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Age differences in night-time metabolic rate and body temperature in a small passerine

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Abstract

Spending the winter in northern climes with short days and cold ambient temperatures (T_a) can be energetically challenging for small birds that have high metabolic and heat loss rates. Hence, maintaining body temperature (T_b) in T_a below thermoneutrality can be energetically costly for a small bird. We still know little about how increased heat production below thermoneutrality affects the level at which T_b is maintained, and if these patterns are age specific. To test this, we measured subcutaneous body temperature (T_s) and resting metabolic rate (RMR) simultaneously in blue tits (*Cyanistes caeruleus*) during winter nights in T_a 's ranging from 25 to -15 °C. RMR increased below the lower critical temperature (LCT, estimated at 14 °C) and was 6% higher in young (birds in their first winter) compared to old birds (birds in their second winter or older). The higher RMR was also mirrored in higher T_s and thermal conductance (C) in young birds, which we suggest could be caused by age differences in plumage quality, likely driven by time constraints during moult. Reduction in nightly predicted T_b was modest and increased again at the coldest ambient temperatures, suggesting that either heat retention or heat production (or both) improved when T_a reached levels which are cold by the standards of birds in our population. Our results show that levels of heat production and T_b can be age specific. Further studies should address age-specific differences on quality, structure, and thermal conductivity of plumage more explicitly, to investigate the role of variation in insulation in age-linked metabolic phenotypes.

Keywords Thermoregulation · Hypothermia · Age effects · Thermogenesis · Bird · Metabolic rate

Introduction

Animals that overwinter at northern latitudes face several energetic challenges. It is often cold, days are short, and food supply can be scarce. Such conditions are especially challenging for small birds that have high surface area to volume ratio (which increases heat loss rate), high mass-specific metabolic rate, and limited capacity to store energy as fat (e.g., Haftorn 1992). Thus, a small bird in winter needs to minimize energy expenditure while simultaneously

maximizing energy intake and heat production capacity. Several behavioral adaptations, such as communal roosting (DuPlessis and Williams 1994), choosing optimal roosting sites (Elkins 2004), and ptiloerection (Hill et al. 1980; Hohola et al. 1980) reduces heat loss and energy expenditure in thermally challenging environments. Physiologically, one way to conserve energy is to reduce body temperature, T_b , during resting periods, which is typically referred to as shallow hypothermia, rest-phase hypothermia or night-time hypothermia (McKechnie and Lovegrove 2002). Hypothermia can amount to significant energy savings (Reinertsen and Haftorn 1986; Cooper and Gessaman 2005) and is predicted to substantially increase winter survival even when energy savings are moderate (Brodin et al. 2017). Despite the potential energetic savings and benefits to winter survival, birds seem to attenuate or avoid nightly hypothermia in milder ambient temperatures (T_a), when food is plentiful and when body condition is high (Reinertsen and Haftorn 1986; Dolby et al. 2004; Nord et al. 2009, 2011). This indicates that there are costs associated with the use of

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hypothermia, e.g., impaired quality of sleep (Mueller et al. 2012), impaired immune function (Nord et al. 2013; Sköld-Chiriac et al. 2015), and increased predation risk (Laurila and Hohtola 2005; Carr and Lima 2013; Andreasson et al. 2019).

The energy expenditure of any resting bird or mammal (assuming a post-absorptive state) is a function of its metabolic rate which in turn is related to its T_b and thermal conductance, C . When T_a falls below the lower critical temperature (LCT), i.e. below the thermoneutral zone (TNZ) where basal heat metabolism is enough to maintain preferred T_b , heat must be produced to defend T_b . In birds, heat is believed to be produced mainly by shivering thermogenesis (Marsh and Dawson 1989; Hohtola 2004), although there is evidence for non-shivering thermogenesis in a few species (Duchamp et al. 1992; Duchamp and Barré 1993; Teulier et al. 2010). The thermal conductivity of the plumage has a large effect on total conductance. This probably explains why plumage insulation changes as part of a seasonal acclimatization to winter conditions (e.g., Dawson and Carey 1976; Swanson 1991; Cooper 2002) and is more developed in birds residing further north (Pap et al. 2017; Osvath et al. 2018). Yet, plumage quality is still a somewhat static trait that is mainly modulated during moult, and so not amenable for variation on a night-to-night basis. Metabolic rate and nocturnal hypothermia, on the other hand, are open for dynamic and strategic changes that potentially depend on T_a and internal states such as size, age and nutritional state. However, although several studies have investigated nocturnal hypothermia in small, northern passerines (e.g., Palmgren 1944; Udvardy 1955; Steen 1958; Haftorn 1992; Hill et al. 1980; Reinertsen and Haftorn 1983; Reinertsen and Haftorn 1986) and the proximate mechanisms that determine the depth and use of hypothermia (i.e., ambient conditions, body condition/reserves, food availability and predation risk; Reinertsen and Haftorn 1986; Dolby et al. 2004; Nord et al. 2009, 2011; Andreasson et al. 2019), we still know relatively little about how increased heat production below thermoneutrality actually affects at which level T_b is maintained. Some studies have measured both metabolic rate and T_b continuously (black-capped chickadees (*Poecile atricapillus*): Chaplin 1976; Grossman and West 1977; willow tits (*Poecile montanus*): Reinertsen and Haftorn 1983; Reinertsen and Haftorn 1984; greenfinches (*Chloris chloris*): Saarela et al. 1995) in T_a below thermoneutrality. Those studies have provided valuable information on annual and circadian variation in T_b and metabolic rate and their relationship with food availability, T_a and shivering. However, to the best of our knowledge, the effect of state variables, such as age, on T_b and metabolic rate in “the little bird in winter” (sensu Brodin 2007) is yet to be tested.

