Journal of Comparative Physiology B (2019) 189:451–462
https://doi.org/10.1007/s00360-019-01218-8

ORIGINAL PAPER

Within-winter flexibility in muscle and heart lipid transport and catabolism in passerine birds

David L. Swanson[1] · Marisa O. King[1,2] · William Culver III[1,3,4] · Yufeng Zhang[1,5]

Received: 17 January 2019 / Revised: 11 April 2019 / Accepted: 22 April 2019 / Published online: 10 May 2019

Abstract

Small birds in cold climates may show within-winter metabolic flexibility to match metabolic capacities to prevailing weather conditions. This flexibility may occur over periods of days to weeks, but the underlying mechanisms for such flexibility are not well understood. Because lipids are the primary fuel for sustained thermogenesis, we examined whether lipid transport and catabolism can mediate within-winter metabolic flexibility in two small temperate-zone wintering passerine birds, dark-eyed juncos (*Junco hyemalis*) and house sparrows (*Passer domesticus*). We used simple and multiple regression analyses to test for correlations of several lipid transporters in pectoralis muscle (plasma membrane-bound and cytosolic fatty acid-binding proteins, FABP; fatty acyl translocase, FAT/CD36) and regulatory enzymes (carnitine acyl transferase, CPT; β-hydroxyacyl CoA dehydrogenase, HOAD) in pectoralis and heart with short-term (ST, 0–7 days), medium-term (MT, 14–30 days) and long-term (LT, 30-year mean daily and extreme minimum temperatures, day of winter season) temperature variables. We hypothesized negative correlations between these regulators and temperature variables. Juncos showed negative correlations for FABPs with ST or MT temperature variables, but other lipid transporters and regulatory enzymes showed positive correlations with ST or MT temperatures for juncos, suggesting no consistent pathway-wide response to within-winter temperatures. LT temperature variables showed several significant associations with lipid transporters and enzymes for juncos, but also not in consistent directions. House sparrows showed the expected negative correlations with LT temperatures for FABPpm, but positive correlations with temperature variables for FABPc, CPT and HOAD. Different species-specific patterns of variation and the absence of consistent pathway-wide responses to temperature suggest that the lipid transport and catabolism pathway is not a uniform mediator of within-winter metabolic flexibility among small birds.

Keywords Thermogenesis · Metabolic flexibility · Birds · Winter · Lipid metabolism · Fatty acid-binding protein · Enzymes

Introduction

Small birds inhabiting cold winter climates exhibit flexible seasonal metabolic phenotypes to better match phenotypes to prevailing weather conditions. One prominent aspect of this seasonal metabolic flexibility is a winter increase in summit metabolic rate (M_{sum}; maximum cold-induced metabolic rate) (Swanson 2010; Petit et al. 2014; Swanson and Vézina 2015), which describes the maximum capacity for heat production. This winter increase in M_{sum} is associated with an increase in cold tolerance (Swanson 2001; Swanson and Liknes 2006) and high M_{sum} also promotes overwinter survival in small birds (Petit et al. 2017; Latimer et al. 2018). M_{sum} not only varies seasonally, but can vary among and within winters, in response to shorter term (days to weeks) temperature variation (Swanson and Olmstead 1999; Petit et al. 2013; Dubois et al. 2016). This within-winter variation

Communicated by F. Geiser.

✉ David L. Swanson
david.swanson@usd.edu

[1] Department of Biology, University of South Dakota, 414 East Clark Street, Vermillion, SD 57069, USA

[2] Present Address: Department of Biology, Grand View University, 1200 Grandview Ave, Des Moines, IA 50316, USA

[3] Department of Wildlife, Humboldt State University, Arcata, CA 95521, USA

[4] Present Address: Virginia-Maryland College of Veterinary Medicine, 205 Duck Pond Drive, Blacksburg, VA 24061, USA

[5] Present Address: School of Health Studies, University of Memphis, Memphis, TN 38152, USA

typically acts to elevate M_{sum} during cold winters or during cold periods within winters (Swanson and Olmstead 1999; Petit et al. 2013; Dubois et al. 2016).

Thermogenesis in adult birds is accomplished primarily, if not exclusively, through shivering in skeletal muscles (Hohtola 1982; Marsh and Dawson 1989), so winter increases in organismal M_{sum} are a product of the muscles, such that changes in thermogenic capacity are associated with changes in muscular performance (i.e., aerobic capacity). Two primary options are available to elevate muscular thermogenic performance, increases in muscle size and/or increases in cellular aerobic metabolic intensity, with supply of substrates and oxygen to shivering muscles also potentially contributing to variation in thermogenic performance (Marsh and Dawson 1989; Swanson 2010). Winter increases in the size of muscles, particularly the pectoralis muscle, and heart are consistent contributors to the winter phenotype and are positively correlated with variation in M_{sum} (Swanson and Vézina 2015; but see Milbergue et al. 2018). Nevertheless, neither the size of pectoralis muscles or heart nor expression patterns of the myostatin system (a pathway involved in regulation of muscle size; Lee 2004; Rogers and Garikipati 2008) varied consistently with short-term (0–7 days), medium-term (14–30 days), or long-term (30-year averages) temperature variables in dark-eyed juncos (*Junco hyemalis*) and house sparrows (*Passer domesticus*), suggesting that within-winter variation in M_{sum} is not primarily accomplished via changes in muscle or heart masses in these species (Swanson et al. 2017).

