Metabolic Rate and Prehibernation Fattening in Free-Living Arctic Ground Squirrels

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ABSTRACT

Hibernating mammals become sequestered and cease foraging during prolonged seasonal periods of reduced or unpredictable food availability and instead rely on cached food and/or endogenous reserves of fat and protein accumulated during the previous active season. The gain in weight is due to increased food consumption, but it also has been hypothesized that hibernators maximize rates of fattening by decreasing costs of maintenance before weight gain, reflected in reduced resting metabolic rate (RMR). We recorded repeated measures of total body, lean, and fat mass in individual adult male and female arctic ground squirrels across their active season and found that squirrels increased body mass by 42% (males) and 62% (females). This gain was achieved through a 17% increase in lean mass and a 7–8-fold increase in fat mass; however, mass gain was not linear and patterns differed between sexes. Contrary to our hypothesis, decreases in RMR were not associated with rapid mass gain. We found RMR of males increased (whole-animal RMR or lean-mass-specific RMR) or remained constant (mass-specific RMR) for most of the active season and decreased only after the majority of mass had been gained. In females, although RMR (whole-animal, mass-specific, and lean-mass RMR) generally decreased across the active season, the greatest decrease occurred late in the active season after the majority of mass had been gained. In conclusion, arctic ground squirrels do not trade off metabolism to facilitate rates of weight gain before hibernation, but they do use energy sparing strategies before hibernation that help maintain peak mass.

Introduction

Hibernation is a dramatic example of recurring phenotypic plasticity displayed by mammals in a variety of orders and species. Annually, mammalian hibernators undergo a complex suite of adaptive morphological, physiological, and behavioral changes to conserve energy in anticipation of seasonal periods of reduced or unpredictable food availability (e.g., poor resource availability during winter). Hibernation includes reduced activity and periodically or continuously depressed metabolism and body temperature (T_b) while typically remaining sequestered within a hibernaculum (Geiser 2004). Extensive research has been conducted on physiological, comparative, and molecular aspects of hibernation (Carey et al. 2003; Geiser 2004; Dark 2005; Drew et al. 2007). However, far less is known about physiological and behavioral changes that occur in preparation for hibernation, particularly in free-living animals (Arnold et al. 2011).

For hibernators that rely on endogenous fuels, hibernation is primarily fueled by fat metabolism (Dark 2005), although catabolism of lean tissue is also important in certain species (Buck and Barnes 2000). These animals typically do not eat during hibernation (but see Munro et al. 2005 for a discussion of chipmunks) and must rely on energy reserves accumulated during a discrete interval after reproduction has ceased. In highly seasonal environments such as the Arctic, with short growing seasons and early winters, this interval may be as short as 6 wk in reproductively successful females (Sheriff et al. 2011). Although the mechanisms responsible for the accumulation of energy reserves are not completely understood, investigations suggest increased foraging time, decreased activity, selection or availability of higher quality food items, changes in assimilation efficiency, or reduced levels of baseline or maintenance energy expenditure as possible mechanisms that create or facilitate the increase in mass involved in preparing for hibernation. In free-living arctic ground squirrels (AGS), Sheriff et al. (2012b) found significant decreases in daily minimum, maximum and average T_b beginning 45 d before hibernation, which should save energy because of the reduced temperature gradient between the animal and its environment. Delehanty and Boonstra (2011) and Sheriff et al. (2012a) showed that free-cortisol concentrations decreased during the active season in plasma and feces of AGS and suggest that this promotes tissue anabolism as ground squirrels add lean as well as fat mass in preparation for hibernation. Kronfeld-Shor et al. (2000) found that during the pre-

hibernation period free-living little brown bats reduced whole-animal basal metabolic rate (MR) and had reduced sensitivity to the satiety hormone, leptin, both of which may facilitate body mass gain and increase adiposity of hibernators (see also Concannon et al. 2001). In laboratory studies on captive animals, reduced mass-specific resting MR has also been found late in the active season of woodchucks (Rawson et al. 1998) and yellow-bellied marmots (Kilgore and Armitage 1978; Ward and Armitage 1981). Concurrent with reduced energy expenditure, laboratory studies have found food consumption also declines; however, this typically occurs well before peak body mass is achieved and provides evidence that additional factors besides energy intake significantly contribute to patterns and rate of body mass gain (Pengelley and Fisher 1963; Ward and Armitage 1981; Dark 1984, 2005). Similarly, hibernators usually decrease aboveground activity (suggested to be associated with foraging time) before hibernation (Johns and Armitage 1979; Körtner and Geiser 1995; Long et al. 2005). Thus, it has been hypothesized that decreased MR (as opposed to solely increased food intake) may be a key component of an animal's ability to rapidly fatten, shifting the energy balance equation away from maximizing input toward maximizing savings to provide the surfeit of energy needed to increase body and fat mass before hibernation (Armitage and Shulenberger 1972; Ward and Armitage 1981; Concannon et al. 1993; Thorp et al. 1994; Geiser 2004; reviewed in Dark 2005; Florant and Healey 2011).

Arctic ground squirrels (*Urocitellus parryii*) are an excellent species in which to investigate this hypothesis in free-living animals and assess the interrelationships among body mass and composition and concurrent changes in MR and T_b. AGS are the northern most hibernator in North America (MacDonald and Cook 2009) and experience an extremely short active season (∼4–6 mo) during which adults must breed, molt, and then accumulate sufficient reserves to survive a long hibernation season characterized by the lowest known hibernacula temperatures (as low as −23°C; Buck and Barnes 1999*b*). Timing of active season events is sex (and age) specific (MacLean and Towns 1981; Buck et al. 2008; Sheriff et al. 2011) and the differences in phenology and behavior between the sexes create a platform to investigate how animals balance energy expenditure against energy savings as they prepare for hibernation. At our study site in northern Alaska, adult males emerge in early April 3–4 wk before snowmelt (Sheriff et al. 2011) when foraging opportunities are limited and food quality is poor. In mid- to late April males engage in scramble-competition breeding that includes intense agonistic encounters and wounding (Buck and Barnes 2003); males reduce foraging during this time and lose considerable body mass (Carl 1971; Buck and Barnes 1999*a*). Males continue to show aboveground activity until late September when the ground is once again snow covered. In mid-August onwards males engage in a second period of highly agonistic behavior while they gather food that they cache in their hibernacula. Males feed from their caches after ending torpor the next spring during a 2–3-wk period of reproductive maturation before emergence (Barnes 1996), appearing aboveground with body masses not significantly different from pre-

hibernation measures (Buck and Barnes 1999*a*). Females emerge from hibernation about 10 d after males in mid- to late April. Conception occurs on average 4 d after emergence and females give birth 25 d later (Sheriff et al. 2011). Offspring are then weaned after ∼25 d in late June or early July. Females have the shortest amount of time to gain mass in preparation for hibernation and begin hibernation in early to mid-August and may enter as early as late July.

Here, using free-living AGS, we tested the hypothesis that, similar to what has been suggested in captive animals, free-living hibernators decrease thermoneutral resting metabolic rate (RMR) before peak body mass to facilitate mass gain. To test this, we measured total body mass, lean mass, and fat mass concomitantly with RMR and nocturnal T_b throughout the active season. This study is the first to provide repeated measures of total-body, lean-mass, and fat-mass rates of overall energy expenditure, and metabolic fuel use of a free-living hibernator across the entire active season. Previously in AGS, we have shown: a significant body mass gain during the active season in which patterns of gain differ between the sexes (Buck and Barnes 1999*a*); a significant increase in percent body fat immediately before hibernation (Buck and Barnes 1999*a*); a significant decrease in T_b in preparation for hibernation (Sheriff et al. 2012*b*). Given these observations and those from laboratory-based studies we make the following predictions:

1. Total body, fat, and lean mass will increase following reproduction, but the patterns of gain will differ between the sexes.

2. RMR will decrease before and concurrent with mass gain.

3. Metabolism will progressively shift away from fat-based fuel to facilitate fattening and conserve fat mass as animals near the beginning of hibernation.

4. Nocturnal T_b will decrease in preparation for hibernation, further decreasing energy expenditure and facilitating mass gain.