Age is a particularly interesting state variable as young and old birds may differ in both heat producing and heat

retaining capacity, likely as a consequence of different plumage properties, and age-related differences in metabolic rate and T_b . Juveniles need to finish growing and attain a certain body condition before energy-consuming moult can begin (Ginn and Melville 1983; Vágási et al. 2012), while adult blue tits (*Cyanistes caeruleus*) can commence their complete post-nuptial moult earlier (sometimes already while feeding their chicks: Svensson and Nilsson 1997). Thus, a more time-constrained, delayed moult of juveniles could lead to plumage of inferior insulating capacity (Nilsson and Svensson 1996). Moreover, great tits (*Parus major*) from a northerly population, being more time constrained during moult due to shorter day lengths, produce contour feathers that are likely of inferior insulating capacity compared to birds from a more southern population (shorter, and with a lower proportion of plumulaceous barbs: Broggi et al. 2011). This pattern is also clear in Svalbard ptarmigan (*Lagopus muta hyperborea*) where young birds have a higher resting metabolic rate, RMR, and C compared to older conspecifics in winter after post-juvenile moult (where they are time constrained), but not the following summer where onset of moult is the same for both age groups (Nord and Folkow 2018). In addition, young blue tits keep a higher T_b in cold ambient temperatures when faced with a higher perceived predation risk compared to older birds (Andreasson et al. 2019), which would require either amplified heat production, reduced heat loss or both. Thus, physiological and behavioral age-related differences could potentially shape the relation between metabolic rate and T_b differently during a cold winter night.

Our aim was to study patterns of T_b regulation and metabolic rate in the “little bird in winter”, with a specific focus on potential differences between young and old birds. Given the potential age-related variation in both plumage quality and metabolic capacity, we predicted that LCT and metabolic rate below LCT would be higher in birds that were in their first winter (young) compared to those that had experienced at least one winter before (old). To test this, we measured metabolic rate and nightly subcutaneous body temperature, T_s , simultaneously during sliding cold exposure (+25 to –15 °C) in young and old, wild, blue tits in winter.

Methods

Instrumentation for T_b measurement

The blue tit is a small (11–13 g), hole-nesting passerine with a high mass-specific metabolic rate. In southern Sweden, the blue tits that remain resident over winter (Smith and Nilsson 1987) and routinely use nest boxes for non-communal night roosts, making them well suited for this kind of study. All birds were free-living blue tits captured when roosting

in nest boxes around Lake Krankesjön, 20 km east of Lund in southern Sweden (55° 42' N, 13° 28' E) from 23 Nov 2017 to 30 Jan 2018. When a roosting blue tit was found, we weighed (± 0.1 g), ringed, and aged the bird based on plumage characteristics (Svensson 1992) and also measured tarsus- (± 0.1 mm) and wing length (± 0.5 mm). Then, we implanted a temperature-sensitive passive integrated transponder tag (PIT tag; BioTherm13-Biomark, Boise ID, USA; height: 13.0 mm; diameter: 2.1 mm) subcutaneously in the neck under aseptic conditions (following Nord et al. 2013; Sköld-Chiriac et al. 2015). The incision hole was sealed using cyanoacrylate (Loctite Power Easy Gel, Henkel AG & Company, Düsseldorf, Germany) before the bird was returned to the nest box. In total, 65 birds were implanted. Three additional birds that were already tagged during the breeding season in 2017 were also used in this study.

Metabolic rate measurements

PIT-tagged birds were recaptured when roosting in nest boxes just after sunset 32 ± 14 (\pm SD) days after instrumentation (range 6–78 days). Two birds per night were brought to Lund University for measurement of metabolic rate and T_b starting 1.6 ± 0.4 h after sunset (mean start time 17:57 GMT + 1) and lasting throughout the night (1.2 ± 0.4 h before sunrise; mean end time 06:58 GMT + 1), after which the birds were returned to the nest boxes in which they had been caught. All measurements took place between 19 Dec 2017 and 15 Feb 2018.

We put birds individually in cylindrical 0.6 L, hermetic, polyvinyl chloride (PVC) chambers placed in a dark, temperature-controlled cabinet (Weiss Technik WK3 C180, Reiskirchen, Germany). An identical chamber was left empty for baselining. Chamber temperature, T_a , was measured continuously with a thermocouple (36 gauge, type T, copper–constantan) connected to a thermocouple meter (TC-2000, Sable Systems). The climate cabinet temperature changed in incremental steps of 5 °C, so that birds were subjected to a temperature regime starting at 25 °C in the evening and ending at – 15 °C in the morning (standard order, $n = 20$) or starting at – 15 °C in the evening and ending at 20 °C in the morning (reversed order, $n = 10$). All birds were placed in the climate cabinet, set at the starting temperature of the program (25 °C or – 15 °C) for 80 min acclimation before data collection commenced. Each temperature step lasted 80 min and during this time each animal was measured for 20 min which was preceded and succeeded by 20 min of baselining via the empty chamber. Baseline gas concentrations were calculated as the mean of the last minute of baselining before and after each animal measurement. This protocol was adequate to obtain stable RMR and T_b . Subcutaneous body temperature, T_s , was measured each min by implanted PIT

tags, and was recorded by a Biomark HPR Plus data logger (Biomark, Boise ID, USA) connected to an antenna placed directly beneath the metabolic chambers.

