The Journal of Experimental Biology 213, 3988-3997

doi:10.1242/jeb.046821

The energy budget, thermogenic capacity and behavior in Swiss mice exposed to a consecutive decrease in temperatures

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Accepted 10 September 2010

SUMMARY

The limitation on sustainable energy intake (SusEI) is important because it establishes the upper energetic limit on the ability of animals to disperse, survive and reproduce. However, there are still arguments about what factors impose that limitation. Thermoregulation in cold environments imposes great energy demands on small mammals. A cold-exposed animal has been suggested to be a model suitable for testing these factors. Here, we examined the changes in food intake and digestible energy intake (DEI) as measures of SusEI, thermogenic capacity and behavioral patterns in Swiss mice exposed to consecutively lower ambient temperatures from 23 to –15°C. Cold-exposed mice showed significant decreases in body mass, fat content of the carcass and body temperature, and increases in DEI compared with controls. The time spent on feeding significantly increased with decreasing temperatures, and time spent on general activity decreased following cold exposure. Resting metabolic rate, nonshivering thermogenesis and serum tri-iodothyronine levels significantly increased in mice exposed to lower temperatures in comparison with controls, whereas these thermogenic variables were not significantly different between 0 and –15°C. It might suggest that SusEI in cold exposed Swiss mice was constrained peripherally by the capacity to produce heat and also by the ability to dissipate body heat, but to a different extent. Moderate cold exposure might result in a relaxation of the heat dissipation limit (HDL), allowing the animals to increase food intake to meet cold stress. When animals are exposed to severe cold, the thermogenenic capacity might reach a ceiling, failing to compensate for the heat loss and which would finally result in lower body temperature and considerable weight loss. This might indicate that the HDL was set at a higher level than peripheral limits for Swiss mice exposed to a consecutive decrease in ambient temperatures.

Key words: behavior, nonshivering thermogenesis (NST), resting metabolic rate (RMR), sustainable energy intake (SusEI), Swiss mice, thyroid hormones.

INTRODUCTION

It is a fundamental tenet of physiology that animals must take in energy to fuel their energy use. For animals maintaining constant body mass and body composition, time-averaged energy intake (i.e. sustainable energy intake, SusEI) equals time-averaged energy use (Hammond and Diamond, 1997). What factors set the limit to SusEI remain somewhat controversial.

The limit to SusEI may be imposed either centrally or peripherally (Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman and Król, 2005; Speakman, 2007; Speakman and Król, 2010), although it is also at least conceivable that both central and peripheral limitations can apply if central supply and peripheral consumption are perfectly matched. The central limitation hypothesis advocates the constraint on SusEI may exist in shared central machinery for acquiring, processing and distributing energy to any and all energy consuming organs (Kirkwood, 1983; Karasov, 1986; Root, 1988; Bozinovic and Rosenmann, 1989; Weiner, 1989; Peterson et al., 1990; Weiner, 1992; Thompson, 1992; Hammond and Diamond, 1997; Bryan and Bryant, 1999; Thompson and Nicol, 2002). However, when lactating animals are exposed to cold, they increase their food intake beyond that observed in the individuals lactating at room temperature, indicating that SusEI is not limited centrally (Hammond et al., 1994; Rogowitz, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001; Zhang and Wang, 2007).

The peripheral limitation hypothesis, in contrast, suggests that the limits to SusEI are imposed by the expenditure capacities of the energy-consuming organs, such as work properties of skeletal muscle during physical exercise, the performance of mammary glands during lactation or the capacity of brown adipose tissue (BAT) to produce heat during cold exposure (Hammond and Diamond, 1997; Speakman and Król, 2005; Speakman, 2007; Speakman, 2008; Speakman and Król, 2010). Lactation is the most energetically demanding period encountered by small mammals, and therefore used for testing this hypothesis (Speakman and Król, 2005). For example, Swiss mice in which half of the mammary glands are surgically removed during late lactation do not increase productivity in the remaining glands (Hammond et al., 1996). Zhao (Zhao, 2010) studied the energy budget in the same strain of mouse and found that in mothers supporting more pups than they gave birth to, milk production did not increase. These data may suggest that the mammary glands are the point at which the system is peripherally limited. However, MF1 mice lactating at cold temperatures show higher food intake and also milk energy output (MEO) than that observed in females lactating at room temperature, indicating that SusEI and MEO are actually not peripherally limited (Król and Speakman, 2003b).

Król and Speakman (Król and Speakman, 2003a; Król and Speakman, 2003b) presented a novel hypothesis that the limits to SusEI during peak lactation were imposed by the capacity to

dissipate body heat generated as a by-product of processing food and producing milk, i.e. the heat dissipation limit hypothesis (HDL). This hypothesis suggests that the increased obligatory heat production during lactation, combining with a decreased ability to dissipate heat as a result of mother–pup contact may contribute to a chronic maternal hyperthermia. When maternal hyperthermia approaches the upper lethal body temperature, lactating mothers are forced to interrupt pup contact and leave the nest area to dissipate heat (Król and Speakman, 2003a; Król and Speakman, 2003b; Speakman and Król, 2005; Speakman and Król, 2010). Frequent and prolonged maternal nest absence would affect the suckling behavior involved in stimulation of milk production, and consequently decrease the amount of milk produced (Król and Speakman, 2003b).When an animal is exposed to the cold, it is not an additional demand, but a relaxation of the heat dissipation limit, allowing the animals to elevate not only their food intake but also their milk production. Similarly, the mice lactating in the hot would reduce their capacity to dissipate heat, restrict their food intake and milk production (see also Speakman and Król, 2005; Speakman and Król, 2010; Speakman and Król, in press). MF1 mice export significantly less energy as milk (87.7 kJ day^{-1}) at 30°C than mice at 21°C (166.7 kJ day^{-1}) but more at 8°C (288.0 kJ day^{-1}), which is paralleled by lower asymptotic food intake at 30°C and higher food intake in the cold, providing strong support for the HDL hypothesis (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007).

However, Rogowitz (Rogowitz, 1998) demonstrated that levels of milk production in cotton rats at 21°C and 8°C were similar. Zhao and Cao (Zhao and Cao, 2009b) found that in lactating Swiss mice dorsal fur removal increased the thermal conductance and thus increased body heat dissipation, but had no effect on milk production. This suggests that the data from other rodent species or strains like Swiss mice are consistent with the peripheral limitations hypothesis (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Zhao and Cao, 2009b). However, cold-exposure or shaving increases food intake during peak lactation beyond that observed in controls, indicating that it is not in conflict with the HDL hypothesis. Therefore it may be assumed that SusEI is imposed by both peripheral limitation and HDL, but to different extents (Zhao and Cao, 2009b; Zhao et al., 2010; Speakman and Król, in press). As the data from the performance of mammary glands during peak lactation are less conclusive, further investigation into the capacity of brown adipose tissue (BAT) to produce heat during cold exposure is necessary to establish if this is the case.

