

## RESEARCH ARTICLE

# Environmental temperature effects on adipose tissue growth in a hibernator

Amanda D. V. MacCannell<sup>1,\*</sup>, Kevin J. Sinclair<sup>2</sup>, Charles A. McKenzie<sup>2</sup> and James F. Staples<sup>1</sup>

## ABSTRACT

Obligate hibernators express circannual patterns of body mass and hibernation, which persist under constant laboratory conditions. Brown adipose tissue (BAT) is important for thermogenesis during arousals from hibernation, whereas white adipose tissue (WAT) serves as energy storage and thermal insulation. The goal of this study was to investigate the effects of environmental temperature on BAT and WAT. We hypothesized that changes to environmental temperature would not influence the pattern of mass gain or BAT and WAT volume in the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*). To test this, we housed animals at thermoneutral 25°C (warm-housed) or 5°C (cold-housed), with the same photoperiod (12 h light:12 h dark) over an entire year. Throughout the year we measured the volume and water:fat ratio of WAT and BAT using magnetic resonance imaging (MRI). We found no evidence of torpor in the warm-housed animals, indicating that this species might not be an obligate hibernator, as previously assumed. Regardless of ambient temperature, BAT volume increased prior to winter, then decreased in late winter with no change in water:fat ratio. By contrast, both body mass and WAT volume of cold-housed animals declined throughout the winter and recovered after hibernation, but thermoneutral housing produced no circannual pattern in body mass, even though WAT volume declined in late winter. Cold exposure appears to be a primary regulator for WAT but BAT may exhibit an endogenous circannual rhythm in terms of depot volume.

**KEY WORDS:** Circannual endogenous rhythm, MRI, Hibernation, Brown adipose tissue, White adipose tissue

## INTRODUCTION

Mammals typically maintain a constant core body temperature ( $T_b$ ) over a broad range of ambient temperatures ( $T_a$ ), but this strategy can be challenging in the winter when  $T_a$  is low and food resources are limited. Hibernation evolved in some small mammals, such as the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), presumably to conserve energy during the winter. During hibernation, animals spend the majority of their time in torpor, where both metabolic rate and  $T_b$  are significantly reduced. These periods of torpor are spontaneously interrupted by periods of interbout euthermia (IBE), where body temperature and metabolic rate return to similar levels to those in the summer. Hibernation is classified as either facultative, occurring only under environmental

conditions that are particularly unfavourable, or obligate, occurring at approximately the same time each year regardless of environmental conditions (Strijkstra, 2009). Facultative hibernators include the Syrian hamster, which only hibernates after acclimation for approximately 74 days to cold  $T_a$  (5°C) and short (8 h light:16 h dark) photoperiods (Chayama et al., 2016). In contrast, obligate hibernators, such as the golden-mantled ground squirrel (*Callospermophilus lateralis*), are thought to show an endogenous circannual rhythm (Pengelley and Asmundson, 1969). Juveniles of this species housed under constant environmental conditions of either 3 or 12°C with a 12 h light:12 h dark photoperiod for more than 4 years showed free-running patterns of body mass, food intake, fat storage and hibernation that corresponded with patterns seen in wild populations (Pengelley and Asmundson, 1969). Similar patterns were observed in golden-mantled ground squirrels housed constantly at 22 or 5°C (Florant et al., 2012). Some researchers believe such patterns suggest that obligate hibernation is controlled genetically, without the requirement for environmental cues (Pengelley and Asmundson, 1969).

Obligate hibernators seasonally regulate fat deposition, specifically by increasing lipid mass prior to hibernation. Endogenous lipid stores are increased by enlarging brown adipose tissue (BAT) (Hindle and Martin, 2014; MacCannell et al., 2017) and white adipose tissue (WAT) depot sizes prior to hibernation (MacCannell et al., 2017). WAT is the main driver of body mass gain prior to hibernation, and exhibits an endogenous circannual rhythm in obligate hibernators such as the Arctic ground squirrel (*Spermophilus parryii*) (Sheriff et al., 2013). BAT is a mitochondria-dense tissue that increases metabolic heat production during arousal, the rapid transition from torpor to IBE. BAT mitochondria express uncoupling protein 1 (UCP-1), which facilitates thermogenesis by uncoupling electron transport from ATP synthase activity. BAT is found in small eutherian mammals and is typically distributed in thoracic depots, primarily superior to the heart, axial to the ribcage and between the scapulae (Oelkrug et al., 2015).

The volume and lipid content of BAT and WAT can be measured repeatedly within individuals using non-invasive magnetic resonance imaging (MRI). The MRI pulse sequence ‘iterative decomposition of water and fat with echo asymmetry and least-squares estimation’ (IDEAL) (Fuller et al., 2006; Reeder et al., 2005) allows identification of the location and proton density fat fraction (PDFF, the amount of lipid signal over total signal) (Reeder et al., 2012; Hernando et al., 2017) of each tissue throughout the entire volume of an animal, calculated as in Eqn 1.

$$\text{PDFF} = \frac{\text{lipid } ^1\text{H signal}}{\text{lipid } ^1\text{H signal} + \text{water } ^1\text{H signal}} \quad (1)$$

This quantitative approach can non-invasively determine the amount of lipid within a tissue. The PDFF of BAT (30–70%) (Hu et al., 2010; Rasmussen et al., 2013; Prakash et al., 2016) is lower

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than that of WAT (80–100%), allowing IDEAL MRI to differentiate between WAT and BAT and quantify the volume of each tissue non-invasively. A lower PDFF of BAT reflects a high proportion of mitochondria and blood vessels within the tissue (increased water content throughout the tissue), whereas the high PDFF of WAT reflects its role in metabolic energy storage (increased lipid and lower water content throughout the entire tissue).