We pushed (mean flow \pm SD) 287 ± 9 mL min⁻¹ (STP) pressurized air (measured by a FlowBar-8 multichannel mass flow meter, Sable Systems) over each channel. Effluent air was measured for relative humidity (RH-300, Sable Systems) before water vapor was removed by Drierite (W.A. Hammond Drierite Company, Xenia, OH) and the gas stream was analyzed sequentially for carbon dioxide (CA-10A, Sable Systems) and oxygen (FC-10A, Sable Systems). Gas concentrations, relative humidity, flow rate, and T_a were recorded every second, and were digitized by a UI2 data acquisition interface (Sable Systems). Switching between channels was achieved automatically using a RM8 multiplexer (V3, Sable Systems).

We calibrated the carbon dioxide analyzer using 100% N₂ and 1% CO₂ in N₂. The oxygen analyzer was calibrated using 100% N₂ and incoming air (20.95% O₂). Calibration of the carbon dioxide analyzer, and zero calibration of the oxygen analyzer, was performed immediately before the start of the experiment. Span calibration of the oxygen analyzer was performed daily. In addition, we calibrated the oxygen analyzer using a gas of known O₂ concentration (20.4%, in N₂) on three separate occasions during and directly after the measurement series. The RH meter had been factory calibrated shortly before the experiment started.

Calculation of metabolic rate

We calculated O₂ consumption rate (V_{O_2}) in mL min⁻¹ following Eq. 10.6 in Lighton (2008):

$$V_{O_2} = f_{STPD} \times \frac{(F_{I_{O_2}} - F_{E_{O_2}}) - F_{E_{O_2}} \times (F_{E_{CO_2}} - F_{I_{CO_2}})}{1 - F_{E_{O_2}}}, \quad (1)$$

where $F_{I_{O_2}}$, $F_{I_{CO_2}}$, $F_{E_{O_2}}$, $F_{E_{CO_2}}$ are influent and effluent fractional concentrations of O₂ and CO₂, respectively, and f_{STPD} is standard temperature and pressure, dry (STPD)-corrected flow rate (mL min⁻¹) calculated by subtracting f_{H_2O} (Eq. 2 following Nord and Folkow 2018) from standard temperature- and pressure-corrected flow, f_{STP} (mL min⁻¹), as measured by the flow meter. In Eq. 2, T_{gas} is gas temperature, RH is relative humidity (here we used fixed values of 13.17625 °C and 8.60821% based on mean T_{gas} , and RH measured over a whole night (18:00–06:00 GMT + 1) on 21 Feb 2018) and BP is barometric pressure (mmHg) during the experimental night, measured at Malmö Airport, 22 km from Lund University (Swedish Meteorological and Hydrological Institute 2018, unpublished data)

$$f_{\text{H}_2\text{O}} = f_{\text{STP}} \times \frac{(\text{RH}/100) \times 4.588 \times 10^{(7.59 \times T_{\text{gas}})/(240.73 + T_{\text{gas}})}}{\text{BP}} \quad (2)$$

We converted oxygen consumption to energy consumption (W) assuming an oxyjoule equivalence of 20 J mL⁻¹ O₂ (Kleiber 1961). Wet thermal conductance, C (W °C⁻¹), was calculated following Aschoff (1981):

$$C = \text{RMR}/(T_s - T_a) \quad (3)$$

Sample sizes

All analyses of RMR, C , T_s and T_a were made on mean values per minute for each individual of the last 10 min in each temperature interval (to obtain the most stable readings). Thus, total sample size was data from 260 temperature intervals from 30 individuals (20 individuals with 9 temperature intervals—standard, 10 individuals with 8 temperature intervals—reversed). We wanted to exclude data that were indicative of either stress and/or activity. Since we did not record behavior, we excluded data where the standard deviation in oxygen consumption during the 10 min of measurement were > 2 SD larger than the mean standard deviation ($n = 11$). One additional observation was excluded as the temperature profile clearly suggested substantial activity. Thus, final sample size was 248 observations based on 30 individuals (young: $n = 16$, old: $n = 14$). Due to technical malfunction, we were unable to record T_s for three of these observations.

