



Energy regulation in context: Free-living female arctic ground squirrels modulate the relationship between thyroid hormones and activity among life history stages



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ARTICLE INFO

Article history:

Received 30 May 2015

Revised 13 September 2015

Accepted 22 September 2015

Available online 28 September 2015

Keywords:

Thyroid hormone

Triiodothyronine

T₃

Thyroxine

T₄

Polar

Rodent

Reproduction

Energetics

Female

Activity

ABSTRACT

Thyroid hormones (THs), key regulators of lipid and carbohydrate metabolism, are likely modulators of energy allocation within and among animal life history stages. Despite their role in modulating metabolism, few studies have investigated whether THs vary among life history stages in free-living animals or if they exhibit stage-specific relationships to total energy expenditure and activity levels. We measured plasma total triiodothyronine (tT₃) and thyroxine (tT₄) at four, discrete life history stages of female arctic ground squirrels from two different populations in northern Alaska to test whether plasma THs correlate with life history stage-specific changes in metabolic rate and energy demand. We also tested whether THs explained individual variation in aboveground activity levels within life history stages. T₃ peaked during lactation and was lowest during pre-hibernation fattening, consistent with known changes in basal metabolism and core body temperature. In contrast, T₄ was elevated shortly after terminating hibernation but remained low and stable across other life-history stages in the active season. THs were consistently higher in the population that spent more time above-ground but the relationship between THs and activity varied among life history stages. T₃ was positively correlated with activity only during lactation ($r^2 = 0.50$) whereas T₄ was positively correlated with activity immediately following lactation ($r^2 = 0.48$) and during fattening ($r^2 = 0.53$). Our results support the hypothesis that THs are an important modulator of basal metabolism but also suggest that the relationship between THs and activity varies among life history stages.

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1. Introduction

Seasonal environments are characterized by annual changes in ambient conditions that result in predictable transitions between periods of relatively high food availability and food scarcity. Organisms inhabiting cyclical environments can take advantage of high energy availability for growth and reproduction, but also must cope with seasonal scarcity (Nelson et al., 2002). In long-lived organisms, this is typically achieved by expressing season-specific behaviors, morphologies, and physiologies that, together, characterize or define specific life history stages. Constraining the expression of life history stages to specific seasons or environmental conditions is adaptive for longer-lived animals because it allows them to match energy needs with energy availability as well as enabling them to temporally segregate energetically-intensive physiological and behavioral processes (King and Murphy, 1985; Payne, 1972).

The expression of multiple life history stages is also modulated by internal machinery (hormones and other chemical signals) that precipitates and maintains stage-specific behavior and physiology (Jacobs and Wingfield, 2000; Romero, 2002). For example, in males of several species, high circulating testosterone concentrations are necessary for the expression of territoriality, but also are an important contributor to the suppression of the immune system and parenting behaviors (Hau, 2007; Ketterson and Nolan, 1992). Circulating testosterone therefore tends to be higher when animals are establishing and defending territories but declines while rearing offspring (Hegner and Wingfield, 1987; Nunes et al., 2001).

Hormones not only coordinate the transitions between annually recurring life history stages, but also mediate energy allocation among competing demands within life history stages.

Steroids, including androgens, estrogens, and glucocorticoids, have been well studied and play important roles in mediating behavioral and physiological trade-offs within and between life history stages (Hau, 2007; Ketterson and Nolan, 1992; Ricklefs and Wikelski, 2002), in addition to playing an indirect role in the control of metabolism. Thyroid hormones (THs), in contrast, can act directly and indirectly to

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influence metabolism through both peripheral and central actions (Kim, 2008; Mullur et al., 2014), yet are surprisingly understudied in free-living mammals. We are only aware of one study examining the role of THs as modulators of metabolic rate, activity or total energy expenditure within or across life-history stages in a free-living mammal (Lachiver et al., 1978).

Uncertainty surrounding the role of THs in mediating energy use within and across life history stages is due, in part, to the variety of mechanisms that control their availability within target tissues and their diverse physiological effects. The production and release of THs are regulated via negative-feedback in the hypothalamic-pituitary-thyroid (HPT) axis. In addition, we now know that hypothalamic TRH release is affected by cold exposure via adrenergic input and that the set-point for TRH inhibition via THs can be modulated by catecholamines (Chiamolera and Wondisford, 2009). Fasting and other metabolic cues also directly regulate the HPT axis via effects of leptin on neuronal stimulation in the hypothalamus (Chiamolera and Wondisford, 2009). These alternate mechanisms render the HPT axis responsive to metabolic cues as well as classic, homeostatic negative feedback.

THs themselves are produced as thyroxine (T_4), and activated by deiodinases (*Dio1* or *Dio2*) that convert T_4 to triiodothyronine (T_3). Conversion of T_4 to T_3 can occur in the thyroid gland prior to secretion into the circulatory system; alternatively, THs can be converted to T_3 within peripheral tissues (Darras and Van Herck, 2012). The vast majority of THs circulate as T_4 whereas the amount of circulating T_3 depends on enzymatic conversion of T_4 in the thyroid gland and peripheral tissues, particularly the liver and kidneys. The relative contribution of different tissues to circulating T_3 levels varies among taxa (Darras and Van Herck, 2012). Most TH in the circulation is bound by thyroid binding globulin (TBG), which extends TH half-life, with only the free (unbound) hormone available for active transport across cell membranes into target tissues (Visser et al., 2011).

The effects of thyroid hormones on metabolism have been known for more than 100 years, however many of the molecular processes underlying these effects are still unclear. THs can act through the binding of T_3 to nuclear receptors that regulate gene expression to modulate metabolism directly and through cross-talk with other pathways (Brent, 2012); TH also has non-genomic effects via membrane receptors and in the cytoplasm (Mullur et al., 2014). The expression of the two primary TH receptor (TR) genes, $TR\alpha$ and $TR\beta$ is regulated, in part, by T_3 itself (Cheng et al., 2010). These two genes can be differentially spliced such that there are four thyroid hormone binding isoforms which vary in their tissue distribution and likely have different biological functions (Cheng et al., 2010; Williams, 2000).