In a previous experiment (MacCannell et al., 2017), we used MRI to quantify changes in volume and PDFF of BAT and WAT over an entire year in thirteen-lined ground squirrels, which are thought to be obligate hibernators (Drew et al., 2007). That experiment showed that BAT depots increase in size even without exposure to cold  $T_a$ , which is unusual for mammals. In that study, however, animals were housed at a photoperiod mimicking their natal origin (Carman, MB, Canada), and it is possible that the changes in BAT were triggered by a changing photoperiod.

In the current study, we investigated the effects of environmental temperature and photoperiod on changes in BAT and WAT. We hypothesized that, if the cyclical changes in BAT and WAT are regulated by an endogenous circannual pattern, then changes to environmental temperature would not influence the pattern of mass gain or volume of BAT and WAT. To test this, we held two groups of thirteen-lined ground squirrels over a full year under different temperature conditions. One group was housed at 25°C (thermoneutral) and the other at 5°C, but photoperiod was the same for both groups (12 h light:12 h dark). We used water–fat MRI to locate and quantify changes in the relative volume and PDFF of both WAT and BAT.

## MATERIALS AND METHODS

### Experimental animals

All procedures were approved by the University of Western Ontario Animal Care Committee (protocol 2012-016) and followed Canadian Council on Animal Care guidelines. Details of ground squirrel trapping and husbandry can be found in our recent publication (MacCannell et al., 2017).

Eight juvenile males from the same litter were housed at 22°C until weaning, at which point they were divided randomly into two conditions: 5°C ('cold-housed') and thermoneutral 25°C ('warm-housed'). After the initial MRI of the cold-housed squirrels on 19 August 2016, the temperature was decreased 1°C day<sup>-1</sup> until the  $T_a$  reached 5°C (6 September 2016). On 26 August 2016, immediately after the first MRI of the warm-housed squirrels, temperature was increased to 25°C. Both groups were held on a 12 h light:12 h dark photoperiod. Rat chow (LabDiet 5P00), dry dog food (Iams) and water were provided *ad libitum*, with sunflower seeds and corn provided three times a week. Animals were weighed approximately every week during cage changes. Cage changes did not occur if animals appeared to be in torpor, to minimize disturbance. Torpor bouts were confirmed by the sawdust technique (Pengelley and Fisher, 1961), in which sawdust is placed on the back of a torpid squirrel and animals were observed daily for the presence of the sawdust. We used this technique because instrumenting these animals with  $T_b$  telemeters is incompatible with MRI.

### MRI scanning

MRI scans for T1-weighted images and IDEAL water–fat images were conducted on each group approximately every 3 weeks, using isoflurane anaesthesia when necessary to prevent movement within the scanner. Animals in torpor were kept cool using circulating water blankets and remained torpid throughout the scan without

requiring anaesthesia. The two treatment groups were scanned on subsequent weeks. MRI scanning details can be found in our recent publication (MacCannell et al., 2017).

### Segmentation of images

BAT volumes were segmented using OsiriX v5.6 (Bernex, Switzerland) 2D threshold region growing algorithm tool with segmentation parameters set to a lower threshold of 30% PDFF and an upper threshold of 70%, i.e. a minimum of 30% and a maximum of 70% of the tissue volume consisted of lipid, based on segmentation guidelines adapted from earlier studies (Hu et al., 2010; Prakash et al., 2016; Rasmussen et al., 2013). WAT volumes were segmented with parameters set to a lower threshold of 80% PDFF. Visceral and subcutaneous adipose tissue were measured separately. T1-weighted images were used to determine the location of the abdominal wall, which was used as the division between visceral and subcutaneous WAT.

### Statistical analyses

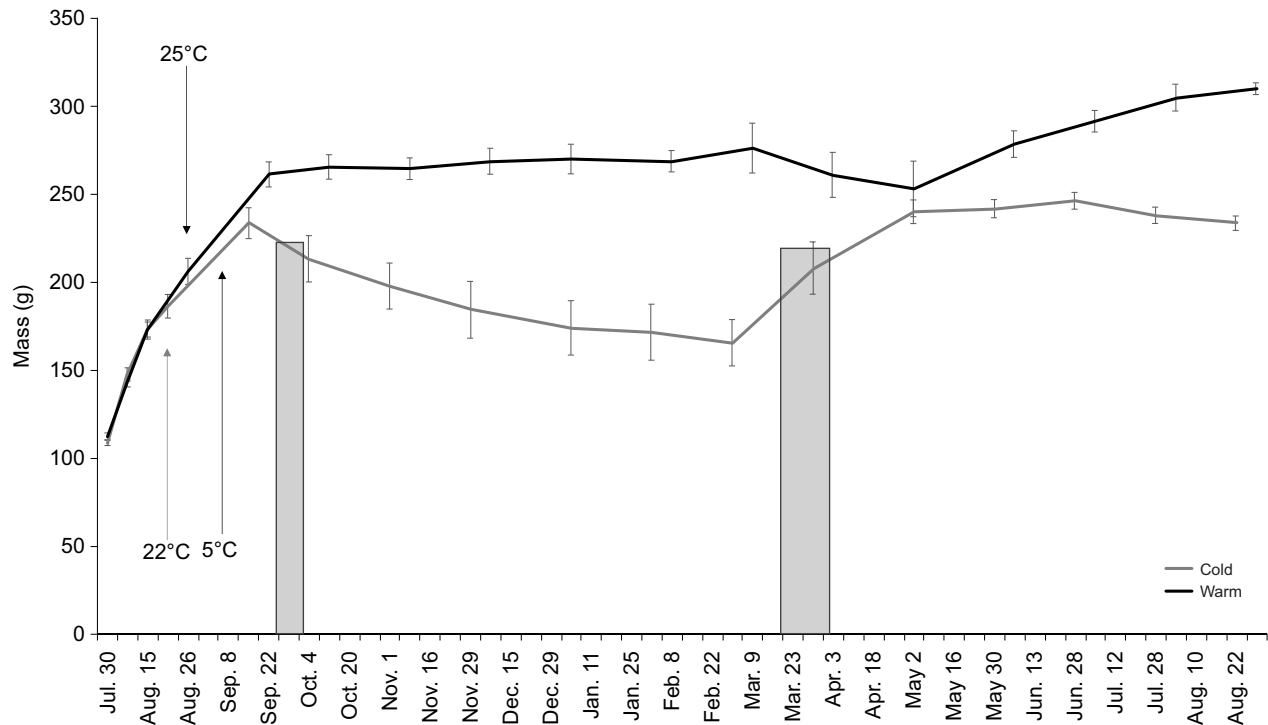
All values are presented as means±s.e.m. We examined how the measured parameters were affected by time throughout the year and whether this temporal pattern was affected by ambient temperature. Mass, PDFF and volume- or mass corrected BAT or WAT of cold- and warm-housed animals were compared using a repeated measures ANOVA and Greenhouse–Geisser correction on SPSS (SPSS Statistics for Windows, v22.0, released 2013, IBM Corp., Armonk, NY, USA). Reliability of MRI segmentation volumes was confirmed by calculation of the interclass correlation coefficient (ICC) between values determined by A.D.V.M. and a second reader (P. Parthasarathy); ICC values higher than 0.9 are indicative of excellent reliability (Koo and Li, 2016).