Predicted T_b

To evaluate the relationship between T_s and cloacal temperature, T_c , we measured these two parameters simultaneously in four birds, outside of the main experiment. The birds were placed in the metabolic chambers in the climate cabinet and subjected to the same temperature regime (reversed order from -15 to 20 °C) over the full night. T_s was recorded each minute as in the main experiment, and T_c was recorded each s by inserting a 36-gauge type T thermocouple attached to a thermocouple meter (TC-2000, Sable Systems) 12 mm through the cloaca. The thermocouple was fixed to two tail feathers using surgical tape. We excluded data from one bird where the thermocouple had been dislodged during the night. The three full profiles of T_s and T_c (see Fig. 1a for a representative example) were used to calculate mean difference (ΔT) between T_s and T_c at each temperature intervals (Fig. 1b). These temperature-specific values were used to calculate predicted T_b for all experimental birds.

Statistical analyses

All statistical analyses were performed in R ver. 3.5.1 (R Development Core Team 2018). To determine LCT, we used a mixed “broken-stick” model (lmer package: Bates et al. 2015) with RMR as the dependent variable with a variable break point (see Supporting Information for R code), T_a as a covariate and bird ID as a random factor. Two identical models were also fitted to estimate LCT in the two age groups.

We then used linear mixed models (lmer package) to evaluate the effect of age and T_a on RMR both below and above (overall) LCT, respectively. We also included the two-way interaction age \times T_a . Bird ID was added as a random factor (random intercept for models above LCT and random intercept + slope for models below LCT). An identical model was also fitted for thermal conductance below LCT. To account for the effect of body mass on metabolic rate, we also ran these models with body mass as a covariate, avoiding the use of mass-specific indices (see Packard and Boardman 1999; Hayes 2001). Body mass was corrected for time of measurement, i.e. we calculated the body mass of the bird at the actual time of measurement assuming a linear decrease over time from evening to morning body mass. Mean (\pm SD) evening and morning body mass was 12.1 ± 0.9 and 11.3 ± 0.9 g, respectively. Estimated regression lines and predicted values from these models are presented in text and figures as mass-adjusted RMR and mass-adjusted conductance.

For subcutaneous temperature, T_s , and predicted body temperature, T_b , we fitted similar models but with centered T_a . In addition, we also added T_a^2 and the interaction with age to account for the quadratic pattern.

All lmer models were fitted using restricted maximum likelihood (REML) and interactions with $p > 0.10$ were removed from final models. Estimates for factors (\pm SE) presented in text and tables are estimated marginal means (emmeans package: Lenth 2019) unless otherwise stated.

Results

Resting metabolic rate

Based on total RMR, LCT over both age groups was 14.2 °C (95% CI 13.3 – 15.0 °C) (Fig. 2a) and age-specific estimates of LCT were 14.2 °C (95% CI 13.1 – 15.2 °C) and 14.3 °C (95% CI 12.9 – 15.5 °C) for young and old birds, respectively. Below the overall LCT, RMR increased with 0.0093 ± 0.0002 W for every 1 °C decrease in T_a ($P < 0.0001$, Table 1, Fig. 2a). Young birds had a 6% higher RMR compared to old birds throughout the T_a -range below LCT ($P = 0.013$, Table 1, Fig. 2a), but the difference between the age groups did not change with T_a (age \times T_a