Material and Methods

Study Area

This study was conducted in the Alaskan Arctic along the Atigun River (68°27′N, 149°21′W) in the northern foothills of the Brooks Range. This river bank area is south facing and composed of gradually sloping sandy substrate with an active-layer thaw depth of 1–2 m that provides excellent burrowing habitat for AGS. The flora is dominated by low-growing willow shrubs (*Salix* spp. [<30 cm], *Dryas octopetala*, *Rhododendron lapponicum*, *Arctostaphylos alpine*, and *Vaccinium uliginosum* (Sheriff et al. 2011).

Trapping and Handling

Adult (>1 yr) AGS (32 males and 23 females) were (re)captured at each time period using Tomahawk live traps (Tomahawk Live Trap, Tomahawk, WI) baited with carrot from May to September 2007. Trapping was conducted during three periods: April 29–May 17, July 12–August 29, September 20–22; every

individual was recaptured every 7–11 d within each period (females had begun to hibernate and were not caught in the last trapping periods of late August and September). Animals were not trapped during late May, June, and early July to avoid disturbing females during late gestation and lactation. Traps were set in the early morning and checked every 1.5 h until closure in midafternoon. Trapped animals were transported by truck in covered traps 20 km to the Toolik Field Station of the University of Alaska Fairbanks. There, animals were anesthetized by a 3–5-min exposure to isoflurane vapors and uniquely tagged (on first capture with Monel no. 1 ear tags and AVID MUSICC passive integrated transponder [PIT] tags), weighed, sexed, and a 1–2-mL blood sample was taken via cardiac puncture. Animals were then analyzed for body composition estimates via a deuterium-labeled water technique and allowed to recover from anesthesia in a quiet, dark room before overnight measures of RMR. The following morning, animals were released at their site of capture (within 24 h of capture). All procedures were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee.

Body Composition Estimates

Body composition (lean and fat mass) was estimated by calculating the dilution of a known mass of injected deuterium-labeled water that provided a measure of total body water, from which an estimate of lean mass was derived (Reid et al. 1963). This method has been specifically validated for use with AGS (Lee et al. 2011). Briefly, we used a subsample of blood, 75 μL, collected in a heparinized Natelson capillary tube from the initial cardiac puncture. Animals then received an intraperitoneal injection of deuterium-enriched water (27,400 ppm; Cambridge Isotope Laboratories, Andover, MA, and Alfa Aesar, Ward Hill, MA; diluted with sterile water for injection) corresponding to an average of 0.0893% (range: 0.066%–0.115%) of total body weight to achieve the desired enrichment within animals (~40 ppm). Animals were then moved to a dark, quiet room for 1 h for deuterium equilibration with body water (Lee et al. 2011). Animals were then briefly anesthetized, and a second blood sample (75 μL) was collected by capillary after a claw clip. Capillary tubes were flame sealed within 5 min of collection and stored at 4°C until assayed for deuterium (Speakman 1997).

Before analysis, water from blood samples was distilled using a modified version of Nagy's (1983) pipette distillation procedure. Briefly, blood samples were transferred from capillary tubes into the large end of a Pasteur pipette. Pipettes were flame sealed at both ends and placed on a hot plate at ca. 60°C until water had distilled into the tip (2–3 d). Pipettes were cracked and the distilled water was transferred into autosampler vials containing microinserts and resealed until analysis 1–14 d later. Samples were analyzed in triplicate using a high-temperature conversion/elemental analyzer (TC/EA, Thermo Finnigan, Bremen, Germany) interfaced (ConFlo II; Thermo Finnigan) to an isotope ratio mass spectrometer (Delta Plus XP; Thermo Finnigan) at the University of Alaska Anchorage En-

vironmental and Natural Resources Institute Stable Isotope Laboratory. Samples were analyzed in one assay against International Atomic Energy Agency standards V-SMOW, SLAP, and GISP. Anchorage tap water (intra-assay coefficient of variation [CV] = 0.126%) and one background AGS sample (CV = 0.077%) were used as internal standards for background, and Houston tap water (CV = 0.045%) and one enriched AGS sample (CV = 0.127%) were used as internal standards for enriched sample runs. Total body water was calculated following the methods of Speakman (1997). Lean mass was then calculated as in Lee et al. (2011):

$$\text{lean mass}_{\text{deuterium}} \text{ (g)} = 1.062 \times \text{total body water}_{\text{deuterium}}$$

$$+ \; 0.14 \times \text{body mass (g)} + 48.151.$$

Estimates of RMR and Respiratory Quotient

After recovery from the deuterium dilution experiment, animals were held overnight while RMR was estimated by whole-animal respirometry. Room temperature ranged between 16.7°C and 26.4°C, mean 20.9°C; temperature measured in one metabolic chamber (out of four) per experiment stayed between 19°C and 27°C, which is considered thermoneutral in this species (Hock 1960). Rates of O_2 consumption and CO_2 production of AGS were measured from approximately 2000 hours to 0800 hours the following morning with an open, flow-through respirometry system (Oxzilla oxygen analyzer and two CA-2A CO_2 analyzers, Sable Systems, Las Vegas) at a flow rate of 2.5 L min^{-1} (Brooks 5850E mass flow controllers, range 5L/min, Coastal Instruments, Burgaw, NC). AGS were held in covered polycarbonate chambers (15 L) with a layer of clean, dry wood shavings provided for bedding. Depending on time of capture, animals were fasted between 3 and 10 h before the start of metabolic measurements.

Ambient room air was drawn through individual chambers and automatic calibrations for O_2 concentration using room air were performed at 1-h intervals and for CO_2 concentrations using gas scrubbed of CO_2 by soda lime and span gas (tank gas of known CO_2 and O_2 concentration) every 3 h. The software (LabGraph; Tøien 2013) interpolated between calibrations and calculated O_2 consumption according to the principles of the Haldane transformation (Haldane 1912; Wagner et al. 1973). Measurements were conducted on four animals semi-continuously on a two-channel system. Analyzers alternatively sampled between chambers every 5 min with the first 2 min of data discarded to allow for washout. Minimum RMR was determined as the lowest running average of O_2 consumption recorded over a 30-min block during the approximate 12-h measurement interval. Average CO_2 production was determined during the same period and used in combination to calculate respiratory quotient (RQ). Rates of O_2 consumption were transformed to kcal h^{-1} accounting for changes in RQ values across the season (using the equation for a mixture of fat and carbohydrate metabolism; table 7.4 of Kleiber 1975) and results are expressed in watts (W). RQ values were also used to infer

changes in metabolic fuel use across the active season (Lusk 1924). RMR was compared on a whole-animal-mass-specific and lean-mass-specific basis. The integrity and accuracy of the respirometry system as a whole was validated at least weekly through controlled ethanol burns with a small lamp in the respirometry chamber. Results were considered satisfactory if measured O_2 consumption was within 3% of the calculated O_2 consumption and expected RQ deviated by less than 0.015.

Nocturnal T_b and Lean-Mass-Specific RMR Adjustments

A subsample of AGS (9 males and 8 females) were abdominally implanted with temperature loggers that recorded core T_b each 20 min (modified StowAway Tidbit, Onset Computer, Bourne, MA) in 2006–2007 and explanted in 2007–2008 (see Long et al. 2007 for details). The minimum levels (T_{bmin}) that were reached during metabolic rate measurements were regressed against ordinal date to determine whether levels changed significantly across the active season. Concurrent T_{bmin} values were used to transform lean-mass-specific RMR (LM-RMR) assuming a Q_{10} of 2, following the equation $R_2 = R_1^* Q_{10}^{(T2-T1)/10}$ (Schmidt-Nielson 1997). The earliest date of metabolic measurement and concurrent temperature were set as R_1 and T_1 and $R_1 = 1$, and T_1 is equal to that date's T_{bmin} as determined by regression.

Statistical Analysis

We used repeated-measures ANOVA with Holm-Sidak post hoc tests to compare changes in mass and RMR of AGS across the active season. Comparisons were made within, but not between, sexes as the life-history characteristics of AGS reveal that males and females are in different physiological states during most of their active season (Buck and Barnes 1999a). Data were not normally distributed (as tested by Shapiro-Wilk) and standard transformations did not normalize the distribution. However, ANOVA is generally robust to nonnormal data and our data did not violate homogeneity of variance assumptions. Most reported differences were at P values <0.001 (with the exception of seasonal changes in LM-RMR of males, $P = 0.032$); thus, we believe that the differences we report are significant and we report P values throughout. The T_{bmin} data conformed to model assumptions of normality and variance. Analyses were performed using SigmaPlot v.11.0 (containing SigmaStat v.3.5). Data are presented as mean $\pm$ SE unless otherwise specified.

