



Diet affects resting, but not basal metabolic rate of normothermic Siberian hamsters acclimated to winter

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ABSTRACT

We examined the effect of different dietary supplements on seasonal changes in body mass (m_b), metabolic rate (MR) and nonshivering thermogenesis (NST) capacity in normothermic Siberian hamsters housed under semi-natural conditions. Once a week standard hamster food was supplemented with either sunflower and flax seeds, rich in polyunsaturated fatty acids (FA), or mealworms, rich in saturated and monounsaturated FA. We found that neither of these dietary supplements affected the hamsters' normal winter decrease in m_b and fat content nor their basal MR or NST capacity. NST capacity of summer-acclimated hamsters was lower than that of winter-acclimated ones. The composition of total body fat reflected the fat composition of the dietary supplements. Resting MR below the lower critical temperature of the hamsters, and their total serum cholesterol concentration were lower in hamsters fed a diet supplemented with mealworms than in hamsters fed a diet supplemented with seeds. These results indicate that in mealworm-fed hamsters energy expenditure in the cold is lower than in animals eating a seed-supplemented diet, and that the degree of FA unsaturation of diet affects energetics of heterotherms, not only during torpor, but also during normothermy.

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1. Introduction

Seasonally changing environments affect physiology and behavior of animals. Non-migrating mammals living in the temperate zone of the northern hemisphere have to prepare for winter; a time of food shortage and low ambient temperatures. In summer heterothermic rodents, such as marmots and some ground squirrels, begin to accumulate fat that will serve as the predominant source of energy during hibernation (Armitage et al., 1976; Davis, 1976; Körtner and Heldmaier, 1995). Others, for example chipmunk species and most ground squirrels, both gain fat and gather hoardable food (Davis, 1976). Conversely, Siberian hamsters *Phodopus sungorus*, that enter torpor daily in winter (henceforth—daily heterotherms), begin to lose body fat and decrease body mass (m_b) at the onset of winter, are active on a daily basis, and do not store food (Ruf and Heldmaier, 1992, 2000).

Independent of overwintering strategy, all animals face seasonal variations in food type and availability. As winter approaches, green vegetation is gradually replaced with hay, seeds, nuts and tubers. Availability of invertebrates as a food source also decreases. Seasonal changes in diet composition may result either from active selection of particular food or solely from the availability of particular food at a given time of year (Fine and Bartness, 1996). Selection of a diet rich in polyunsaturated fatty acids (PUFA) can bring significant benefits

to heterothermic animals by enabling maintenance of the fluidity of fat depots and membrane phospholipids during torpor (Munro and Thomas, 2004). Before winter, yellow-bellied marmots *Marmota flaviventris* even extend their home ranges to find their preferred plants that are rich in PUFA and they eat flowers of other PUFA-rich plants, which are generally avoided because of their high secondary compound content (Armitage, 1979; Florant et al., 1990). Moreover, essential fatty acids, namely linoleic (n–6) and α -linolenic (n–3) acids cannot be synthesized by animals and must be obtained from the diet, mainly from flowers and seeds (Florant et al., 1990; Hulbert et al., 2005).

All seasonal changes in diet composition are reflected in the composition of body fat (Geiser et al., 1994; Frank and Storey, 1995; Florant, 1998; Hill and Florant, 2000; Harlow and Frank, 2001; Dark, 2005). Increased amounts of dietary PUFA lead to increased unsaturation of body fat, resulting in a decrease in melting point of body lipids, enabling their mobilization even at body temperatures (T_b) as low as $\sim 0^\circ\text{C}$ (Frank, 1991). Moreover, high PUFA content in the cell and organelle membranes increases their fluidity at low temperature (Lenaz et al., 1975; Brown and London, 1998; Hulbert and Else, 1999). Many studies indicate that dietary PUFA incorporated into body fat affects energy expenditure of heterothermic animals by lowering minimum T_b and metabolic rate (MR) during torpor (Geiser and Kenagy, 1987; Geiser, 1991; Frank, 1992; Geiser et al., 1992, 1994; Thorp et al., 1994; Florant, 1998; Harlow and Frank, 2001; Munro and Thomas, 2004; Dark, 2005; Geiser et al., 2007). However, both insufficiency and superabundance of PUFA in an animal's diet, and hence in its body lipids are disadvantageous for

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hibernating mammals (Florant et al., 1993; Frank and Storey, 1995; Frank, 2002). It is generally accepted that the negative effect of surplus dietary PUFA results from higher production of toxic lipid peroxides (Frank and Storey, 1995; Frank et al., 1998; Munro and Thomas, 2004). Several researchers have suggested that heterothermic animals prefer a diet with an intermediate content of PUFA, compromising between the risk of lipid peroxidation (autooxidation) and energy savings due to hibernation with lower T_b and MR (Frank and Storey, 1995; Frank et al., 1998; Munro and Thomas, 2004; Munro et al., 2005; Frank et al., 2008). There is also some evidence that diet rich in PUFA can enhance capacity for nonshivering thermogenesis (NST) (Takeuchi et al., 1995; Kawada et al., 1998) and, as a result, improve animal's ability to cope with cold. However, the results of experiments on the correlation between fatty acid unsaturation and NST efficiency are not consistent (Bartness et al., 1993; Takeuchi et al., 1995; Rafael et al., 1988). Different fatty acid composition in cell membranes may also affect membrane permeability and thus it may change MR of animals (Hulbert, 1993; Hulbert and Else, 1999; Hulbert et al., 2005; but see Brzęk et al., 2007). Many studies have reported the effects of diet on thermoregulation and energetics during torpor, mostly during hibernation, but diet-induced changes of MR in heterothermic animals during normothermy have received less attention (e.g. Geiser, 1991; Thorp et al., 1994; Withers et al., 1996).