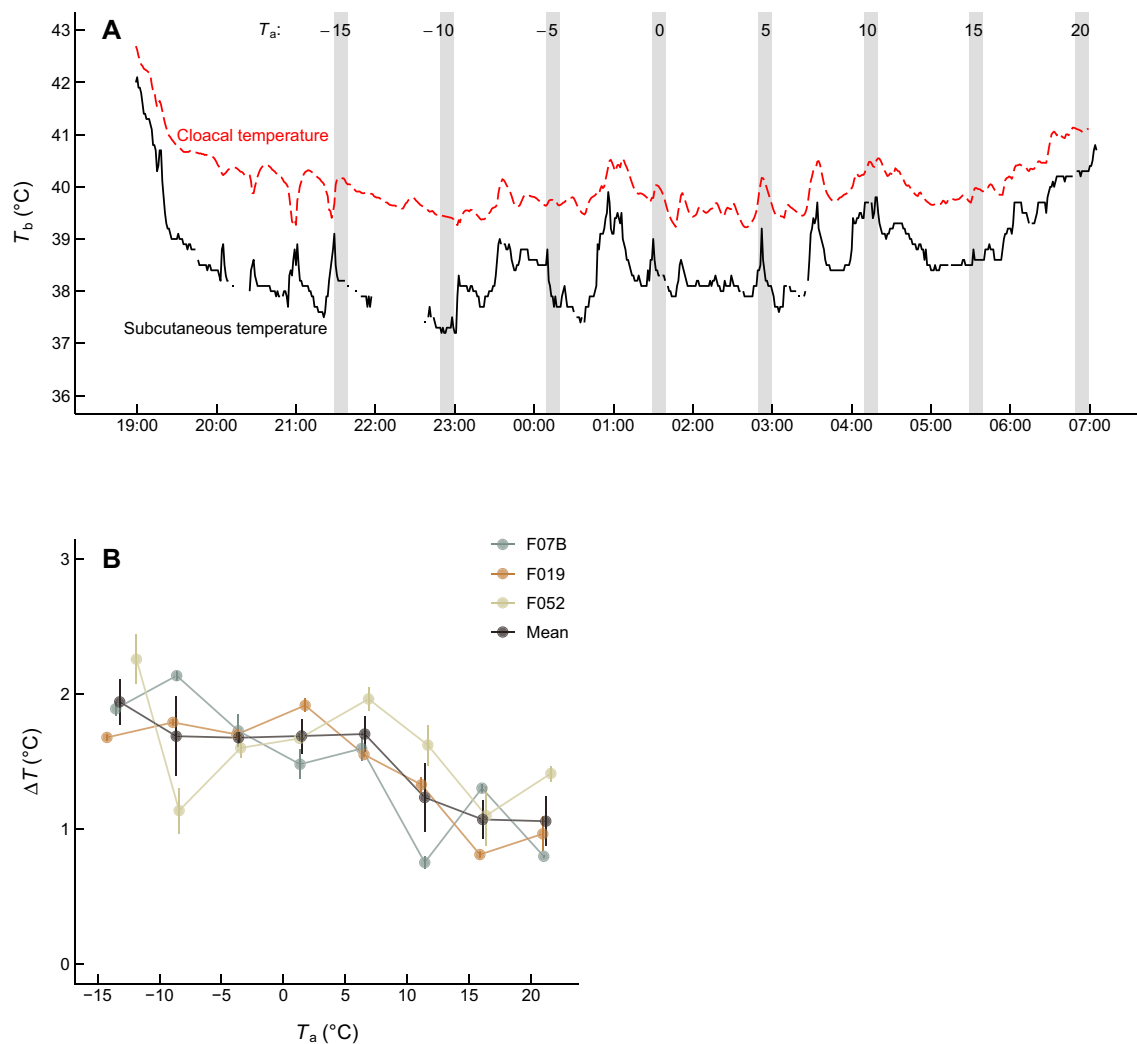


Fig. 1 **a** Example of T_b during a whole night of measurement, where T_b was continuously measured both subcutaneously (black), T_s , and in the cloaca (red), T_c . Grey bars indicate incremental increases in T_a and where data were extracted to create **b**. **b** Mean (\pm SE) difference,

ΔT , between subcutaneous temperature, T_s , and cloacal temperature, T_c , as a function of T_a . Measurements are based on three individuals ($n=3$) (color figure online)

interaction: $P=0.18$). RMR above LCT (BMR) was also 7% higher in young birds compared to old birds ($P=0.028$, Table 1, Fig. 2a) and increased with increasing T_a ($P=0.005$, Table 1, Fig. 2a). Body mass correlated positively with RMR, both below ($P=0.0002$) and above ($P=0.003$) LCT, and when body mass was accounted for, mass-adjusted RMR also tended to increase more with decreasing T_a in young birds compared to old birds (age $\times T_a$ interaction: $P=0.054$, Fig. 2b, Table 1) below LCT. However, when body mass was accounted for, RMR above LCT did not differ between young and old birds ($P=0.13$). Mean (\pm SD) respiratory quotient across the whole T_a gradient was 0.715 ± 0.005 , indicating that fat was the main substrate being catabolized during experiments (Fig. S1).

Subcutaneous and predicted body temperature

Both T_s and predicted T_b showed a quadratic relationship with T_a , where T_s and predicted T_b initially decreased as T_a decreased, but subsequently increased again at the lowest T_a (Fig. 3). This effect was most pronounced in old birds, both for T_s (age $\times T_a^2$ interaction $P=0.005$, Table 1, Fig. 3a) and for predicted T_b (age $\times T_a^2$ interaction $P=0.006$, Table 1, Fig. 3b).

Thermal conductance

Overall, thermal conductance decreased with decreasing T_a below the LCT ($P<0.0001$), was higher in young

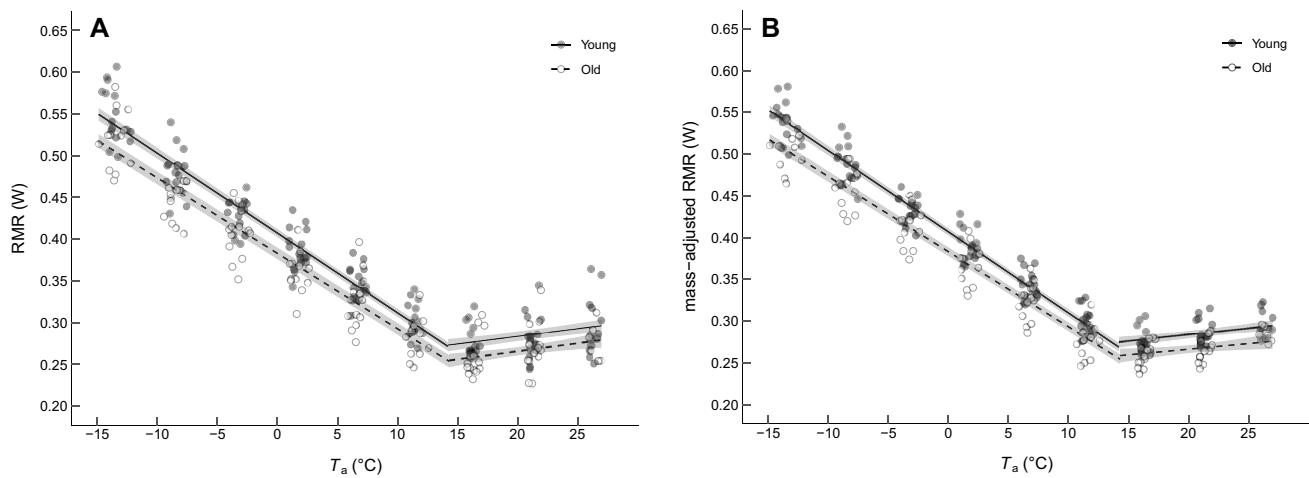


Fig. 2 **a** Whole animal resting metabolic rate (raw data) and **b** mass-adjusted resting metabolic rate (fitted values) in young and old blue tits as a function of T_a . Age-specific regressions are model estimates (\pm SE) from separate linear mixed models above and below LCT