Results

Body Composition

Total body mass increased by 42% in males (from 639.1 ± 23.3 g to 904.8 ± 30.2 g) and by 62% in females (from 492.1 ± 21.4 g to 815.9 ± 50.9 g) from the end of reproduction to the end of the active season (males: $F_{31,8} = 51.00$, $P < 0.001$; females: $F_{22,6} = 106.01$, $P < 0.001$; figs. 1, 2). Males increased mass by 26% between mid-May and mid-July and continued to increase mass until entrance into hibernation at the

end of September. In contrast, females completed their entire mass gain between mid-May and mid-July and then maintained a relatively constant mass until they entered hibernation in mid-August.

Lean mass increased by 16% in males (from 610.1 ± 21.1 g to 710.2 ± 23.1 g) and 18% in females (from 455.0 ± 16.4 g to 537.5 ± 18.1 g) from the end of reproduction to the end of the active season (males: $F_{31,8} = 28.81$, $P < 0.001$; females: $F_{22,6} = 25.11$, $P < 0.001$; figs. 1A, 2A); however, unlike total body mass, increases in lean mass were not linear across the season. Males increased lean mass by 18% between mid-May and mid-July and continued to increase lean mass peaking in mid- to late- August (22% gain) and then declining until entrance into hibernation at the end of September. Females increased lean mass by 23% between mid-May and mid-July; however, from late July until entrance into hibernation in mid-August females lost 8% of their peak lean mass.

Fat mass increased 7–8-fold in both males (from 29.0 ± 6.7 g to 194.6 ± 14.7 g) and females (from 37.1 ± 6.7 g to 278.5 ± 37.6 g) from the end of reproduction to the end of the active season (males: $F_{31,8} = 27.66$, $P < 0.001$; females: $F_{22,6} = 55.89$, $P < 0.001$; figs. 1B, 2B). Furthermore, both sexes significantly increased in percent body fat throughout the active season (males: $F_{31,8} = 23.41$, $P < 0.001$; females: $F_{22,6} = 49.69$, $P < 0.001$). Males increased in percent body fat from 3.3% in May to 21.4% by the end of September. Females increased in percent body fat from 5.3% in May to 35.4% in mid-August. Fat mass gain accounted for the majority of total body mass gain over the active season in both males (lean mass gain = 100.1 g, fat mass gain = 165.7 g) and females (lean mass gain = 82.5 g, fat mass gain = 241.4 g).

Estimates of Resting Metabolic Rate and RQ

Whole-animal RMR varied significantly across the active season in both sexes (males: $F_{31,8} = 19.63$, $P < 0.001$; females: $F_{22,6} = 8.95$, $P < 0.001$; figs. 3A, 4A). Male RMR increased between mid-May (2.26 ± 0.06 W) and mid-July (2.89 ± 0.07 W) and continued to gradually increase to a seasonal peak in mid-August (3.15 ± 0.07 W). In September RMR significantly declined until entrance into hibernation (2.76 ± 0.11 W). Female RMR increased between mid-May (2.17 ± 0.06 W) and mid-July (2.74 ± 0.08 W) and decreased thereafter returning to early season May values by mid-August (2.27 ± 0.13 W) just before entrance into hibernation.

Mass-specific RMR decreased across the active season in both sexes (males $F_{31,8} = 4.89$, $P < 0.001$; females $F_{22,6} = 23.86$, $P < 0.001$; figs. 3B, 4B). Male mass-specific RMR was relatively constant until mid-August (average 3.68 ± 0.09 W kg^{-1}) and did not significantly decrease until the end of September (3.06 ± 0.09 W kg^{-1}). Female mass-specific RMR decreased across the active season from a maximum in May (4.74 ± 0.15 W kg^{-1}) to a minimum in mid- August (2.95 ± 0.23 W kg^{-1}).

Seasonal patterns of LM-RMR differed between sexes. Male LM-RMR remained relative constant across the active season with an increase in values until mid-August and a decline there-
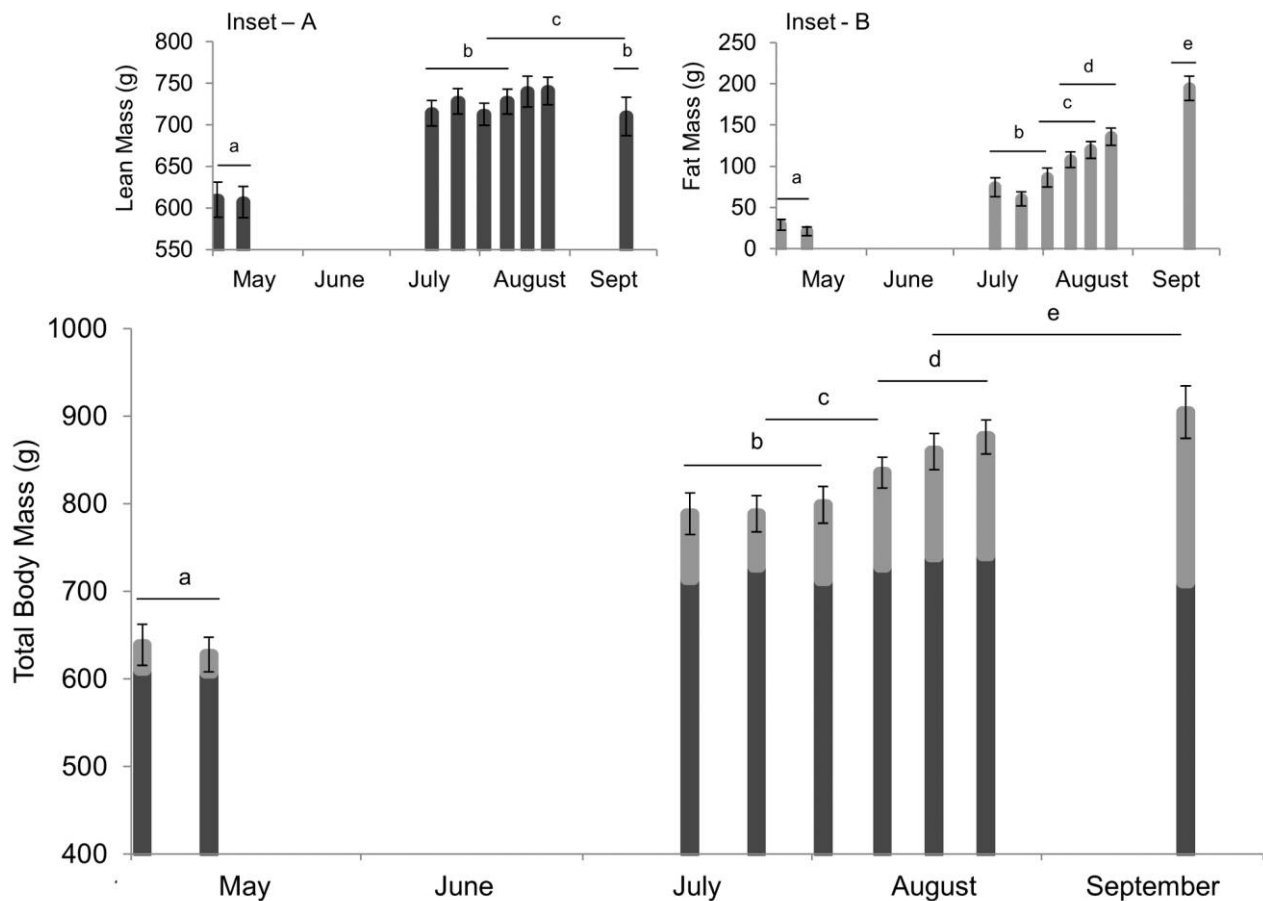
Figure 1. Total body mass (mean ± SE) of free-living male arctic ground squirrels throughout the active season, broken into lean and fat mass components. Inset A shows change in lean mass across the active season; inset B shows change in fat mass across the active season. Letters denote significant differences using a Holm-Sidak post hoc comparison. Measurements were taken repeatedly on the same animals ($n = 32$). A color version of this figure is available online.

after ($F_{31,8} = 2.24$, $P = 0.032$; fig. 3C). Female LM-RMR varied significantly across the active season ($F_{22,6} = 3.35$, $P = 0.009$; fig. 4C), with maximum values occurring in early May (4.98 ± 0.14 W kg^{-1}) and declining thereafter to minimum values in mid-August (4.14 ± 0.24W kg^{-1}).