THs increase the number of mitochondria in cells and modulate global mitochondrial gene expression (Cheng et al., 2010). Basal proton leak, which contributes to mitochondrial heat generation, is also modulated by THs (Brand, 2005), though the contribution of thyroid hormones to this process outside of brown adipose tissue is unknown.

TRs can homodimerize and bind directly to thyroid hormone receptor response elements (TREs) to modulate gene transcription; however, TRs can also heterodimerize and compete with other nuclear receptor proteins for alternative response elements that modulate metabolic pathways (reviewed in Cheng et al., 2010). THs also affect metabolism nongenomically via calcium pump activity and direct effects on glucose uptake in a variety of cell types (Segal, 1989). In addition to the wide variety of peripheral effects, THs also exhibit central effects on metabolism. For example, THs can stimulate hepatic glucose production via sympathetic projections to the liver (Fliers et al., 2010).

Based on the broad effects of THs on various metabolic pathways and tissue types, peripheral T_3 is thought to primarily act as the direct regulator of basal metabolic rate (BMR; the minimum rate of energy expenditure at rest, but not sleeping, while in a post-absorptive state) in adult mammals. Circulating levels of T_3 are therefore associated with the cost of self-maintenance in free-living animals. Circulating T_3 and BMR

usually exhibit a strong, positive relationship in both free-living and laboratory animals (Bobek et al., 1977; Chastel et al., 2003; Elliott et al., 2013; Vezina et al., 2009; Welcker et al., 2013) such that T_3 has been used as a proxy measurement for BMR (Welcker et al., 2014). In contrast, the role of circulating T_4 is less clear; plasma T_4 is not correlated with BMR in free-living birds (Elliott et al., 2013; Vezina et al., 2009), but is positively correlated with BMR in some rodents (Banta and Holcombe, 2002) and humans (Al-Adsani et al., 1997; Johnstone et al., 2005).

THs also contribute to metabolic expenditure above that of basal metabolic rate, such as facultative thermogenesis (Silva, 2003; Seebacher, 2009; Brent, 2012); in fact, the contribution of thyroid hormones to energy expenditure above that of BMR underlies the common assumption that BMR and total energy expenditure and activity levels of individuals are physiologically linked. (We use “activity” here to refer to the compilation of all external physical movements made by an organism with the “level” of activity being a direct reflection of the energetic cost of this movement). THs have been predicted to act as critical, physiological modulators of both activity and overall metabolism (Biro and Stamps, 2011). Indeed, manipulation of thyroid hormones via exogenous supplementation or thyroidectomy alters activity levels of some laboratory rodents (McEachron et al., 1993; Pilhatsch et al., 2010; Schull et al., 1989). However, thyroid hormone levels can also change in response to training or exercise in dogs and humans (Hill et al., 2001; Panciera et al., 2003). Thus, thyroid hormones appear to both potentiate metabolic change in addition to responding to metabolic demands related to activity of an individual.

Currently, two conceptual models are used to describe the linkages between basal metabolism and total energy expenditure: the *performance* model and the *allocation* model. The *performance* model assumes that BMR reflects the size of an individual's internal machinery (e.g., organ size), which subsequently determines the amount of energy available for other energetically-demanding processes, including activity (Careau et al., 2008). According to this model, BMR should positively correlate with daily energy expenditure and activity levels. In contrast, the *allocation* model, assumes that energy must be “divided” between BMR and other energetic outputs, such that BMR is predicted to negatively correlate with activity levels (Mathot and Dingemans, 2015).

Studies testing these hypotheses in free-living individuals have primarily found support for the *performance* model; basal metabolism tends to positively correlate with activity levels (Biro and Stamps, 2011; Mathot and Dingemans, 2015). However, few studies have examined whether these relationships hold across life history stages. Instead, many studies focus on reproductive individuals during a single life history stage. Given the dynamic nature of energy availability, utilization, and storage across the annual cycle of individuals, further investigations are needed to establish whether these energetic models are relevant across life history stages. Similarly, despite the fact that TH is a clear physiological correlate for activity based on its strong causal relationship with BMR, whether a relationship between TH, as a proxy for BMR, and activity exists has not been tested in any life history stage of a free-living mammal.

We were interested in testing the *performance* and *allocation* models described above in a novel system using THs and activity levels of individuals. However, in testing these relationships, we wanted to also assess the interrelationships among THs (which contribute to both basal metabolism and facultative thermogenesis), and energy expenditure. Furthermore, we hypothesized that the correlation between THs and activity levels in free-living animals would be context-dependent. Our hypothesis is based on prior evidence that the relationship between daily energy expenditure, which is in large part a function of activity, and RMR, which is likely to correlate strongly with THs, changes across the annual cycle in other small mammals (Careau et al., 2013). Our hypothesis is also based on the premise that effects of peripheral THs will change with life-history stage due to modulation of TH availability or

binding at the tissue level; similar processes are already well-established within the brain (reviewed in [Dardente et al., 2014](#)).

We investigated this hypothesis in free-living, female arctic ground squirrels (*Urocitellus parryii*), a semi-fossorial rodent living in a highly seasonal environment. Female arctic ground squirrels experience an extremely long hibernation season (7–9 months), and a conversely short 3–5 month active season, and therefore must be able to tightly control physiology associated with energy utilization as they rapidly transition between life history stages. Moreover, females exhibit stage-specific changes in body composition, body temperature, and metabolic rate ([Williams et al., 2011](#); [Sheriff et al., 2013](#)), substantiating that significant changes in energy acquisition and expenditure occur across their annual cycle. Therefore, if THs significantly contribute to modulation of energy allocation among life history stages, female arctic ground squirrels should exhibit dynamic regulation of THs consistent with other known changes in their physiology.