birds ($P=0.012$) and positively affected by body mass ($P=0.0001$) (Fig. 4).

Discussion

Metabolic rate increased linearly below LCT. Mean (\pm SD) resting metabolic rate above LCT (i.e. BMR, assuming a post-absorptive state) was 0.269 ± 0.024 W across age groups which align well with previous studies in winter. For example, BMR reported here was 4% higher and 11% lower than BMR in blue tits previously measured in this population (55° N) and in a more northerly population in Oulu, Finland (65° N) (Broggi et al. 2019), and 6% lower and 2% higher compared to similar sized black-capped chickadees in South Dakota, USA (42° N; Cooper and Swanson 1994) and in Quebec, Canada (48° N; Petit and Vézina 2014), respectively. RMR (\pm SD) at -15° C was 0.536 ± 0.036 W (i.e., twice that of BMR), suggesting that the blue tits in our experiment were far from using their full thermogenic capacity as summit metabolic rate (M_{sum} —maximum MR during cold exposure) usually range from 5 to $8 \times$ BMR in other passerines (Dutenhoffer and Swanson 1996).

As predicted, young birds had a higher metabolic rate below thermoneutrality. This was accompanied by higher T_s and predicted T_b . However, RMR above LCT was also higher in young birds and consequently LCT did not differ between the age groups. Using the mean predicted T_b above LCT (39.4° C) and mean evening body mass (12.1 g), predicted LCT for a resting blue tit is expected to be somewhere between 21.1° C (Eq. 3 in Weathers and Van Riper 1982) and 25.9° C (Eq. 18 in Calder and King 1974). Thus, the observed value of 14.2° C is well below

allometric predictions. It is also lower than empirical data from similarly sized, phylogenetically related, black-capped chickadees in South Dakota (18.3° C, Cooper and Swanson 1994), but aligns well with LCT in Alaskan black-capped chickadees (14.4° C, Grossman and West 1977) and redpolls *Acanthis flammea* (11.5° C, Pohl and West 1973) which are naturally exposed to very low ambient temperatures.

In general, most birds did not reduce predicted T_b more than $4\text{--}5^\circ$ C from daytime levels (42.6° C: Nord et al. 2009). Minimum predicted T_b (\pm SD) was attained when T_a was around -5° C and averaged $38.3 \pm 0.7^\circ$ C for young birds and $37.6 \pm 1.1^\circ$ C for old birds. These measures of predicted T_b align well with previous studies of night-time hypothermia in undisturbed birds from the same population (Nord et al. 2009, 2011; 2013; Andreasson et al. 2019). A few individuals ($n=3$) reduced T_s substantially to levels below 35° C, but most birds exhibited a rather modest predicted T_b reduction over a 40° C gradient from 25 to -15° C, with an initial decrease in T_s below LCT and a slight increase again at the lowest T_a (Fig. S2). Thus, for most birds, increased heat production below LCT was sufficient to maintain a relatively shallow hypothermic T_b with modest variation across the considered T_a -range. It follows that the slight decrease in T_b below LCT might be a result of high heat loss rate (i.e., poor insulation) more than a reflection of change in set point.

Increased T_s and predicted T_b at the two coldest ambient temperatures was achieved without any detectable change in the rate of RMR increase. However, the ca. 1° C change in gradient between T_s and T_a would not necessarily be easily detected as a deviation from linearity in RMR. Nevertheless, increasing T_s in the two coldest temperatures suggests that the birds were able to further reduce heat loss and/or increase heat production. The fact that these processes were

Table 1 Resting metabolic rate, body temperature, and thermal conductance in blue tits