## RESULTS

Upon daily observation, there was no indication that the warm-housed squirrels ever entered torpor, given the daily disappearance of sawdust placed on the back or the inability to place sawdust on the back because of animal movement. In contrast, cold-housed animals began entering torpor on 28 September 2016 and maintained consistent torpor bouts (with periodic arousals) until 18 March 2017, with the last animal arousing on 3 April 2017. This indicates ~1 month longer torpor period than typical for the thirteen-lined ground squirrel – males in the wild will enter torpor in late October, emerging in late March (Kisser and Goodwin, 2012).

Both cold- and warm-housed ground squirrels increased body mass until mid-September (Fig. 1). Subsequently, the two groups diverged, as indicated by the significant interaction between the effects of time and  $T_a$  on body mass ( $F_{3,1,18,8}=15.0$ ,  $P<0.001$ ). The warm-housed squirrels maintained a mean body mass of 266.9±1.8 g until April. In contrast, the body mass of the cold-housed squirrels declined steadily to mid-March, falling 29.1% from 233.5±8.8 g to 165.5±13.2 g. After torpor bouts stopped in April 2017, the cold-housed squirrels steadily gained mass until the end of April. Between April and August 2017, the warm-housed squirrels increased their body mass steadily, but the cold-housed squirrels did not (Fig. 1).

All MRI segmentation measurements used in this study were conducted by A.D.V.M. and confirmed by a second reader (P. Parthasarathy) with an interclass correlation coefficient of 0.99 between the two readers. Changes in percentage body mass composed of WAT or absolute WAT volume did not follow the same pattern as body mass (Fig. 2). There was a significant effect of  $T_a$  ( $F_{1,6}=12.6$ ,  $P=0.012$ ) on the percentage of body mass composed



**Fig. 1. Ground squirrel body mass over an entire year.** The black line represents animals housed at 25°C (warm-housed) and the grey line represents animals housed at ~5°C (cold-housed); both groups were held under a 12 h light:12 h dark photoperiod. Data are presented as means±s.e.m.,  $n=4$  for each group. The arrow labelled 25°C indicates the initial MRI scan of warm-housed animals and the day they were transferred to an ambient temperature ( $T_a$ ) of 25°C (26 August 2016). The light grey arrow labelled 22°C indicates the initial scan for the cold-housed squirrels, and the day the decrease in  $T_a$  began (1°C day<sup>-1</sup>; 19 August 2016). The dark grey arrow labelled 5°C indicates the day  $T_a$  for the cold-housed squirrels reached 5°C (6 September 2016). The first box indicates the range of initial torpor bouts for the cold-housed squirrels (beginning 28 September 2016). The second box indicates the range of terminal arousal dates (18 March–3 April 2017). There was a significant effect of time ( $F_{3.1,18.8}=76.2$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=27.8$ ,  $P=0.002$ ), and an interaction between time and temperature ( $F_{3.1,18.8}=15.0$ ,  $P<0.001$ ) on mass.

of WAT (assuming a density of 0.9 g ml<sup>-1</sup>), with the warm-housed squirrels showing consistently higher levels than the cold-housed squirrels after the first MRI in August 2016 (Fig. 2A). The warm-housed squirrels maintained a consistent relative WAT content of approximately 60% body mass from 22 September 2016 to 8 February 2017. Between February and March, however, this value fell to the lowest point of 34.4±3.1%. In contrast, the cold-housed animals showed a steady decline from 42.6±0.9% in September to 18.7±2.1% in March. Between March and August 2017, warm-housed animals significantly increased their WAT content to values that did not differ significantly from those of the previous early winter, 55.5±2.7%, whereas in the cold-housed group, WAT remained steady at 21.8±0.6%. The pattern seen in the percentage body mass composed of WAT was also reflected in the absolute volume of WAT (Fig. 2B). There was a significant interaction between time and temperature for both the mass-corrected WAT ( $F_{3.1,18.4}=6.1$ ,  $P=0.004$ ) and absolute volume of WAT ( $F_{2.3,13.8}=7.7$ ,  $P=0.005$ ).