In the present study we examined the effect of different dietary supplements on seasonal changes in m_b and body fat composition, MR and NST capacity in normothermic Siberian hamsters. Since a diet rich in PUFA brings about lower MR during torpor, we hypothesized that it also leads to lower resting metabolic rate (RMR) during normothermy. We predicted that hamsters fed a diet supplemented with sunflower and flax seeds (a source of PUFA) would have lower RMR than hamsters fed a diet supplemented with mealworms, a source of saturated (SFA) and monounsaturated fatty acids (MUFA). To test our prediction we housed Siberian hamsters under semi-natural conditions in Central Poland from May 2009 to February 2010. Animals were fed standard food, once a week supplemented with sunflower and flax seeds or mealworms. In late summer and winter we measured RMR at ambient temperatures ranging from 10 to 35 °C. We also expected that a diet-induced decrease in RMR would result in a higher capacity for NST in hamsters fed a PUFA-supplemented diet. To test this prediction we measured noradrenaline-induced changes in oxygen consumption at $T_a = 25$ °C. After respirometry measurements animals were killed and body fat composition was examined. Since mealworm supplementation could increase cholesterol intake, we also measured total cholesterol concentration in serum.

2. Materials and methods

2.1. Animals and housing

This study was done at the Nicolaus Copernicus University in Toruń, Poland (53.02°N, 18.57°E). Siberian hamsters (*P. sungorus*) used in these experiments originated from a commercial colony and from March to May 2009 were kept under laboratory conditions ($T_a \sim 22$ °C, natural photoperiod). Starting from May 2009, 18 male hamsters were transferred to an outdoor enclosure located on the university campus, where animals were kept individually in 90 L plastic bins covered with wire mesh. The bins were filled with wood shavings and dug into the ground to a depth of 35 cm. The enclosure was covered with a transparent roof to protect the hamsters from rain and snow as described by Jefimow et al. (2004). Air temperature (T_a) in the outdoor enclosure was recorded every 30 min with a DataHog 2 data logger (Skye Instruments, UK) placed ~20 cm above the ground. Additionally, temperatures at the ground surface and inside the bin (at a depth of ~30 cm) were recorded every 20 min with pre-calibrated data loggers (DS 1921G, iButton, Maxim Integrated Products, Inc., USA). Once a

month all hamsters were weighed to ± 0.1 g with an electronic balance (SPU402, Ohaus, USA).

2.2. Experimental procedures

Throughout the study hamsters were fed standard rodent chow (Ziarnko, Jelcz-Laskowice, Poland) and provided with water *ad libitum*. From May to September 2009, once a week their standard food was supplemented with a mixture of mealworms (larvae of *Tenebrio molitor*) (8 g) and sunflower (*Helianthus annuus*) seeds (8 g). In September 2009, after ~5 months under semi-natural conditions and of eating this diet, we measured resting metabolic rate (RMR) and non-shivering thermogenesis (NST) capacity in 6 hamsters. After measurements hamsters were killed by cervical dislocation, blood samples (~300 μ L) were taken, and then total body fat content and fatty acids composition were analyzed in the Department of Food Chemistry, Technology and Biotechnology, Gdańsk University of Technology, Poland. Total lipids were extracted using the modified Folch method (Folch et al., 1957; Christie, 1987) and total fatty acid composition was determined using gas chromatography (see below). Briefly, frozen hamster carcasses were lyophilized and then homogenized in a chloroform:methanol solution (2:1 v/v). Lipids recovered in the chloroform phase were transferred to a separate glass vial and dried in a stream of N_2 and then weighed. Recovered fats were converted into fatty acid methyl esters (FAME, EN: ISO 5509:2000). Fatty acids composition was analyzed by HG-RC gas chromatography (Hewlett-Packard 5890 Series II) and the column Rtx 2330 (100 m \times 0.25 mm, Restek, USA). Analysis was done using standard solutions of FAME (Supelco Bellefonte, Pennsylvania, USA; Larodan Fine Chemicals, Malmö, Sweden). For a more detailed description of the procedure see Zalewski et al. (2009).

Blood was centrifuged and serum was frozen at < -20 °C for later cholesterol analysis. Serum total cholesterol concentration was measured with a COBAS INTEGRA 400 Plus Biochemical Analyzer (Roche Diagnostics Lt., Switzerland).

In the middle of September 2009, the remaining 12 hamsters were randomly divided into two diet groups of 6 animals. Once a week, on each Monday, the diet of one group was supplemented with 16 g of mealworms (no seeds) while the diet of another group was supplemented with 8 g of sunflower and 8 g of flax (*Linum usitatissimum*) seeds (no mealworms). The detailed composition of dietary fat is presented in Table 1. When MR was measured on the day of food supplementation, mealworms and seeds were given to the hamsters after the measurements to avoid the potential immediate effect of dietary supplementation on MR.

RMR and NST capacity of hamsters fed a diet supplemented with mealworms or seeds were measured in January and February 2010. After measurements, animals were killed and their serum cholesterol concentration, total body fat content and fatty acids composition were analyzed using the same method as described above.

All experimental procedures were approved by the Local Committee for the Ethics in Animal Research in Bydgoszcz, Poland.

2.3. Respirometry

RMR of normothermic hamsters was measured at a range of T_a between 10 °C and 35 °C (only one T_a per day; in a random order) by

Table 1
The total fat content and its composition in standard food and food supplements.

%	Standard diet	Mealworms	Sunflower seeds	Flax seeds
Fat	2.7	6.7	51.0	31.6
SFA	22.5	21.4	12.2	11.7
MUFA	28.5	44.7	34.5	22.1
PUFA	49.0	33.9	53.3	66.2

indirect calorimetry using an open flow respirometry system. The same respirometry system and procedures were used in the autumn and winter series of experiments. Measurements were commenced at 08:00 h, i.e. during animals' rest-phase of the day, and lasted for 6–7 h. At least 0.5 h before measurements, six hamsters were brought from the outdoor enclosure to the laboratory. To avoid an effect of specific dynamic action of food, RMR was determined based on the minimum rate of oxygen consumption ($\dot{V}O_2$) measured for 6 min and 40 s (400 s) within the last hour of a 6 h long measurement period. In preliminary experiments we found that RMR calculated for that time did not differ from RMR of hamsters that were additionally deprived of food for 5 h prior to MR measurements, in either mealworm-supplemented or seed-supplemented animals (RM ANOVA, $p=0.90$ and $p=0.14$, respectively). Thus, we consider that the hamsters were post-absorptive during MR measurements.