RQ varied significantly across the active season in both sexes (males: $F_{31,8} = 15.00$, $P < 0.001$; females: $F_{22,6} = 5.69$, $P < 0.001$; fig. 5). Male RQs increased from a seasonal nadir in early May (0.80 ± 0.01) to a peak in late August (0.97 ± 0.02) and then declined until the end of September (0.86 ± 0.02). Female RQs were lowest in early May (0.79 ± 0.01) and remained relatively low and constant until early August when values increased reaching a peak in mid-August (0.90 ± 0.06).

Nocturnal T_b and Lean-Mass-Specific RMR Adjustments

Seasonal patterns of T_{bmin} differed between males and females. Male T_{bmin} did not change ($P = 0.26$), whereas female T_{bmin} decreased throughout the active season ($P = 0.03$) and followed the equation: T_{bmin} (°C) $= 39.571$ (°C) $- (0.0175 \times$

ordinal date). Adjusting LM-RMR for T_{bmin} in females resulted in a LM-RMR$_{Tbmin}$ that did not change across the active season ($F_{22,6} = 1.14$, $P = 0.36$; fig. 6).

Discussion

In this study, we tested the hypothesis that before entering hibernation free-living ground squirrels decrease RMR to facilitate mass gain. We found that AGS exhibited large changes in body mass, metabolic fuel use, and RMR across their 3–6-mo active season. Consistent with our first prediction, AGS increased total body mass from shortly after the breeding season (late April and early May) until the end of their active season (mid-August for females, late September for males); however, patterns of mass gain were not linear and differed between sexes (figs. 1, 2). Body mass was gained through increases in both lean mass and, to a much larger extent, in fat mass. Patterns of RMR differed between sexes and, contrary to our second prediction, RMR of males increased (whole-animal RMR or LM-RMR) or remained constant (mass-specific RMR) for
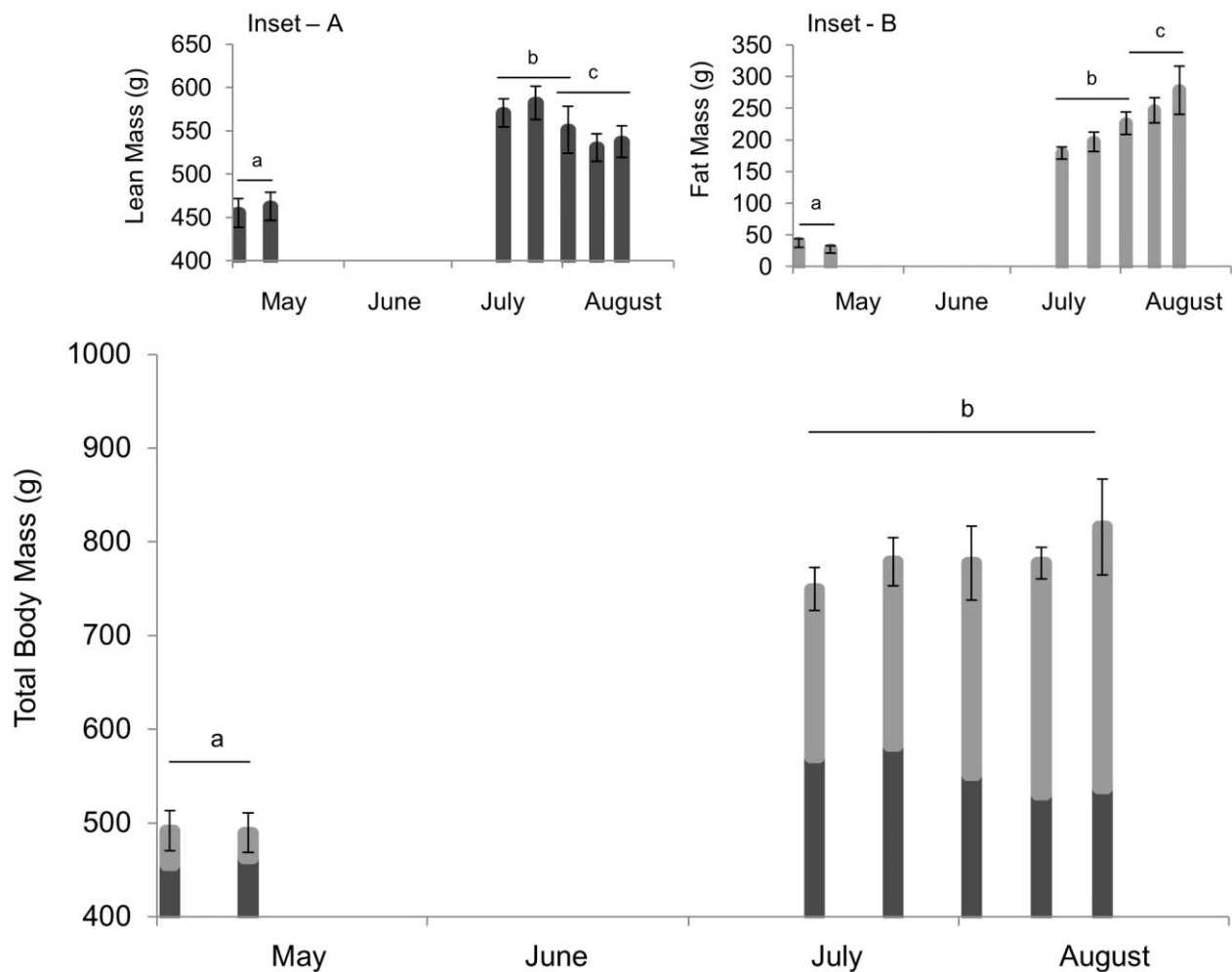
Figure 2. Total body mass (mean ± SE) of free-living female arctic ground squirrels throughout the active season, broken into lean and fat mass components. Inset A shows change in lean mass across the active season; inset B shows change in fat mass across the active season. Letters denote significant differences using a Holm-Sidak post hoc comparison. Measurements were taken repeatedly on the same animals ($n = 23$). A color version of this figure is available online.

most of the active season and decreased only after the majority of mass had been gained and shortly before entrance into hibernation (fig. 3). In females, more consistent with our prediction, RMR generally decreased across the active season (fig. 4); however, the greatest decrease occurred late in the active season after the majority of mass had been gained. Consistent with our third prediction, an upward shift in RQ across the active season indicated that metabolic fuel use shifted from primarily fat-based in spring to more mixed fuel and most likely dietary-based later in the active season (fig. 5). Finally, our results support our prediction that AGS decrease T_{bmin}, which is linked to a decrease in energy expenditure (fig. 6). Thus, unlike captive hibernators, free-living AGS attained the majority of mass gain before reducing RMR, though they continued to fatten until entrance into hibernation. These results suggest that reduced RMR is not the major driver facilitating mass and fat gain during the early portions of the active season

but may be critical in maintenance of high levels of adiposity during the latter parts of the active season.