In addition to quantifying total WAT, we analysed visceral and subcutaneous WAT separately. This analysis showed that the patterns for both depots generally followed that of total WAT, with subcutaneous WAT comprising the majority (approximately 65%) of total WAT, in both warm- and cold-housed squirrels (Fig. 3). However, the PDF of the visceral WAT in the warm-housed animals was 3.1±0.1% higher than subcutaneous values throughout the year (Fig. 4A). In the cold-housed animals, the PDF of visceral WAT was also 2.8±0.1% higher than the subcutaneous WAT for most of the year (Fig. 4B). There was a significant effect of time ( $F_{2.0,12.1}=9.4$ ,  $P=0.003$ ) and temperature ( $F_{1,6}=67.3$ ,  $P<0.001$ ),

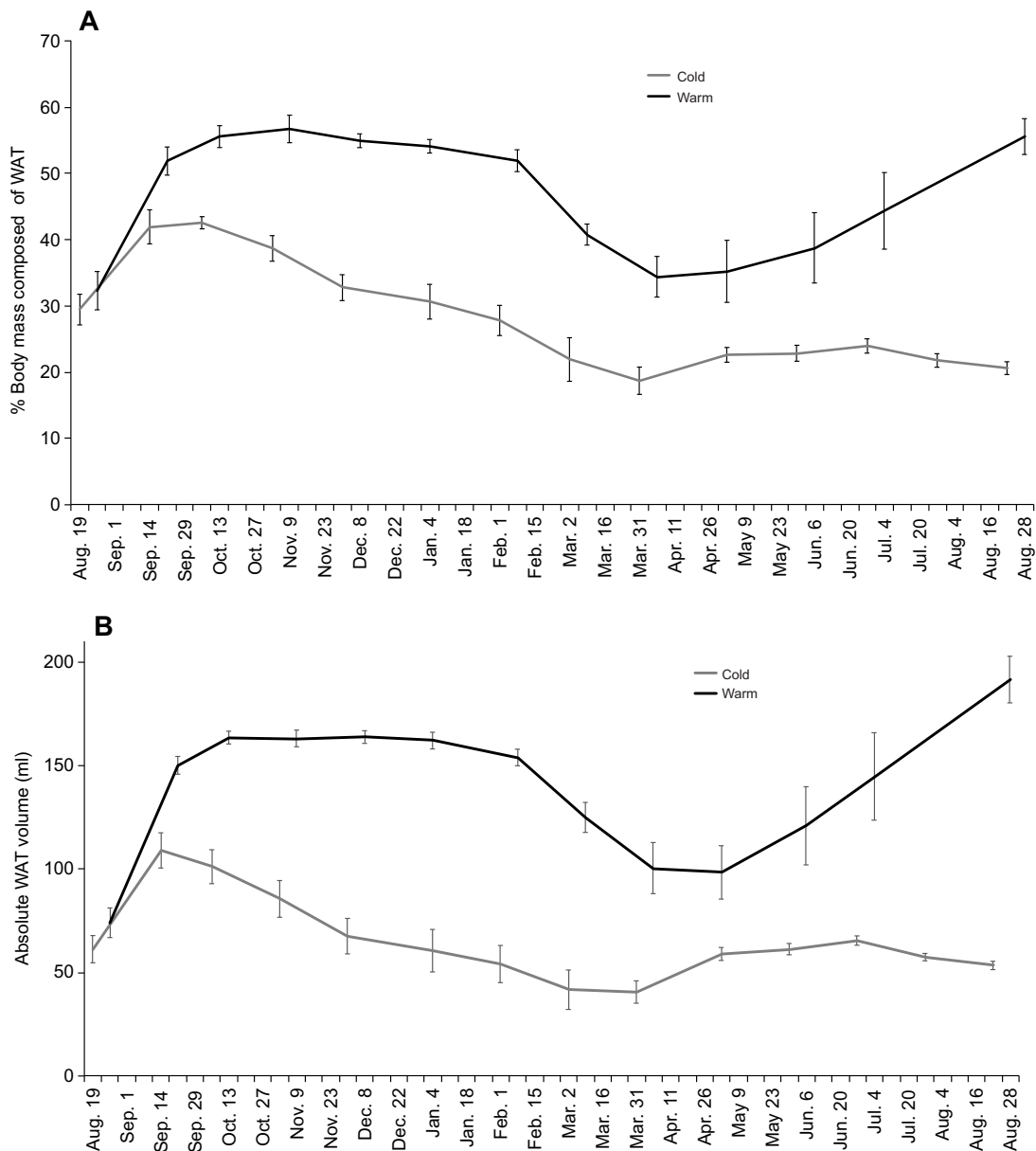
with no interaction ( $F_{2.0,12.1}=0.7$ ,  $P=0.504$ ) for both the visceral and subcutaneous WAT in the cold- and warm-housed squirrels.

Between August and October 2016, the relative content of BAT increased at least twofold in both cold- and warm-housed animals (Fig. 5A). Subsequently, BAT content remained fairly constant in both groups until February 2017, comprising 2.0±0.0% and 1.7±0.8% of body mass for the cold- and warm-housed squirrels, respectively. Between February and August 2017, BAT content decreased by almost half in the cold-housed squirrels, but in the warm-housed squirrels BAT fell as low as 0.5±0.0%. The absolute volume of BAT followed a similar pattern to that of the percentage body mass composed of BAT, except that the cold-housed squirrels had a smaller BAT volume in the winter, between November 2016 and February 2017, than the warm-housed squirrels (Fig. 5B). There was no significant effect of  $T_a$  on the absolute volume of BAT ( $F_{1,6}=0.3$ ,  $P=0.599$ ), but there was a significant effect on the percentage body mass composed of BAT ( $F_{1,6}=12.6$ ,  $P=0.012$ ).

The PDF of thoracic BAT ranged from 35.6% to 54.6%, which is typical for small mammals (Rasmussen et al., 2013). These values did not change significantly over time nor did they differ significantly between warm- or cold-housed ground squirrels (Fig. 5C).