We predicted that animals would exhibit the highest annual plasma THs during lactation, because female rodents near-universally experience annual peaks in metabolic expenditure during lactation ([Johnson and Speakman, 2001](#); [Kenagy et al., 1989](#); [Velo and Bozinovic, 2000](#); [Zhang and Wang, 2007](#)). We predicted that females would exhibit their lowest annual plasma THs during pre-hibernation fattening, to limit lipid metabolism and promote energy storage. During fattening, female arctic ground squirrels decrease both lean-mass specific resting metabolic rate (RMR) and body temperature to prepare for hibernation ([Sheriff et al., 2013](#)).

2. Methods

2.1. Species life-history

Arctic ground squirrels are the northern-most hibernating mammal. They spend 7–9 months of the year sequestered in belowground hibernacula, and emerge for 3–5 months of the summer to reproduce and fatten ([Buck and Barnes, 1999](#)). During the active season, perennial burrow systems are used for thermoregulation ([Long et al., 2005](#); [Williams et al., 2014](#)), refuge from predators ([Carl, 1971](#)), and for rearing young ([Carl, 1971](#)). Animals must come above ground for foraging and social interactions. Ground squirrels will spend up to 12 h above ground per day ([Williams et al., 2014](#)), and body temperature data suggest that most of the time below ground is spent inactive ([Long et al., 2005](#)). Across this short active season, ground squirrels are exposed to appreciable fluctuations in temperature and weather patterns, with the majority of their active season in northern Alaska occurring under 24-h of daylight above the Arctic Circle.

Female arctic ground squirrels emerge from their hibernacula in late April at their lowest seasonal body weight and become pregnant, on average, within 4 days ([Buck and Barnes, 1999](#); [Williams et al., 2012](#)). Gestation is approximately 26 days. In female arctic ground squirrels, lactation occurs concurrently with annual lows in body weight and adiposity ([Buck and Barnes, 1999](#)). Pups remain in the burrow for another 4–5 weeks until they are weaned. After their pups are weaned, females continue foraging extensively as they store energy endogenously as fat in preparation for hibernation ([Buck and Barnes, 1999](#)). Females enter hibernation in early to mid-August. Arctic ground squirrels also undergo two pelage molts during the active season — a spring molt and a fall molt prior to hibernation ([Butterworth, 1958](#)).

2.2. Data collection

Female arctic ground squirrels were trapped on the North Slope of the Brooks Range, AK, using Tomahawk live-traps (Tomahawk Trap Co.) baited with carrot. Individuals were trapped at two sites that differed in population density. Our low-density site is located adjacent to Toolik Field Station (68° 38'N, 149° 38'W, elevation 719 m), while the high-density site is located approximately 20 km south along the

northern bank of the Atigun River (68° 27'N, 149° 21'W, elevation 812 m). These sites are further characterized elsewhere ([Sheriff et al., 2011](#)), but differ in vegetation and soil types resulting in greater habitat availability (i.e., more areas suitable for burrowing) at the high-density site. However, whether other environmental components contribute to site-differences in ground squirrel density is currently unknown.

Animals were trapped at four intervals across the active season in 2014. We offset trapping between sites by approximately one week to account for known differences in phenology ([Sheriff et al., 2011](#)). Traps were usually set in the mid-morning (~10:00), checked every 1–3 h, and closed in the early evening (~17:00). Animals at both sites are uniquely ear-tagged and PIT-tagged for individual identification. Immediately prior to sampling, squirrels were anesthetized using a brief exposure (3–5 min) to isoflurane vapors and then weighed and assessed for reproductive status (mating, pregnant, lactating, post-lactation, fattening). In the mating season (April 20 to May 1), animals from both sites were trapped and brought back to the lab prior to sampling and released at their site of capture the following day. The lab is directly adjacent to our Toolik site such that hold times prior to sampling were not substantially longer during the mating season at this site. During lactation (May 30 to June 13), post-lactation (June 28 to July 8), and fattening (August 1 to 8), all animals were sampled and released in the field.

Activity data were collected using collar-mounted light loggers which provide information about when squirrels are active above-ground (exposed to light) vs. below-ground (dark). Throughout the study, we used two types of light loggers: BAS model MK7290 light loggers (1 g; Biotrack Ltd., Dorset, UK) which record light levels every 2 min, or Intigeo-C56 light loggers (1 g; Migrate Technology Ltd., Cambridge, UK), which record light every minute and then save the highest value per 5-min interval. For detailed methods of light logger collar deployment and calculation of above-ground activity see [Williams et al. \(2014\)](#).

At each capture, we also collected blood samples for hormone analysis via cardiac puncture using 26 G needles in 3 mL syringes. No more than 1.5 mL was collected at a single capture (<1% body mass for all animals). Blood was placed on ice in EDTA-coated tubes in the field. Samples were centrifuged in the lab for 10 min at 6500 rpm within 7 h of collection to separate plasma. Plasma was stored in microcentrifuge tubes at approximately –18 °C until transport to Anchorage, AK, where they were stored at –80 °C until assayed for total T₃ (tT₃) and total T₄ (tT₄). Altogether, we collected 91 plasma samples across the season from 40 individual females ([Table 1](#)). We collected activity data from 22 of these individuals using collar-mounted light loggers ([Table 1](#)).

2.3. Thyroid hormone assay

We measured tT₄ using a commercially-available tT₄ EIA kit for human serum (MP Biomedicals, Solon, OH). The kit was validated using arctic ground squirrel plasma prior to use by both the standard addition method and using tests of parallelism. Samples were randomized

Table 1

Sample sizes for plasma and activity data across the 2014 season by site and life history stage. Animals with activity data were only included if they also had plasma samples collected during that life history stage.