Body Mass Gain

Hibernating ground squirrels and marmots gain significant body mass during their active seasons (Pengelley and Fisher 1963; Galster and Morrison 1976; Ward and Armitage 1981; Lyman et al. 1982; Humphries et al. 2003; Klingenspor 2003; Dark 2005), and timing of entrance into hibernation is delayed until a suitable body mass is reached (Morton et al. 1974). A number of studies have also found that the amplitude of mass gain in the active season is positively correlated with overwinter survival (Michener 1974, 1978; Slade 1974; Murie and Boag 1984; French 2000; Humphries et al. 2003). Previous work with free-living AGS shows that squirrels increase body mass by 40%–55% between emergence in May and entrance into hi-
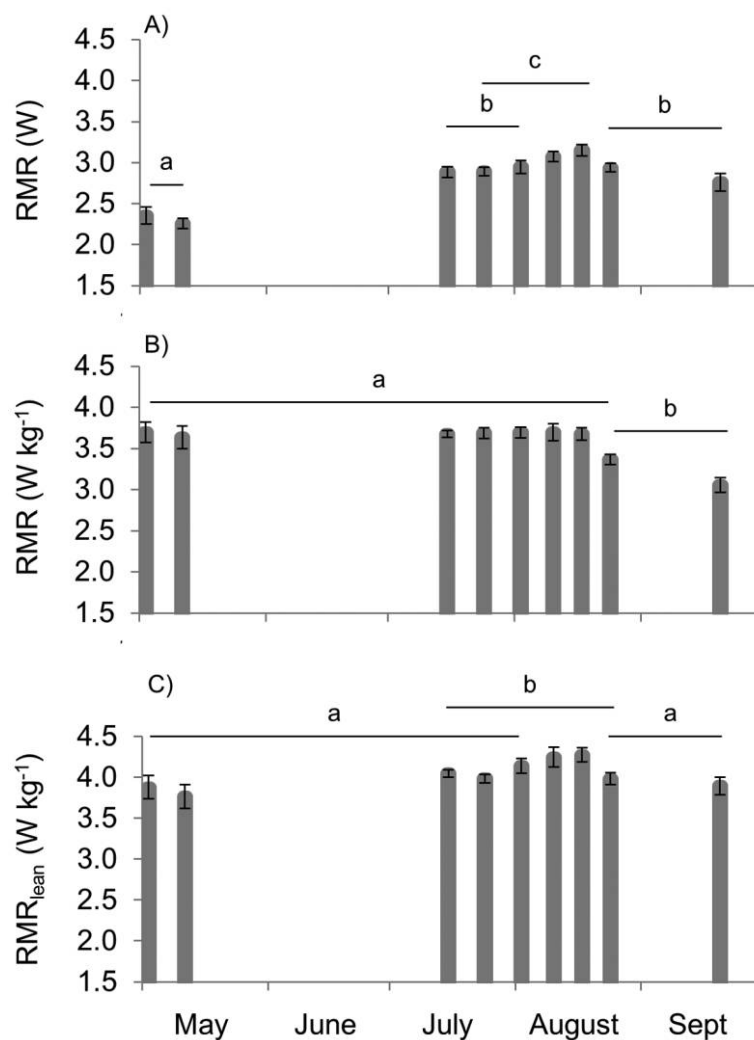
Figure 3. Resting metabolic rate (RMR; mean $\pm$ SE) of free-living male arctic ground squirrels throughout the active season. *A*, Whole-animal RMR (W); *B*, total-body-mass-specific RMR (W kg^{-1}); and *C*, lean-mass-specific RMR (RMR$_{lean}$, W kg^{-1}). Letters denote significant differences using a Holm-Sidak post hoc comparison. Measurements were taken repeatedly on the same animals ($n = 32$). A color version of this figure is available online.

bernation (Buck and Barnes 1999*a*), which is consistent with our results of total body mass gain of 42% (males) and 62% (females; figs. 1, 2). In the laboratory, animals can gain a significantly higher proportion of mass before hibernation. For example, both golden-mantled ground squirrels (Klingenspor 2003) and AGS (Galster and Morrison 1976) doubled their body mass before hibernation. However, Ward and Armitage (1981) found a relatively smaller weight gain of between 30% and 45% in yellow-bellied marmots. Differences between free-living and laboratory-housed animals in patterns and extent of mass gain are expected since free-living animals are necessarily engaged in energy requiring activities throughout the day (e.g., foraging, interacting with conspecifics, avoiding predators, thermoregulation, etc.) whereas captive animals are greatly limited in their activities and are generally provided food ad li-

bitum. Furthermore, there may be large differences in the quality of the food between nature and the laboratory.

Unlike many laboratory studies, we found that mass gain in free-living AGS was not linear across the active season and that patterns of gain differed between sexes. In males, the majority of mass is gained by mid-July with smaller increases thereafter until the end of the active season. The majority of early season mass gain is accounted for by gains in lean mass, which peaks in mid-August. This substantial increase in lean mass may be important at this time because of the highly aggressive territorial defense that occurs in late summer and fall (Carl 1971; Lacey 1991; Buck and Barnes 2003), and greater lean mass may confer a competitive advantage when males defend food caches, territories, and hibernacula, especially those near females. Between late August and late September, despite decreases in lean
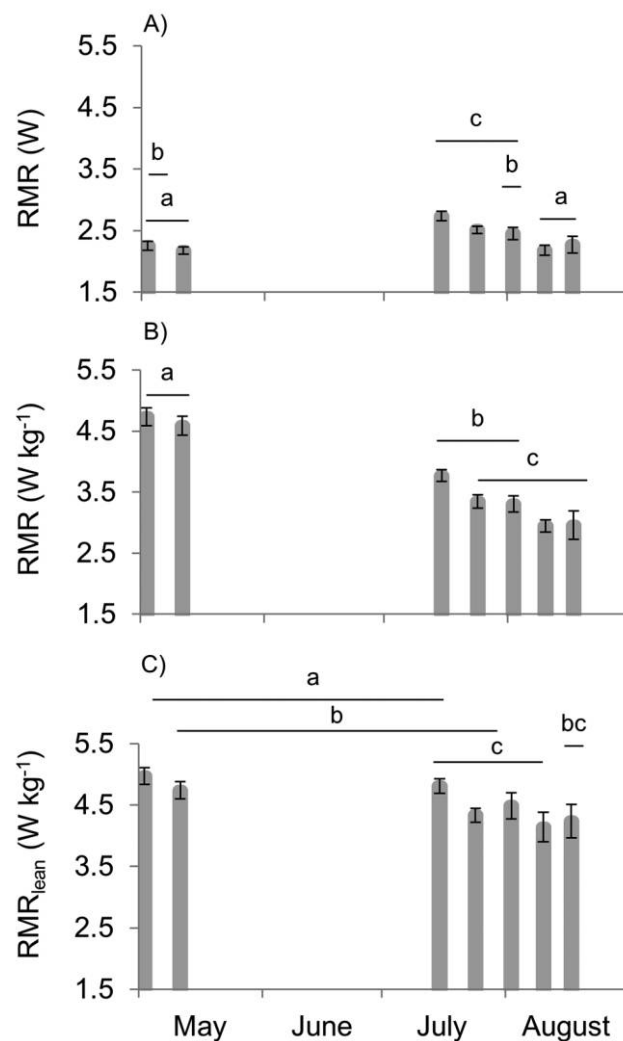
Figure 4. Resting metabolic rate (RMR; mean ± SE) of free-living female arctic ground squirrels throughout the active season. *A*, Whole-animal RMR (W); *B*, total-body-mass-specific RMR (W kg^{-1}); *C*, lean-mass-specific RMR (RMR$_{lean}$, W kg^{-1}). Letters denote significant differences using a Holm-Sidak post hoc comparison. Measurements were taken repeatedly on the same animals ($n = 23$). A color version of this figure is available online.

mass, males continue to increase total body mass through a substantial gain in fat mass. In females, mass gain is completed by mid-July approximately 1 mo before hibernation. Similar to males, the majority of early season mass gain can be attributed to increased lean mass, which then decreases from mid-July to mid-August, just before hibernation. Females maintain a constant body weight by balancing the loss of lean mass with a substantial gain in fat mass. Thus, in both sexes, lean mass significantly increases early in the active season just after breeding and then declines thereafter until hibernation. Fat mass, on the other hand, contributes the most to mass gain during the latter part of the active season. Moreover, although both sexes lose significant lean mass in late summer (females) or early fall (males), lean mass is still significantly greater at the end of the active season than the beginning.