It has been well established that cold exposure is able to enhance the thermogenic capacity for endotherms and is also capable of increasing thermal conductance and consequently heat dissipation (Toloza et al., 1991; Konarzewski and Diamond, 1994; Kotaja et al., 1994; Kotaja, 1996). Many small mammals can increase heat production by non-shivering thermogenesis (NST) to cope with moderate cold exposure, whereas they usually die after exposure to severe cold conditions (Wunder et al., 1977; Heldmaier et al., 1982; Toloza et al., 1991; Hammond and Wunder, 1995; Klingenspor et al., 1996; Li and Wang, 2005; Zhang and Wang, 2006). For example, laboratory mice die after exposure to ambient temperature below –15°C (Toloza et al., 1991; Konarzewski and Diamond, 1994). If the central systems are not the factors limiting SusEI, these animals may die from the hypothermia caused by the limited thermogenic capacity. Limits to SusEI in Swiss mice have been previously reported to be acted peripherally (Hammond and Diamond, 1992; Hammond and Diamond, 1994; Hammond et al., 1994; Hammond et al., 1996; Hammond and Diamond, 1997; Zhao and Cao, 2009b; Zhao et al., 2010). So the study in the same strain of mouse exposed to consecutively decreasing temperatures is helpful for testing if it is only peripherally limited or also constrained simultaneously by the HDL. In the present study, by systematically measuring a variety of physiological and hormonal markers indicative of thermogenic capacity, as well as energy budget and behavior in Swiss mice exposed to consecutively lower temperatures from 23 to –15°C, we tested the components of the peripheral limit hypothesis in Swiss mice exposed to consecutive decreasing temperatures, and then compare these to what other studies have found regarding the HDL hypothesis. We hypothesized that if cold-exposed mice increase energy intake with decreasing ambient temperatures, SusEI may not be limited centrally but probably constrained by the capacity to dissipate heat, and if the animals are not capable of increasing thermogenesis to meet heat loss during exposure to the most severe cold and show hypothermia and weight loss, peripheral limitation may be the case.

MATERIALS AND METHODS

Animals and experimental protocol

All experimental procedures were in compliance with the Animal Care and Use Committee of Liaocheng University. Adult male Swiss mice aged 10–11 weeks were obtained from a laboratory colony at the Experiment Animal Center of Shandong University, and housed individually in plastic cages (29 cm×18 cm×16 cm) with fresh sawdust bedding. Food (standard rodent chow; Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum* and temperature was kept constant at 23±1°C with a 12 h:12 h light:dark cycle (lights on at 08:00 h).

Experiment 1

In order to examine the effects of consecutive decreases in ambient temperatures on body mass, energy budget and behavior in Swiss mice, we randomly assigned 18 mice to either a control (CTRL, N=9) or a cold group (COLD, N=9). The mice in the cold group were transferred from 23°C to 15°C to 0°C to –8°C then to –15°C, with 2 weeks at each temperature. Body mass and food intake were measured throughout the experiment on a daily basis. After exposed to –15°C, one mouse died in the cold group and so only eight were measured in this group.

Behavior observation

Behavioral observations on the mice were made before the experiment started and at the end of the exposure to each temperature: 15, 8, 0, –8 and –15°C. The behavior of the mice was observed over a 24 h period using computer-connected infrared monitors (SONY, 420 TVL) and automatically stored in the computer, and then analyzed. The dominant behavior of each mouse over the 24 h period was classified into one of four categories: general activity, feeding, grooming and resting behavior (Speakman and Rossi, 1999; Speakman et al., 2001; Zhao et al., 2009a). Briefly, general activity included any active movement such as walking around the cage and climbing on the cage bars; feeding was referred to as eating when the animal was at the hopper instead of drinking; grooming was referred to as self-grooming; resting was being inactive in any location of the cage (Speakman and Rossi, 1999; Speakman et al., 2001). Observations were made on four mice from each ambient temperature, and the time spent on each behavior described above was recorded using a stopwatch and expressed in min per hour (min h^{-1}). The accumulative time spent on activity, feeding, grooming and resting behavior was also separately calculated over the day (min 24 h^{-1}).

Body temperature

Rectal body temperature (T_b) was measured between 09:00 and 09:30 h using a digital mouse thermometer (produced by the Beijing Normal University). The probe of the thermometer was inserted 3 cm into the rectum and a reading was taken after 1 min.

Energy intake and digestibility

At the end of each period of temperature exposure, from 23 to –15°C, energy intake and 'digestibility' were measured in controls (N=9) and cold mice (N=9 from 23 to –8°C, but N=8 at –15°C). As described previously (Grodzinski, 1975; Liu et al., 2003; Zhao and Wang, 2006; Zhao and Wang, 2007), the spillage of food mixed with bedding and feces were collected from each cage over the last 2 days of each temperature and separated manually after they were dried at 60°C to constant mass. The gross energy contents of the food and feces were determined using a Parr 1281 oxygen bomb calorimeter (Parr Instrument, Moline, IL, USA). Dry matter intake (DMI), gross energy intake (GEI), digestible energy intake (DEI), and apparent energy assimilation efficiency (digestibility) were calculated as follows (Grodzinski, 1975; Liu et al., 2003; Zhao and Wang, 2006; Zhao and Wang, 2007):

$$\text{DMI (g day}^{-1}\text{)} = \text{food intake (g day}^{-1}\text{)} \times \text{dry matter content of food (\%)} - \text{dry spillage of food ;} \quad (1)$$

$$\text{GEI (kJ day}^{-1}\text{)} = \text{DMI (g day}^{-1}\text{)} \times \text{gross energy content of food (kJ g}^{-1}\text{) ;} \quad (2)$$

$$\text{DEI (kJ day}^{-1}\text{)} = \text{GEI} - [\text{dry feces mass (g day}^{-1}\text{)} \times \text{gross energy content of feces (kJ g}^{-1}\text{)] ;} \quad (3)$$

$$\text{Digestibility (\%)} = \text{DEI/GEI} \times 100\% . \quad (4)$$

Experiment 2

To examine the time-course changes in resting metabolic rate (RMR), nonshivering thermogenesis (NST), serum thyroid hormones levels and body fat, 56 mice were assigned randomly into the following seven groups with eight animals of each: (1) two control groups, in which the mice were housed at 23°C for 2 or 10 weeks (hereafter, described as 2w-CTRL or 10w-CTRL groups); (2) five cold groups in which the mice were exposed to reducing temperatures (i.e. 15, 8, 0, –8 and then –15°C) with 2 weeks of each (referred to as 15, 8, 0, –8 or –15°C groups, respectively). The protocol of cold exposure was the same as in experiment 1.