We used two parallel respirometry systems that allowed us to measure gas exchange in six hamsters at once. The system was set up so that respiratory gas exchanges of two animals were measured simultaneously. In one system $\dot{V}O_2$ and $\dot{V}CO_2$ were measured and in the other only $\dot{V}O_2$. Measurements were made for 10 min with reference air being sampled for 3 min between animals. Thus, MR was measured in each hamster every 40 min throughout the experiment. Outside air was continuously pushed through six air-tight metabolic chambers (1.7 L each) with a constant flow rate of $\sim 600 \text{ mL} \cdot \text{min}^{-1}$ per chamber. All chambers were placed in a custom modified temperature controlled cabinet (INCUDIGIT, Selecta, Spain). Airflow was regulated upstream of the respirometry chambers using precise needle valves. Gases leaving the respirometry chambers were selected sequentially with a computer-controlled multiplexer (MUX-4, Sable Systems Int., USA) and flow rate through a selected chamber was measured downstream using a mass flow meter (FlowBar-4, Sable Systems Int., USA). A multiplexer was set up so that two parallel, independent air streams leading to two gas analyzers were selected at the same time. After flow measurement, air from each gas stream was subsampled at a rate of $\sim 100 \text{ mL} \cdot \text{min}^{-1}$ and water vapor pressure of the subsampled air was measured with a water vapor analyzer (RH-300, Sable Systems Int., USA). Air was then dried in a column of magnesium perchlorate (Sigma-Aldrich, USA), and subsequently fractional concentrations of CO_2 (F_{CO_2}) and O_2 (F_{O_2}) were measured using a FoxBox-C integrated CO_2 and O_2 analyzer, or only F_{O_2} with a FC-10a O_2 analyzer (Sable Systems Int., USA).

All electronic elements of the respirometry system were connected to a PC computer via an analog-to-digital interface (UI2, Sable Systems Int., USA). Respirometry data were acquired using ExpeData software (Sable Systems Int.) at 0.5 Hz. Ambient temperature (T_a) in each respirometry chamber was measured using a type-T thermocouple connected to the thermocouple reading interface (USB-4718 Advantech EUROPE, Germany) and acquired using WaveScan 2.0 software (Advantech EUROPE). $\dot{V}O_2$ of animals for which both F_{O_2} and F_{CO_2} were measured was calculated using equation 11.7 of Lighton (2008). For the calculation of $\dot{V}O_2$ of animals for which only F_{O_2} in the exhaled air was measured we used eq. 11.2 of Lighton (2008) assuming $RQ=0.8$ (Koteja, 1996). For animals for which $\dot{V}O_2$ and $\dot{V}CO_2$ were known energy expenditure was calculated using energy equivalent of oxygen for a given RQ following Lighton (2008) while for animals for which only F_{O_2} was measured, we assumed energy equivalent 20.087 kJ per L O_2 (Koteja, 1996).

Non-shivering thermogenesis capacity was measured as noradrenaline (NA)-induced changes in $\dot{V}O_2$. We used the respirometry system described above but $\dot{V}O_2$ was measured only in two animals at the same time. Measurements were done at $T_a=25^\circ\text{C}$. After 2 h, NA (Levonor 1 mg $\cdot \text{mL}^{-1}$, Polfa, Poland) was injected subcutaneously at a dose of $2.54 m_b^{-0.4} \text{ mg} \cdot \text{kg}^{-1}$ (Wunder and Gettinger, 1996). In each hamster, NST capacity was calculated as the difference between BMR and maximum metabolic rate (average of the continuous maximum MR over 5 min) after NA injection. Control animals were

injected with the same volume of saline (0.9% NaCl) as NA in the experimental animals. Half of the animals were injected first with NA and then with saline. The sequence of injections in the other half was the reverse.

2.4. Data analysis

All data fulfilled the assumptions of parametric tests. Seasonal changes of m_b were analyzed with 2-way RM ANOVA (with month and diet supplement type as independent variables) followed by Tukey's post-hoc test. In summer (from May to September) all animals were fed the same diet supplemented with both sunflower seeds and mealworms, while in winter (from September to February) the hamsters' diet was supplemented with sunflower and flax seeds or mealworms. Therefore, we were not able to do ANOVA on all groups at once. Instead we used a Student's t-test with the Bonferroni correction for multiple comparisons. First, we analyzed the effect of diet on measured variables, i.e. mean fat content, fatty acids composition, BMR and NST capacity and serum cholesterol concentration in winter-acclimated hamsters and then we tested for an effect of season on these variables.

To interpret objectively the relationship between T_a and MR we followed Pinshow et al. (1976). MR at T_a s below the lower critical temperature (T_{lc}) were compared by analysis of covariance (ANCOVA) with T_a as a covariate. Effects of diet supplementation and season on RMR below the T_{lc} of winter and summer acclimated hamsters were tested as in the case of other variables, i.e. we applied the Bonferroni correction to correct for the effect of multiple comparisons. Data were considered statistically significant at $p \leq 0.05$. All values are presented as mean \pm SD.

3. Results

3.1. Environmental conditions

In summer (August) the mean ambient temperature (T_a) measured 20 cm above the ground was $20.1 \pm 4.7^\circ\text{C}$ (min = 9.2°C , max = 32.1°C) and in winter (January) mean T_a was $-6.1 \pm 2.9^\circ\text{C}$ (min = -32.1°C , max = 9.1°C). Mean ground surface temperature in summer was $21.5 \pm 3.7^\circ\text{C}$ (min = 11.9°C , max = 35.3°C), while in winter it was $-7.0 \pm 4.1^\circ\text{C}$ (min = -16.2°C , max = -0.9°C). Mean temperatures recorded in the hamster's bin, at a depth of 30 cm were much more stable than air temperatures and equaled $22.3 \pm 0.4^\circ\text{C}$ (min = 21.4°C , max = 22.8°C) in summer and $0.7 \pm 0.5^\circ\text{C}$ (min = 0.2°C , max = 1.7°C) in winter (Fig. 1).