The significant 20% increase in lean mass that we show by

the end of the active season may be critical to overwinter survival of AGS. Typically, hibernators overwinter in sites with ambient temperatures > 0°C (e.g., Young 1990; Arnold et al. 1991), but AGS living in arctic regions must hibernate at sites well below 0°C (AGS hibernacula can reach ∼−23°C; Buck and Barnes 1999b). Although lipid is the primary metabolic fuel for hibernation, torpid AGS in the laboratory progressively increase RQ with ambient temperatures decreasing < 0°C, suggestive of increased reliance on nonlipid fuel at these temperatures (Buck and Barnes 2000). Although it is not clear why torpid AGS switch metabolic fuels at low ambient temperatures, it has been hypothesized that fat catabolism alone may not provide sufficient gluconeogenic substrates and that gluconeogenesis from muscle catabolism is required (Galster and Morrison 1976; Buck and Barnes 2000). Glucose is required by various tissues (e.g., brain, heart, erythrocytes) and for a num-
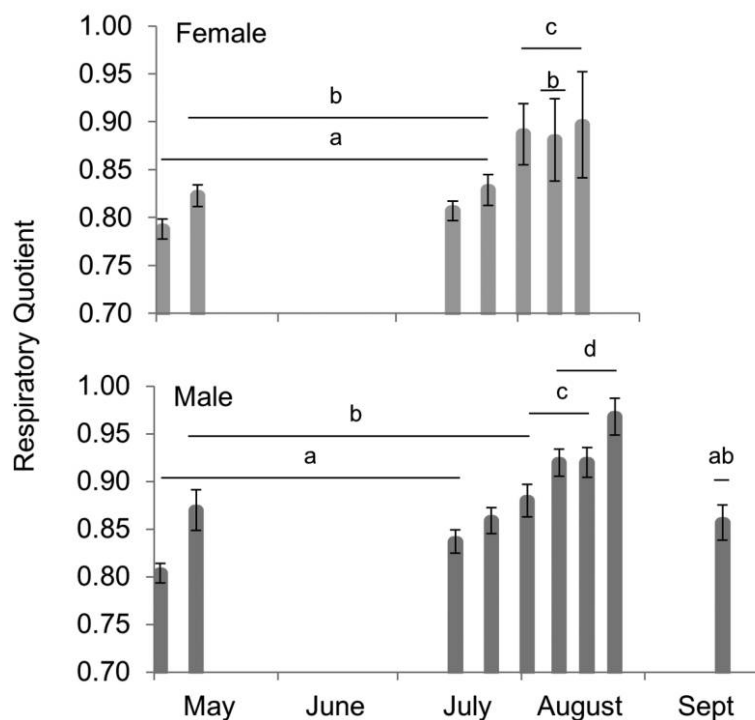
Figure 5. Respiratory quotient (mean ± SE) of free-living arctic ground squirrels throughout the active season. Measurements were taken overnight during resting metabolic trials after 3–10 h fasting. Letters denote significant differences using a Holm-Sidak post hoc comparison. Measurements were taken repeatedly on the same animals ($n = 23$ females, 32 males). A color version of this figure is available online.

ber of functions (e.g., brown adipose tissue thermogenesis) to permit survival during hibernation (Gibbons et al. 1985; Cooney et al. 1986; Dienel and Hertz 2001;). Laboratory observations of switching from fat to mixed fuel catabolism are corroborated by the significant decrease in both lean (20.8%) and fat (66.0%) mass of free-living AGS over their hibernation season at soil temperatures averaging −8.9°C (Buck and Barnes 1999b). Recently, Boonstra et al. (2011) found that both male and female AGS have high levels of plasma androgens after reproduction and proposed that androgens are necessary to promote lean mass growth late in the active season.

RMR and Mass Gain

A circannual cycle in feeding and metabolism underlies the changes in body mass during a hibernator's active season (e.g., Armitage and Shulenberger 1972; Ward and Armitage 1981). In captivity, under constant conditions of food availability, photoperiod, and temperature, many studies have shown that MR and food consumption peak in early spring and decline thereafter until entrance into hibernation, leading to the conclusion that for energy storage to occur as energy input decreases, energy involved in daily maintenance must decrease (Pengelley and Fisher 1963; Armitage and Shulenberger 1972; Ward and Armitage 1981; Thorp et al. 1994; Dark 2005). This has led to the hypothesis that hibernators must first decrease their MR

to facilitate body mass gain. Using free-living animals, we tested but did not find support for this hypothesis.

First, using whole-animal RMR (figs. 3A, 4A), we found that males and females increased RMR from a nadir in the early part of the season, just after breeding, to a peak in the middle of their active season (mid-August for males and mid-July for
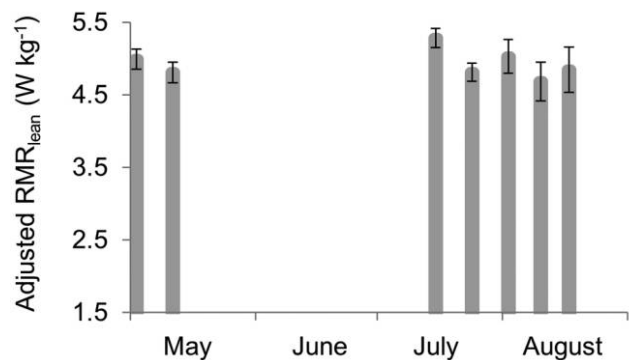

Figure 6. Lean-mass-specific resting metabolic rate (RMR_{lean}; W kg^{-1}) adjusted for Q_{10} of 2 following the regression of nocturnal body temperature (T_{bmin}) of 8 free-living female arctic ground squirrels throughout the active season (mean ± SE). There was no difference in rates following the adjustment (Holm-Sidak post hoc comparison). A color version of this figure is available online.

females). This change in RMR can be explained by changes in body size. Animals were at their lowest body mass during early May and greatly increased mass throughout the active season. Body mass gain was nearly complete in males and complete in females at peak RMR. Interestingly, RMR declined from peak levels until entrance into hibernation despite continued increase in body mass of males and maintenance in body mass of females.

Second, to correct for changes in mass throughout the season (discussed in Ward and Armitage 1981) we calculated mass-specific RMR. In males, mass-specific RMR remained relatively mass independent until it declined late in the active season (fig. 3B). This suggests that males maximize energy input during the early and middle portion of the season to increase mass, and once the majority of mass is gained they minimize energy expenditure. Males may need to engage in this energy expenditure strategy to accumulate and defend their cache and territory during the aggressive territorial defense period in August (Carl 1971; Lacey 1991; Buck and Barnes 2003). During the latter part of the season, males may spend less time above ground and remain closer to their hibernacula (Long et al. 2005), thus reducing energy expenditure.

In females, mass-specific RMR was highest during early May and declined thereafter to a nadir just before entrance into hibernation (fig. 4B). Mass-specific RMR significantly declined between early May and mid-July when females completed their mass gain, suggesting that this decrease may facilitate mass gain. Furthermore, mass-specific RMR continued to decline until hibernation, potentially enabling the maintenance of body mass. This is similar to the findings of captive hibernators (e.g., Bailey 1965; Armitage and Shulenberger 1972; Ward and Armitage 1981; Thorp et al. 1994; Dark 2005) and provides our only support of the hypothesis that reduced RMR before hibernation enables body mass gain. However, as we discuss below, these changes in mass-specific RMR may be driven by changes in body composition of lean and fat mass rather than decreases in tissue metabolism.

Finally, the use of whole animal or mass-specific MR estimates are inappropriate measures for seasonal comparisons in animals that dramatically change in fat mass, because lipid is relatively inactive metabolically (Blaxter 1989; Scott and Evans 1992). In animals that double or triple their fat mass, as many hibernators do (e.g., Klingenspor 2003), mass-specific MR should, by virtue of increasing metabolically inactive tissue, decline. If mass-specific RMR varies due to changes in body composition alone a more appropriate comparison would use LM-RMR. Using this metric, we found that LM-RMR of males changed throughout the active season with peak levels occurring in mid- to late August and declining thereafter (fig. 3C) although this relationship is weak ($P = 0.03$) and the change, if real, is slight. Female LM-RMR, however, remained relatively constant in the early part of the active season and did not begin to decline until late July (fig. 4C). In both sexes, LM-RMR did not decline until late in the active season after males had gained nearly 90% of their total peak body mass and females had completed their mass gain. Col-

lectively, our results indicate that reduced RMR does not facilitate mass gain in free-living AGS but may help to maintain body mass late in the active season.

RQ and Mass Gain

By the end of the active season (August), RQ values increased from 0.80 to >0.90 (fig. 5) indicating a switch from fat-based fuels in the early active season to one primarily composed of forage and of very low fat. During most of the active season AGS have an RQ which hovers around 0.85, indicating they likely rely on mixed fuels of fat, protein, and carbohydrate. The reliance on fat metabolism in the early season, just after breeding, when animals are at their body-condition nadir, emphasizes the importance of fall fattening for both males and females (and food caching in males), not only to survive winter but also to fuel activity during the beginning of the active season when food availability is limited. This switch in fuel use at the end of the active season may increase an animal's ability to store fat and facilitate late season accumulation of fat mass (figs. 1, 2).