Life history stage	Number of individuals			
	Plasma samples		Activity	
	High density	Low density	High density	Low density
Mating	22	7	5	4
Lactation	15	9	9	2
Post-lactation	18	6	12	6
Fattening	11	3	6	2
Unique individuals	28	12	14	8

across plates and assayed in triplicate when possible (100/116). A small number of small volume samples were run in duplicate (10/116) or singlet (6/116). tT_4 concentrations were calculated using a fitted 4-parameter line. The mean intra-assay CV was 5.52% and mean inter-assay CV was 12.5%.

Plasma was extracted prior to tT_3 assay using a standard barbital buffer protocol (Crane et al., 2004). The mean extraction efficiency ($85.4 \pm 1.6\%$) was calculated using I^{125} -labeled T_3 . Reported values were not corrected for extraction efficiency. Extracted samples were stored at -80°C and resuspended in RIA zero standard at room temperature immediately prior to assay. We measured tT_3 using a commercially-available tT_3 RIA kit for serum (MP Biomedicals, Solon, OH) which we validated on extracted plasma using the standard addition method and tests of parallelism. Samples were run in duplicate (90/113) with the exception of some small volume samples which could only be run in singlet (23/113). Concentration of tT_3 was calculated using a 2-parameter log-fit. All samples were run in a single assay; the intra-assay CV was 7.1%.

2.4. Data analysis

All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC). We used log-transformed tT_3 and tT_4 values for all analyses to normalize residuals for the model analysis (see below); this also normalized the raw data. We evaluated the relationship between tT_3 and tT_4 using a Pearson's product moment correlation analysis for each life history stage (mating, lactation, post-lactation, and fattening). We investigated the factors affecting tT_3 and tT_4 using separate linear mixed models (LMMs) that included site and life history stage (LHS) as categorical predictors. Individual identity (ID) was included as a random effect to control for non-independence (repeated-measures of individuals) in the dataset. For LMM of T_3 , the g matrix was not positive definite and the estimated variance attributable to the random effect of individual was zero; removal of the random effect did not alter the results for fixed effects. Significant LHS effects were evaluated with post-hoc least-squares analysis using a Tukey–Kramer adjustment for multiple comparisons.

We used a one-way ANOVA to test whether above-ground activity duration differed among LHSs. Pearson's product moment correlation analyses were used to examine the relationship between aboveground activity and TH levels within each life history stage. We did not include activity data in our initial LMMs because this data was only collected for a subset of the animals for which we had TH data. However, we also examined the factors influencing tT_3 and tT_4 using LMMs on this subset of data. Our LMMs included site, LHS, and activity levels (mean daily activity in minutes during the week preceding blood sampling) as fixed effects. We performed all activity analyses on data collected in the week immediately prior to avoid any effect of capture on daily activity subsequent to sampling. Capture and handling of free-living animals can impact total activity and displacement for several days following intervention (e.g. Dennis and Shah, 2012; Morellet et al., 2009). We also tested for interactions between activity and LHS to account for LHS-dependent effects of activity on TH levels, with ID included as a random effect. All values are expressed as mean \pm SEM and differences considered significant at $P < 0.05$.

3. Results

3.1. T_3 and T_4 vary among life-history stages and site

Plasmas T_3 and T_4 levels both varied among life history stage (LHS) and site (T_3 , LHS: $F_{3,47} = 16.33$, $P < 0.0001$, site: $F_{1,47} = 7.62$, $P < 0.008$; T_4 , LHS: $F_{3,46} = 12.85$, $P < 0.0001$, site: $F_{1,46} = 5.33$, $P < 0.026$). The variance component attributable to the random effect of individual was $4.8 \pm 13\%$ for T_4 . Throughout the active season, female ground squirrels at the high-density site exhibited higher plasmas T_3 and T_4 compared to individuals at the low-density site (Figs. 1, 2). Regardless

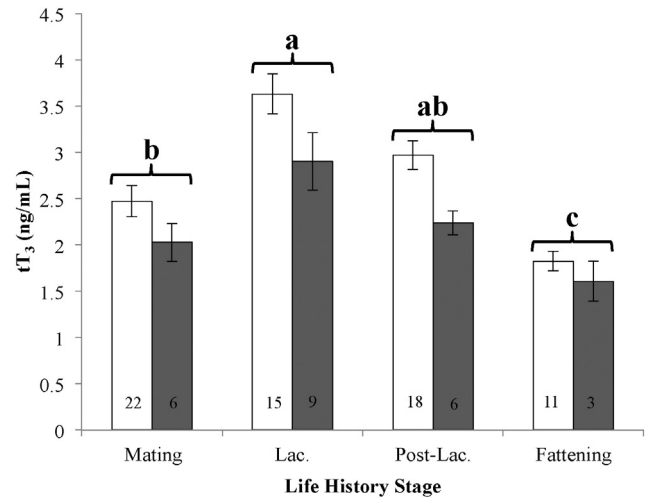


Fig. 1. Total T_3 of female arctic ground squirrels across the active season. Different letters represent significantly different tT_3 among times of the year as determined by post-hoc tests ($P < 0.05$). Open bars represent samples from the high-density field site. Filled bars were collected from the low-density field site. Numbers within the bar indicate sample size. tT_3 levels were highest during lactation (Lac.), but dropped post-lactation (Post-Lac.). tT_3 levels were lowest during mating and fattening.

of site, T_3 was highest during lactation, with lower circulating levels during the mating season, and the lowest circulating T_3 during fattening (Fig. 1). T_3 post-lactation was intermediate between levels observed during lactation and mating (Fig. 1). T_4 was highest in females at both sites during the mating season, but did not significantly differ among the later life history stages (Fig. 2).

Plasmas T_3 and T_4 were not significantly correlated with each other during the mating season ($r^2 = 0.04$, $P = 0.34$) nor during lactation ($r^2 = 0.17$, $P = 0.061$), but had a weak, positive correlation during both the post-lactation ($r^2 = 0.27$, $P = 0.011$) and fattening ($r^2 = 0.31$, $P = 0.037$) intervals.