3.2. Body mass (m_b)

Independent of diet, m_b in all hamsters decreased in winter (2-way RM ANOVA $F_{8,89}=23.028$, $p<0.001$; Fig. 2). The highest m_b s were recorded in August and September, while the lowest in winter (January/February). Body mass in hamsters fed the diet supplemented with mealworms dropped from $53.4 \pm 6.7 \text{ g}$ in late summer (September) to $33.3 \pm 1.8 \text{ g}$ in winter (HSD post hoc test; $p<0.001$). In hamsters fed a diet supplemented with seeds, m_b decreased from $50.3 \pm 4.6 \text{ g}$ to $35.9 \pm 2.4 \text{ g}$ (HSD post hoc test; $p<0.001$). There was no difference in winter m_b s between hamsters fed different diets. Maximum m_b of hamsters that were killed in late summer ($54.6 \pm 6.8 \text{ g}$) was similar to maximum m_b s of hamsters assigned for the two dietary groups.

3.3. Fat content and fatty acids composition

In late summer total body fat content was $23.3 \pm 8.6\%$ of total wet m_b . In winter body fat content was much lower than in summer (Fig. 3), both in hamsters fed a diet supplemented with mealworms

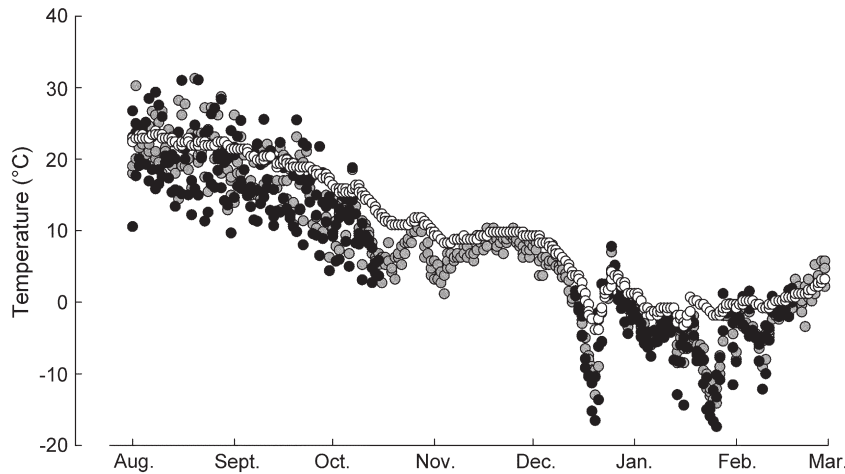


Fig. 1. Air temperature (●), ground surface temperature (○) and temperature recorded in the hamster's bin at a depth of ~30 cm (○) in the outdoor enclosure, recorded from August 2009 to March 2010. Data are presented as means of 10 h for air temperature and 6.7 h for temperature of the ground surface and temperature inside the bin. Due to technical problems there are no air temperature data from October 16th to December 14th.

($10.9 \pm 2.7\%$; $t_9 = 3.082$, $p = 0.013$) or sunflower and flax seeds ($12.3 \pm 1.7\%$; $t_9 = 2.795$, $p = 0.021$).

Fatty acid composition depended on diet and season (Table 2). In late summer SFA accounted for $19.4 \pm 4.8\%$ of all body fatty acids, MUFA accounted for $46.6 \pm 2.0\%$ and PUFA for $33.5 \pm 4.5\%$. In winter, in hamsters fed a diet with addition of mealworms PUFA content was lower than in summer ($25.3 \pm 1.4\%$, $t_9 = 3.863$, $p = 0.004$), while SFA and MUFA levels did not change significantly (25.0 ± 2.7 and $49.2 \pm 1.6\%$, respectively). Conversely, in hamsters supplemented with sunflower and flax seeds a significant increase in PUFA content ($44.7 \pm 3.8\%$, $t_9 = 4.368$, $p = 0.002$) and a decrease in MUFA content ($34.5 \pm 2.7\%$, $t_9 = 8.536$, $p < 0.001$) were recorded. Proportions of SFA remained unchanged ($20.5 \pm 1.9\%$). In winter, hamsters fed two different diets differed in total content of SFA ($t_8 = 3.047$, $p = 0.0016$), MUFA ($t_8 = 10.434$, $p < 0.001$) and PUFA ($t_8 = 10.596$, $p < 0.001$). The level of the most abundant saturated fatty acid, the palmitic acid (C16:0) was higher in animals fed a diet containing mealworms than those fed a diet supplemented with seeds ($t_8 = 3.008$, $p = 0.017$). Among all MUFAs, the amount of oleic acid (C18:1 n-9) was highest, but in hamsters fed with the addition of seeds, its level in winter was lower than in late summer ($t_9 = 7.364$, $p < 0.001$) and

lower than in hamsters fed with the addition of mealworms ($t_8 = 12.481$, $p < 0.001$). We recorded a significant increase in the content of linoleic ($t_8 = 8.602$, $p < 0.001$) and α -linolenic acids in hamsters supplemented with seeds compared to hamsters fed a diet supplemented with mealworms ($t_8 = 7.051$, $p < 0.001$). Detailed data on fatty acids content and differences between dietary groups are presented in Table 2.

3.4. Serum cholesterol

No difference was observed in serum cholesterol concentration between late summer ($1.55 \pm 0.4 \text{ mg}\cdot\text{mL}^{-1}$) and winter ($1.8 \pm 0.4 \text{ mg}\cdot\text{mL}^{-1}$) in the seed-supplemented group, but in the mealworm-supplemented group it was lower and equaled $1.2 \pm 0.1 \text{ mg}\cdot\text{mL}^{-1}$ ($t_6 = 2.652$, $p = 0.038$).