Q_{10} Effects on RMR and Mass Gain

A decrease in T_b is an effective way in which animals can reduce rates of energy expenditure. This phenomenon is widely studied in hibernators (Geiser 2004) and recently, we (Sheriff et al. 2012b) found that free-living AGS reduce T_b beginning 45 days before hibernation and suggest that adjustments in T_b are preparatory for hibernation. Here we tested whether a decline in T_b provides additional energy savings as animals increase body mass and fat in preparation for hibernation. In males, we did not find a significant decline in T_{bmin} before hibernation, which may be due to the small animal number, the lower number of temperature samples as animals were only recorded during the metabolic rate trials, or a disruption in T_{bmin} due to being held in captivity and thermoneutral during sampling. In females, similar to results in Sheriff et al. (2012b), we found a decrease in nocturnal T_{bmin} in the latter parts of the active season. Assuming a Q_{10} of 2 (as is commonly referenced for biological reactions; Schmidt-Nielsen 1997), we found the decline in T_{bmin} was sufficient to explain the seasonal decrease in female LM-RMR (fig. 6). Thus, animals quite likely decreased their nocturnal T_b during the latter portion of the active season, reducing RMR and energy expenditure and enabling maintenance of mass accumulated earlier in the active season in preparation for hibernation. Alternatively, animals may actively reduce MR, resulting in the decrease in T_b we observed. However, the correspondence between the decreased T_b, MR, and Q_{10} support the former explanation over the latter.

Conclusion

In this study, we tested the hypothesis that AGS decrease their RMR before and in order to facilitate mass gain. However, our
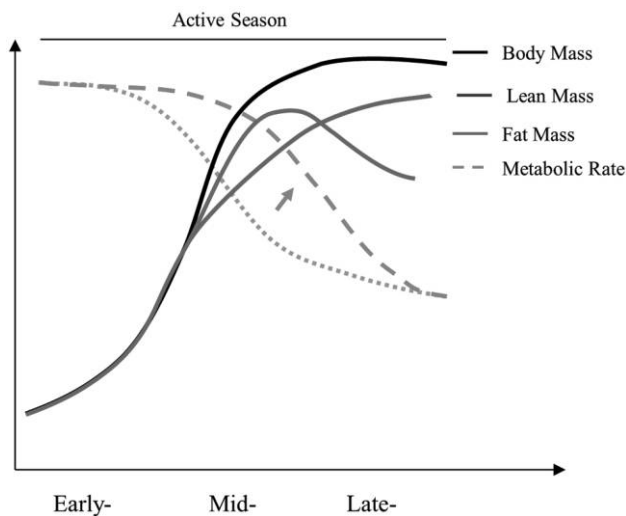
Figure 7. Schematic of change in body mass and composition (lean and fat mass) and metabolic rate across the active season in a free-living hibernator, as exemplified by arctic ground squirrels (this study). Note that unlike previously suggested metabolic rate (dotted line) for lab-kept rodent hibernators (Pengelley and Fisher 1963; Armitage and Shulenberger 1972; Ward and Armitage 1981; Thorp et al. 1994; Dark 2005; Florant and Healey 2011), metabolic rate in the current study (hashed line) does not decline before body mass gain but after mass gain is nearly complete, suggesting that in free-living hibernators changes in metabolic rate may be more important in the late active season and play a role in maintaining body mass rather than facilitating mass gain. A color version of this figure is available online.

results did not support this hypothesis, and in general we found that RMR declined well after the majority of body mass gain. Furthermore, we found that AGS may facilitate fat gain and minimize energy expenditure in the latter parts of the active season by switching from a metabolic fuel source primarily fat-based in spring to one primarily carbohydrate-based and by decreasing their nocturnal T_b. As opposed to the facilitative effects of reduced RMR on body mass gain as previously hypothesized, we suggest a maintenance role of the RMR decline (fig. 7). Unlike their laboratory counterparts, free-living hibernators may not be able to afford the trade-offs associated with a decrease in energy expenditure. During the early part of the season, high levels of energy expenditure are necessary for breeding and lactation. During the middle portion of the active season, animals need to recover from the high costs of breeding, actively forage, defend territories, and avoid predation. Further, processing large quantities of food may cause MR to remain relatively high. Only later in the active season, when body mass is maximal and territorial threats are reduced (juveniles have stopped dispersing) can animals reduce activity budgets and energy expenditure. Thus, free-living hibernators may employ an energy intake strategy for the majority of their active season in order to gain sufficient body mass while maintaining necessary activity levels, switching to an energy savings strategy to maintain previously gained body mass as they near hibernation (fig. 7).

Literature Cited

Armitage K. and E. Shulenberger. 1972. Evidence for a circannual metabolic cycles in *Citellus tridecemlineatus*, a hibernator. Comp Biochem Physiol 42:667–688.

Arnold W., G. Heldmaier, S. Orlmann, H. Pohl, T. Ruf, and S. Steinlechner. 1991. Ambient temperatures in hibernacula and their energetic consequences for the alpine marmot (*Marmota marmot*). J Thermal Biol 16:223–226.

Arnold W., T. Ruf, F. Frey-Roos, and U. Bruns. 2011. Diet-independent remodeling of cellular membranes precedes seasonally changing body temperature in a hibernator. PLoS ONE 6:e18641, doi:10.1371/journal.pone.0018641.

Bailey E.D. 1965. Seasonal changes in metabolic activity of non-hibernating woodchucks. Can J Zool 43:905–909.

Barnes B.M. 1996. Relationships between hibernation and reproduction in male ground squirrels. In F. Geiser, A.J. Hulbert, and S.C. Nicol, eds. Adaptations to the Cold: Tenth International Hibernation Symposium. University of New England Press, Armidale, Australia

Blaxter K.L. 1989. Energy Metabolism in Animals and Man. Cambridge University Press, Cambridge.

Boonstra R., A.J. Bradley, and B. Delehanty. 2011. Preparing for hibernation in ground squirrels: adrenal androgen production in summer linked to environmental severity in winter. Funct Ecol 25:1348–1359.

Buck C.L. and B.M. Barnes. 1999a. Annual cycles of body composition and hibernation in free-living arctic ground squirrels. J Mammal 80:430–442.

———. 1999b. Temperature of hibernacula and changes in body composition of arctic ground squirrels over winter. J Mammal 80:1264–1276.

———. 2000. Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. Am J Physiol 279:R255–R262.

———. 2003. Androgen in free-living arctic ground squirrels:

seasonal changes and influence of staged male-male aggressive encounters. Horm Behav 43:318–326.

Buck C.L., A. Breton, Ø. Tøien, and B.M. Barnes. 2008. Overwinter body temperature patterns of free-living arctic ground squirrels (*Spermophilus parryii*). Pp. 317–326 in B.G. Lovegrove and A.E. McKechnie, eds. Hypometabolism in Animals: Torpor, Hibernation and Cryobiology. University of KwaZulu-Natal, Pietermaritzburg.

Carey H.V., M.T. Andrews, and S.L. Martin. 2003. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol Rev 83:1153–1181.

Carl E.A. 1971. Population control in arctic ground squirrels. Ecology 52:395–413.

Concannon P., K. Levac, R. Rawson, B. Tenant, and A. Bensadoun. 2001. Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. Am J Physiol 281:R951–R959.

Concannon P., P. Roberts, B. Baldwin, H. Erb, and B. Tennant. 1993. Alteration of growth, advancement of puberty, and season-appropriate circannual breeding during 28 mo of photoperiod reversal in woodchucks (*Marmota monax*). Biol Reprod 48:1057–1070.

Cooney G., R.M.A. Curi, P. Newsholme, M. Simpson, and E.A. Newsholme. 1986. Activities of some key enzymes of carbohydrate, carbohydrate, ketone body, adenosine and glutamate metabolism in liver, and brown and white adipose tissues of the rat. Biochem Biophys Res Commun 138:687–692.

Dark J. 1984. Seasonal weight gain is attenuated in food-restricted ground squirrels with lesions of the suprachiasmatic nuclei. Behav Neurosci 98:830–835.

———. 2005. Annual lipid cycles in hibernators: integration of physiology and behavior. Ann Rev Nutri 25:469–497.

Delehanty B. and R. Boonstra. 2011. Coping with intense reproductive aggression in male arctic ground squirrels: the stress axis and its signature tell divergent stories. Physiol Biochem Zool 84:417–428.