Resting metabolic rate and nonshivering thermogenesis

RMR was measured as the rate of oxygen consumption in a closed circuit respirometer (Beijing Glass Instruments Ltd, Beijing, China) as described previously (Gorecki, 1975; Zhao and Wang, 2005; Zhao and Cao, 2009a). Briefly, the chamber was 3.6 liters, and carbon dioxide and water in the chamber were absorbed with KOH and silica gel, respectively. The chamber temperature was controlled within ±0.5°C by immersion in a water bath. RMR was determined at a thermoneutral temperature of 30±0.5°C (Speakman and Rossi, 1999; Zhang et al., 2007). Subjects were fasted for 5 h in a temperature of 30°C prior to being put into the metabolic chamber. After a 1-h adaptation to the chamber, oxygen consumption was recorded for 60 min at 5 min intervals. We calculated RMR based on the average of the two continuous stable minimum recordings, in which the minimal difference between the two recordings is considered to be stable. On the next day, maximum NST (NST_{max}) was defined as the maximum rate of oxygen consumption in response to norepinephrine (NE) (Heldmaier et al., 1982) and was induced by a subcutaneous injection of NE at 25±0.5°C. The mass-dependent dosage of NE (Shanghai Harvest Pharmaceutical Co., Shanghai, China) was calculated according to the equation: NE (mg kg^{-1}) = 6.6 Mb$^{-0.458}$(g) (Heldmaier, 1971). Maximum rate of O_2 consumption in response to norepinephrine (NE) occurred around 20–25 min after dorsal subcutaneous injection and reached a plateau for 15–25 min. NST_{max} was calculated using the same methods as for RMR, but instead two continuous stable maximal recordings were used. NST was calculated as NST_{max} minus RMR. RMR and NST_{max} were finally corrected to standard temperature and air pressure (STP) conditions. All measurements were made between 14:00 h and 18:00 h (n=8 for each group) (Wang et al., 2000; Zhao and Wang, 2005).

Serum thyroid hormones

All subjects were killed by decapitation between 09:00 and 11:00 h, after each cold temperature acclimation (N=8 for each group). Trunk blood was collected for tri-iodothyronine (T_3) and thyroxine (T_4) measurements. Serum was separated from each blood sample and stored at –75°C. T_3 and T_4 concentrations were quantified by radioimmunoassay using RIA kits (China Institute of Atomic Energy, Beijing, China). This RIA kit was previously validated and used for Swiss mice following the standard kit instructions. Intra- and inter-assay coefficients of variation were 2.4 and 8.8% for T_3 and 4.3 and 7.6% for T_4, respectively.

Fat content of carcass and body composition

After trunk blood was collected, the gastrointestinal tract was removed and the stomach, small and large intestine and caecum were separated. These were weighed separately (to 1 mg) after gut content and mesentery had been removed. Brown adipose tissue (BAT), liver, heart, lung, spleen and kidneys were then removed and weighed (to 1 mg). The remaining carcass (including the brain, but excluding thyroid, pancreas and urinary bladder) was weighed to determine wet mass, dried in an oven at 60°C to a constant mass, and then weighed (to 1 mg) again to determine dry mass. Total fat of the carcass was extracted from the dried carcass by ether extraction in a Soxhlet apparatus. The fat content of carcass (FCC) was calculated as follows: FCC = total fat of carcass/wet carcass mass × 100% (Zhao and Wang, 2006).

Statistics

Data were analyzed using SPSS 13.0 software. In experiment 1, repeated-measures analysis of variance (RM-ANOVA) was used to determine the significance of changes in body mass, food intake, behavior, T_b, GEI, DEI and digestibility over time. The least-significant difference (LSD) *post hoc* tests were used when differentiation between days of cold exposure was required. Direct comparisons of body mass and food intake of control and cold mice on the same day were made using two-sample *t*-tests. The differences in the above variables, except for body mass, between the control and cold groups were evaluated using ANOVA or ANCOVA with body mass as a covariate where appropriate. In experiment 2, effects of cold exposures on RMR, NST_{max}, NST, T_3, T_4, FCC and body composition were examined using ANOVA or ANCOVA (with body mass as a covariate) followed by LSD *post hoc* tests. For percentage data, arcsine-square-root transformation was performed prior to analysis to normalize the data. Data were expressed as means ± s.e.m. Data were regarded as statistically significant at P<0.05.

RESULTS

There were no significant differences in body mass, food intake, T_b, GEI, DEI or digestibility between CTRL and COLD mice prior to the experiment.

Experiment 1

Body mass

Body mass of control mice increased significantly throughout the experiment, by 15.4% on day 70 compared with day 0 (RM-ANOVA, $F_{69,552}$=25.65, P<0.001; Fig. 1A). Cold mice showed a significant decrease in body mass over the period of cold exposure, during which weight loss occurred at –8°C, and again at –15°C relative to 0°C (RM-ANOVA, $F_{69,483}$=15.16, P<0.001). There was no significant difference in body mass between control mice and mice exposed to 15, 8 or 0°C (15°C, day 14, t_{16}=0.10, P=0.92; 8°C, day 28, t_{16}=0.66, P=0.52; 0°C, day 42, t_{16}=1.18, P=0.26). After being exposed to –8°C (on day 43 and thereafter), cold mice had a significantly lower body mass than controls (–8°C, day 56, t_{16}=3.32, P<0.01). At the end of experiment, body mass of cold mice was 21.9% lower than that of control individuals (–15°C, day 70, t_{15}=4.77, P<0.001; Fig. 1A).

Food intake

Food intake of cold mice significantly increased throughout the experiment, and averaged 14.1±0.5 g day^{-1} on day 70, which was a 116.5% increase relative to that on day 0 (RM-ANOVA, $F_{69,483}$=68.32, P<0.001, Fig. 1B). Cold-treated mice had significantly higher food intake than controls on day 1 and thereafter (ANOVA, day 1, $F_{1,15}$=44.55, P<0.01; day 70, $F_{1,14}$=141.27, P<0.01). Mean food intake was higher by 31.3%, 55.0%, 76.2%, 116.2% and 125.5% in cold mice exposed to 15°C, 8°C, 0°C, –8°C and –15°C than that of controls (ANOVA, 15°C, $F_{1,15}$=50.30, P<0.01; 8°C, $F_{1,15}$=110.12, P<0.01; 0°C, $F_{1,15}$=133.57, P<0.01; –8°C, $F_{1,15}$=306.62, P<0.01 and –15°C, $F_{1,15}$=308.36, P<0.01). Mean food intake was not significantly different between control groups (ANOVA, $F_{5,48}$=1.30, P=0.17; Fig. 2). Cold mice significantly increased their mean food intake with decreasing ambient temperatures (RM-ANOVA, $F_{5,48}$=86.50, P<0.001), whereas, the difference between –8°C and –15°C was not significant (*post hoc*, P=0.73; Fig. 2).