## DISCUSSION

The findings from this study suggest that environmental temperature does influence changes in mass and WAT, seen through the differences in volume between warm- and cold-housed animals, but not BAT, as the pattern of BAT absolute volume did not differ between the temperature treatments. Moreover, we found that

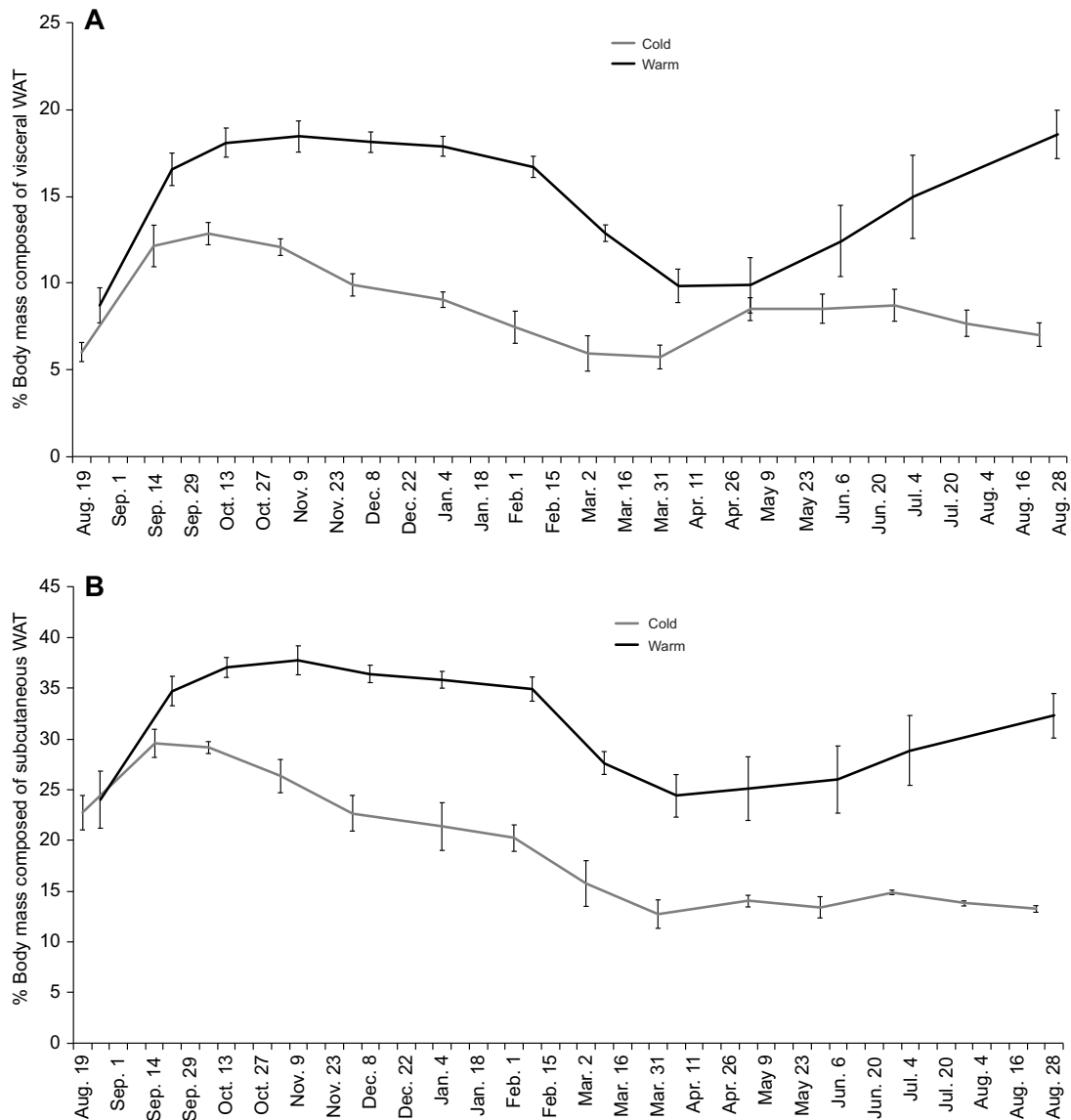


**Fig. 2. Changes in contribution of white adipose tissue (WAT) to body mass and WAT volume over 1 year.** (A) Percentage of body mass composed of WAT (assuming a density of  $0.9 \text{ g ml}^{-1}$ ). (B) Absolute volume of WAT. The black line represents animals housed at  $25^\circ\text{C}$  and the grey line represents animals housed at  $\sim 5^\circ\text{C}$ ; both groups were maintained on a 12 h light:12 h dark photoperiod. Data are presented as means  $\pm$  s.e.m.,  $n=4$  for each group. There was a significant effect of time ( $F_{3,1,18,4}=24.9$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=12.6$ ,  $P=0.012$ ) on the percentage of body mass composed of WAT, with an interaction between time and  $T_a$  ( $F_{3,1,18,4}=6.1$ ,  $P=0.004$ ). There was also a significant effect of time ( $F_{2,3,13,8}=14.0$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=98.1$ ,  $P<0.001$ ) on the absolute volume of WAT, with an interaction between time and  $T_a$  ( $F_{2,3,13,8}=7.7$ ,  $P=0.005$ ).

housing thirteen-lined ground squirrels at a thermoneutral temperature appears to inhibit hibernation, suggesting that this species is not an obligate hibernator. The design of the current study cannot confirm this suggestion; as we could only monitor hibernation status visually, short overnight torpor bouts could have been missed. We are expanding on these findings in a separate study using implanted data loggers. At the very least, the findings of the current study suggest that environmental parameters have significant effects on multiple circannual rhythms in these hibernators.