Dienel G.A. and L. Hertz. 2001. Glucose and lactate metabolism during brain activation. J Neurosci Res 66:824–838.

Drew K.L., C.L. Buck, B.M. Barnes, S.L. Christian, B.T. Rasley, and M.B. Harris. 2007. Central nervous system regulation of mammalian hibernation: implications for metabolic suppression and ischemia tolerance. J Neurobiochem 102:1713–1726.

Florant G.L. and J.E. Healey. 2011. The regulation of food intake in mammalian hibernators: a review. J Comp Physiol B, doi:10.1007/s00360-011-0630-y.

French A.R. 2000. Interdependency of stored food and changes in body temperature during hibernation of the eastern chipmunk, *Tamias striatus*. J Mammal 81:979–985.

Galster W. and P.R. Morrison. 1975. Gluconeogenesis in arctic ground squirrels between periods of hibernation. Am J Physiol 228:325–330.

Galster W. and P.R. Morrison. 1976. Seasonal changes in body composition of the arctic ground squirrel, *Citellus undulates*. Can J Zool 54:740–778.

Geiser F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. Ann Rev Physiol 66:239–274.

Gibbons J.M., R.M. Denton, and J.G. McCormack. 1985. Evidence that noradrenaline increases pyruvate dehydrogenase activity and decreases acetyl-CoA carboxylase activity in rat intrascapular brown adipose tissue in vivo. Biochem J 228:751–755.

Haldane J.S. 1912. Methods of Air Analysis. Griffin, Lippincott, Philadelphia.

Hock, R.J. 1960. Seasonal variation on physiologic functions of arctic ground squirrels and black bears. Proc. First Int. Symp. Nat. Hibernation. Bull Mus Comp Zool 124:155–173.

Humphries M.M., D.W. Thomas, and D.L. Kramer. 2003. The role of energy availability in mammalian hibernation: a cost-benefit approach. Physiol Biochem Zool 76:165–179.

Johns D.W. and K.B. Armitage. 1979. Behavioral ecology of alpine yellow-bellied marmots. Behav Ecol Sociobiol 5:133–157.

Kilgore D.L. and K.B. Armitage. 1978. Energetics of yellow-bellied marmot populations. Ecology 59:78–88.

Kleiber M. 1975. The Fire of Life. Kreiger, Huntington, NY.

Klingenspor M. 2003. Cold-induced recruitment of brown adipose tissue thermogenesis. Exp Physiol 88:141–148.

Körtner G. and F. Geiser. 1995. Effects of photoperiod and ambient temperature on activity patterns and body weight cuces of mountain pygmy possums, *Burramys parvus* (Marsupialia) J Zool 235:311–322.

Kronfeld-Schor N., C. Richardson, B.A. Silvia, T.H. Kunz, and E.P. Widmaier. 2000. Dissociation of leptin secretion and adiposity during prehibernatory fattening in little brown bats. Am J Physiol 279:R1277–R1281.

Lacey E.A. 1991. Reproductive and dispersal strategies of male arctic ground squirrels (*Spermophilus parryii plesius*). PhD diss. University of Michigan, Ann Arbor.

Lee T.N., R.W. Fridinger, B.M. Barnes, C.L. Buck, and D.M. O'Brien. 2011. Estimating lean mass over a wide range of body composition: a calibration of deuterium dilution in the arctic ground squirrel. Rapid Commun Mass Spectrom 25:3491–3496.

Long R.A., R.A. Hut, and B.M. Barnes. 2007. Simultaneous collection of body temperature and activity data in burrowing mammals: a new technique. J Wildl Manag 71:1375–1379.

Long R.A., T.J. Martin, and B.M. Barnes. 2005. Body temperature and activity patterns in free-living arctic ground squirrels. J Mammal 86:314–322.

Lyman C.P., J.S. Willis, A. Malan, and L.C.H. Wang. 1982. Hibernation and torpor in mammals and birds. Academic Press, New York.

Lusk G. 1924. Animal calorimetry. Twenty-fourth paper. Analysis of the oxidation of mixtures of carbohydrate and fat. A correction. J Biol Chem 59:41–42.

MacDonald S.O. and J.A. Cook. 2009. Recent mammals of Alaska. University of Alaska Press, Fairbanks.

McLean I.G. and A.J. Towns. 1981. Difference in weight changes

and the annual cycles of male and female arctic ground squirrels. Arctic 34:249–254.

Michener G.R. 1974. Annual cycle of activity and weight changes in Richardson's ground squirrel, *Spermophilus richardsonii.* Can Field Nat 88:409–413.

———. 1978. Effects of age and parity on weight gain and entry into hibernation in Richardson's ground squirrels. Can J Zool 56:2573–2577.

Morton M.L., C.S. Maxwell, and C.E. Wade. 1974. Body size, body composition, and behavior of juvenile Belding's ground squirrels. Great Basin Natural 34:121–134.

Munro D., D.W. Thomas, and M.M. Humphries. 2005. Torpor patterns of hibernating eastern chipmunks *Tamias striatus* vary in response to the size and fatty acid composition of food hoards. J Anim Ecol 74:692–700.

Murie J.O. and D.A. Boag. 1984. The relationship of body weight to overwinter survival in Columbian ground squirrels. J Mammal 64:688–690.

Nagy K.A. 1983. The Doubly Labeled water (^{3}HH^{18}O) Method: A Guide To Its Use. Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles.

Pengelley E.T. and K.C. Fisher. 1963. The effect of temperature and photoperiod on the yearly hibernating behavior of captive golden-mantled ground squirrels (*Citellus lateralis tescorum*). Can J Zool 41:1103–1120.

Rawson R.E., P.W. Concannon, P.J. Roberts, B.C. Tennant. 1998. Seasonal differences in resting oxygen consumption, respiratory quotient, and free thyroxine in woodchucks. Am J Physiol 274:R963–R969.

Reid J.T., A. Bensadoun, O.L. Paladines, B.D.H. Van Niekerk. 1963. Body water estimation in relation to body composition and indirect calorimetry in ruminants. Ann NY Acad Sci 110:327–342.

Schmidt-Nielsen K. 1997. Animal Physiology: Adaptations and Environment. Cambridge University Press, New York.

Scott I. and P.E. Evans. 1992. The metabolic output of avian (*Sturnus vulgaris, Calidris alpina*) adipose tissue liver and skeletal muscle: implications for BMR/body mass relationships. Comp Biochem Physiol 103:329–332.

Sheriff M.J., G.J. Kenagy, M. Richter, T. Lee, Ø. Tøien, F. Kohl, C.L. Buck, and B.M. Barnes. 2011. Phenological variation in annual timing of hibernation and breeding in nearby population of arctic ground squirrels. Proc R Soc B 278:2369–2375.

Sheriff M.J., H. Wheeler, S.A. Donker, C.J. Krebs, R. Palme, D.S. Hik, and R. Boonstra. 2012*a*. Mountain-top and valley-bottom experiences: the stress axis as an integrator of environmental variability in arctic ground squirrel populations. J Zool 287:65–75.

Sheriff M.J., C.T. Williams, G.J. Kenagy, C.L. Buck, and B.M. Barnes. 2012*b*. Thermoregulatory changes anticipate hibernation onset by 45 days: data from free-living arctic ground squirrels. J Comp Physiol B 182:841–847.

Slade N.A. and D.F. Balph. 1974. Population ecology of Uinta ground squirrels. Ecology 55:989–1003.

Speakman J.R. 1997. Doubly Labeled Water: Theory and Practice. Chapman & Hall, London.

Thorp C.R., P.K. Ram, and G.L. Florant. 1994. Diet alters metabolic rate in the yellow-bellied marmot (*Marmota flaviventris*) during hibernation. Physiol Zool 67:1213–1229.

Tøien, Ø. 2013. Automated open flow respirometry in continuous and long-term measurements: design and principles. J Appl Physiol 114:1094–1107.

Wagner J.A., S.M. Horwath, T.E. Dahms, and S. Reed. 1973. Validation of open-circuit method for the determination of oxygen consumption. J Appl Physiol 34:859–863.

Ward J.M. and K.B. Armitage. 1981. Circannual rhythms of food consumption, body mass, and metabolism in yellow-bellied marmots. Comp Biochem Physiol 69A:621–626.

Young P.J. 1990. Hibernating patterns of free-ranging Columbian ground squirrels. Oecologia 83:504–511.