Variable	Estimate (SE)	<i>df</i>	<i>F</i> / χ^2	<i>P</i>
Total RMR (W) below LCT				
Age		1, 28.2	7.1	0.013
Young	0.414 (0.006)			
Old	0.392 (0.006)			
T_a	−0.0093 (0.0002)	1, 28.0	2353.7	< 0.0001
ID (random intercept and slope)		3	89.8	< 0.0001
Mass-adjusted RMR (W) below LCT				
Age		1, 27.7	7.3	0.012
Young	0.413 (0.005)			
Old	0.393 (0.005)			
T_a	−0.0094 (0.0002)	1, 27.2	2504.7	< 0.0001
Age $\times T_a$		1, 28.0	4.1	0.054
Young $\times T_a$	−0.0098 (0.0003)			
Old $\times T_a$	−0.0090 (0.0003)			
Body mass	0.015 (0.004)	1, 32.4	16.9	0.0002
ID (random intercept and slope)		3	57.3	< 0.0001
Total RMR (W) above LCT				
Age		1, 28.5	5.4	0.028
Young	0.285 (0.005)			
Old	0.267 (0.006)			
T_a	0.0018 (0.0006)	1, 50.5	8.5	0.005
ID (random intercept)		1	9.4	0.002
Mass-adjusted RMR (W) above LCT				
Age		1, 26.1	2.4	0.13
T_a	0.0015 (0.0006)	1, 51.4	5.5	0.023
Body mass	0.012 (0.004)	1, 28.0	10.6	0.003
ID (random intercept)		1	4.3	0.037
Subcutaneous temperature, T_s (°C)				
Age		1, 35.8	7.6	0.009
T_a		1, 26.9	92.8	< 0.0001
T_a^2		1, 194.5	166.7	< 0.0001
Age $\times T_a$		1, 26.9	6.2	0.019
Age $\times T_a^2$		1, 194.5	8.2	0.005
Young $\times T_a^2$	0.106 (0.020)			
Old $\times T_a^2$	0.179 (0.021)			
ID (random intercept and slope)		3	126.9	< 0.0001
Predicted body temperature, T_b (°C)				
Age		1, 38.1	7.8	0.008
T_a		1, 29.5	37.0	< 0.0001
T_a^2		1, 169.4	81.9	< 0.0001
Age $\times T_a$		1, 29.5	8.5	0.007
Age $\times T_a^2$		1, 169.4	7.8	0.006
Young $\times T_a^2$	−0.005 (0.003)			
Old $\times T_a^2$	−0.015 (0.003)			
ID (random)		3	104.9	< 0.0001
Conductance (W °C ^{−1})				
Age		1, 28.2	7.1	0.012
Young	0.0110 (0.0001)			
Old	0.0105 (0.0001)			
T_a	3.5e−05 (4.3e−06)	1, 29.9	64.3	< 0.0001
ID (random)		3	75.7	< 0.0001

Table 1 (continued)

Variable	Estimate (SE)	<i>df</i>	F/χ^2	<i>P</i>
Mass-adjusted conductance ($W\ ^\circ C^{-1}$)				
Age		1, 28.3	6.4	0.017
Young	0.0109 (0.0001)			
Old	0.0106 (0.0001)			
T_a	3.2e-05 (4.1e-06)	1, 76.7	61.3	<0.0001
Body mass	0.0004 (0.00009)	1, 36.1	18.2	0.0001
ID (random)		3	48.8	<0.0001

Test statistics, degrees of freedom, parameter estimates, and levels of significance derived from linear mixed models on metabolic rate, body temperature, and thermal conductance in blue tits. For factors, estimates are least-square means (emmeans package; Lenth 2019). For continuous variables (and their interactions with factors), estimates represent the slope of the regression between the dependent variable and the continuous variable (SE represents the fit of the regression). Significant effects ($P < 0.05$) are given in bold and effects $0.05 < P < 0.1$ in italics

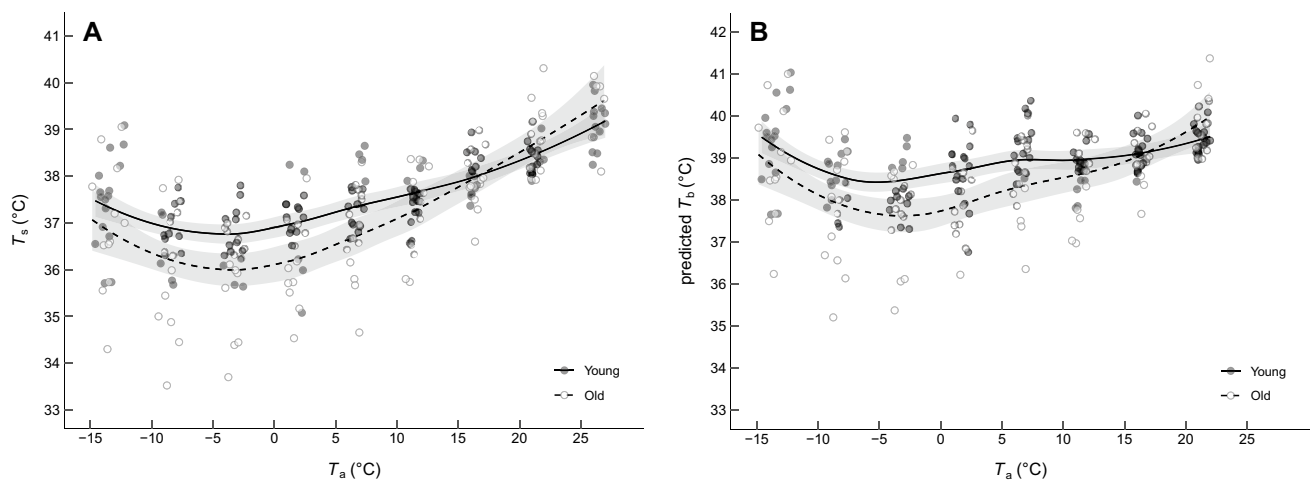


Fig. 3 **a** Subcutaneous body temperature, T_s , and **b** predicted body temperature, T_b , in young and old blue tits as a function of T_a . Age-specific fitted curves are LOESS (locally estimated scatterplot smoothing) \pm 95% CI