Indices of pectoralis muscle or heart cellular aerobic metabolic capacity (usually measured by citrate synthase or cytochrome *c* oxidase activities) are less consistently associated with seasonal M_{sum} variation, with some cold-climate wintering small birds showing winter increases or increases with cold acclimation, and others not (American goldfinch, *Spinus tristis*, Marsh and Dawson 1982; Yacoe and Dawson 1983; house finch, *Haemorhous mexicanus*, O'Connor 1995; black-capped chickadee, *Poecile atricapillus*, white-breasted nuthatch, *Sitta carolinensis*, and house sparrow, Liknes and Swanson 2011; rufous-collared sparrow, *Zonotrichia capensis*, Peña-Villalobos et al. 2014; hwamei, *Garrulax canorus*, Zhou et al. 2016). In addition, transcriptomic studies of pectoralis muscles of passerine birds show that some pathways associated with cellular aerobic metabolic capacity (e.g., Krebs cycle, oxidative phosphorylation) may be upregulated in winter or with cold acclimation, but again, variation among species of passerine birds is evident (Stager et al. 2015; Cheviron and Swanson 2017). Within-winter variation in pectoralis and heart citrate synthase (a key regulatory enzyme of the Krebs cycle) was not strongly or consistently correlated with short-, medium- or long-term temperature variables in dark-eyed juncos and house sparrows (Swanson et al. 2017). These data suggest that cellular aerobic metabolic capacity, at least as measured by citrate synthase activity, is also not a prominent driver of within-winter variation in organismal M_{sum}.

Prolonged exercise and shivering in birds are primarily fueled by catabolism of lipids from adipose tissue (McWilliams et al. 2004; Vaillancourt et al. 2005; Vaillancourt and Weber 2007). Because the lipid transport and catabolism pathways from adipose tissue to the mitochondrial matrix involve several steps, theoretically any of these steps could limit overall lipid catabolic capacity (Guglielmo 2010). Limits to lipid transport to the mitochondrion and catabolism within the mitochondrial matrix could thus potentially limit organismal exercise or thermogenic capacities (Dawson et al. 1983; Guglielmo 2010). Many aspects of pectoralis lipid catabolism and transport increase consistently with migration in birds, namely transport across muscle plasma membrane via fatty acyl translocase (FAT/CD36) and plasma membrane fatty acid-binding protein (FABPpm), and intramyocyte transport via cytosolic fatty acid-binding protein (FABPc) (Guglielmo 2010). Transport across the pectoralis mitochondrial membrane by carnitine acyl transferase (Driedzic et al. 1993, semipalmated sandpiper, *Calidris pusilla*; Guglielmo et al. 2002, western sandpiper, *Calidris mauri*; McFarlan et al. 2009, white-throated sparrow, *Zonotrichia albicollis*; but see Zhang et al. 2015a, house sparrow) and catabolism within the mitochondrial matrix via β-oxidation (often measured by β-hydroxyacyl coA dehydrogenase (HOAD) activity) (see Dawson et al. 1983; Guglielmo 2010; Swanson 2010 for reviews) also often, although not always, increase with migration in a variety of small birds. Some of these same steps are upregulated during winter for birds wintering in cold climates, but such seasonal variation appears less consistent than that for migration (Guglielmo 2010; Swanson and Vézina 2015). Pectoralis FABPpm and FABPc (black-capped chickadee and white-breasted nuthatch, Liknes et al. 2014; American goldfinch, house sparrow, Zhang et al. 2015b, c) and HOAD (American goldfinch, Marsh and Dawson 1982; Yacoe and Dawson 1983; Zhang et al. 2015b; black-capped chickadee, Liknes and Swanson 2011) often, although not always (house finch, Carey et al. 1989; O'Connor 1995; black-capped chickadee, house sparrow, Zhang et al. 2015b, c), increase with winter acclimatization or cold acclimation in a variety of passerine bird species. Pectoralis mitochondrial membrane lipid transport (measured as carnitine acyl-CoA transferase activity), however, shows little evidence for seasonal variation associated with the winter phenotype (Zhang et al. 2015b, c). Transcriptomic studies revealed that the fatty acid metabolism pathway was upregulated during cold acclimation in dark-eyed juncos (Stager et al. 2015) and during winter relative to summer for black-capped chickadees, but not for American goldfinches (Cheviron and

Swanson 2017). Thus, some steps in lipid transport and catabolism might be expected to vary with temperature to help promote within-winter metabolic flexibility in birds.

