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RESEARCH ARTICLE

Metabolic reduction after long-duration flight is not related to fat-free mass loss or flight duration in a migratory passerine

Alexander R. Gerson[1,*], Joely G. DeSimone[1], Elizabeth C. Black[1], Morag F. Dick[2] and Derrick J. Groom[1]

ABSTRACT

Migratory birds catabolize large quantities of protein during long flights, resulting in dramatic reductions in organ and muscle mass. One of the many hypotheses to explain this phenomenon is that decrease in lean mass is associated with reduced resting metabolism, saving energy after flight during refueling. However, the relationship between lean body mass and resting metabolic rate remains unclear. Furthermore, the coupling of lean mass with resting metabolic rate and with peak metabolic rate before and after long-duration flight have not previously been explored. We flew migratory yellow-rumped warblers (*Setophaga coronata*) in a wind tunnel under one of two humidity regimes to manipulate the rate of lean mass loss in flight, decoupling flight duration from total lean mass loss. Before and after long-duration flights, we measured resting and peak metabolism, and also measured fat mass and lean body mass using quantitative magnetic resonance. Flight duration ranged from 28 min to 600 min, and birds flying under dehydrating conditions lost more fat-free mass than those flying under humid conditions. After flight, there was a 14% reduction in resting metabolism but no change in peak metabolism. Interestingly, the reduction in resting metabolism was unrelated to flight duration or to change in fat-free body mass, indicating that protein metabolism in flight is unlikely to have evolved as an energy-saving measure to aid stopover refueling, but metabolic reduction itself is likely to be beneficial to migratory birds arriving in novel habitats.

KEY WORDS: Avian migration, Basal metabolic rate, Peak metabolic rate, Metabolic scope, Flight

INTRODUCTION

Each year, billions of songbirds (Passeriformes) travel extraordinary distances between their wintering grounds and their more temperate breeding grounds (Dokter et al., 2018; Horton et al., 2019). In songbirds, migration typically comprises a series of non-stop flights lasting from 6 h to 12 h in the case of overland migration, but non-stop flight duration in songbirds can exceed 60 h when crossing ecological barriers such as the Gulf of Mexico (DeLuca et al., 2019; Deppe et al., 2015; McKinnon et al., 2017). During flight, the metabolic rate is ∼10-fold higher than basal levels, and rate of water loss can exceed 4% of body mass per hour, which is similar to that of birds exposed to high temperatures at rest in the hottest deserts on earth (Butler, 1991; Butler et al., 2000; Engel et al., 2006; Smith et al., 2017; Wikelski et al., 2003). Therefore, it is clear that non-stop migratory flight places extreme demands on the ability of birds to manage energy and water budgets (Carmi et al., 1995; Klaassen, 1995, 1996).

In preparation for migration, birds accumulate large fat stores, and it was long thought that fat was the sole fuel for long-duration flights (Odum et al., 1964). Because fat has the highest energy density of all the metabolic fuels (37.6 kJ g^{-1} wet mass), it is the best possible fuel for volant animals, and migratory birds seasonally upregulate fat transporters and enzymes that allow for rapid mobilization and catabolism of fats (Guglielmo, 2010; Guglielmo et al., 2002; McFarlan et al., 2009). Protein was long thought to be spared during flight because of its functional role in organs, tissues and enzymes; however, there is now a large and convincing body of evidence showing that migratory birds display dramatic reductions in lean mass (fat-free mass consisting of muscle and organ masses) during flight, which then must be rebuilt between flights during refueling at stopovers (Battley et al., 2000; Bauchinger and Biebach, 2001; Biebach and Biebach, 1998; Piersma, 1990; Schwilch et al., 2002). Therefore, 80–90% of the energy for flight is derived from fat, and the remaining 10–20% comes from the breakdown and oxidation of proteins, which occurs simultaneous to fat metabolism (Gerson and Guglielmo, 2011b, 2013; Jenni and Jenni-Eiermann, 1998). Carbohydrates contribute very little during long-duration flight, and only during the initial minutes of flight (Rothe et al., 1987).

The catabolism of lean tissues during flight can result in dramatic reductions in organ masses upon arrival at stopover destinations, imposing a physiological limit on stopover refueling. Nevertheless, birds must rebuild the organs that were catabolized in flight before they can replenish the fat deposits required to complete the next flight (Gannes, 2002; Karasov and Pinshow, 2000). As a consequence, the magnitude of protein catabolized aloft could constrain refueling rate, extending stopover duration and potentially delaying arrival to the breeding or wintering grounds (Alerstam and Lindstrom, 1990; Møller, 1994). However, numerous benefits to protein catabolism during flight have been proposed (Bauchinger and Biebach, 1998; Klaassen, 1996), one of which is that lower lean mass upon arrival would result in lower metabolism (Battley et al., 2000, 2001; Biebach and Bauchinger, 2003). For example, Biebach and Bauchinger (2003) estimated metabolic savings of reduced lean mass in a migratory songbird, resulting from both reduced flight costs and mass-dependent reductions in basal metabolic rate (BMR), to be 21%. Battley et al. (2000, 2001) empirically found a 42% reduction in BMR after a long-distance migratory flight in great knots (*Calidris tenuirostris*), and these savings were attributed to reduced lean body mass, although these birds displayed mass-specific metabolic reduction as well. It has been widely assumed that the breakdown of tissues does not result in functional deficits to the organ or to whole-animal metabolism, and that by reducing lean body mass, BMR reductions would simply follow allometric scaling principles. However, the basic premise that lean mass loss is

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[1]Department of Biology, University of Massachusetts Amherst, Amherst, MA 01003, USA. [2]Advanced Facility for Avian Research, Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7.