The differing patterns in mass gain and loss between our warm- and cold-housed squirrels differ from measurements made from the golden-mantled ground squirrel, an obligate hibernator which

demonstrates a strict endogenous rhythm of mass regardless of  $T_a$  or photoperiod (Pengelley and Asmundson, 1969; Florant et al., 2012). The greatest contributor to mass change in most mammals is WAT. WAT volume decreased in the warm-housed squirrels from February to April 2017, but this change was not reflected in changes to total body mass. By contrast, a previous study showed that mass did reflect changes in WAT in thirteen-lined ground squirrels housed with a photoperiod mimicking that of Carman, MB, Canada, and a temperature of  $\sim 20^\circ\text{C}$  until October, then transferred to  $5^\circ\text{C}$  during the winter months (MacCannell et al., 2017). This difference suggests that circannual rhythms of WAT are influenced by  $T_a$  and photoperiod, and that WAT gain and loss are not regulated solely by an endogenous rhythm in this species.

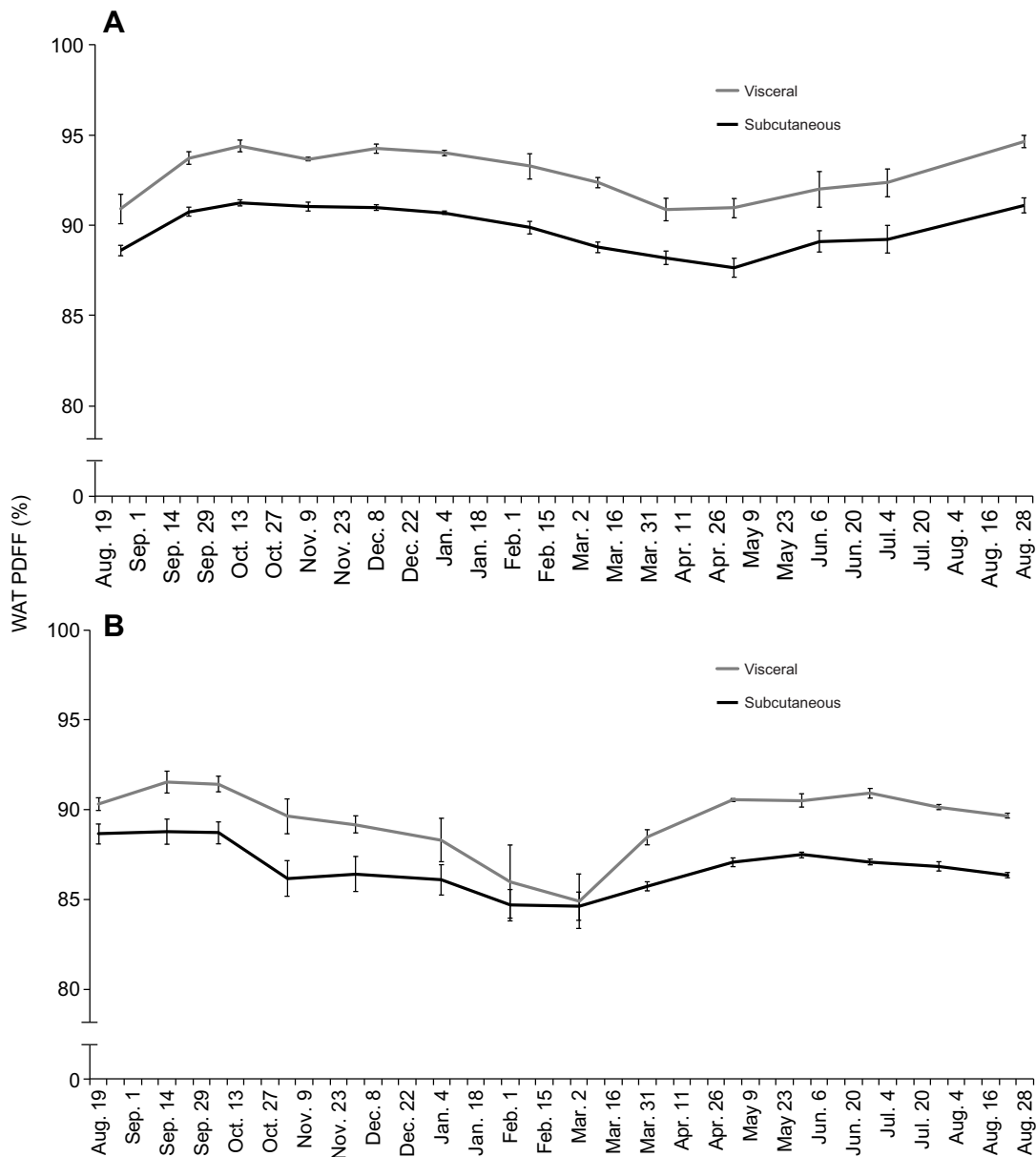


**Fig. 3. Quantity of visceral and subcutaneous WAT relative to body mass.** Data are the percentage of body mass composed of visceral (A) and (B) subcutaneous WAT (assuming a density of  $0.9 \text{ g ml}^{-1}$ ). The dark line represents animals housed at  $25^\circ\text{C}$  and the grey line represents animals housed at  $\sim 5^\circ\text{C}$ ; both groups were held on a 12 h light:12 h dark photoperiod. Data are presented as means  $\pm$  s.e.m.,  $n=4$  for each group. There was a significant effect of time ( $F_{3,2,19,0}=18.7$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=47.8$ ,  $P=0.001$ ) on the percentage of body mass composed of visceral adipose tissue, with an interaction between time and  $T_a$  ( $F_{3,2,19,0}=5.5$ ,  $P=0.006$ ). There was also a significant effect of time ( $F_{2,9,17,3}=34.7$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=36.2$ ,  $P=0.001$ ) on the percentage of body mass composed of subcutaneous adipose tissue with an interaction between time and  $T_a$  ( $F_{2,9,17,3}=6.8$ ,  $P=0.003$ ).