3.5. Metabolic rate

In late summer the thermoneutral zone (TNZ) of Siberian hamsters ranged between 26.2°C and 30.7°C . TNZ of winter-acclimated hamsters ranged from 24.5°C to 29.5°C in animals fed a diet supplemented with seeds, and from 23.0°C to 29.7°C in hamsters with supplementary mealworms (Fig. 4).

Regression coefficients of the equations describing the relationship between T_a and mass specific-RMR (RMR/m_b) below the T_{lc} in

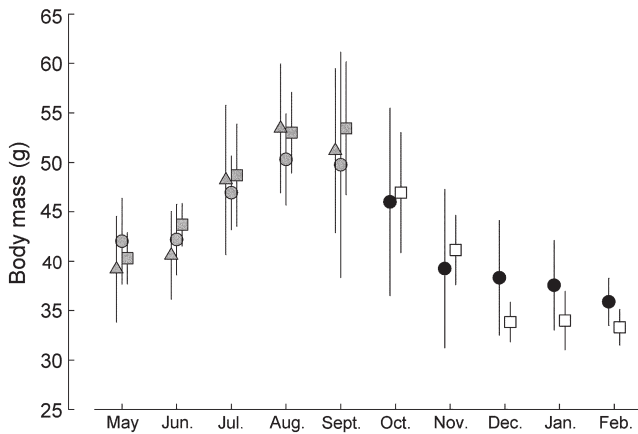


Fig. 2. Seasonal changes in body mass (m_b , mean \pm SD) of Siberian hamsters housed under semi-natural conditions from May 2009 to February 2010. Data from May to September (gray symbols) represent m_b of hamsters fed the same diet. One group (Δ) was killed in September, after measurements of MR in late summer. Starting from October, ● represents m_b of hamsters fed a diet supplemented with sunflower and flax seeds, while □ represents m_b of hamsters fed a diet supplemented with mealworms. From May to September these animals were fed the same diet (mean m_b s presented as ○ and □, respectively).

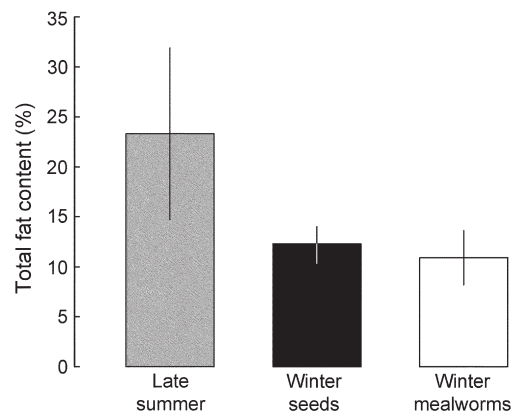


Fig. 3. Total body fat content (mean \pm SD) as a percentage of total wet m_b in Siberian hamsters. Gray bar indicates fat content in late summer, black bar—winter fat content of animals fed a diet supplemented with seeds and white bar—winter fat content of animals fed a diet supplemented with mealworms.

Table 2

Proportion of different fatty acids in the total body fat of Siberian hamsters: 1) in late summer, animals fed a standard diet, once a week supplemented with a mixture of seeds and mealworms and 2) in winter, animals fed the standard diet, once a week supplemented with seeds or with mealworms. Statistical significance was accepted at $p < 0.017$. *—significant difference between two dietary groups in winter; #—significant difference between winter and late summer. "Other" fatty acids indicates fatty acids which accounted for 0.01–0.2% of all fatty acids identified (other SFA—10:0, 12:0, 15:0, 17:0, 20:0, 24:0; MUFA—14:1, 17:1, 20:1; PUFA—20:2, 20:3 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-6, 22:5 n-3, 22:6 n-3).

Fatty acid	Late summer	Winter	
		Seed-supplemented	Mealworm-supplemented
C14:0	0.7 ± 0.15	0.5 ± 0.2	1.3 ± 0.2*#
C16:0	14.4 ± 3.4	12.9 ± 1.7	17.3 ± 2.7*
C18:0	3.7 ± 1.2	6.2 ± 1.3#	5.3 ± 0.4#
Other SFA	0.6 ± 0.3	0.9 ± 0.3	1.1 ± 0.3*
Σ SFA	19.4 ± 4.8	20.5 ± 1.9	25.0 ± 2.7
C16:1, n-9	4.4 ± 1.2	2.1 ± 1.2#	3.5 ± 0.6*
Other C16:1	0.4 ± 0.2	0.5 ± 0.3	1.2 ± 0.5
C18:1, n-9	39.3 ± 2.2	30.1 ± 1.8#	41.6 ± 1.0*
C18:1, n-11	1.9 ± 0.6	1.5 ± 0.2	2.3 ± 0.4
Other C18:1	0.2 ± 0.1	0.1 ± 0.1	0.042 ± 0.05
Other MUFA	0.4 ± 0.1	0.2 ± 0.1	0.7 ± 0.3
Σ MUFA	46.6 ± 2.0	34.5 ± 2.7#	49.3 ± 1.6*
C18:2, n-6	29.8 ± 4.5	34.1 ± 3.2	21.5 ± 0.7*#
C18:3, n-3	2.3 ± 1.1	8.7 ± 2.4#	1.0 ± 0.1*
C20:4, n-6	0.6 ± 0.4	0.7 ± 0.1	1.2 ± 0.3*#
Other PUFA	0.8 ± 0.5	1.2 ± 0.1	1.6 ± 0.5
Σ PUFA	33.5 ± 4.5	44.7 ± 3.8#	25.3 ± 1.4*#