*Author for correspondence (argerson@bio.umass.edu)

A.R.G., 0000-0002-9082-1907; J.G.D., 0000-0002-7486-6443; E.C.B., 0000-0001-5861-0299; D.J.G., 0000-0001-9300-9116

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directly and primarily responsible for reductions in metabolism has not been evaluated. Furthermore, the effects of lean mass loss in flight on peak metabolic rate (PMR) have not been evaluated and would provide insight into the detrimental effects of lean mass loss on ability to perform high-intensity exercise, while also investigating the coupling of overnight resting metabolic rate (RMR) and PMR in this context.

PMR is typically defined as the maximal metabolic rate of an animal and can be used as a proxy for performance-related traits, such as predator avoidance or flight performance in birds. PMR has been shown to be correlated with BMR in inter-individual and inter-species comparisons, but this is not always the case (Barceló et al., 2017; Chappell et al., 1999; Wiersma et al., 2007). Nonetheless, the potential impact of lean mass breakdown during long-duration migratory flight on performance after flight has not been evaluated, and therefore measuring PMR before and after long-duration flight could shed light on performance deficits that may arise as a result of lean mass catabolism. Furthermore, in order to evaluate the proposed adaptive benefit of lean mass catabolism to migratory birds, it is essential to measure both RMR and PMR before and after flight within individuals, while also investigating the basic premise that metabolic changes demonstrated after flight are, in fact, linked to changes in lean tissue mass.

Flying under low-humidity conditions has been shown to result in increased rates of protein breakdown in flight in migratory songbirds, and this phenomenon has been replicated in multiple studies in numerous species of birds, both in flight and at rest (Gerson and Guglielmo, 2011a,b; Groom et al., 2019). The costs and benefits of this metabolic response to the environment are still being evaluated, but shifting the fuel mixture to include a greater proportion of protein increases the rate of endogenous water production by up to 20% (Gerson and Guglielmo, 2011b). Nonetheless, this approach has provided a means by which the rate of protein metabolism can be manipulated independent of flight duration, allowing the effects of lean mass loss to be decoupled, to a degree, from the effects of long-duration flight.

Here, we measured overnight resting metabolism under standard BMR conditions and PMR using a hop-hover wheel in yellow-rumped warblers (*Setophaga coronata*), a Nearctic migratory passerine bird, before and after long-duration flight (up to 10 h) in a wind tunnel. We used quantitative magnetic resonance body composition analysis (QMR) (Guglielmo et al., 2011) to measure changes in fat and lean body mass in flight, to determine the influence of changes in lean body mass and flight duration on changes in metabolism that may occur after long flight in migratory birds, while also investigating the intra-individual coupling of overnight resting metabolism and PMR, and how this can change after long-duration flight in a wind tunnel.

MATERIALS AND METHODS

Bird capture and care

Yellow-rumped warblers, *Setophaga coronata* (Linnaeus 1766), were captured near Long Point Bird Observatory (Long Point, ON, Canada) during their southbound migration in early October 2016, banded with a unique color band combination for identification in captivity and transported to the Advanced Facility for Avian Research at Western University, London, ON, Canada. Birds were held on a 12 h light:12 h dark photoperiod (lights on at 06:00 h) at 20°C and ~60% relative humidity in indoor free-flight aviaries (2.3×2.4×3.5 m) or individual cages (121×68×185 cm). A synthetic diet (Dick and Guglielmo, 2019a; Guglielmo et al., 2017) and water were provided *ad libitum*, and approximately two to three *Tenebrio* mealworms per bird were provided daily. Birds were held for at least 2 weeks before beginning experimental procedures. All procedures were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee (2015-0019) and the Western University Animal Use Subcommittee (2010-216) and collected under Canadian Wildlife Service permit #CA-0256.

Experimental design

Birds were moved to individual cages 3 days before experimental procedures. We measured overnight RMR under thermoneutral conditions to approximate BMR. Because birds were in migratory disposition, and the post-flight birds were recovering from a long-duration flight, we likely did not measure true BMR in these birds, but repeated measures of RMR under controlled repeatable conditions allowed us to investigate changes in resting metabolism due to changes in lean mass and flight duration. We measured PMR 2 days before the scheduled long-duration flight. PMR was measured in the morning, after which birds were returned to their cages until evening when RMR measurements began (see below). Birds were then allowed 2 days of recovery before being flown in the wind tunnel. Birds were flown in the wind tunnel (see below) for up to 10 h during the evening and into the night. After flight, birds were returned to their cages for the remainder of the night and provided with fresh food and water. At approximately 10:00 the morning after a flight, PMR was measured (post-flight). Birds were then allowed to feed for the rest of the day, and in the evening, RMR was measured. All RMR and PMR measurements were performed on at least 1 h post-absorptive animals (Afik and Karasov, 1995). Body condition was determined using QMR to determine body composition before and after flight and RMR, but only before PMR.