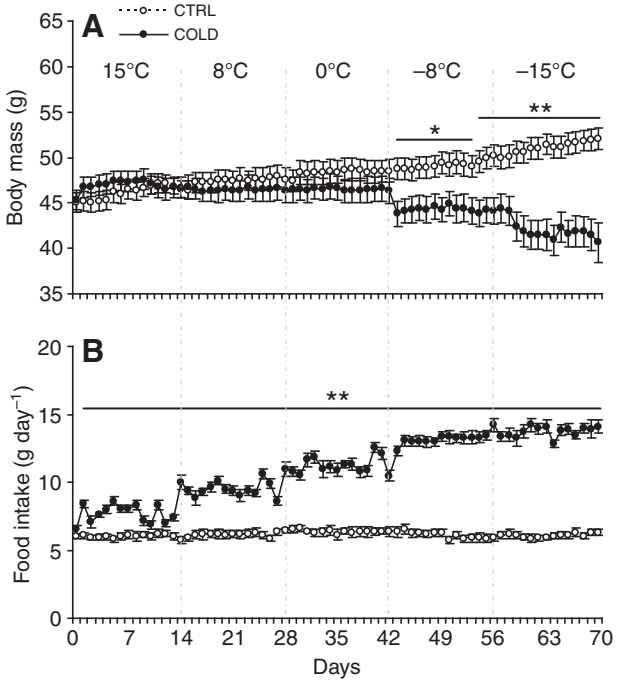

Fig. 1. Body mass (A) and food intake (B) in Swiss mice exposed to a decrease in ambient temperature. CTRL: control mice housed at 23°C (N=9); COLD: the mice were exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15°C until –8°C and N=8 at –15°C). Data are means ± s.e.m. Significant difference between the two groups is indicated by asterisks (*P<0.05, **P<0.01).

Behavior

General active behavior of control mice showed significant changes throughout a day (RM-ANOVA, $F_{23,69}$=2.92, P<0.01, Fig. 3A), during which the mice was active over two peaks: 21:00–22:00 h (*post hoc*, P<0.05) and 08:00–11:00 h (*post hoc*, P<0.05). After exposure to cold, they did not show the two active peaks, and the magnitude of activity tended to be reduced with decreasing ambient temperatures. In contrast to activity, the time spent on feeding behavior increased with decreasing ambient temperatures (Fig. 3B). There were no changes in time spent on grooming behavior between the temperatures (Fig. 3C). The mice at 23°C generally spent less time resting during 19:00–21:00 h and 07:00–09:00 h, whereas this was not observed in the cold-exposed mice (Fig. 3D).

Cold exposures led to a significant decrease in accumulative time spent on activity (ANOVA, $F_{7,23}$=15.11, P<0.01), which decreased by 62% at –15°C compared with 23°C (*post hoc*, P<0.05; Fig. 4). Accumulative time spent feeding increased significantly with decreasing ambient temperatures (ANOVA, $F_{7,23}$=22.35, P<0.01). Accumulative time on feeding averaged 277±8 min over 24 h at –15°C, which was 138% higher than 23°C (*post hoc*, P<0.05). Accumulative time spent on grooming behavior did not show significant differences between CTRL and COLD groups (ANOVA, $F_{7,23}$=2.01, P=0.09). These mice tended to spend more time resting after being exposed to cold (ANOVA, $F_{7,23}$=6.00, P<0.01; Fig. 4).

Body temperature

Control mice had a constant T_b throughout the experiment (ANOVA, $F_{5,40}$=0.72, P=0.49, Fig. 5). In cold-exposed mice T_b decreased with

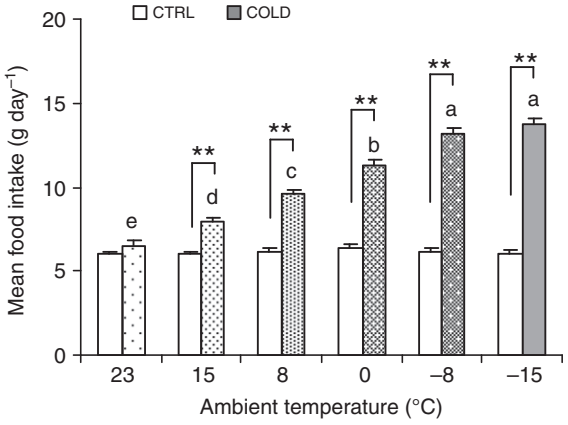

Fig. 2. Mean food intake of Swiss mice exposed to a decrease in ambient temperatures. CTRL: control mice housed at 23°C (N=9); COLD: mice exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15 to –8°C and N=8 at –15°C). Data are means ± s.e.m. **Significant difference between the two groups (P<0.01). Different letters above the columns indicate significant difference between cold groups (P<0.05).

decreasing temperatures, such that T_b at –8°C and –15°C was significantly lower than at 23°C (ANOVA, $F_{5,40}$=7.52, P<0.01). In addition, cold mice showed a significantly lower T_b at 0°C, –8°C and –15°C than controls, respectively (ANOVA, 0°C, $F_{1,15}$=8.06, P<0.05; –8°C, $F_{1,15}$=12.22, P<0.01; –15°C, $F_{1,15}$=4.63, P<0.05, Fig. 5).

Energy intake and digestibility

Control mice did not show significant changes in GEI, DEI or digestibility throughout the experiment (RM-ANOVA, GEI, $F_{5,20}$=1.30, P=0.26; DEI, $F_{5,20}$=0.78, P=0.53; digestibility, $F_{5,20}$=0.35, P=0.76; Fig. 6A–C). GEI and DEI significantly increased with decreasing ambient temperatures (RM-ANOVA, GEI, $F_{5,20}$=197.72, P<0.001; DEI, $F_{5,20}$=147.17, P<0.001); the maximum GEI and DEI averaged 222.4±8.2 kJ day^{-1} and 168.1±6.2 kJ day^{-1} at –15°C increases of 124% and 126%, respectively, compared with 23°C (*post-hoc*, GEI, P<0.05; DEI, P<0.05; Fig. 6A,B). In the cold groups, GEI and DEI at 15°C, 8°C, 0°C, –8°C and –15°C were significantly higher than controls (ANOVA, 15°C, GEI, $F_{1,7}$=8.84, P<0.05; DEI, $F_{1,7}$=6.87, P<0.05; –15°C, GEI, $F_{1,7}$=115.70, P<0.01; DEI, $F_{1,7}$=112.57, P<0.01). No significant difference in digestibility between control and cold mice was observed at any temperatures (ANOVA, –15°C, $F_{1,7}$=0.19, P=0.78, Fig. 6C).