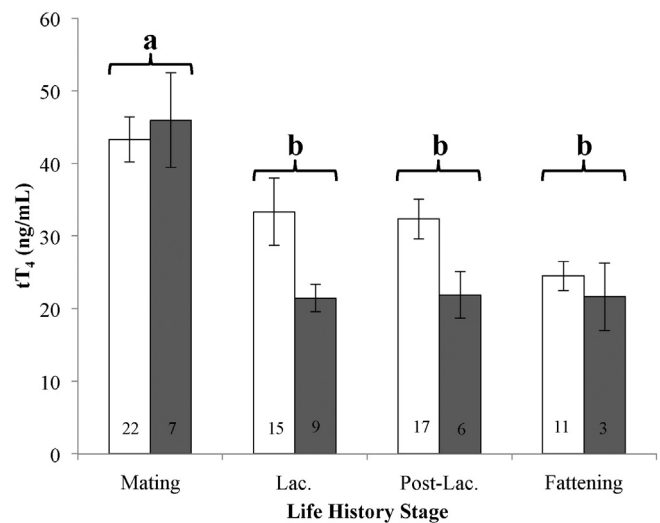


Fig. 2. Total T_4 of female arctic ground squirrels across the active season. Different letters represent significantly different tT_4 among times of the year as determined by post-hoc tests ($P < 0.05$). Open bars are individuals from the high-density field site. Gray bars are from the low-density field site. Numbers within the bar indicate sample size. tT_4 levels were highest immediately following mating, and remained lower during lactation (Lac.), post-lactation (Post-Lac.), and fattening. Circulating tT_4 levels are consistently much higher than levels of circulating tT_3 .

3.2. Thyroid hormones and activity co-vary, but only during certain life history stages

Average duration of above-ground activity of female arctic ground squirrels also varied among life history stages ($F_{3,43} = 69.80$, $P < 0.0001$). Activity duration was lowest during mating (193 ± 42 min.; $P < 0.01$), but did not differ among lactation (708 ± 17 min.), post-lactation (615 ± 27 min.), and fattening (621 ± 28 min) stages. Our model revealed a significant interaction between LHS and above-ground activity, indicating that the effect of activity duration on T_3 varies with LHS (Table 2). T_3 was positively correlated with activity during lactation ($r^2 = 0.50$, $P = 0.015$). Circulating T_3 was not correlated with activity during mating ($r^2 = 0.12$, $P = 0.33$), post-lactation ($r^2 = 0.07$, $P = 0.30$), or fattening ($r^2 = 0.04$, $P = 0.64$; Fig. 3).

Similar to T_3 , the LMM for T_4 showed that the relationship between above-ground activity and T_4 was dependent on LHS (significant interaction term, Table 2). The variance component attributable to the random effect of individual was $21.6 \pm 22.5\%$. T_4 was also not correlated with activity during mating ($r^2 = 0.30$, $P = 0.10$). However, in contrast to T_3 , T_4 did not correlate with activity during lactation ($r^2 = 0.04$, $P = 0.56$), but did exhibit significant, positive correlations during the post-lactation ($r^2 = 0.48$, $P = 0.002$) and fattening ($r^2 = 0.53$, $P = 0.039$; Fig. 4) periods.

4. Discussion

Surprisingly few studies have investigated variation in hormonal levels across life history stages in free-living mammals, and therefore little is known about the functional or behavioral consequences of such stage-specific variation. We found clear, significant changes in circulating levels of THs across life-history stages during the active season of a free-living rodent hibernator. More notably, we also found that activity levels were correlated with circulating THs. However, the relationship between THs and activity was clearly context-dependent and limited to specific life history stages. More studies utilizing free-living animals are clearly needed in order to create a broad understanding regarding the physiological mechanisms that modulate energetics in free-living animals.

The observed changes in circulating TH concentrations among life history stages are largely consistent with known changes in the physiology and energy expenditure of free-living, female arctic ground squirrels. The highest T_3 levels were measured during lactation, which coincides with peak MR levels in many rodent species (Johnson and Speakman, 2001; Veloso and Bozinovic, 2000; Zhang and Wang, 2007), including ground squirrels (Kenagy et al., 1989). Furthermore, female arctic ground squirrels exhibit elevated body temperature during lactation (Williams et al., 2011). Core body temperature makes up a substantial portion of RMR (Aschoff and Wever, 1958), so elevated T_3 coincident with elevated body temperature in these females suggests that T_3 may contribute to the increase in RMR through modulation of core body temperature.

Decreased plasma T_3 concentrations post-lactation and during fattening are consistent with previous work demonstrating that lean-mass-specific resting metabolic rate significantly decreases in the late

season for these animals (Sheriff et al., 2013). Interestingly, late-season changes in RMR in females are largely explained by, again, changes in core body temperature (Sheriff et al., 2012; Sheriff et al., 2013), supporting our argument that T_3 is likely related to changes in core body temperature. Late in the active season, decreased circulating T_3 probably also contributes to reduced lipid catabolism and enhanced lipid deposition.

In contrast to T_3 , T_4 showed relatively consistent levels during gestation, lactation, and through pre-hibernation fattening indicating peripheral T_4 may not be a relevant correlate for changes in RMR or body temperature across life history stages in this species. Female arctic ground squirrels exhibited contrastingly high T_4 during the early active season (mating stage); high T_4 during this period may be related to the transition from heterothermy of hibernation to active season homeothermy. Immediately following hibernation, animals also experience organ-specific growth, especially of the gastrointestinal tract (Hume et al., 2002), and recovery of the microbial community of the gut (Carey et al., 2013; Stevenson et al., 2014). These major physiological fluxes likely impact energy intake and energy allocation, and therefore may be associated with the unanticipated high T_4 levels in this period. However, very little is known regarding the role THs play, if any, in mediating the physiological transition from hibernation. Females become receptive and are impregnated by males within days of terminating hibernation and it's possible elevated T_4 is associated with reproductive physiology, rather than reflecting a transitional state from hibernation to activity. Elevated circulating T_4 is associated with increased circulating levels of estrogen, and TH administration can elicit reproductive onset in rats (Doufas and Mastorakos, 2000), suggesting that elevated T_4 during the mating season may be associated with activation of the reproductive axis.