Flight protocol

Birds were flown in the wind tunnel at the Advanced Facility for Avian Research (for a description of the wind tunnel, see Gerson and Guglielmo, 2011b; Pennisi, 2011). Birds were flown at 8 $\mathrm{m\,s^{-1}}$ at 18°C in either 12 g H_2O $\mathrm{m^{-3}}$ (low evaporative water loss; LEWL) or 2 g H_2O $\mathrm{m^{-3}}$ (high evaporative water loss; HEWL) humidity as in Gerson and Guglielmo (2011b) and Groom et al. (2019). Individual birds were randomly assigned to flight treatments. All flights began 30 min after lights off, because yellow-rumped warblers are nocturnal migrants.

Birds were fasted for 1 h before flight, with only access to fresh water. Immediately before flight, the first candidate flight bird was weighed (to the nearest 0.001 g), and body composition was determined using QMR, which measures fat mass, lean body mass and total body water. The second candidate bird was also scanned with the QMR but then placed into a covered cage within the plenum of the wind tunnel, and the flight bird was released into the air stream. We aimed for each bird to achieve steady-state flight for a minimum of 30 min. If a bird was unable to achieve steady-state flight during this time, the bird was removed from the wind tunnel and replaced with the second candidate flier, which was weighed and QMR scanned immediately prior to its release into the air stream.

Once a bird achieved steady flight, we followed a ‘three strikes’ rule to determine maximum voluntary flight duration (Dick and Guglielmo, 2019b), where a flight was ended if a bird stopped flying three times within 5 min. Birds were allowed to fly up to a maximum flight duration of 10 h. Upon completion of the flight, the bird was weighed, QMR scanned, blood sampled and returned to its cage. Blood sampling occurred within 10 min of the conclusion of the flight. Plasma was separated by centrifugation at 2000 ***g*** for 10 min and frozen at −80°C.

Overnight RMR

Because RMR was measured overnight, all recordings started at lights off (similar to Dick and Guglielmo, 2019b). Following a 1-h fast to ensure that the birds were post-absorptive before lights off, birds were scanned in the QMR, weighed and placed on a perch in a 1.3 l sealable chamber. The bottom of the respirometry chambers was lined with ∼1 cm of mineral oil to prevent feces from adding water vapor to the excurrent air, and the birds were separated from the mineral oil by a metal grate. The chambers were housed in a 30°C incubator (Sanyo Incubator MIR-154, Sanyo Scientific) for the duration of the RMR measurement. Incurrent air was dried with a peltier gas dryer (PC-4, Sable Systems International, North Las Vegas, NV, USA) followed by passage through two Drierite columns (W. A. Hammond Drierite, Zenia, OH, USA). The dried air was split into eight streams, and flow rate into the chambers was controlled and measured by a flow meter (FB-8, Sable Systems International) at ∼700 ml min^{-1}. Excurrent flow was directed by an eight-channel multiplexer (MUX, Sable Systems International), which switched chambers in 5 min intervals with a baseline once every cycle and was subsampled at 250 ml min^{-1} to measure water vapor (RH-300, Sable Systems International), CO_2 (CA-2A, Sable Systems International) and O_2 (FC-1B, Sable Systems International). Data were logged using Expedata (v1.8.4, Sable Systems International) at 1 sample s^{-1}. Rates of O_2 consumption ($\dot{V}_{O_2}$) and CO_2 production ($\dot{V}_{CO_2}$) were calculated using standard push-respirometry equations (Eqns 10.6 and 10.7 from Lighton, 2008), after mathematical corrections for the presence of water vapor were applied (Eqns 6 and 7 from Chapter 9 in Lighton, 2008) and the lowest consecutive mean $\dot{V}_{O_2}$ over a 60 s interval was taken as the RMR (Lighton and Halsey, 2011). Respiratory quotient (RQ) was calculated as the ratio of $\dot{V}_{CO_2}/\dot{V}_{O_2}$, and converted to watts using an oxyjoule equivalent of [16+5.164×(RQ)] (Lighton, 2008).

PMR

PMR was measured using a hop-hover wheel, as described in Dick and Guglielmo (2019b). Briefly, birds were placed in a 7.7 l wheel receiving 1.5 l min^{-1} of air, and the wheel was covered for 5 min to allow birds to adjust to their new environment and to measure resting $\dot{V}_{O_2}$. The wheel was then manually spun until the bird was unable to maintain hovering flight, which usually occurred within 10 min. The O_2, CO_2 and water signals were *Z*-transformed following smoothing as previously described (Bartholomew et al., 1981; Lighton, 2008; Lighton and Halsey, 2011), and $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated as described above after mathematically scrubbing water. PMR was taken as the highest consecutive 60 s of recorded $\dot{V}_{O_2}$ after the instantaneous transform. Data were recorded using Expedata as above, except the sampling rate was increased to 10 samples s^{-1}.