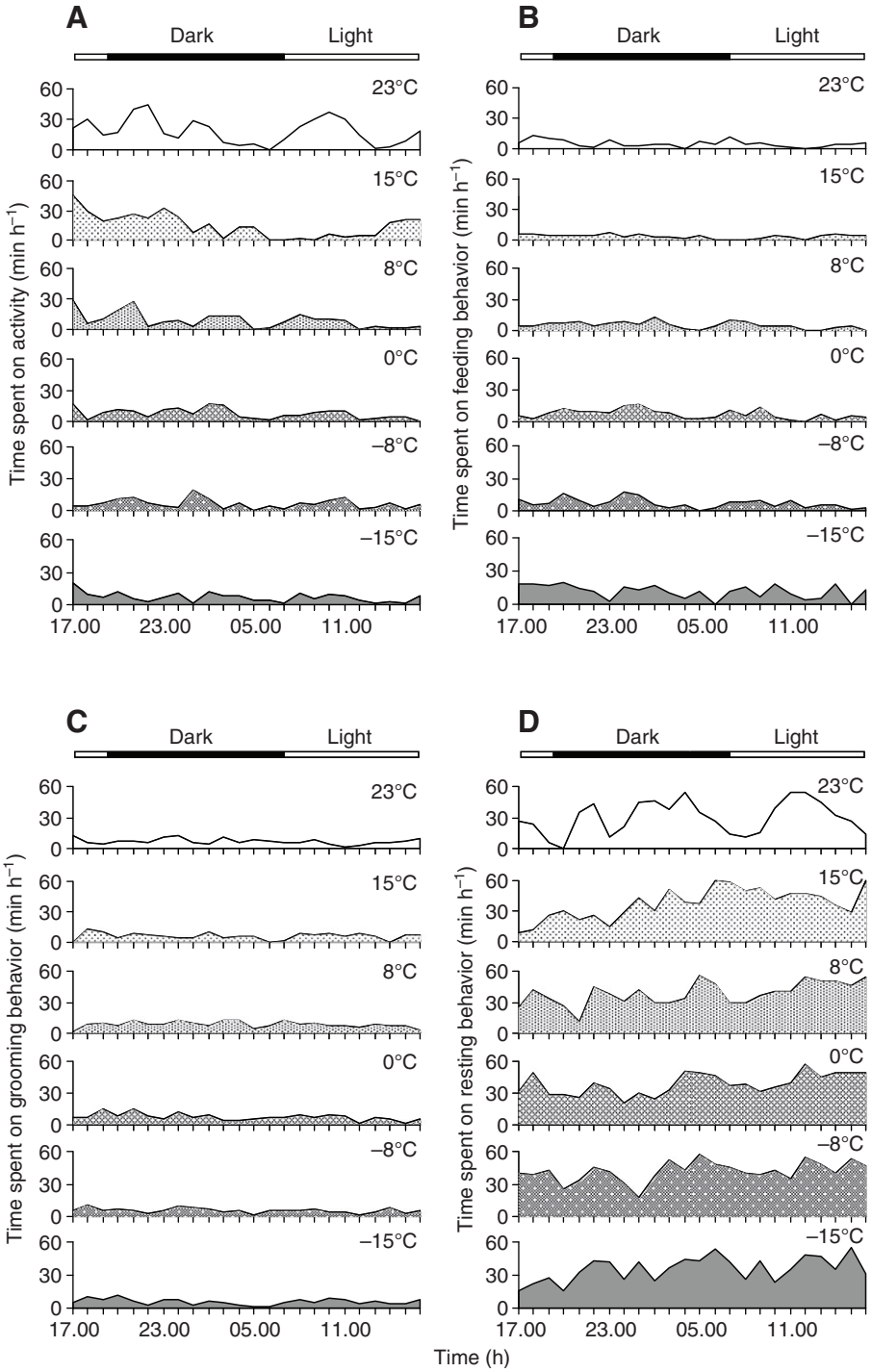

Fig. 3. Time spent on activity (A), feeding (B), grooming (C) and resting behavior (D) throughout a day by Swiss mice exposed to a decrease in ambient temperatures from 23 to –15°C (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15 to –8°C and N=8 at –15°C). Data are means.

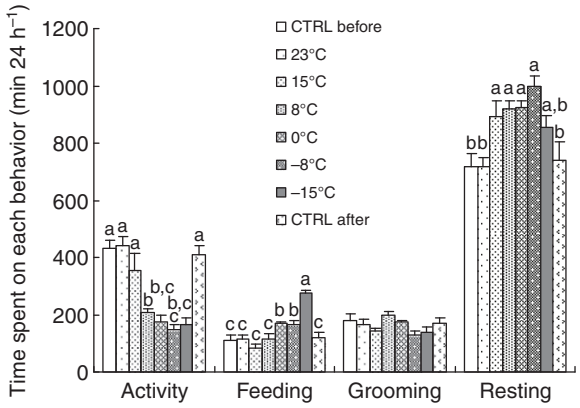

Fig. 4. Time spent on activity, feeding, grooming and resting by Swiss mice exposed to a decrease in ambient temperatures. CTRL before or after: behavioral observation was made before the experiment started and at the end of this experiment on control mice housed at 23°C (N=9); COLD: mice exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15 to –8°C and N=8 at –15°C). Data are means ± s.e.m. Different letters above the columns indicate significant difference between cold groups (P<0.05).

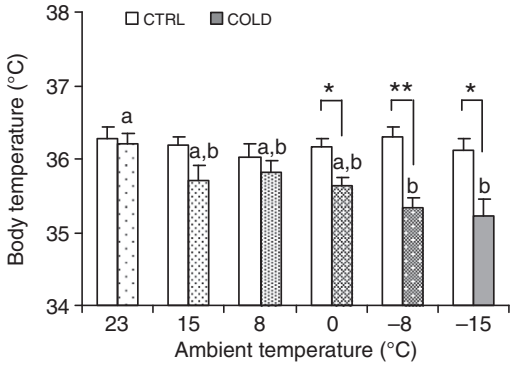

Fig. 5. Body temperature (T_b) in Swiss mice exposed to a decrease in ambient temperatures. CTRL: control mice housed at 23°C (N=9); COLD: mice exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15 to –8°C and N=8 at –15°C). Data are means ± s.e.m. Significant difference between the two groups is indicated by asterisks (*P<0.05, **P<0.01). Different letters above the columns indicate significant difference between cold groups (P<0.05).

Experiment 2

Resting metabolic rate and nonshivering thermogenesis

Cold exposures had significant effects on RMR and thermogenesis. Cold-exposed mice had significantly higher RMR, NST_{max} and NST than control mice even when corrected for body mass (ANOVA, RMR, $F_{5,50}$=2.13, P<0.05, NST_{max}, $F_{5,50}$=4.88, P<0.01; NST, $F_{5,50}$=6.28, P<0.01; ANCOVA, RMR, $F_{5,49}$=7.38, P<0.01, NST_{max}, $F_{5,49}$=8.14, P<0.01; NST, $F_{5,49}$=7.67, P<0.01; Fig. 7), whereas the difference in RMR within cold groups (15°C, 8°C, 0°C, –8°C and –15°C) was not significant (*post hoc*, P=0.15). In addition, neither NST_{max} nor NST showed significant difference within cold mice between 8°C, 0°C, –8°C or –15°C (ANOVA, *post hoc*, NST_{max}, P=0.25; NST, P=0.21; ANCOVA, *post hoc*, NST_{max}, P=0.13; NST, P=0.10; Fig. 7).