In the present study, we measured protein expression throughout the winter season for several key lipid transporters (FABPpm, FAT/CD36, and FABPc) in pectoralis muscles of dark-eyed juncos and house sparrows (*P. domesticus*). We also measured enzyme activities in pectoralis and heart for carnitine palmitoyl transferase (CPT, transport across the mitochondrial membranes) and HOAD as indices of mitochondrial lipid transport and catabolism capacities in these same two species. Both of these study species winter in cold-temperate climates and show seasonal variation in summit metabolic rates (Swanson 1990; Arens and Cooper 2005) and aspects of fat transport and catabolism (Swanson 1991; Liknes and Swanson 2011; Zhang et al. 2015b). In addition, within-winter variation in M_{sum} has also been documented for juncos (Swanson and Olmstead 1999). We used simple and multiple regression analyses to test for correlations of fat transport and catabolism indices with short-term (0–7 days), medium-term (14–30 days) and long-term (30-year averages) temperature variables. We hypothesized that values for the different indices would increase in response to cold periods during the winter to help mediate winter adjustments in M_{sum} in response to temperature variation, although we do not expect that all indices will vary with temperatures on a similar time frame for the two study species.

Materials and methods

Bird collection and tissue dissection

We collected birds in the morning (0700–1100 CST) during winters of 2010–2011 (9 December–26 February) and 2011–2012 (6 December–22 February) near Vermillion, Clay County, South Dakota (approximate latitude 42°50′N). We collected totals of 38 dark-eyed juncos (18 in 2010–2011 and 20 in 2011–2012) and 35 house sparrows (14 in 2010–2011 and 21 in 2011–2012) over the two winters. Upon capture, we weighed birds to the nearest 0.1 g on an Ohaus Model LS200 (Parsippany, NJ 07054, USA) top-loading balance, wing chord to the nearest 0.5 mm on a wing ruler, and tarsus length to the nearest 0.1 mm with calipers. Within 1 h of capture, we transported birds back to the laboratory (< 15 min transport time) where we euthanized birds by cervical dislocation followed by immediate dissection of pectoralis muscle and heart on ice. After dissection, we immediately immersed tissues in liquid nitrogen for flash-freezing and stored tissues frozen at − 70 °C until later assays, which we conducted between 30 and 38 months from sample collection.

Enzyme assays

We conducted enzyme assays for pectoralis and heart CPT (EC 2.3.1.21) and HOAD (EC 1.1.1.35) activities according to Zhang et al. (2015a). Briefly, after we removed tissues from storage we excised small samples while tissues were still frozen, minced samples on ice, and weighed them to the nearest 0.1 mg. We next homogenized samples in 10–40 volumes ice-cold homogenizing buffer (see below) on a Tekmar Model SDT-1810 Tissumizer (Cincinnati, OH, USA). Following mechanical homogenization, we sonicated tissues on ice for three 10-s periods with 30 s between sonication treatments. The homogenizing buffer for CPT contained 10 mM Tris (hydroxyl-methyl) aminomethane and 1 mM EDTA at pH 7.5 (Guglielmo et al. 2002). The HOAD homogenizing buffer consisted of 100 mM phosphate and 2 mM EDTA at pH 7.3 (Liknes and Swanson 2011).

We conducted spectrophotometric assays at 39 °C to measure activities for both enzymes with a Beckman DU 7400 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). We used wavelengths of 412 nm and 340 nm for CPT and HOAD assays, respectively. The buffer for CPT assays contained 50 mM Tris buffer at pH 8.0, with 5 mM carnitine, 0.15 mM DTNB, 0.035 mM palmitoyl CoA, and 10 µL of homogenate in 1 mL total volume (Guglielmo et al. 2002). The assay buffer for HOAD contained 100 mM triethanolamine–HCl, 5 mM EDTA, 0.225 mN $NADH_2$, 0.1 mM acetoacetyl CoA and 5 µL of homogenate in 1.0 mL total volume at pH 7.0. For both assays, we measured background activity for 2–3 min before addition of the substrate to initiate the reaction, and continued measurements for 7–8 min after substrate addition. We subtracted background activity from reaction rates after the addition of substrate to calculate enzyme reaction rates. We conducted duplicate samples for each individual tissue and used the average in subsequent analyses. We report all enzyme activities as mass-specific values (µM min^{-1} g wet tissue $mass^{-1}$).

Western blots

We conducted western blots to determine protein expression levels in pectoralis muscle of the two study species using antibodies for FAT/CD36, FABPpm and FABPc according to the methods of Zhang et al. (2015a). Briefly, we removed pectoralis samples from the tissue while it was still frozen and sonicated samples on ice, as previously described, in a sonication buffer of 50 mM Tris, 100 mM NaCl, and 2% SDS at pH 7.5. After sonication, we decanted supernatants from homogenates for analysis with a WesternBreeze kit (Invitrogen, Carlsbad, CA, USA). We used a modified DC Lowry assay to determine protein concentrations and loaded 20 µg protein from homogenates into each well for SDS-polyacrylamide gel electrophoresis to separate proteins. We