Statistical analysis

All statistics were performed in R (https://www.r-project.org/) with significance of α=0.05. All comparisons of body composition before and after flight, and changes in body composition with flight duration and in response to humidity treatments were evaluated using general linear models. For body composition, we used the fat measurement from the QMR, as this value can be easily calibrated and was validated using precisely weighed canola oil standards before and after each scan. We then calculated fat-free mass instead of using the lean measure from the QMR by subtracting fat mass from total mass. This provided a more accurate and validated measure of lean body mass. Flight costs were calculated using the change in fat mass and fat-free mass during flight, as determined using QMR, assuming 37.6 kJ g^{-1} for fat and 5.3 kJ g^{-1} for wet lean tissue (Jenni and Jenni-Eiermann, 1998). Changes in RMR and PMR from before to after flight were assessed using general linear mixed models using the nlme package (https://cran.r-project.org/web/packages/nlme/index.html) in R to account for repeated measures on individuals in an unbalanced design, because not all animals that were tested before flight completed a flight of sufficient length to warrant post-flight testing, and some individuals that did complete a flight were not measured post-flight. This approach allowed all tests to be included in the analysis, even if they did not have a corresponding measurement (pre- or post-flight). For change in RMR and PMR, only individuals with paired measurements were included in the analysis. For metabolic measurements, initial models included body size (wing and tarsus) and mass as a covariate, as well as the main effects of flight duration or change in body composition and all two-way interactions. Non-significant terms were removed until only significant terms remained using backward stepwise model selection (Crawley, 2005).

RESULTS

Flights

Birds successfully completed 20 flights (11 HEWL, nine LEWL) ranging in duration from 28 min to 600 min (10 h), and flight durations were not significantly different (t=−0.30, d.f.=16.56, P=0.77) between the treatment groups [HEWL, mean 246 min (47–600 min); LEWL, mean 373.7 min (28–600 min)].

Body composition changes in flight

Before flight, birds had a mean (±s.d.) mass of 12.67±1.33 g (10.37–15.04 g) with average fat load of 1.98±0.98 g (0.533–3.68 g), which corresponds to a mean percentage fat of 15.16% (4.84–26.40%) and an average fat-free mass of 10.68±0.77 g (9.66–12.19 g). There were no significant differences in mass ($F_{1,18}$=0.448, P=0.512), fat mass ($F_{1,18}$=0.858, P=0.367) or fat-free mass between the treatment groups prior to flight ($F_{1,18}$=0.001, P=0.991). After flight, birds had an average mass of 11.53±1.24 g (10.04–14.19 g), with an average fat load of 1.32±0.91 g (0.26–3.59 g), which corresponds to a mean percentage fat of 11.50% (2.60–26.75%) and an average fat-free mass of 10.12±0.70 g (9.04–11.40 g).

During flight, birds under the HEWL conditions lost significantly more mass than birds under the LEWL conditions ($F_{1,17}$=6.99, P=0.017), and mass losses increased with flight duration ($F_{1,17}$=128.57, P<0.001). Total fat loss increased with flight duration ($F_{1,17}$=282.58, P<0.001), and there were no significant differences in total fat loss in response to different humidity treatments ($F_{1,17}$=0.410, P=0.530). Total fat-free mass loss increased with increasing flight duration overall ($F_{1,17}$=10.27, P=0.005), and birds under the HEWL treatment lost more fat-free mass than those under the LEWL treatment ($F_{1,17}$=9.75, P=0.006; Fig. 1; post fat-free HEWL, 10.05 g; post fat-free LEWL, 10.26 g). Birds under the HEWL conditions lost fat-free mass at a rate of 0.249±0.16 g h^{-1}, whereas birds under the LEWL conditions lost fat-free mass at a rate of 0.227±0.27 g h^{-1}. Overall, the rate of fat mass loss was 0.144±0.05 g h^{-1}. Mean flight cost was 1.85±0.58 W (1.19–3.36 W) and there were no significant differences in flight costs between treatments ($F_{1,17}$=0.176, P=0.680), but flight costs did decrease with increasing flight duration ($F_{1,17}$=6.043, P=0.024).

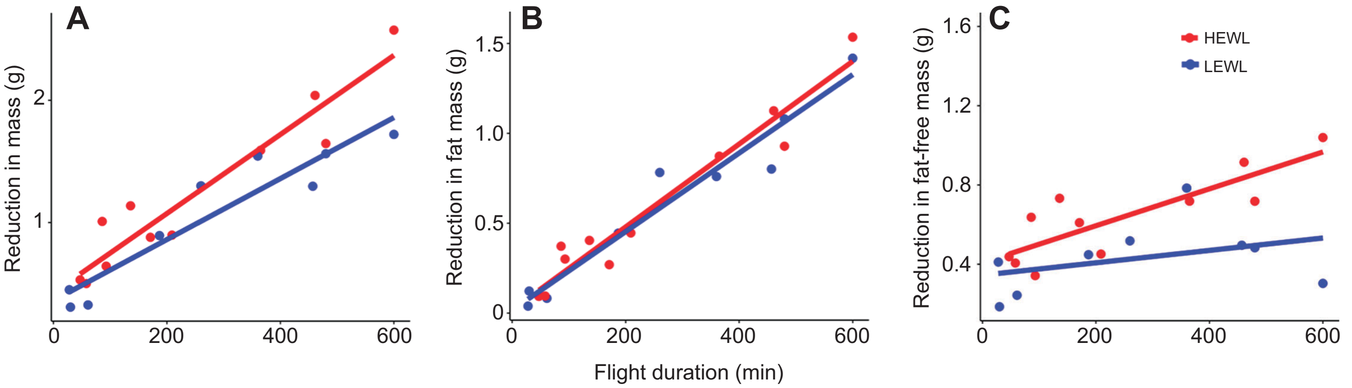

Fig. 1. Reduction in mass, fat mass and fat-free mass with increasing flight duration in *Setophaga coronata*. (A–C) Reductions in mass (A), fat mass (B) and fat-free mass (C) increased as flight duration increased. Mass loss and fat-free mass loss were significantly greater in the high evaporative water loss (HEWL; red; *N*=11 individuals) treatment group compared with the low evaporative water loss (LEWL; blue; *N*=9 individuals) treatment group ($P<0.05$), but there was no significant interaction between treatment groups in C and no significant difference in fat mass loss between the treatment groups.