Serum tri-iodothyronine and thyroxine

Concentrations of serum T_3 and T_4 were affected significantly by the cold exposures (Table 1): cold-exposed mice showed higher T_3, but lower T_4. Levels of T_3 significantly increased with decreasing ambient temperatures, which were 40.7% and 144.3% higher at –15°C than that of controls (*post hoc*, T_3, P<0.05). By contrast, the concentration of serum T_4 significantly reduced after cold exposures, and was 39.9% lower at –15°C than controls (*post hoc*, P<0.05; Table 1).

Fat content of carcass (FCC) and body compositions

Exposure to cold caused a significant decrease in body mass, which was paralleled by a change of carcass mass (Table 1). There were significant effects of cold exposure on FCC, in that there was a significant decrease in FCC with decreasing ambient temperatures (*post hoc*, P<0.05). FCC averaged 4.6±0.3% in cold mice at –15°C, which was 64.0% lower than that of controls (*post hoc*, P<0.05). However, no significant difference was observed between –8°C and –15°C (*post hoc*, P=0.43). Cold exposures imposed significant effects on most components of body composition, and cold mice had heavier BAT, liver, heart, lung, kidneys as well as the gastrointestinal tract than control groups (Table 1).

DISCUSSION

In the present study, consecutively lower ambient temperatures imposed significant effects on body mass, fat content of the carcass and body temperature, which decreased significantly in the mice exposed to –8 to –15°C. Food intake, GEI and DEI increased significantly during exposure to all the lower temperatures. Cold-exposed mice exhibited significant increase in the time spent feeding, and spent less time on general activity with decreased ambient temperatures. RMR, NST and serum T_3 levels significantly increased in cold-exposed mice, whereas the differences were not significant between 0 and –15°C.

Body mass and body composition

It has been reported that many small mammals such as Brandt's voles (*Lasiopodomys brandtii*), meadow voles (*Microtus pennsylvanicus*), prairie voles (*M. ochrogaster*), Djungarian hamster (*Phodopus sungorus*), South American field mice (*Abrothrix andinus*) and laboratory mice (Iverson and Turner, 1974; Steinlechner et al., 1983; Bozinovic et al., 1990; Toloza et al., 1991; Konarzewski and Diamond, 1994; Voltura and Wunder, 1998; Li and Wang, 2005) respond to low temperature environments by reducing body mass. Consistently, in the present study Swiss mice showed significant decreases in body mass after being exposed to –8°C to –15°C, during which body mass was lower by 21.9% in the mice at –15°C than controls. It has been well established that changes in body mass are the result of the imbalance between energy intake and expenditure, and decrease in body mass is generally due to a negative energy state (Voltura and Wunder, 1998; Speakman, 2000; Merritt et al., 2001; Li et al., 2005). Here, body fat mass was, on average, 3.3 g in mice exposed to 8°C and 1.3 g in –15°C, suggesting that 2 g fat was proximately mobilized. One gram of adipose tissue contains about 0.8 g lipid (39 kJ g^{-1}), and thus contains 31.2 kJ energy (Speakman et al., 2002). Thus, during the 6 weeks from 8 to –15°C, about 62.4 kJ in total and 1.5 kJ per day was mobilized. NST_{max} averaged 300.2 ml O_2 h^{-1} in mice exposed to –15°C, which equaled 150.6 kJ day^{-1} after it was converted to energy expenditure

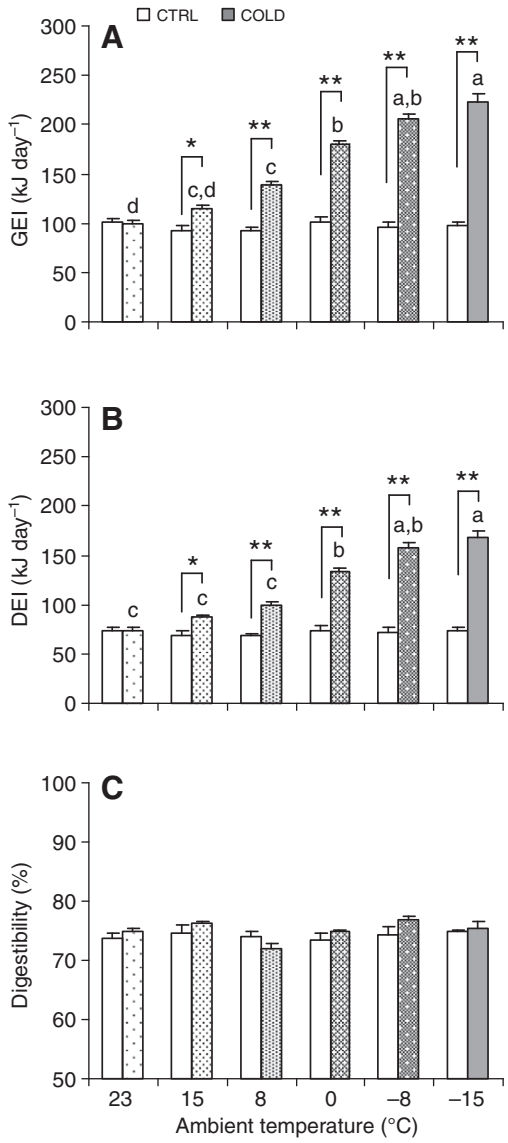

Fig. 6. Gross energy intake (GEI; A), digestible energy intake (DEI; B) and digestibility (C) in Swiss mice exposed to a decrease in ambient temperatures. CTRL: control mice housed at 23°C (N=9); COLD: mice exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=9 at 15 to –8°C and N=8 at –15°C). Data are means ± s.e.m. Significant difference between the two groups is indicated by asterisks (*P<0.05, **P<0.01). Different letters above the columns indicate significant difference between cold groups (P<0.05).

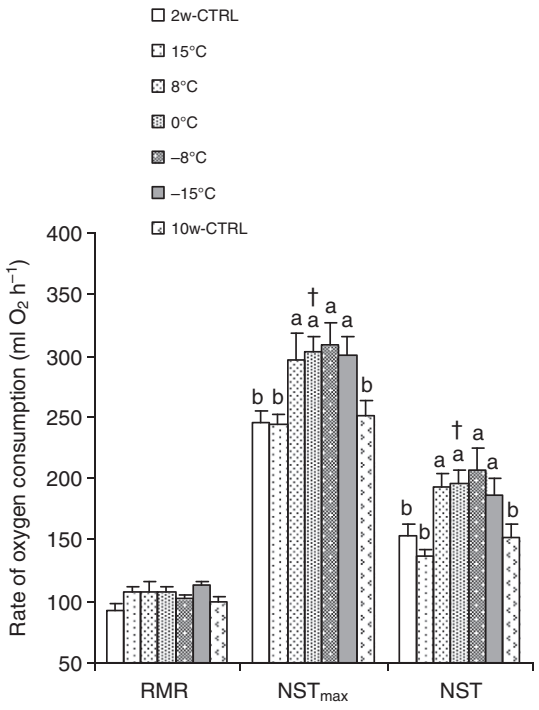

Fig. 7. Resting metabolic rate (RMR), maximal nonshivering thermogenesis (NST_{max}) and NST in Swiss mice exposed to a decrease in ambient temperatures. 2w- or 10w-CTRL: control mice housed at 23°C for 2 (N=8) or 10 weeks (N=8); COLD: the mice exposed to a consecutive decrease in ambient temperatures (15, 8, 0, –8 and –15°C, 2 weeks of each, N=8 for each temperature). Data are means ± s.e.m. †Significant effects of cold exposure on NST_{max} or NST (P<0.01). Different letters above the columns indicate significant difference between groups (P<0.05).