all three groups did not differ (1-way ANCOVA: $F = 0.77$, $p = 0.47$; Fig. 4). In winter, RMR/m_b below the T_{lc} was lower in hamsters fed a diet supplemented with mealworms ($MR (mW \cdot g^{-1}) = 30.69 - 0.97 \cdot T_a$, $r^2 = 0.90$, $p < 0.001$) than in hamsters fed a diet supplemented with seeds ($MR (mW \cdot g^{-1}) = 29.92 - 0.83 \cdot T_a$, $r^2 = 0.90$, $p < 0.01$; 1-way ANCOVA $F_{1,26} = 9.54$, $p = 0.0049$). However, only the RMR/m_b of the mealworm-supplemented group was lower than RMR/m_b of summer-acclimated hamsters ($MR (mW \cdot g^{-1}) = 30.38 - 0.85 \cdot T_a$, $r^2 = 0.87$, $p < 0.001$; 1-way ANCOVA $F_{1,44} = 12.58$, $p < 0.001$). In winter, mass-specific basal metabolic rate (BMR/m_b) did not differ between hamsters fed two different diets ($t_9 = 0.953$, $p = 0.365$) and equaled $8.8 \pm 0.7 mW \cdot g^{-1}$. BMR/m_b of summer-acclimated hamsters ($8.0 \pm 1.2 mW \cdot g^{-1}$) was not different from the individuals fed the mealworm-supplemented diet in winter ($t_9 = 0.971$, $p = 0.357$) as well as from the animals fed the seed-supplemented diet ($t_{10} = 1.675$, $p = 0.125$; Fig. 5).

3.6. Non-shivering thermogenesis (NST)

In winter, the maximum increase in MR after NA injection did not differ between hamsters fed a diet with additional seeds or mealworms (seed-supplemented: $33.7 \pm 5.6 mW \cdot g^{-1}$, mealworm-supplemented: $37.1 \pm 3.3 mW \cdot g^{-1}$, $t_{10} = -1.283$, $p = 0.229$; Fig. 6). NST capacity was lower in summer-acclimated hamsters ($23.9 \pm 3.6 mW \cdot g^{-1}$) than NST of winter animals fed a diet enriched with mealworms ($t_{10} = 6.654$, $p < 0.001$) or with seeds ($t_{10} = 3.605$, $p = 0.005$).

4. Discussion

In the present study we examined the effect of increasing dietary MUFA and SFA, or PUFA on m_b , fat composition, total serum cholesterol concentration, MR and NST capacity in normothermic Siberian hamsters during acclimation to winter. Because of technical limitations, we were unable to keep an additional group of hamsters on an unaltered diet for the duration of this study that might have served as a control. However, since our goal was to address questions about the specific effects of different diet supplementation on seasonal changes in hamster physiology, we chose to have two groups, one

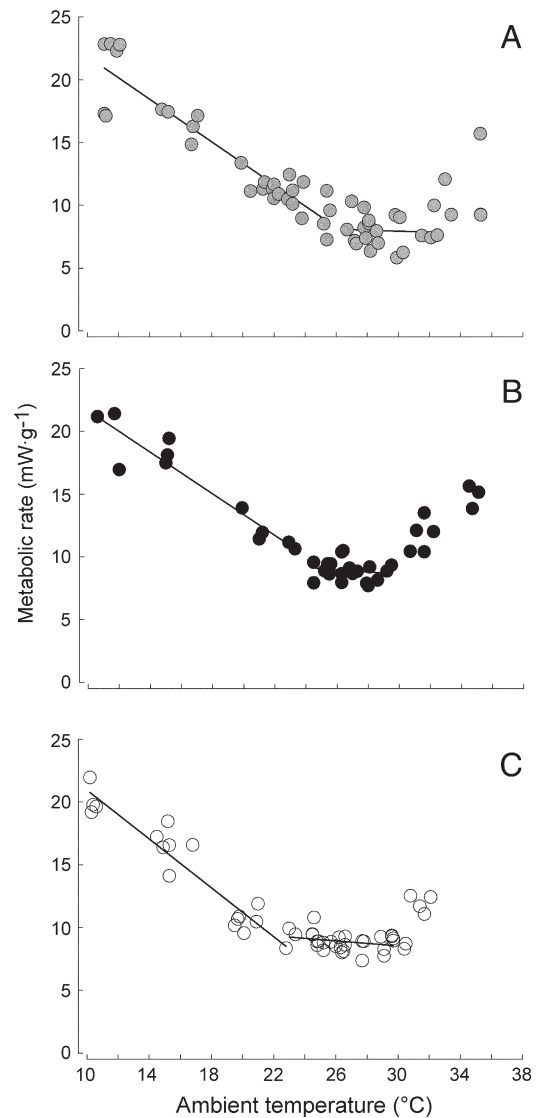


Fig. 4. Mass specific resting metabolic rate of Siberian hamsters measured at ambient temperatures between 10 °C and 35 °C. A) Summer-acclimated Siberian hamsters fed a diet supplemented with a mixture of mealworms and sunflower and flax seeds; B) winter-acclimated hamsters fed a diet supplemented with sunflower and flax seeds; C) winter-acclimated hamsters fed a diet supplemented with mealworms.

supplemented with insects and the other with oilseeds. Consequently, throughout the paper we discuss only the effects of different dietary supplementation in a between-group context, where we have clear, supportive data.

All animals, independent of dietary supplementation, underwent clear seasonal changes in m_b . Hamsters were heaviest in late summer (September) and lightest in winter (January/February). The addition of mealworms, or sunflower and flax seeds, to standard rodent food did not affect the natural winter decrease in m_b of hamsters. In both groups, m_b s were approximately 30% lower in winter than in late summer. A similar decrease in m_b was recorded in Siberian hamsters kept under a natural photoperiod by Heldmaier and Steinlechner (1981).