Overnight RMR

There was a significant reduction in whole-animal RMR after flight ($F_{1,9}=8.04$, $P=0.020$) and fat-free mass was a significant covariate ($F_{1,9}=5.79$, $P=0.039$; Fig. 2), whereas structural size was not significant ($P=0.628$). Before flight, birds had an average RMR of 0.256±0.038 W, which is greater than that predicted allometrically (0.189 W; McKechnie and Wolf, 2004); and there was no significant relationship between RMR and mass ($P=0.23$), fat-free mass ($P=0.49$) or structural size ($P=0.80$). After flight, RMR was 0.220±0.034 W, which represents a 14.35% reduction compared to pre-flight levels (Fig. 2), and corresponds with a 9.0% reduction in total mass and a 4–6% loss of fat-free mass (see above). Post-flight RMR was significantly related to mass ($F_{1,12}=12.968$, $P=0.004$) and to fat-free mass [$F_{1,12}=8.07$, $P=0.015$; logRMR, −4.633+1.27 log(mass); logRMR, −4.423+1.23 log(fat-free mass)]. The reduction in RMR was not related to flight duration ($F_{1,9}=1.61$, $P=0.236$; Fig. 3A), did not differ between humidity treatments ($F_{1,9}=2.67$, $P=0.136$) and was not related to change in fat-free mass ($F_{1,9}=1.41$, $P=0.265$; Fig. 3B).

PMR

Birds had a mean PMR of 1.54±0.33 W before flight and a mean PMR of 1.49±0.31 W after flight, and these values were not significantly different ($F_{1,17}=0.23$, $P=0.637$; Fig. 4). There was no effect of humidity treatment on PMR ($F_{1,18}=0.42$, $P=0.525$), and overall PMR was significantly related to fat-free mass ($F_{1,18}=22.638$, $P<0.001$). Change in PMR was unaffected by flight duration ($F_{1,15}=0.50$, $P=0.4877$), humidity ($F_{1,15}=0.006$, $P=0.940$) or change in fat-free mass during flight ($F_{1,15}=0.129$, $P=0.725$).

Relating PMR to RMR

PMR was 6.27±1.25-fold higher than RMR before flight, and 6.91±1.67-fold higher after flight, but this difference was not significant ($F_{1,10}=0.90$, $P=0.366$), and differences between the metabolic rates after flight were not explained by flight duration, humidity treatment, change in mass or change in fat-free mass (all $P>0.05$).

PMR was significantly related to RMR overall [$F_{1,10}=25.79$, $P<0.001$; PMR (W)=5.98 × (RMR W)+0.157; Fig. 5], and there was no difference in slope or intercept from before flight to after flight ($P>0.05$), indicating a functional linkage between RMR and PMR.

DISCUSSION

Here, we clearly demonstrate a significant reduction in metabolism after long-duration flight that is not solely explained by reductions in fat-free body mass, indicating mass independent metabolic reduction, and the lack of relationship between change in fat-free body mass and change in RMR indicates that protein catabolism in flight is unrelated to post-flight metabolic reduction per se. Therefore, it is unlikely that protein metabolism in flight has been

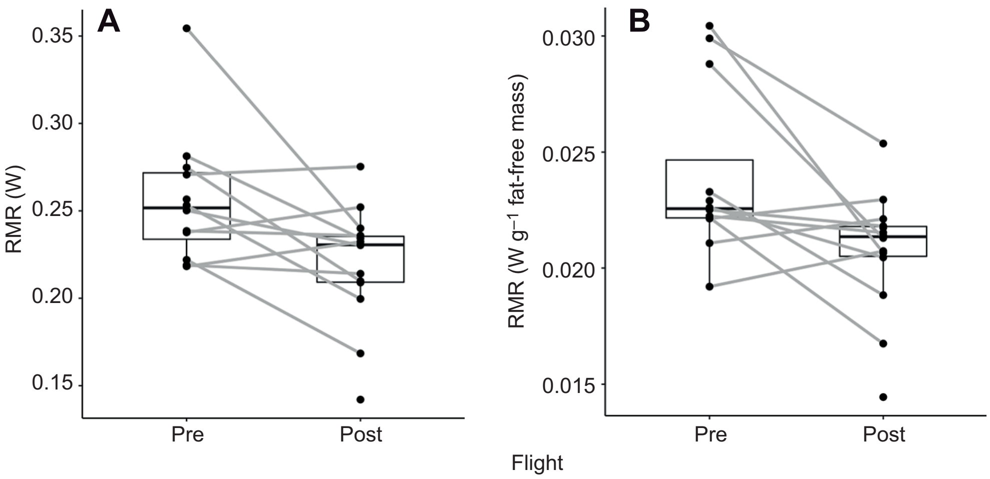

Fig. 2. Reduction in whole-animal and mass-specific resting metabolic rate (RMR) after long-duration flight in *S. coronata*. (A,B) Whole-animal RMR (A) and mass-specific RMR (B) were significantly reduced ($P<0.05$) after long-duration flight in a wind tunnel (pre-flight *N*=12, post-flight *N*=14) (see text for details). Gray lines connect measurements within each individual. In boxplots, the box represents the interquartile range and the line represents the median; whiskers indicate 95% confidence interval.