probed protein blots with primary antibodies for FAT/CD36 (rabbit polyclonal; from C.G. Guglielmo, 1:8000 dilution), FABPpm (rabbit polyclonal; from C.G. Guglielmo, 1:10,000 dilution) and FABPc (rabbit polyclonal; from C.G. Guglielmo, 1:8000 dilution; McFarlan et al. 2009) and GAPDH (chicken polyclonal, Millipore, Temecula, CA, USA). We then washed membranes with TBS-T and incubated them with horseradish peroxidase-conjugated secondary antibodies; anti-rabbit (1:2000 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) for FAT/CD36, FABPpm and FABPc, and anti-chicken (1:1500 dilution; Abcam, Cambridge, MA, USA) for GAPDH. We used the ECL Plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK) for visualizing blots and a VersaDoc 3000 Molecular Imager (Bio-Rad, Hercules, CA, USA) to capture chemiluminescent images to quantify protein levels. We divided protein expression values for FAT/CD36, FABPpm and FABPc by protein expression values for GAPDH (as a housekeeping protein) and used these values corrected for GAPDH expression in subsequent analyses.

Statistical analyses

We present data as mean ± SE unless otherwise indicated. Mean temperatures for Vermillion, Clay County, SD, were colder during the 2010–2011 winter than the 2011–2012 winter (Swanson et al. 2017). Mean December–February daily average temperatures were − 8.8 °C for 2010–2011 and − 3.2 °C for 2011–2012. Similarly, mean December–February daily minimum temperatures were − 14.3 °C for 2010–2011 and − 9.2 °C for 2011–2012 (South Dakota Office of Climatology, http://climate.sdstate.edu/climate_site/archive_data.htm). Consequently, we compared mean values for enzyme activities and protein expression for each of the two study species between the two winters with Student's *t* test or Mann–Whitney *U* test, if parametric assumptions were not met.

We used simple and multiple (forward-stepwise) regression analyses to analyze the effects of temperature variables on enzyme activities and protein expression for both study species according to Swanson and Olmstead (1999) and Swanson et al. (2017). Temperature variables for the regression analyses included short-term (ST; running means for daily minimum and daily average temperatures for 0-, 1-, 3-, 5- and 7-day periods prior to capture), medium-term (MT; 14- and 30-day running means), and long-term [LT; 30-year means for daily average and minimum temperature, extreme minimum temperature for the day of capture, and day of winter season (1 = 1 December)] temperature variables. We obtained daily temperature data for Vermillion, Clay County, South Dakota from the South Dakota Office of Climatology, http://climate.sdstate.edu/climate_site/archive_data.htm). We first conducted simple linear regression analyses of enzyme activities and protein expression against all ST, MT and LT temperature variables. Following Swanson et al. (2017), we did not adjust α-levels for multiple simple regression tests because such tests may be too conservative in detecting significant results (Moran 2003; Nakagawa 2004) and because the pattern of *P* value variation around the most significant variables was always consistent and in the same direction (e.g., if a 3-day variable showed the highest *P* value and a negative trend, other ST temperature variables showed similar negative relationships and ST variables had lower *P* values than MT variables).

If more than one temperature variable gave significant results for simple linear regression analyses, we used forward-stepwise multiple regression analyses (Swanson and Olmstead 1999; Swanson et al. 2017) to detect which temperature variables were the best predictors of variation in enzyme activities or protein expression. We considered *P* values ≤ 0.05 as significant. We conducted all analyses with SigmaStat Version 3.5 (Systat, Point Richmond, CA, USA).

Results

Between-winter comparisons

Dark-eyed juncos showed significantly greater pectoralis protein expression for FABPpm and FABPc in the colder winter of 2010–2011 than in the warmer winter of 2011–2012 (Fig. 1), but FAT/CD36 protein expression did not vary significantly between winters. Enzyme activities,

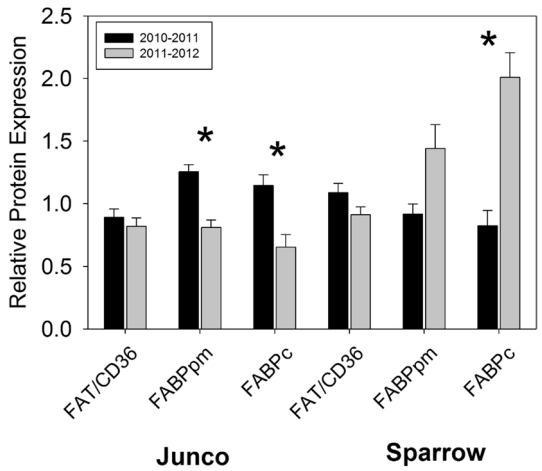

Fig. 1 Winter-to-winter variation in protein expression for pectoralis sarcolemmal lipid transporters, fatty acyl translocase (FAT/CD36) and plasma membrane fatty acid-binding protein (FABPpm), and cytosolic fatty acid-binding protein (FABPc) for dark-eyed juncos and house sparrows. Temperatures during the winter of 2010–2011 averaged approximately 5 °C colder than in 2011–2012. Asterisks indicate significant differences between winters