One could argue that the degree of unsaturation of dietary FA in the present study was too small to affect seasonal changes in m_b . However, previous studies also reported no changes in m_b loss in Siberian hamsters fed a diet rich in SFA (67%), MUFA (64%) or PUFA (69%) (Geiser and Heldmaier, 1995). Similarly, sunflower seeds added to standard rodent chow did not affect the hamsters' m_b either (Ruf et al., 1991). This supports our contention that the degree of

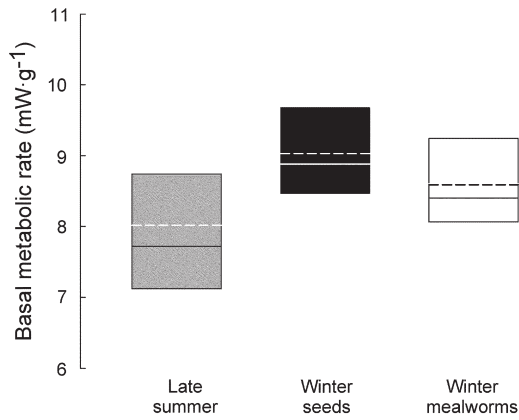


Fig. 5. Mass specific basal metabolic rate of Siberian hamsters in late summer (animals fed a diet supplemented with a mixture of sunflower and flax seeds and mealworms) and in winter (2 dietary groups: one supplemented only with seeds—“winter seeds” and the other supplemented only with mealworms—“winter mealworms”). Box indicates 25th and 75th percentiles, solid line stands for median and dashed line stands for mean.

dietary fat unsaturation does not affect seasonal changes in m_b of Siberian hamsters. Similarly, unsaturation of dietary FA does not affect m_b changes in hibernators that are increasing their m_b for winter, such as yellow-pine chipmunks *Eutamias amoenus* (Geiser et al., 1994), yellow-bellied marmots *M. flaviventris* (Florant et al., 1993; Thorp et al., 1994), ground squirrels *Spermophilus saturatus* (Geiser and Kenagy, 1993), 13-lined ground squirrels *Spermophilus tridecemlineatus* (Staples et al., 2008) and golden-mantled ground squirrels *Callospermophilus lateralis* (Frank and Storey, 1996).

In the present study, the winter decrease in m_b was related to the decrease in body fat content (from ~23% of wet body mass in late summer to ~11% in winter) independent of the dietary supplements. Total body fat was composed mainly of three FAs: palmitic, oleic and linoleic. Linoleic acid is the most abundant of all PUFAs in animals (Carneheim et al., 1989; Frank, 1994; Harlow and Frank, 2001; Frank et al., 2008). As expected, diet composition was reflected by the composition of body lipids. The amounts of linoleic and α -linolenic acids were higher in Siberian hamsters fed a diet supplemented with oilseeds than in hamsters fed supplementary insects. Conversely, hamsters fed a mealworm-supplemented diet had a higher proportion of SFA (myristic and palmitic acids), MUFA (palmoleic and oleic acids) and also of arachidonic acid (PUFA).

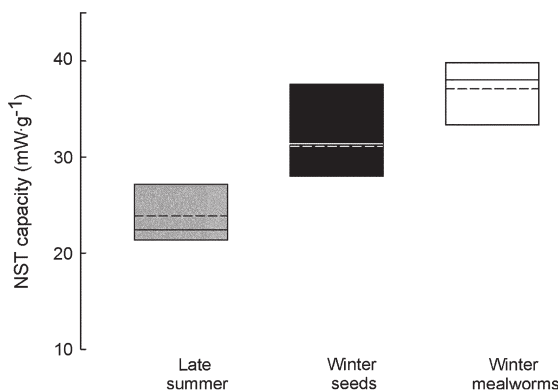


Fig. 6. Non-shivering thermogenesis capacity (presented as a difference between maximum mass-specific metabolic rate after NA injection and mass-specific BMR) in Siberian hamsters acclimated to summer and winter. Independent of diet, NST capacity was higher in winter than in summer ($p < 0.001$) while diet alone did not affect capacity for NST. Box indicates 25th and 75th percentiles, solid line stands for median and dashed line stands for mean.

We supplemented hamsters' diet every week but it was impossible to verify precisely the amount of seeds or mealworms eaten. However, if something remained the next day it was only some flax seeds. Composition of body fat indicates however that both mealworms and seeds were definitely consumed. We observed that in summer hamsters ate sunflower seeds willingly while mealworms were eaten willingly throughout the year. These observations correspond to the results obtained by Hiebert et al. (2000, 2004) who reported that Siberian hamsters acclimated to a short (winter-like) photoperiod and exposed to cold preferred a diet rich in unsaturated fat even when daily torpor was not used (Hiebert et al., 2000, 2004). Their other study also showed that cold exposed animals acclimated to a long (summer-like) photoperiod preferred a diet rich in unsaturated fatty acids (Hiebert et al., 2003). Thus, one could conclude that diet selection depends on ambient temperature. However, according to Fine and Bartness (1996), diet preference is driven by photoperiod because Siberian hamsters exposed to short days only did not select a diet rich in PUFA but preferred a diet rich in carbohydrates and proteins, compared to hamsters exposed to long days. As a result hamsters would prevent a winter decrease in m_b and maintain lean body mass (Fine and Bartness, 1996).

In the present study, different diet did not bring about changes in BMR/ m_b despite different body fat composition. We cannot exclude the possibility that the lack of difference in BMR/ m_b between seed- and mealworm-supplemented hamsters results from the fact that FA composition of cell membranes may not fully reflect FA composition of the dietary fat (Hulbert et al., 2005). In our study BMR/ m_b in winter was similar in both dietary groups (mean $8.8 \pm 0.7 \text{ mW} \cdot \text{g}^{-1}$) and was not different from BMR/ m_b measured in summer ($8.0 \pm 1.2 \text{ mW} \cdot \text{g}^{-1}$). Lower critical temperature was shifted from ~26 °C in late summer to ~23 °C in winter in hamsters fed a diet supplemented with mealworms, and ~24.5 °C in animals fed a diet supplemented with seeds. These values concur with results of Heldmaier and Steinlechner (1981) who found that the T_{lc} of Siberian hamsters shifted from ~26 °C in summer to ~20 °C in winter. The higher values of T_{lc} recorded in the present study in winter might result from the conditions in which the animals were housed and from the availability of extra insulation. We kept our hamsters in large 90 L bins filled with sawdust, giving them the opportunity to burrow in well-insulated surroundings. This suggests that thermal properties of the environment may affect seasonal adjustments in physiology of small mammals, indicating that acclimatization is a flexible trait rather than a programmed response to a changing photoperiod or air temperature.