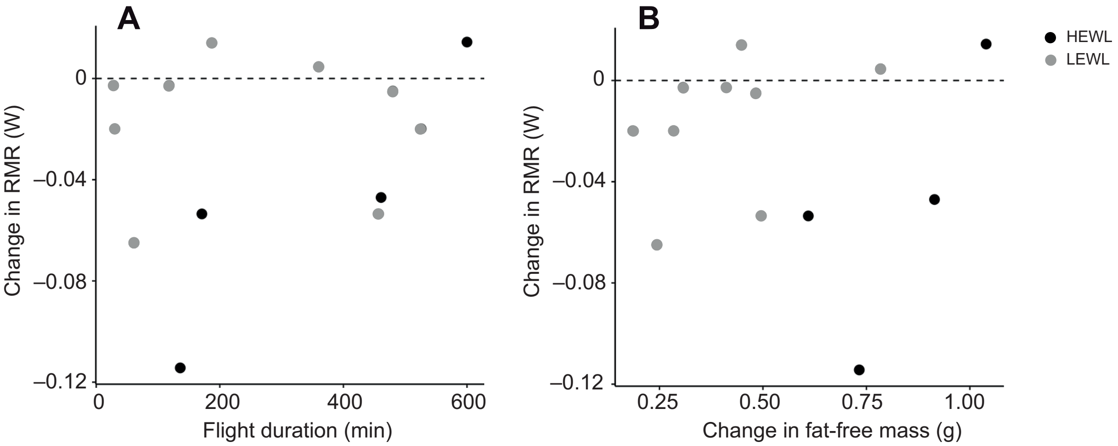

Fig. 3. Changes in RMR after long-duration flight are not associated with the flight duration or changes in fat-free mass in *S. coronata*. (A,B) The reduction (post-flight RMR – pre-flight RMR) in RMR after flight was not explained by flight duration (A) or changes in fat-free mass (B) after long-duration flight in a wind tunnel (*N*=13 total, *N*=9 LEWL, *N*=4 HEWL).

selected for as a mechanism to reduce metabolism during refueling after long-duration flight. We manipulated the rate of fat-free body mass loss in flight by varying humidity in the wind tunnel, successfully decoupling changes in fat-free body mass loss from flight duration, allowing us to investigate the impact of changes in body composition on both peak and resting metabolism under thermoneutral conditions, independent of the impact of flight duration. Through this novel approach, we clearly demonstrate that reduced RMR (measured under BMR conditions) after long-duration flight is independent of changes in fat-free body mass and flight duration. However, this reduction in RMR did not correspond with a reduced PMR. We show a strong correlation between RMR and PMR, although PMR is not significantly impacted by flight among individuals, whereas RMR is reduced. This indicates a context-dependent regulation of metabolism, where metabolism is suppressed at rest, perhaps through reduced metabolism of tissues associated with digestion, without a concomitant suppression of peak metabolism, which may be governed by muscle metabolism (Barceló et al., 2017). This response could result in substantial energy savings during rest phase, at night, when animals are unable to forage, while not compromising predator escape, foraging or settling movements within a stopover habitat that would require high metabolism to power flight.

After long-duration flight, the birds in the present study demonstrated a 14% reduction in RMR, on average, which is less than what was observed in great knots after a ∼4 day flight, where birds showed dramatic reductions in both body mass and lean mass-specific BMR (∼33%) (Battley et al., 2001). The substantial reduction in whole-animal BMR in these ultra-long-distance migrants was explained by both a dramatic reduction in lean body mass and active metabolic suppression, as indicated by reduction in mass-specific metabolic rate as well. A separate study (Lindström et al., 1999) did not measure pre-flight BMR, but tracked the recovery of BMR over 3 days after a 12 h flight in two birds and showed a recovery of BMR of ∼20%, which was mostly explained by recovery of mass. In contrast to these two studies, we show comparable reduction in resting thermoneutral metabolism, but we demonstrate that metabolic suppression under these conditions