In addition to investigating seasonal changes in peripheral concentrations of THs, we examined whether circulating THs were correlated with the level of above-ground activity within and across life history stages. We anticipated that average plasma levels of T_3 , which has previously been suggested as a proxy measure for BMR (Welcker et al., 2014), could also act as a physiological correlate of activity levels in ways that would be valuable for evaluating current conceptual models that link energetics and behavior. Positive correlations between THs and activity levels would provide some support the *performance* model, which proposes that increases in the metabolic machinery that drives basal metabolism ought to facilitate increases in other metabolically expensive processes, including activity. In contrast, negative correlations would support the *allocation* model, which is based on the idea that animals have limited energy available to allocate towards disparate processes.

Although we anticipated that T_3 , the active form of TH, would play an important role in modulating metabolism beyond BMR, we found no evidence for a consistent relationship between T_3 and our measure of activity levels across the active season in female arctic ground squirrels, albeit with small sample sizes, especially during the fattening period. T_3 was not correlated with activity within three of the four life history stages (mating, post-lactation, & fattening). However, T_3 was positively ($r^2 = 0.50$) correlated with above-ground activity during lactation, which is consistent with the *performance* model. In contrast, we were surprised to find that T_4 was significantly correlated with activity during two of the life history stages we evaluated (post-lactation and during fattening). These correlations between T_4 and activity levels were positive, which also provides support for the *performance* model of energy management. Interestingly, high levels of T_4 in the mating season were combined with relatively low activity levels, which would produce a negative correlation between activity and T_4 across the active season if life history stages were excluded. However, the significant interaction between life history stage and above-ground activity suggests that such an analysis would produce spurious results that are being driven by unique thyroid hormone levels seen during the mating season.

Table 2

Models testing the relationship between THs and above-ground activity (AGA) duration in free-living female arctic ground squirrels among life history stages (LHS). Location refers to high- versus low-density field sites. Significant P-values are italicized.

Effect	tT_3			tT_4		
	DF	F	P	DF	F	P
LHS	3,18	2.55	0.088	3,17	13.7	<i><0.0001</i>
Location	1,18	1.26	0.28	1,17	4.28	0.054
AGA	1,18	1.24	0.28	1,17	5.08	<i>0.038</i>
LHS × AGA	3,18	3.61	<i>0.034</i>	3,17	8.39	<i>0.0012</i>

