:1	Oelic acid	18.9	19.2	60.0	20.6
C20:1	Eicosenoic acid	0.4	–	–	0.8
C18:2	Linoleic acid; n-6	62.0	14.3	17.1	45.9
C18:3	α-Linolenic acid; n-3	5.2	55.1	5.3	6.3
C20:4	Arachidonic acid	–	–	–	1.1
C20:5	Eicosapentaenoic acid	–	–	–	0.5
Ratio					
C18:2 to C18:3		11.8	0.3	3.2	7.3
(C18:2+C18:3) to C18:1		3.7	3.6	0.4	2.5

H6L3, L6H3 and HOA diets were from ssniff; control diet was from Altromin (see Materials and methods). C:D, number of carbon atoms and double bonds.

Experimental diets

The three diets (ssniff Spezialdiäten, Soest, Germany) differed in the composition of fatty acids, whereas macronutrients and caloric content were kept constant (Tables 1, 2). All diets provided basic nutritional requirements regarding minerals, trace elements, vitamins and essential fatty acids. The differences in the amounts of the essential PUFA (LA and ALA) and the monounsaturated OA were achieved by different combinations of vegetable oils (soybean, safflower, linseed, olive) as the lipid source.

The first diet (H6L3) contained high amounts of LA as the n-6 PUFA source and a low amount of ALA, the n-3 PUFA source. The second diet (L6H3) contained a low n-6 to n-3 PUFA ratio, and the third diet was low in LA and ALA, but high in monounsaturated OA (HOA).

Torpor monitoring

Hamsters were provided with a small wooden nest box (7×5×5 cm, l×w×h, inner size) for measurement of T_s. A small infrared thermometer (MLX90614ESF-BAA; Melexis Microelectronic Systems, Ieper, Belgium) with a coverage angle of 90 deg was fixed to a hole in the middle of the top (distance of ~2.5 cm between the sensor and the hamster's back) of each nest box and was connected to a microcontroller board (Leonardo Arduino). The measurement resolution and accuracy of the thermometers were 0.02 and 0.5°C, respectively. T_s values were stored every minute on a PC receiving the data from the microcontroller board. Torpor registrations were validated by using hamsters from another experiment that had been implanted i.p. with temperature-sensitive transmitters (TA11TA-F10, Data Science International, St Paul, MN, USA). Thus, core T_b could be directly compared with T_s (Fig. 1). This non-invasive method allowed easy and reliable detection of torpor bouts via continuous registration of T_s while leaving the animals undisturbed (cf. Geiser and Heldmaier, 1995; Warnecke, 2012). A torpor bout was characterized by a gradual reduction of T_s (moderate slope) that could be clearly distinguished from a rapid change in T_s evoked by a hamster leaving the nest box (Fig. 1; supplementary material Fig. S1). After the hamsters were provided with the nest box, they received no more tissue for nest building to allow unhindered measurement of T_s.

Experimental setup

Experiment 1

At the summer solstice, 34 adult female hamsters with an age of about 3 months were transferred to a temperature-controlled chamber (18±1°C) with a LP of 16 h of light and 8 h of darkness [lights on 04:00 h–20:00 h, Central European Time (CET)] where they were kept singly in plastic cages (type II).

Before the animals had access to the experimental diets, their daily intake of the standard diet (Altromin) and their M_b were determined for 30 days. Eleven females were then provided with the H6L3 diet, and 12 and 11 females were supplied with the L6H3 or HOA diet, respectively. The pellet food was supplemented by a slice of apple every 10 days. After 20 days of acclimation to lowered T_a and new food, the lighting regime was switched to SP with 8 h of light and 16 h of darkness (lights on 06:00 h–14:00 h, CET).

M_b, food intake and fur index were assessed once per week for 26 weeks of SP exposure. The fur index was determined according to Figala and colleagues with index 1 for dark summer fur and index 6 for white winter fur (Figala et al., 1973).

Beginning in week 8 (day 56), torpor expression was continuously monitored until the end of the experiment by providing each animal with a wooden nest box equipped with an infrared thermometer.

Experiment 2

Adult female hamsters (7–8 months old) were kept singly in plastic cages (type II) lined with wood shavings. Both tap water and food (hamster breeding diet, Altromin 7014) were available *ad libitum* and tissue was provided for nest building. Between the summer solstice and the beginning of the experiment (6 months), the animals were kept under LP (lights on 04:00 h–20:00 h, CET) at 21±1°C. With the beginning of the experiment, T_a was lowered to 18±1°C. One group of hamsters (N=10) still had free access to the standard chow (control), whereas the second group (N=10) was offered three diets differing in fatty acid composition (choice). After 6 weeks in LP, the light–dark cycle was switched to a winter-like SP (lights on 08:00 h–16:00 h, CET) for 22 weeks followed again by 5 weeks of LP exposure. During exposure to SP, T_a was reduced to 8°C twice. The first time (after 6 weeks in SP), T_a was lowered stepwise over 4 days from 18°C to 8°C. Cold exposure lasted until the end of the ninth week in SP before T_a was again elevated to 18°C. For the second period of cold exposure (weeks 18–19), T_a was abruptly decreased to 8°C (over a few hours). Food intake and M_b were determined once per week, coincident with cage changes. Between weeks 13 and 21 during SP exposure (i.e. during the peak torpor period), measurements of food intake and M_b, and cage cleaning were reduced to 2 week intervals to minimize disturbances. Food pellets were provided in small food hoppers that were designed to collect spillage from feeding. For determination of food intake, cages were additionally checked for any small food pellets the hamsters removed from the hopper(s). For the choice group, the arrangement of the three hoppers was randomly changed every week. After about 11 weeks in SP, each cage was equipped with an infrared thermometer integrated in a wooden nest box.

Statistics

Data are presented as means ± s.e.m. except for the probability distribution, which was analysed by using the χ^2-test. Different groups were compared with one-way ANOVA followed by Student–Newman–Keuls test or RM ANOVA and subsequent Tukey *post hoc* test. $P<0.05$ was considered statistically significant.

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Competing interests
The authors declare no competing financial interests.

Author contributions
V.D., F.S. and S.S. conceived and designed the experiments. V.D. performed the first and F.S. the second experiment with data analyses included. V.D., F.S. and S.S. wrote the manuscript.

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