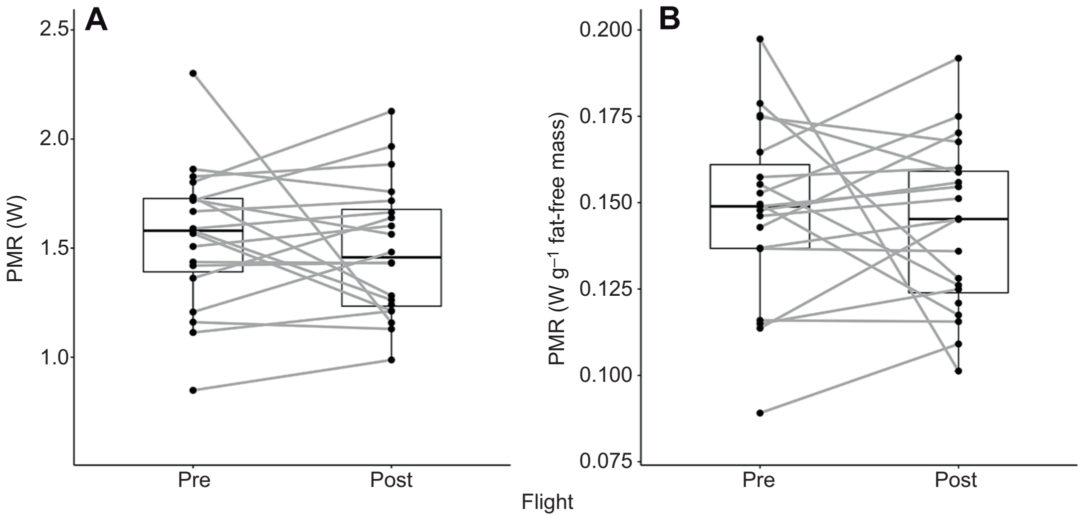

Fig. 4. Whole-animal and mass-specific peak metabolic rates (PMRs) do not change after long-duration flight in *S. coronata*. (A,B) Whole-animal (A) and mass-specific (B) PMRs recorded pre-flight were not different from those recorded after long-duration flight in a wind tunnel (pre-flight *N*=19, post-flight *N*=20). Gray lines connect measurements within each individual. In boxplots, the box represents the interquartile range and the line represents the median; whiskers indicate 95% confidence interval.

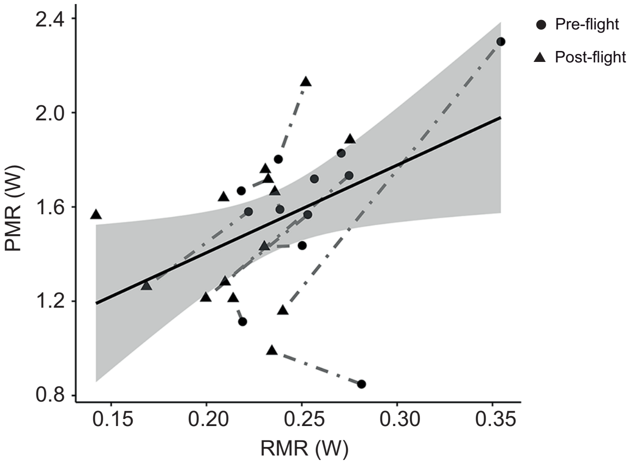

Fig. 5. PMR is significantly associated with RMR after long-duration flight in *S. coronata*. PMR was significantly related to RMR overall ($P<0.001$; see text for statistics and parameter estimates), but the response of PMR was highly variable among individuals. Gray dot-dashed lines connect pre- and post-flight measures within individuals ($N=15$ groups).

occurs in response to migratory flight, regardless of reduction in fat-free body mass or flight duration.

Metabolic suppression after flight could be mechanistically explained by a reduction in body temperature (T_b) setpoint, followed by temperature coefficient over a 10°C temperature range (Q_{10}) effects on metabolic processes, or could be driven by tissue-level metabolic inhibition, which would then lead to reduced T_b. We did not measure T_b, but Wojciechowski and Pinshow (2009) found T_b as low as 33°C in blackcaps (*Sylvia atricapilla*) during migratory stopover through the Negev desert. Assuming a Q_{10} of 2, and no changes in conductance owing to the change in metabolism, the metabolic suppression we measured in our animals would coincide with a maximum reduction in T_b of 5.62°C and a mean reduction of 2.65±1.94°C. This reduction in T_b based on our measured metabolic suppression could range from 4.5±1.9°C at a Q_{10} of 1.5 to 1.68±1.22°C at a Q_{10} of 3 overall. Given the recent and growing literature on heterothermy in birds (Gerson et al., 2019; McKechnie and Lovegrove, 2002; Smit et al., 2013), our data on post-flight metabolic suppression in migratory birds bolster the idea that rest-phase metabolic suppression and the associated hypothermia could be an important physiological mechanism to assist migratory refueling (Wojciechowski and Pinshow, 2009). During stopover, bats and hummingbirds, both of which are heterothermic, use torpor to save energy while roosting to minimize the use of fat, in a migratory strategy termed torpor-assisted migration (Baloun and Guglielmo, 2019; McGuire et al., 2014; Carpenter and Hixon, 1988). This physiological adaptation helps solve the time conflict that arises between active nocturnal migratory flight and nocturnal foraging in bats, and offsets the high cost of foraging in hummingbirds. By entering torpor, these animals minimize the costs of thermoregulation, sparing vital energy reserves to be used in migratory flight. Being diurnal foragers and nocturnal migrants, most migratory birds do not have the same time conflict as bats, but, during a multi-day stopover, birds must rely on fat accrued during the day to fuel night-time thermoregulation, while the pressure still exists to accrue significant fat during the day. This creates a two-steps-forward, one-step-back refueling strategy, as birds accumulate fat during the day, only to lose substantial quantity overnight due to thermoregulatory costs, the magnitude of which depends on environmental temperatures. Therefore, there is significant pressure to minimize thermoregulatory costs at night during stopover, which may account for more than half of the total cost of migration in some species (Wikelski et al., 2003), and rest-phase hypothermia or torpor could therefore result in significant energy savings. How hypothermia impacts other physiological processes, such as digestion and tissue repair, if they occur at all, within migratory birds at night during rest remains an important question.