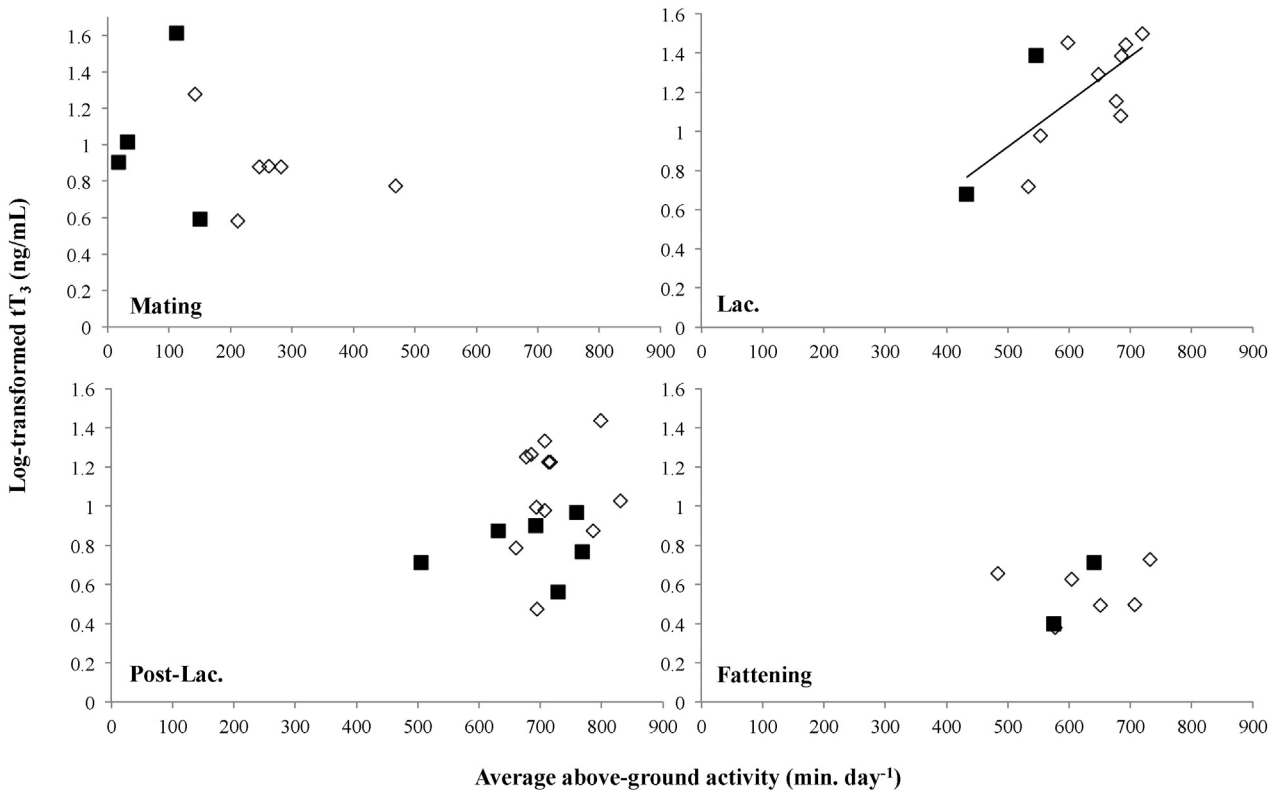


Fig. 3. Correlations between average daily activity, measured as time spent above-ground, and plasma tT₃ of female arctic ground squirrels. Data are blocked by phase of the annual cycle, as indicated in the bottom left of each block: mating; lactation, (Lac.); post-lactation, (Post-Lac.); fattening. Filled-in squares represent individual samples collected at the low-density field site, open diamonds were collected from individuals at the high-density field site. Regression lines are present where significant ($P < 0.05$) relationships exist. Average daily activity was significantly correlated with tT₃ only during lactation ($r^2 = 0.50$).

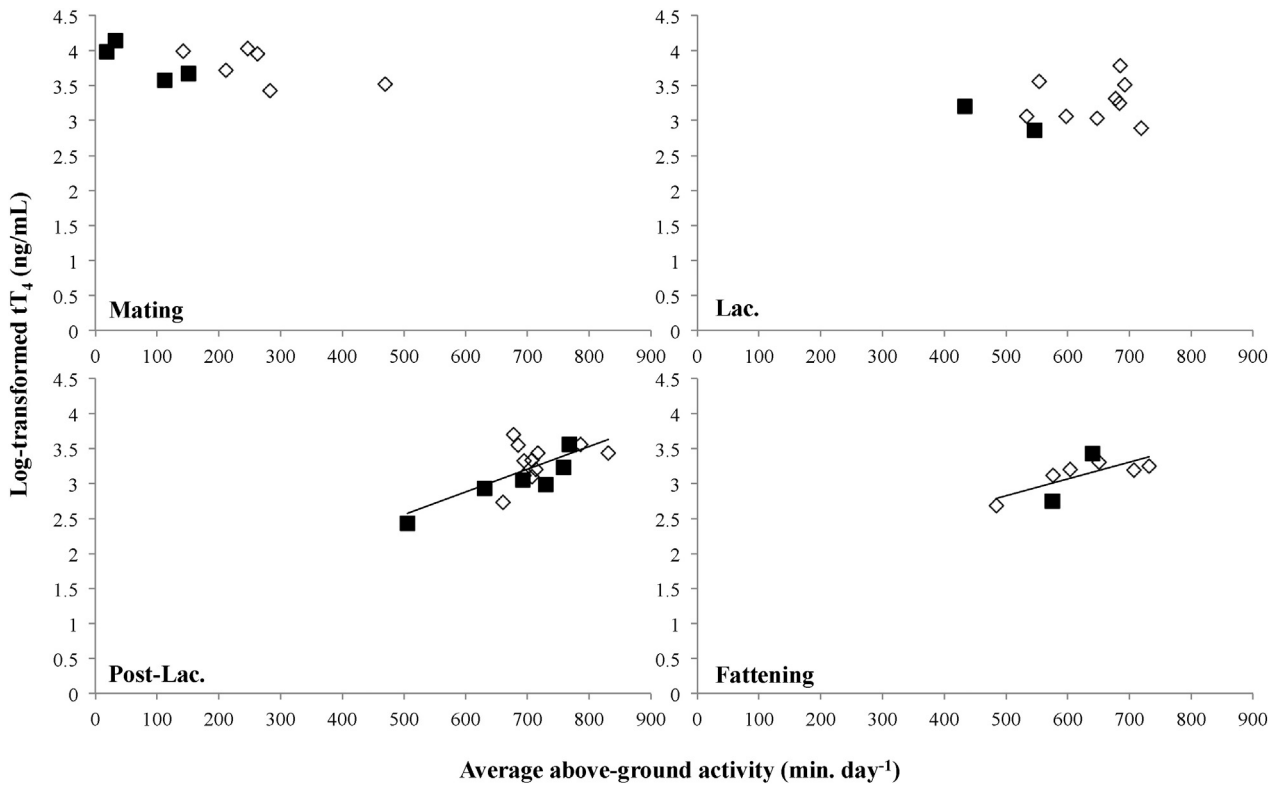


Fig. 4. Correlations between average daily activity, measured as time above-ground, and plasma tT₄ of female arctic ground squirrels. Data are blocked by phase of the annual cycle, as indicated in the bottom left of each block: mating; lactation, (Lac.); post-lactation, (Post-Lac.); fattening. Filled-in squares represent individual samples collected at the low-density field site, open diamonds were collected from individuals at the high-density field site. Regression lines are present where significant ($P < 0.05$) relationships exist. Average daily activity was significantly correlated with tT₄ post-lactation ($r^2 = 0.48$) and during fattening ($r^2 = 0.53$).

The more consistent role of T_4 as a physiological correlate of activity level suggests that measuring circulating T_4 may be more important, at least in mammalian species, than has previously been considered. While we may have a better understanding of the direct relationship between T_3 and metabolic rate, it should not be acceptable to solely measure and interpret T_3 levels. Our study demonstrates that T_4 probably plays unexpected roles, at least in mammals. More extensive sampling, using larger populations and looking across sexes and ages of individuals, will help clarify the extent to which T_4 contributes to metabolism and activity of free-living mammals.

The measurement of TBGs and free (unbound) THs along with total TH availability, when possible, may also contribute to our understanding of how THs influence physiology and behavior. Thus far, TBGs have been primarily implicated in the modulation of thyroid hormone availability during hibernation in ground squirrels (Magnus and Henderson, 1988; Young et al., 1978). The role of TBGs in modulating active season free TH in the periphery of mammals, especially in the context of activity and energetics, is unknown. However, Elliott et al. (2013) demonstrated that, in kittiwakes, total T_3 exhibited a stronger correlation with resting metabolic rate within a single life history stage than did free T_3 , supporting the idea that total TH levels are indeed relevant to activity and metabolic rates. Further investigation is required to determine whether TBGs contribute to the stage-specific modulation of the relationship between activity and thyroid hormone levels by gating availability to peripheral tissues.