WAT does not appear to be the main driver of mass in warm-housed animals during the winter and spring months. WAT comprised almost 60% of body mass throughout most of the winter, a level much higher than that in non-hibernating mice, where WAT typically contributes 2.4% of body mass, and only increases to 22.7% when mice are fed high-calorie, high-fat diets (Sjögren et al., 2001). Hibernating mammals experience restricted muscle movements during the winter months, when they spend the majority of their time immobile during torpid periods. Such conditions are similar to disuse, which, in other mammals, leads to muscle atrophy. Prairie dogs and bears conserve skeletal muscle mass and protein content during hibernation (Cotton and Harlow, 2010), though the precise mechanism underlying this phenomenon is not fully understood. It is possible that the disconnect between body mass and WAT volume that we observed in the warm-housed ground squirrels towards the end of winter is due to activation of such

mechanisms during the winter, even though the animals did not hibernate and, indeed, continued to eat. As a result, instead of simply resisting atrophy, these muscles may have actually hypertrophied, relative to their autumn condition, and contributed a greater proportion to total body mass in the spring. By contrast, the cold-housed animals fasted throughout hibernation and such mechanisms would have simply mitigated any loss of muscle mass, so that WAT would be a greater driver of total mass change.

The PDFF values of both visceral and subcutaneous WAT were within the range expected for mammalian WAT, but we did not expect the higher values in visceral compared with subcutaneous depots. To our knowledge, such a difference is novel among mammals. For example, in humans both visceral and subcutaneous PDFF are approximately 89.7% (Franz et al., 2017). The difference between visceral and subcutaneous WAT PDFF was maintained throughout the year in the warm-housed ground squirrels that did not

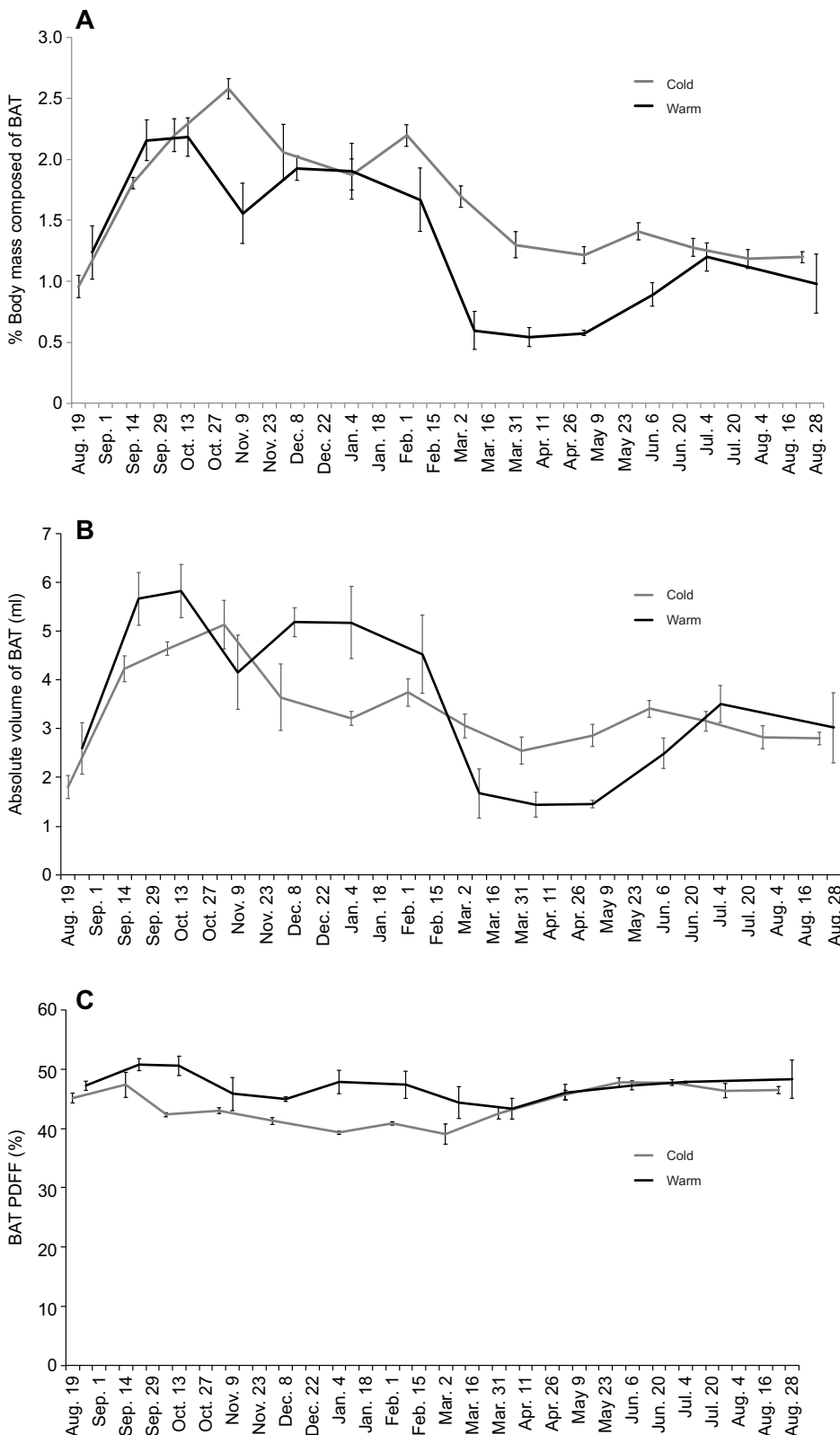


**Fig. 4. Proton density fat fraction (PDFF) values of visceral and subcutaneous WAT.** Values are shown for (A) warm-housed squirrels and (B) cold-housed squirrels. Data are presented as means $\pm$ s.e.m.,  $n=4$  for each group. There was a significant effect of time ( $F_{2,0,12,1}=9.4$ ,  $P=0.003$ ) and  $T_a$  ( $F_{1,6}=67.3$ ,  $P<0.001$ ) on PDFF of visceral and subcutaneous WAT in the warm-housed squirrels, with no significant interaction ( $F_{2,0,12,1}=0.7$ ,  $P=0.504$ ). There was also a significant effect of time ( $F_{2,5,15,3}=11.5$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=23.3$ ,  $P=0.003$ ) on PDFF of visceral and subcutaneous WAT in the cold-housed squirrels, with no significant interaction ( $F_{2,5,15,3}=1.1$ ,  $P=0.380$ ).