(kJ day^{-1}) using the equation of Weir (Weir, 1949; Speakman, 2000). Thus the energy storage that was mobilized in cold-exposed Swiss mice seemed negligible to meet the great energy demands for thermoregulatory cost at severe lower temperatures.

Energy intake

In the current study, cold-exposed Swiss mice showed significantly increases in GEI and DEI. Food intake increased with consecutively lower ambient temperatures: food intake of cold-exposed mice at –8 to –15°C was 116 and 126% higher than controls. However, there was no statistically significant difference between –8 and –15°C (13.2±0.3 g day^{-1} *vs* 13.7±0.4 g day^{-1}, *post hoc*, P=0.73). Konarzewski and Diamond found similar results in mice suddenly exposed to ambient temperatures between 22°C and –15°C (Toloza et al., 1991; Konarzewski and Diamond, 1994). These data might suggest that food intake reached a ceiling around 14 g day^{-1} in the mice exposed to –15°C. However, in several previous studies, the asymptotic food intake during normal lactation averaged 19 g day^{-1} in the same strain of mouse, and even up to 23 g day^{-1} in the dorsally shaved mice during peak lactation (Hammond et al., 1994; Hammond and Diamond, 1997; Zhao and Cao, 2009b). These data suggested that Swiss mice were capable of increasing food intake beyond that observed in the current study, and the SusEI was not centrally constrained. Cold-exposed mice had larger gastrointestinal tracts, suggesting adaptive regulations in gut morphology to accommodate the increased food intake. However, Swiss mice failed to further increase their food intake in response to severe cold exposure, i.e. –15°C, suggesting that the limits to SusEI might be imposed peripherally by the capacities of the mice to produce heat.

Behavior

In terms of energy homeostasis and body weight balance, researchers have increasingly focused on behavior, as animals usually change their behavioral rhythm when environmental variations occur (Johnson and Cabanac, 1982; Geiser and Ruf, 1995; Schwaibold and Pillay, 2006). For example, several small rodents including house mice and rats reduce the time spent on energetically costly behaviors (e.g. activity behavior) in cold temperatures while

Table 1. Fat content of carcass, body composition and serum tri-iodothyronine and thyroxine concentration of Swiss mice exposed to consecutive decreases in temperature

	2w-Con	15°C	8°C	0°C	–8°C	–15°C	10w-Con	*F*	*P*
Body mass	44.5±0.5^{b,c}	46.4±1.8^{a,b,c}	49.0±1.6^{a,b}	47.2±2.3^{a,b,c}	43.3±1.1^{b,c}	40.9±1.2^{c}	52.1±1.0^{a}	6.3	**
Carcass (g)	32.4±0.5^{a,b}	32.9±1.0^{a}	34.0±1.2^{a}	32.0±1.3^{a,b}	28.2±0.6^{b,c}	27.2±1.1^{c}	35.8±1.1^{a}	9.2	**
FCC (%)	12.0±0.8^{a,b}	7.9±0.7^{c}	9.8±0.7^{b,c}	7.7±0.8^{c}	5.0±0.4^{d}	4.6±0.3^{d}	12.7±0.5^{a}	25.8	**
BAT (mg)	101±5^{d}	122±11^{c}	203±15^{a,b}	193±9^{a,b}	194±7^{a,b}	228±14^{a}	121±5^{c,d}	23.8	**
Liver (mg)	2173±58^{a,b}	2333±109^{a,b}	2585±161^{a}	2426±196^{a,b}	1958±47^{b}	2155±119^{a,b}	2570±77^{a}	3.1	*
Heart (mg)	188±4^{c}	201±10^{b,c}	221±13^{a,b,c}	236±15^{a,b}	246±11^{a,b}	254±13^{a,b,c}	214±7^{a,b,c}	14.6	**
Lung (mg)	219±6^{b}	221±14^{b}	316±47^{a}	270±21^{ab}	287±21^{a,b}	267±9^{a,b}	245±17^{b}	3.3	*
Spleen (mg)	103±5	115±6	112±4	105±7	107±19	75±3	124±6	1.5	0.19
Kidneys (mg)	566±26^{b}	678±42^{a,b}	741±50^{a}	710±40^{a,b}	721±36^{a}	657±36^{a,b}	676±30^{a,b}	7.5	**
Stomach (mg)	339±21^{c}	350±13^{b,c}	394±25^{b}	331±26^{c}	373±18^{b,c}	447±28^{a}	356±8^{b,c}	5.1	**
SL (mg)	1235±93^{b}	1288±147^{b}	1343±66^{b}	1310±107^{b}	1517±123^{a}	1678±139^{a}	1335±107^{b}	2.1	**
LL (mg)	448±23^{c}	488±64^{c}	592±30^{b}	535±23^{b}	669±42^{a}	709±25^{a}	452±41^{c}	7.9	**
Caecum (mg)	229±14^{c}	234±22^{c}	216±16^{c}	259±16^{c}	342±27^{b}	412±31^{a}	224±12^{c}	8.2	**
T_3 (ng ml^{-1})	0.61±0.05^{b}	0.58±0.06^{b}	0.62±0.04^{b}	0.89±0.08^{a}	0.91±0.09^{a}	0.82±0.08^{a}	0.58±0.04^{b}	5.6	**
T_4 (ng ml^{-1})	40.2±2.7^{a}	32.3±2.3^{a,b}	32.5±2.6^{a,b}	28.9±1.8^{b}	28.9±2.8^{b}	24.1±2.0^{b}	40.1±2.7^{a}	6.1	**

2w- and 10w-Con, control mice housed at 23°C for 2 or 10 weeks, respectively; BAT, brown adipose tissue; FCC, fat content of carcass; LL, large intestine; SL, small intestine; T_3, tri-iodothyronine; T_4, thyroxine.

Data are means ± s.e.m.