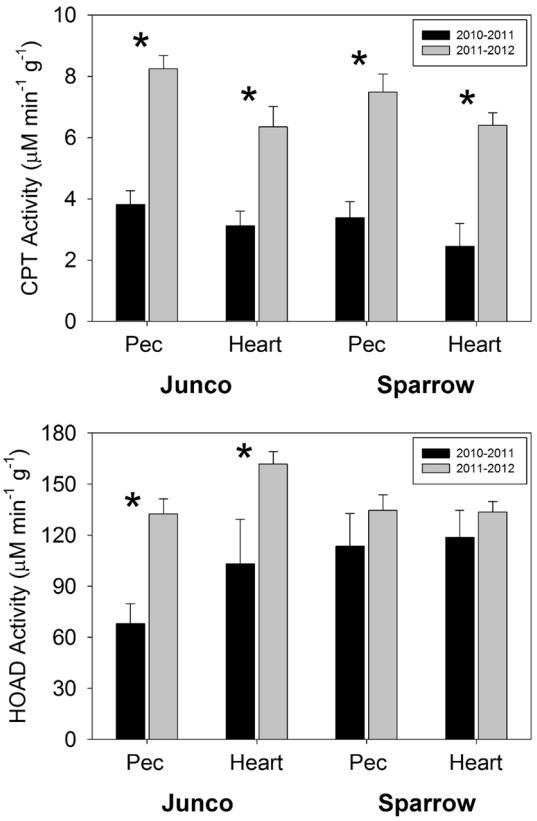

Fig. 2 Winter-to-winter variation in pectoralis (pec) and heart enzyme activities for carnitine palmitoyl transferase (CPT) and β-hydroxyacyl CoA dehydrogenase (HOAD) for dark-eyed juncos and house sparrows. Temperatures during the winter of 2010–2011 averaged approximately 5 °C colder than in 2011–2012. Asterisks indicate significant differences between winters

however, showed the opposite trend for juncos with CPT and HOAD activities in both pectoralis and heart being higher during the warmer winter of 2011–2012 than the colder winter of 2010–2011 (Fig. 2). House sparrows showed significant between-winter differences in pectoralis FABPc protein expression and pectoralis and heart CPT activities, with higher values during the warmer winter of 2011–2012 (Figs. 1, 2). Pectoralis protein expression for FAT/CD36 and FABPpm, and pectoralis and heart HOAD activities, however, did not vary significantly between winters for house sparrows (Figs. 1, 2).

Temperature effects on pectoralis protein expression

Both pectoralis FABPpm and FABPc showed significant negative correlations with short- and medium-term temperature variables for dark-eyed juncos, with medium-term temperatures showing the strongest correlations (Table 1). The only long-term temperature variable showing significant correlations with pectoralis FABPs for simple regression

Table 1 Pearson correlation coefficients for significant temperature variables in simple regressions of pectoralis protein expression from western blots for membrane-bound (FABPpm and FAT/CD36) and cytosolic (FABPc) fat transporters vs. short-, medium- and long-term temperature variables

Species	*Pec FABPpm*	*r*	*F* (*df*)	*P*	*Pec FAT/CD36*	*r*	*F* (*df*)	*P*	*Pec FABPc*	*r*	*F* (*df*)	*P*
Sparrow	LT Min	−0.472	9.461 (1, 33)	0.004	–	–	–	–	14-Day Avg	0.359	4.882 (1, 33)	0.034
	LT Avg	−0.454	8.562 (1, 33)	0.006					30-Day Low	0.545	13.951 (1, 33)	< 0.001
									30-Day Avg	0.557	14.876 (1, 33)	< 0.001
Junco	1-Day Low	−0.325	4.134 (1, 35)	0.050	0-Day Low	0.473	10.100 (1, 35)	0.003	5-Day Avg	−0.342	4.492 (1, 34)	0.041
	1-Day Avg	−0.424	7.690 (1, 35)	0.009	0-Day Avg	0.497	11.461 (1, 35)	0.002	5-Day Low	−0.377	5.647 (1, 34)	0.023
	3-Day Low	−0.547	14.950 (1, 35)	< 0.001	LT Min	0.345	4.719 (1, 35)	0.037	7-Day Low	−0.408	6.802 (1, 34)	0.013
	3-Day Avg	−0.349	4.847 (1, 35)	0.034					7-Day Avg	−0.448	8.552 (1, 34)	0.006
	5-Day Avg	−0.374	5.698 (1, 35)	0.023					14-Day Low	−0.460	9.131 (1, 34)	0.005
	7-Day Low	−0.442	7.574 (1, 35)	0.009					14-Day Avg	−0.472	9.763 (1, 34)	0.004
	7-Day Avg	−0.470	9.900 (1, 35)	0.003					30-Day Low	−0.479	10.134 (1, 34)	0.003
	14-Day Low	−0.701	33.814 (1, 35)	< 0.001					30-Day Avg	−0.513	12.141 (1, 34)	0.001
	14-Day Avg	−0.674	29.147 (1, 35)	< 0.001					LT Avg	0.363	5.161 (1, 34)	0.030
	30-Day Low	−0.689	31.705 (1, 35)	< 0.001								
	30-Day Avg	−0.651	25.785 (1, 35)	< 0.001								