enter torpor. In contrast, the cold-housed animals repeatedly cycled between torpor and IBE throughout the winter, and there was a decrease in WAT PDFF during this time. Metabolism during both torpor and IBE is fuelled predominately by lipid oxidation (Staples, 2016). If visceral WAT is oxidized preferentially, this would account for not only its decreased volume in the winter but perhaps also the decline in visceral PDFF. This would indicate that visceral adipose is used preferentially for energy storage and mobilization whereas subcutaneous adipose is more important for thermal insulation. Once the cold-housed animals emerged from torpor and began eating, lipid vesicles of visceral WAT probably replenished their triglyceride stores, leading to an increase in PDFF and volume of these depots. The segregation of roles within adipose is also seen within the blubber of marine mammals. Marine mammal blubber contains wax esters, not typically found in mammals, which enhance thermal

insulative capacity (Bagge et al., 2012). Wax esters are not uniform across the depth of the blubber and therefore the thermal conductivity of blubber is also not uniform, suggesting the segregation of lipid storage versus insulation (Bagge et al., 2012).

The increase in BAT volume during early autumn in both the warm- and cold-housed squirrels agrees with our recent observation (MacCannell et al., 2017) that BAT growth can occur in a hibernator in the absence of cold exposure. In fact, the largest increase in BAT volume was found shortly after MRI began during late summer in the animals housed at thermoneutral  $T_a$ . Even in the subsequent spring, the warm-housed animals showed a greater increase in BAT volume than their cold-housed counterparts. These results differ from those of non-hibernating mammals, where BAT depot growth only results from either a decrease in  $T_a$  (Nakamura and Morrison, 2007) or high-calorie diets (Rothwell and Stock, 1979).

**Fig. 5. Brown adipose tissue (BAT)**

**dynamics throughout 1 year.** (A) Percentage body mass (assuming a density of  $0.9 \text{ g ml}^{-1}$ ), (B) absolute volume of BAT and (C) corresponding PDFF. The black line represents animals housed at  $25^\circ\text{C}$  and the grey line represents animals housed at  $\sim 5^\circ\text{C}$ ; both groups were held on a 12 h light:12 h dark photoperiod. Data are presented as means  $\pm$  s.e.m.,  $n=4$  for each group. There was a significant effect of time ( $F_{3.1,18.4}=24.9$ ,  $P<0.001$ ) and  $T_a$  ( $F_{1,6}=12.6$ ,  $P=0.012$ ) on the percentage of body mass composed of BAT, with a significant interaction between time and  $T_a$  ( $F_{3.1,18.4}=6.1$ ,  $P=0.004$ ). There was also a significant effect of time ( $F_{2.6,15.6}=18.1$ ,  $P<0.001$ ), but not  $T_a$  ( $F_{1,6}=0.3$ ,  $P=0.599$ ), on the absolute volume of BAT, with a significant interaction between time and  $T_a$  ( $F_{2.6,15.6}=13.5$ ,  $P=0.016$ ). There was no significant effect of time ( $F_{2.4,7.3}=2.7$ ,  $P=0.127$ ) or  $T_a$  ( $F_{1,6}=1.0$ ,  $P=0.382$ ) on the PDFF of BAT, and no interaction ( $F_{2.4,7.3}=2.4$ ,  $P=0.155$ ).

This study revealed influences of environmental temperature on patterns of body mass, WAT and BAT in the thirteen-lined ground squirrel. If these characteristics were regulated entirely by an endogenous rhythm, we would have predicted that the patterns of BAT, WAT and mass would not have differed between the warm- and cold-housed squirrels. Our data show that cold exposure

appears to be a primary regulator for WAT maintenance and is a requirement for torpor entrance. However, this experiment only covered one complete year, and we are not able to assess the long-term circannual effects on this species. Our data indicate that BAT may exhibit an endogenous circannual rhythm in terms of depot size. Nonetheless, our findings suggest that this species may not be

an obligate hibernator. We are working towards clarifying the effect of  $T_a$  on hibernation patterns in this species using  $T_b$  loggers implanted within ground squirrels housed under 12 h light:12 h dark conditions and 25, 16 or 5°C.

#### Acknowledgements

We want to thank Lauren Smith, Stephanie Giza and Daniel McCooney for their assistance in MRI scanning. We thank Prasiddha Parthasarathy for being a second reader on MRI images and Alireza Akbari for assistance with reconstruction of MRIs. We also thank Kate Mathers and Leah Hayward for their animal care support.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: C.A.M., J.F.S.; Methodology: C.A.M., J.F.S.; Software: K.J.S.; Validation: A.D.V.M.; Formal analysis: A.D.V.M.; Investigation: A.D.V.M., K.J.S.; Resources: C.A.M., J.F.S.; Writing - original draft: A.D.V.M., J.F.S.; Writing - review & editing: A.D.V.M., K.J.S., C.A.M., J.F.S.; Supervision: C.A.M., J.F.S.; Project administration: J.F.S.; Funding acquisition: C.A.M., J.F.S.

#### Funding

This research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada and the Faculty of Science, University of Western Ontario (J.F.S.: RGPIN-2014-04860, C.A.M.: RGPIN-2013-356310) and the Canada Research Chairs program (C.A.M.: 950-228038).

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