Different superscripted letters in each row indicate a significant difference between groups. *, $P<0.05$; **, $P<0.01$.

increasing feeding time (Johnson and Cabanac, 1982; Perrigo Bronson, 1985; Schwaibold and Pillay, 2006). Also in deer mice (*Peromyscus maniculatus*), the duration of activity per bout decreased at colder temperatures, and the amount of time spent active in the cold was negatively correlated with ambient temperature (Sears et al., 2009). Consistently, in the current study cold-exposed mice showed a reduction in the time spent on activity and exhibited increases in the time spent on feeding and resting behavior. This might suggest that Swiss mice spent most time in energy acquiring (consuming food) and energy saving (resting) behavior. The increased feeding time would match the elevation in food intake following cold exposures. Consistently, the increased time spent on consuming food during cold weather was also found in other rodents, such as ice rats (*Otomys sloggetti robertsi*) (Schwaibold and Pillay, 2006) and Brant's whistling rat (*Parotomys brantsii*) (Jackson, 1998). The possible explanation would be the higher energy demands when ambient temperature was low, resulting in higher thermogenic demands that are accompanied by greater energy intake through feeding (Hayne et al., 1986; Schultz et al., 1999; Schwaibold and Pillay, 2006).

Thermoregulation and thermogenesis

It has been generally accepted that cold exposure imposes great energy demands for thermoregulation in small endothermic mammals. In the current study, we found a lower T_b in the mice exposed to lower temperatures from 0 to –15°C. On the one hand, the decrease in T_b might be a positive response in energy budget of an animal, which would decrease the energy exported for thermoregulation. On the other hand, the decreased T_b could be a negative response for cold-exposed animals as one mouse died after exposure to a severe low temperature (–15°C). The reason for this death was assumed to be a large reduction in T_b, resulting from the notable increase in heat loss and the failure to increase thermogenesis (Refinetti, 2003). In other words, thermogenesis might not be able to compensate for the heat loss in some mice exposed to severe cold (–15°C or below), which consequently die from hypothermia, suggesting that limitations on thermogenesis might exist where the metabolic and/or adaptive thermogenesis occurred.

Both obligatory and adaptive thermogenesis (RMR and NST) are commonly employed in response to acute cold stress in small mammals living in temperate area (Heldmaier, 1971; Heldmaier and Buchberger, 1985; Hammond and Wunder, 1995; Wang et al., 1999; Li et al., 2001; Hammond and Kristan, 2000; Zhang and Wang, 2006; Zhang and Wang, 2007). In the present study RMR, NST_{max} and NST generally increased with consecutively lower ambient temperatures, whereas the difference in NST_{max} and NST were not statistically different between 8 and –15°C. This suggested that the thermogenic capacity would not be able to meet the increased heat loss in mice at –15°C or below, and finally resulted in a fall in body temperature.

Thyroid hormones can stimulate basal thermogenesis by lowering metabolic efficiency, and thus plays a fundamental role in obligatory and adaptive thermogenesis (Lowell and Spiegelman, 2000). BAT is the primary site of NST in small mammals, and BAT thermogenic response is initiated by NE but must have thyroid hormone present (Ribeiro et al., 2001). Cold-induced thermogenesis also depends on the synergism between the sympathetic nervous system and thyroid hormones (Ribeiro et al., 2001). Metabolic adjustment of thyroid hormones is usually correlated with thermogenic capacity in some cold exposed rodents (Li et al., 2001). In the present study, the serum T_3 concentration increased in the mice exposed to 0°C down to –15°C. Consistent with RMR and NST, we did not find any further increase in T_3 between –8 and –15°C. T_3 is produced locally in BAT from thyroxine by the action of the type 2 5′-deiodinase enzyme (Silva and Larsen, 1983; Ribeiro et al., 2001). Here we also observed that serum T_4 concentration decreased with reducing ambient temperatures, and reached a maximum at 0, –8 and –15°C, which might suggest that T_3 and T_4 conversion worked at maximum. This might be a biochemical mechanism whereby obligatory and adaptive thermogenesis was constrained in the mice exposed to severe lower temperature.

It has been well established that cold exposure results in an increase in heat loss because the thermal conductance will generally increase in animals after being exposed to a colder condition. Consistently, in the present study cold-exposed mice generally showed an elevation in thermogenesis, which was probably used to compensate for heat loss, suggesting that heat dissipation limits on these cold-exposed animals were relaxed. Cold-exposed mice

significantly increased food intake with decreasing ambient temperatures, which was consistent with that predicted by the HDL hypothesis, i.e. limits to SusEI by the capacity to dissipate heat was relaxed, allowing an increase in energy intake (Król and Speakman, 2003a; Król and Speakman, 2003b; Król et al., 2003; Król et al., 2007). This also indicated that these animals would consume as much food as they needed, and produce sufficient heat to compensate for the heat loss under moderately cold conditions and maintain a stable body temperature. However, both digested energy intake and NST_{max} seemed to reach a ceiling in mice exposed to sever cold temperature, which were, on average, 168.1 and 150.6 kJ day^{-1}, respectively, at –15°C. This might suggest that ~90% digested energy contributed to thermogenesis, and only 10% was insufficient to maintain body mass and support daily activity behavior, resulting in a great weight loss. Cold-exposed mice showed significantly lower body temperature at –8 and –15°C, which was probably due to the failure of thermogenic capacity to meet heat loss, providing a support for peripheral limitation hypothesis. These results might suggest that SusEI in cold-exposed Swiss mice was constrained peripherally by the capacity to produce heat and also by the ability to dissipate body heat, but to a different extent. Moderate cold exposure might result in a relaxation on the HDL, allowing the animals to increase food intake to meet cold stress. When animals were exposed to sever cold conditions, their thermogenic capacity might reach a ceiling, which failed to compensate for the heat loss and finally resulted in lower body temperature and greatly weight loss. This might suggest that the HDL was set at a higher level than peripheral limits for Swiss mice when being exposed to consecutive decreases in ambient temperatures.

Conclusion

Cold-exposed Swiss mice showed significant decreases in body mass, fat content of carcass and T_b at consecutively lower ambient temperatures from 23 to –15°C. GEI and DEI increased significantly throughout the experiment, indicating an inconsistency with the central limitation hypothesis, but a consistency with the HDL hypothesis, i.e. cold exposure led to a relaxation of the heat dissipation limit and consequently allowed the animals to elevate their food intake. Cold-exposed mice significantly increased feeding time, but became inactive. The thermogenesis indicative of RMR, NST_{max}, NST, serum T_3 and T_3/T_4 increased in mice exposed to the lower temperatures, whereas the differences were not significant between 0, –8 and –15°C. This might indicate that obligatory and adaptive thermogenesis was constrained in the mice exposed to severe lower temperatures, providing a support for the peripheral limitation hypothesis. These results might suggest that SusEI in cold-exposed Swiss mice was constrained peripherally by the capacity to produce heat and also the ability to dissipate body heat, but to a different extent, by which the HDL might be set on a higher level than peripheral limits.

ACKNOWLEDGEMENTS

We are grateful to the anonymous reviewers for their constructive suggestions and comments on this manuscript. This work was partly supported by grant no. 30800130 from the National Natural Science Foundation of China.

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