analyses was LT Avg for FABPc, which showed a positive correlation (Table 1). Multiple regression analyses identified 14-day Low ($\Delta R^2 = 0.491$) and LT Avg ($\Delta R^2 = 0.189$) temperature variables as significant predictors for FABPpm and 30-day Avg ($\Delta R^2 = 0.263$), LT Avg ($\Delta R^2 = 0.146$), 7-day Avg ($\Delta R^2 = 0.137$) and 3-day Low ($\Delta R^2 = 0.065$) as significant predictors for FABPc (Fig. 3). Pectoralis FAT/CD36 in juncos showed different relationships with temperature variables than the FABPs, with 0-day and LT Min temperature variables being the only significant correlations for simple regression analyses, and the relationship was positive for both of these temperature variables. Multiple regression analyses identified 0-day Avg ($\Delta R^2 = 0.247$) and 5-day Low ($\Delta R^2 = 0.142$) as the only significant predictors of FAT/CD36 protein expression for juncos (Fig. 3).

House sparrow pectoralis fat transporters, in general, showed less variability in response to temperature than juncos. Pectoralis FABPpm for sparrows was significantly correlated only with long-term temperature variables, with both LT Min and LT Avg showing negative relationships with FABPpm (Table 1). In contrast, sparrow pectoralis FABPc showed significant correlations only with medium-term temperature variables and those relationships were positive (Table 1). Multiple regression analyses identified only LT Min as a significant predictor of pectoralis FABPpm protein expression (Fig. 3). Sparrow pectoralis FABPc, however, showed several significant predictors of protein expression in multiple regression analyses, with 30-day Avg ($\Delta R^2 = 0.271$), 0-day Low ($\Delta R^2 = 0.094$), day of winter season ($\Delta R^2 = 0.085$), 30-day Low ($\Delta R^2 = 0.069$), and LT Avg ($\Delta R^2 = 0.061$) all serving as significant predictors of FABPc protein expression (Fig. 3). Pectoralis FAT/CD36 was not significantly correlated with any temperature variables for either simple or multiple regression analyses in house sparrows.

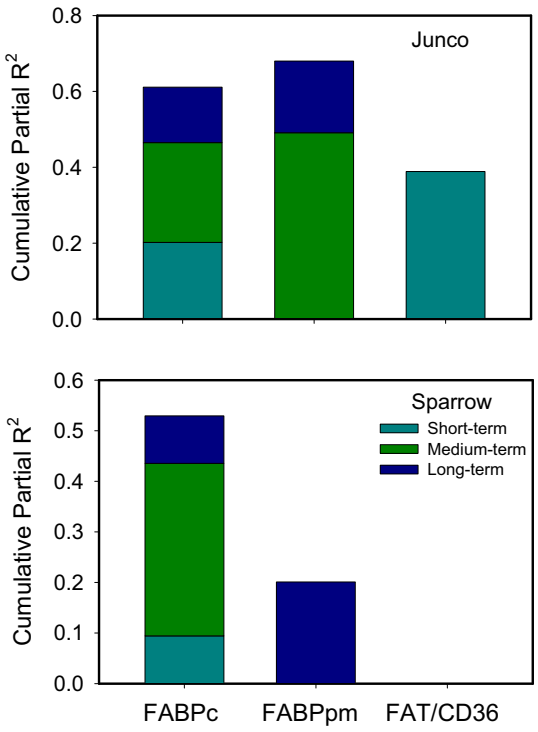

Fig. 3 Cumulative partial R^2 values for forward-stepwise multiple regression of short-term (0–7 days), medium-term (14–30 days), and long-term (30-year averages and day of winter season) temperature variables vs. pectoralis lipid transporter [fatty acyl translocase (FAT/CD36), plasma membrane fatty acid-binding protein (FABPpm), and cytosolic fatty acid-binding protein (FABPc)] protein expression for dark-eyed juncos and house sparrows. The zero value for FAT/CD36 for house sparrows occurred because no temperature variables showed significant correlations

Temperature effects on pectoralis and heart enzyme activities

Pectoralis CPT activity in juncos was significantly positively correlated with medium-term temperature variables and significantly negatively correlated with day of winter season, with 30-day Avg showing the strongest relationship (Table 2). Multiple regression analyses identified 30-day Avg ($\Delta R^2 = 0.537$), LT Avg ($\Delta R^2 = 0.144$), and 30-day Low ($\Delta R^2 = 0.034$) as significant predictors of pectoralis CPT activity in juncos (Fig. 4). CPT activity in junco heart was significantly positively correlated with short- and medium-term temperature variables, with 30-day Avg showing the strongest correlation (Table 2). Multiple regression analyses identified only 30-day Avg as a significant predictor of heart CPT activity in juncos ($\Delta R^2 = 0.423$, Fig. 4).