Although the prospect of daily hypo-metabolism would seem to make sense from an energetics perspective, given the ecological conditions requiring stopover refueling to occur quickly, it does pose challenges from a physiological perspective. Although energy saving, hypo-metabolism could also suspend critical anabolic processes, such as cell proliferation, as is known to occur in daily torpor (Heldmaier et al., 2004). Such processes are required to rebuild tissues such as the epithelial tissue of the intestinal brush border, which is known to be damaged after long-duration fast in passerines (Karasov et al., 2004), or other organs that are so dramatically reduced after flight. Therefore, there appears to be a potential tradeoff between the energy savings afforded by hypo-metabolism and the anabolism of lean tissues during stopover refueling. As such, hypo-metabolism could, in fact, prolong the initial period of stopover when internal organs are being rebuilt, slowing refueling because these essential organs would also not be operating optimally. Although it has been shown that migratory birds with compromised digestion compensate by increasing paracellular absorption of carbohydrates, and increasing retention time, it is unclear how these processes are important to protein digestion and amino acid absorption, which is of utmost importance during initial refueling (Bauchinger et al., 2009; Gannes, 2002; Tracy et al., 2010). How this actually affects the rebuilding of tissues and refueling rate depends on how compromised the system is as a result of the post-flight reduction in the function of particular organs and the circadian rhythms of tissue anabolism. With this in mind, it is possible that the metabolic suppression we have shown here only occurs within 24 h of the completion of a long-duration flight, but does not persist further into the refueling period. This would allow birds energy savings initially upon arrival at a new site, and then allow the rebuilding of tissues once a novel habitat has been explored and food sources acquired. A multi-day metabolic phenotyping approach would thus be most beneficial to understand the energetics of migratory birds after completing long-duration flight.

Total lean mass reduction in migratory birds is caused by significant reductions in organ mass, and the magnitude of the organ mass reduction is determined, in part, by tissue turnover rates (Bauchinger and McWilliams, 2010), and also by environmental conditions experienced (Groom et al., 2019). With this in mind, it is likely that high-turnover tissues, such as the intestine and liver, show the greatest reduction in mass, and also likely have the greatest contribution to BMR (Chappell et al., 1999; Vézina et al., 2017). Pectoralis muscle, by contrast, contributes most to PMR, and reductions in muscle mass in fasting and migrating birds are generally small compared with those in splanchnic organs. Thus, the reduction in RMR without a reduction in PMR could simply be explained by differential organ reduction, but this explanation is insufficient given the data presented here because the metabolic suppression was independent from changes in fat-free body mass. It is possible that there is differential suppression of oxidative metabolism among tissues after flight, where the suppression occurs primarily in visceral organs and not in muscle. Such a mechanism could be detected by investigating changes in oxidative metabolism in permeabilized tissues or isolated mitochondrial preparations after flight in multiple tissue types, but this has not been widely explored in migratory birds. It is noteworthy that the PMR was substantially lower than the flight metabolic rate, and many studies that measure PMR are doing so as a comparative exercise test, but, given the fact that PMR in this study was 20% lower than calculated flight costs, it is important to keep in mind that the PMR is unlikely to be a measure of maximal metabolic rate.

Metabolic reduction after long-duration high-intensity exercise is also unique in the context of exercise physiology in mammalian systems. Flight requires long-duration high-intensity exercise, which is precisely the type of exercise that results in high excess post-exercise oxygen consumption in mammals, which is thought to aid in recovery from oxygen debt (Børsheim and Bahr, 2003). Long-duration high-intensity exercise in humans consistently results in a post-exercise metabolic increase, the magnitude of which increases with increasing duration and intensity of the exercise, and can last for hours to days (Børsheim and Bahr, 2003). The ability of migratory birds to sustain high-intensity exercise for extreme durations aerobically, while relying on fat as the primary fuel, could help explain why birds do not show a post-exercise increase in oxygen consumption, but instead show a metabolic suppression, which warrants further investigation. Sustained flight costs of our birds averaged 1.85 W, which is ~8-fold higher than our measured RMR; however, despite flying at such high intensity for 6–10 h, our birds show significant metabolic suppression rather than metabolic increase after flight, while also undergoing substantial tissue anabolism.

In conclusion, we clearly show that metabolic suppression after flight is independent of fat-free mass losses and flight duration up to 10 h, indicating a metabolic adaptation that would impact thermoregulatory costs, or minimize metabolic costs early during stopover and during non-feeding periods, possibly as a mechanism to maximize overall refueling rates. Furthermore, this metabolic response to high-intensity exercise is unique when compared with alternative exercise models, and investigation into the mechanistic basis for this suppression, as well as further understanding of avian metabolism, could broaden our understanding of heterothermy and post-exercise metabolic processes in vertebrates.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.R.G., J.G.D.; Methodology: A.R.G., J.G.D., E.C.B., M.F.D., D.J.G.; Formal analysis: A.R.G., D.J.G.; Investigation: E.C.B., M.F.D., D.J.G.; Resources: A.R.G.; Data curation: A.R.G., J.G.D., D.J.G.; Writing - original draft: A.R.G., J.G.D.; Writing - review & editing: A.R.G., J.G.D., D.J.G.; Supervision: A.R.G.; Project administration: A.R.G.; Funding acquisition: A.R.G.

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Supplementary information

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