In terms of the proposed conceptual models linking metabolism and behavior, our findings are consistent with recent studies that support the *performance* model (reviewed in Biro and Stamps, 2011; Mathot and Dingemans, 2015). However, we demonstrated this relationship using thyroid hormones, a physiological correlate and potential mediator of metabolic expenditure, and behavioral output in a mammalian system. Moreover, as emphasized by Mathot and Dingemans (2015), exceptionally few studies examine variation across life history stages. Of the studies using direct measures of metabolism, Careau et al. (2013) demonstrated a positive relationship between RMR and daily energy expenditure in reproductive eastern chipmunks, but not in non-reproductive animals, which is consistent with our findings. However, zebra finches exhibit the opposite pattern: a positive relationship between RMR and daily energy expenditure in non-reproductive individuals, and no relationship during reproduction (Vezina et al., 2006). What is still to be determined is whether these patterns across life history stages are species-specific or more universal.

We aim to contribute to the growing framework for further research into thyroid hormone function in free-living animals, though we acknowledge that there are several caveats to our study that need to be addressed. For example, while our light logger data provides quantitative data on time spent above ground, more direct quantitative measures of activity, such as obtaining movement data using accelerometers, may provide a more robust method to further test the hypotheses laid out here. There is also a need to better establish the importance of inter-individual variability in TH levels. In our study, we were able to ascribe very little of the variance in TH levels to individual, but we suspect this is likely due to our difficulty in obtaining repeated measures of all individuals across the reproductive season.

More importantly, data presented here represents a single year with limited sample sizes and validation of our findings is needed. Though short-lived songbirds appear to exhibit stage-specific hormone levels and responsiveness (Jawor et al., 2007; Shultz and Kitaysky, 2008), multi-year datasets in seabird species suggest that, in long-lived species, glucocorticoid concentrations vary in response to environmental conditions but not life history stage (i.e., Kitaysky et al., 2007; Shultz and Kitaysky, 2008). In mammals, a single study found variation in baseline glucocorticoids occurred both among life history stages and between years (Delehanty and Boonstra, 2012). Comparable data are not

available for THs, although variation in food availability did not affect the relationship between daily energy expenditure and basal metabolism in a free-living squirrel (Careau et al., 2013). Explicitly testing whether the relationship between TH and activity is primarily modulated by life-history stage or environmental factors will be important for determining how energy availability might constrain or alter the relationships reported here.

Moreover, further work needs to be done to understand whether these physiological correlates are actually drivers of activity or behavior. While we have primarily focused on peripheral actions of THs, the intriguing possibility remains that changes in activity seen here are a result of TH action in the brain. Elevating peripheral TH levels inhibits estrogen-dependent behaviors such as lordosis in mice and rats (Dellovade et al., 1996; Morgan et al., 2000) and decrease exploratory-measures of anxiety in male mice and rats (Buras et al., 2014; Helmreich and Tylee, 2011). Thus, changes in peripheral TH levels could be modulating behavior through central action. However, thyroidectomized female rats do not change activity patterns (McEachron et al., 1993; Schull et al., 1989), and others have suggested that differential expression of TRs in the brain mediate sexual behavior in females, not changes in peripheral TH levels (Dellovade et al., 2000). Whether peripheral mechanisms act in parallel or complimentary to central actions of THs requires more attention.

As a final note, it is interesting that our two study sites consistently differed in their peripheral TH levels. Very little work has described population-level variation in circulating THs, particularly in populations in close proximity to one another. Elevated levels of THs have been reported in free-living white stork (*Ciconia ciconia*) populations with higher nesting density (Baos et al., 2006), which is consistent with the population-level differences observed in our study. However, whether population density is driving the observed difference in TH levels is unclear. In our study, the higher density population spent, on average, more time above-ground each day compared to the low-density population. In addition to lower activity levels, our low-density site has, on average, slightly lower ambient temperatures across the active season (1.3 ± 0.1 °C difference; unpublished data). Whether the consistent differences in TH levels between the two sites are related to activity levels and/or environmental conditions is currently unclear, although it is notable that our finding of higher TH in more active individuals is consistent within and across sites.

5. Conclusions

We found that plasma levels of T_3 , the biologically-active form of TH, changed across the active cycle in free-living arctic ground squirrels, peaking during the most energetically expensive life history stage (lactation) and reaching its lowest level immediately prior to hibernation, when RMR is known to be depressed. We also found significant relationships between plasma TH levels and activity. T_3 , which may be a useful proxy of RMR, was positively correlated with activity during lactation providing limited support for the concept that the machinery that drives basal metabolism ought to facilitate elevation of other metabolic processes. However, this relationship did not hold true during other life-history stages. Interestingly, T_4 was positively correlated with activity during other life history stages to the same degree as T_3 during lactation and both THs were elevated across the entire active season at the high-density site where animals were more active. Our results suggest that the relationship between TH and activity varies with life history stage. More work across taxa is needed to test if the complex, stage-dependent relationships between THs and activity duration represent universal, physiological links between an endocrine driver and behavioral output. Our findings open several avenues of questioning that will develop a broader understanding of the connection between peripheral physiological mechanisms and behavioral output, especially of free-living animals.

Acknowledgments

The authors thank J. Moore, V. Zhang, and A. Wille for their field assistance, as well as D. Dillon and L. Smayda for their assistance with developing and running thyroid hormone assays. We are also grateful to staff of Toolik Field Station (TFS) for their logistical support.

This research was supported by the National Science Foundation to C.L.B. and C.T.W. (IOS-1147187) and B.M.B. (IOS-1147232). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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