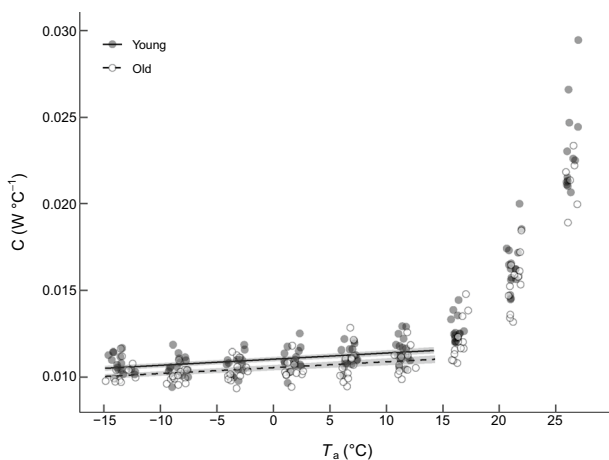


Fig. 4 Thermal conductance (raw data), C , in young and old blue tits as a function of T_a . The regression line is an estimate (\pm SE) from a linear mixed model below LCT

not employed at milder T_a below LCT implies that they could be connected to costs. One possible route for heat loss reduction would be to further constrict peripheral blood flow to retain warm blood in the core. However, as T_s also increased at the lowest ambient temperatures, this mechanism at least did not involve the “shell” of the body. It is possible that some heat could be saved by reducing circulation to the head (which is a main avenue for heat loss in blue tits; Nord et al., unpublished data), at the expense of reduced vigilance and ensuing higher predation risk (Rashotte et al. 1998). Further reductions in peripheral circulation, or any other means to reduce heat loss rate, could also explain the slightly decreasing C below LCT. Whether the increased T_s and predicted T_b at low T_a were related to further vasoconstriction of extremities, reduced circulation to the head region or other thermoregulatory responses (i.e., recruitment of additional muscles for shivering thermogenesis; cf. Saarela et al. 1995) requires further experimentation.

So, why then, do young birds have a higher total RMR in the cold compared to old birds? Much of the variation in total RMR was explained by body mass, but young birds also tended to increase RMR more as T_a decreased compared to old birds when body mass was accounted for. One possible explanation could be differences in plumage quality, supported by the higher thermal conductance in young birds. It is worth noting that the biggest difference in mass-adjusted RMR between age groups occurred at the lowest T_a , $-15\text{ }^\circ\text{C}$, where the difference in T_s and predicted T_b between age groups was smallest. This could indicate that any inferior insulation in younger birds, caused e.g., by time or nutrient constraints during moult, needed to be compensated for by increased heat production, especially at low T_a . Age-related differences in RMR could also be a consequence of a general decline in metabolic rate in older birds.

If young birds had to offset a higher heat loss with increased heat production to maintain T_b below thermo-neutrality, we would expect T_b to be similar between age groups. So why do young birds maintain a higher body temperature in ambient temperatures below LCT compared to old birds? A similar difference between the age groups was found in the same blue tit population in response to increased perceived predation risk (Andreasson et al. 2019). As the confinement within a respirometer chamber may be experienced as a comparable stressor, it seems that young birds keep a higher body temperature in stressful environments. If this difference in T_b between age groups depends on senescence in thermogenic capacity, experience-based variation in risk assessment or variation in life history strategies (see Andreasson et al. 2019 for a discussion of these factors) or other, yet unidentified processes, needs to await further experimentation.

Conclusions

We have shown that metabolic rate in “the little bird in winter” follow a classic Scholander–Irving model (Scholander et al. 1950) where blue tits increase RMR linearly below the lower critical temperature, LCT ($14\text{ }^\circ\text{C}$). Young birds had both higher RMR and T_b below the LCT compared to older conspecifics. Thermal conductance was also higher in young birds, indicating that they were producing more heat to offset increased heat loss compared to older birds. We believe that one likely explanation for this could be differences in plumage quality caused by time constraints during moult. T_b reduction was moderate ($4\text{--}5\text{ }^\circ\text{C}$ reduction from day time levels), though predicted T_b and T_s decreased below LCT and increased again at the coldest T_a . These changes were likely reflections of high heat loss rate, potentially coupled to additional thermoregulatory responses to conserve heat at the lowest T_a . This implies that rest-phase hypothermia

in tits might be mechanistically different compared to species with the capacity for deeper torpor (e.g., swifts, nightjars, and hummingbirds: McKechnie and Lovegrove 2002). Future studies should look to more explicitly evaluate variation in plumage quality and thermal conductivity of plumage between age groups, and how this might affect T_b and RMR. This could help pinpoint mechanisms responsible for the relation between RMR and T_b at cold ambient temperatures.

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Author contribution FA, AN, and J-ÅN conceived the ideas; FA, AN, and J-ÅN designed methodology; FA collected and analyzed the data, and led the writing of the manuscript together with all authors. All authors contributed critically to the drafts and gave final approval for publication.

Data availability Data is attached as supplementary material in the online version of this article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and national guidelines for the care and use of animals were followed.

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