Studies to date indicate that the degree of dietary FA unsaturation has little, if any, effect on MR during normothermy, both in homeo- and hetero-thermic animals. Although focused on diet-induced thermogenesis, experiments on laboratory rats at T_a of 21 °C to 22 °C, revealed that RMR did not differ in animals fed polyunsaturated, monounsaturated or saturated fat diets (Shimomura et al., 1990; Takeuchi et al., 1995). A diet enriched with linoleic acid did not induce any changes in RMR of the rats, either at thermoneutrality or in the cold (Rafael et al., 1988). Normothermic T_b and RMR in deer mice *Peromyscus maniculatus* kept below their T_{lc} and fed diets containing saturated and unsaturated FA did not differ either (Geiser, 1991). Feather-tailed gliders (*Acrobates pygmaeus*) fed diets containing either 10% sunflower oil, or 10% sheep fat had similar RMRs at $T_a = 8 \text{ °C}$ (Geiser et al., 1992). Likewise, BMR of stripe-faced dunnarts (*Sminthopsis macroura*) fed diet rich in either PUFA or SFA did not differ during normothermy (Withers et al., 1996). Even a diet deficient in essential fatty acids did not induce any changes in RMR of hetero-thermic yellow-bellied marmots (*M. flaviventris*) during normothermy (Thorp et al., 1994).

Contrary to the above results, we found that the RMR of Siberian hamsters fed a diet enriched with mealworms was lower below their T_{lc} than hamsters fed a diet supplemented with oilseeds, indicating a lower cost for normothermy in the cold. One could argue that

these differences result from differences in specific dynamic action (SDA) resulting from digestion of foods of different composition. Since the protein content of live mealworms is ~22%, whereas that of sunflower and flax seeds is ~35% and ~25%, respectively (Earle and Jones, 1962; Jones et al., 1972), the differences in overall crude protein content might result in different SDA in both dietary groups, and ultimately in different RMR (Secor, 2009). However, in rats fed saturated fat (lard), diet-induced thermogenesis was much lower than in rats fed a diet containing safflower (*Carthamus tinctorius*), sunflower or linseed (= flax) oil (Takeuchi et al., 1995). Other experiments indicated that the effect of SDA lasts for 3 h after a meal in rats fed either safflower oil or fed beef-tallow, and within the next 3 h $\dot{V}O_2$ did not differ between the groups (Shimomura et al., 1990). Postprandial increase in $\dot{V}O_2$ in the carnivorous sea otter (*Enhydra lutris*) decreases with time after feeding, reaching control levels in 5 h (Costa and Kooyman, 1984).

In light of the above, we argue that lower RMR in hamsters fed a diet supplemented with mealworms did not result from lower SDA compared to hamsters eating the oilseed supplemented diet. First, RMR was calculated based on the data collected within the 6th hour of measurement and it did not differ from RMR of hamsters which were food-deprived for additional 5 h prior to the measurements. Second, the diet was supplemented only once a week, and if MR was measured on that day, food supplements were given after measurements. Third, BMR did not differ between hamsters eating either seed- or mealworm-supplemented diets.

The other nutritional factor that could affect RMR is cholesterol. Geiser et al. (1997) found that a diet enriched with exogenous cholesterol caused longer and deeper hibernation bouts in yellow-pine chipmunks (*Tamias amoenus*). Since cholesterol is produced only in animal cells, one might expect that hamsters fed a supplement of mealworms that contain ~0.3% of cholesterol (of wet m_b , Finkel, 1948), would have lower metabolic rates. Surprisingly, we found that serum cholesterol content was lower in hamster fed a mealworm-supplement than in hamsters fed a seed-supplement (1.2 ± 0.1 and 1.8 ± 0.4 mg·mL⁻¹, respectively).

A possible explanation for lower MR below T_{lc} in hamsters fed a diet supplemented with mealworms could be high content of MUFA in their body fat. Geiser and Heldmaier (1995) reported that the occurrence of torpor in Siberian hamsters kept under natural photoperiod at $T_a = 18$ °C was greater in animals fed diets enriched with PUFA or MUFA than in hamsters fed SFA-diet (Geiser and Heldmaier, 1995). It indicates that not only PUFA, but also MUFA may enhance torpor occurrence (Geiser et al., 1994; Frank and Storey, 1996; Dark, 2005). Frank and Storey (1996) found that in *Spermophilus lateralis*, a diet rich in MUFA enhances hibernation even to a greater extent than PUFA, compared to animals fed a saturated diet, suggesting to us that that MUFA can be more important than PUFA in the expression of heterothermy, and its role is underestimated.

In the present study, different dietary supplementation and body fat composition did not affect NST capacity. As expected, NST capacity was much higher in winter than in summer, but we did not record a significant difference in the NST capacity of hamsters fed the different diets. Similar results were reported for Turkish hamsters (*Mesocricetus brandti*) fed diets enriched with saturated or unsaturated fat, i.e. NST capacity was the same in both groups (Bartness et al., 1993). In rats fed a PUFA-rich diet, noradrenaline turnover in brown adipose tissue (BAT) was higher than in rats fed SFA, indicating higher sympathetic activity in the BAT of the former (Takeuchi et al., 1995). However, Rafael et al. (1988) reported that NST capacity of rats fed PUFA-rich diet was similar to NST capacity of rats fed standard diet, both in the cold (5 °C) and at thermoneutrality (29 °C). Noradrenaline-induced NST and thermogenic properties of brown adipocytes were also unaffected by diet (Rafael et al., 1988).

In conclusion, our data indicate that diet supplementation with mealworms as a source of MUFA and SFA, or PUFA-rich sunflower

and flax seeds, does not affect m_b , BMR and NST capacity in normo-thermic Siberian hamsters housed under semi-natural conditions. Independent of diet, a winter decrease in m_b and an increase in NST capacity were recorded. Surprisingly, serum total cholesterol concentration was higher in hamsters fed a seed-supplemented diet than in hamsters fed mealworms. MR below the T_{lc} was higher in animals fed a diet supplemented with seeds than in the group fed a diet supplemented with mealworms. The lack of seasonal differences in BMR of Siberian hamsters housed under semi-natural conditions, but having access to well insulated living quarters suggest that seasonal changes in their energetics are flexible rather than a programmed response.

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