Junco pectoralis HOAD activity was positively correlated with medium-term temperature variables and negatively correlated with day of winter season (Table 2). Multiple regression analyses identified 30-day Avg ($\Delta R^2 = 0.389$), 1-day Low ($\Delta R^2 = 0.105$), and 30-day Low ($\Delta R^2 = 0.069$) as significant predictors of pectoralis HOAD activity in juncos (Fig. 4). HOAD activity in junco heart was positively correlated with short- and medium-term temperature variables, with 7-day Avg providing the strongest correlation (Table 2). Significant predictors of junco heart HOAD activity in multiple regression analyses included 7-day Avg ($\Delta R^2 = 0.163$) and 5-day Avg ($\Delta R^2 = 0.119$) temperature variables (Fig. 4).

House sparrow pectoralis CPT activity showed positive correlations with short- and medium-term temperature variables in simple regressions, with 30-day Avg showing the strongest correlation (Table 2). Significant predictors of pectoralis CPT activity in house sparrows identified by multiple regression analyses included 14-day Avg ($\Delta R^2 = 0.317$), LT Avg ($\Delta R^2 = 0.117$), 7-day Low ($\Delta R^2 = 0.113$) and 0-day Avg ($\Delta R^2 = 0.085$) (Fig. 4). For heart CPT activity in sparrows, simple regression analyses showed significant positive relationships with

Table 2 Pearson correlation coefficients for significant temperature variables in simple regression analyses of pectoralis and heart carnitine palmitoyl transferase (CPT) and β-hydroxyacyl CoA dehydrogenase (HOAD) activities vs. short-, medium- and long-term temperature variables

Species	*Pec CPT*	*r*	*F* (*df*)	*P*	*Pec HOAD*	*r*	*F* (*df*)	*P*	*Hrt CPT*	*r*	*F* (*df*)	*P*
Sparrow	0-Day Avg	0.368	4.545 (1, 29)	0.042	LT Min	0.570	14.888 (1, 31)	< 0.001	7-Day Avg	0.368	4.703 (1, 30)	0.038
	7-Day Avg	0.363	4.550 (1, 30)	0.041	LT Avg	0.549	14.349 (1, 31)	< 0.001	14-Day Avg	0.473	9.215 (1, 32)	0.005
	14-Day Low	0.344	4.287 (1, 32)	0.047					30-Day Low	0.598	17.803 (1, 32)	< 0.001
	14-Day Avg	0.476	9.369 (1, 32)	0.004					30-Day Avg	0.658	24.477 (1, 32)	< 0.001
	30-Day Low	0.475	9.320 (1, 32)	0.005					Day	− 0.435	7.472 (1, 32)	0.010
	30-Day Avg	0.544	13.438 (1, 32)	< 0.001								
Junco	14-Day Low	0.484	11.023 (1, 36)	0.002	14-Day Avg	0.411	7.304 (1, 36)	0.010	3-Day Low	0.437	8.243 (1, 35)	0.007
	14-Day Avg	0.556	16.146 (1, 36)	< 0.001	30-Day Low	0.553	15.847 (1, 36)	< 0.001	5-Day Avg	0.334	4.380 (1, 35)	0.044
	30-Day Low	0.680	30.970 (1, 36)	< 0.001	30-Day Avg	0.624	22.906 (1, 36)	< 0.001	7-Day Low	0.352	4.937 (1, 35)	0.033
	30-Day Avg	0.733	41.690 (1, 36)	< 0.001	Day	− 0.476	10.526 (1, 36)	0.003	7-Day Avg	0.440	8.390 (1, 35)	0.006
	Day	− 0.400	6.875 (1, 36)	0.013					14-Day Low	0.558	15.840 (1, 35)	< 0.001
									14-Day Avg	0.598	19.529 (1, 35)	< 0.001
									30-Day Low	0.632	23.233 (1, 35)	< 0.001
									30-Day Avg	0.650	25.663 (1, 35)	< 0.001

Species	*Hrt HOAD*	*r*	*F* (*df*)	*P*
Sparrow	5-Day Low	0.360	4.311 (1, 29)	0.047
	5-Day Avg	0.367	4.517 (1, 29)	0.042
Junco	3-Day Low	0.330	4.388 (1, 36)	0.043
	7-Day Low	0.351	5.058 (1, 36)	0.031
	7-Day Avg	0.404	7.017 (1, 36)	0.012
	14-Day Avg	0.347	4.935 (1, 36)	0.033

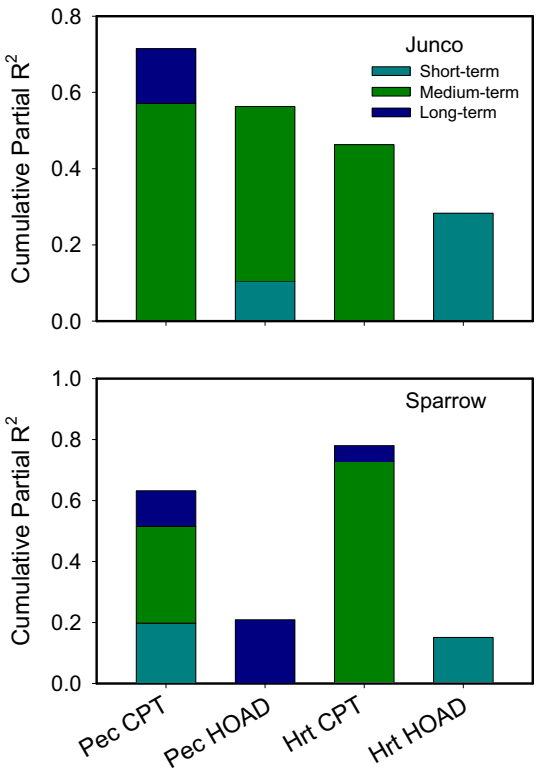

Fig. 4 Cumulative partial R^2 values for forward-stepwise multiple regression of short-term (0–7 days), medium-term (14–30 days), and long-term (30-year averages and day of winter season) temperature variables vs. pectoralis (Pec) and heart (Hrt) enzyme activities for carnitine palmitoyl transferase (CPT) and β-hydroxyacyl CoA dehydrogenase (HOAD) for dark-eyed juncos and house sparrows

short- and medium-term temperature variables, with 30-day Avg showing the strongest correlation, and a significant negative relationship with day of winter season (Table 2). Multiple regression analyses identified 30-day Avg ($\Delta R^2 = 0.637$), 30-day Low ($\Delta R^2 = 0.091$) and LT Avg ($\Delta R^2 = 0.052$) as significant predictors of heart CPT activity in sparrows (Fig. 4).

Temperature variables were relatively poor predictors of HOAD activity for both pectoralis and heart in sparrows. Pectoralis HOAD activity in sparrows showed significant positive relationships only with LT Min and LT Avg temperatures (Table 2). Only LT Min ($\Delta R^2 = 0.209$) remained in the model as a significant predictor of pectoralis HOAD activity for multiple regression (Fig. 4). Only short-term temperature variables were significantly correlated with heart HOAD activity for sparrows, with both 5-day Low and 5-day Avg temperatures positively associated with HOAD activity (Table 2). The only temperature variable retained in the multiple regression model for sparrow heart HOAD activity was 5-day Low ($\Delta R^2 = 0.151$, Fig. 4).

Discussion

Small birds can respond to acute (days to weeks) temperature variation within winters by modifying metabolic capacities (Swanson and Olmstead 1999; Petit et al. 2013; Dubois et al. 2016). This within-winter variation in metabolic capacity, however, is apparently not driven primarily by changes in muscle size or cellular metabolic intensity (Swanson et al. 2017), despite the importance of these factors for seasonal metabolic flexibility (Marsh and Dawson 1989; Swanson and Vézina 2015; but see Milbergue et al. 2018). Consequently, Swanson et al. (2017) suggested that within-winter variation in lipid transport and catabolism might be a candidate for promoting within-winter metabolic flexibility, given the importance of such variation for seasonal and migratory acclimatization in birds (see Marsh and Dawson 1989; Guglielmo 2010; Swanson 2010 for reviews). We addressed this possibility in the present study by examining correlations of short-, medium- and long-term temperature variables with key regulators of lipid transport and catabolism in two species of north-temperate resident passerines, dark-eyed juncos and house sparrows. If variation in lipid transport and catabolism promotes within-winter variation in metabolic capacities, we predicted an upregulation of key regulators of lipid transport and catabolism in response to cold. Responses of these key regulators of lipid transport and catabolism to temperatures were varied, with some showing the expected correlations and others not. Similarly, winter-to-winter variation between the colder winter of 2010–2011 and the warmer winter of 2011–2012 differed among regulators of lipid transport and metabolism, with some being higher during the colder winter and some showing the opposite pattern or not differing significantly between winters.

For juncos, pectoralis fatty acid-binding proteins (both plasma membrane and cytosolic forms) showed the expected negative correlations with short- and medium-term temperature variables, but other fat transporters and catabolic enzymes did not. Protein expression of pectoralis fatty acid-binding proteins was also higher during the colder winter of 2010–2011, but other regulators of lipid transport and catabolism did not show this trend for juncos. House sparrows showed a positive correlation of FABPc with medium-term temperature variables and although FABPpm was negatively related to temperature, it responded only to long-term temperature variables. In addition, responses of the fatty acid-binding proteins in sparrows were generally weaker than those for juncos. Other regulators of lipid transport and catabolism for house sparrows were not negatively correlated with temperature variables. In addition, none of the regulators of lipid transport and catabolism showed higher levels

during the colder winter for house sparrows, suggesting that regulation of lipid metabolism does not play a key role in promoting winter-to-winter metabolic variation in this species. Thus, the data in the present study offer only partial support for our hypothesis and only for juncos. Nevertheless, the data suggest that the fatty acid-binding proteins may be among the most significant contributors to within-winter metabolic flexibility in dark-eyed juncos.

The timing of FABP variation for juncos, where 14- to 30-day temperature variables gave the strongest correlations and medium-term temperatures were the dominant effector of partial R^2 in multiple regressions, fits well with temporal trends in within-winter metabolic variation, which also responded most strongly to 14- to 30-days temperature variables (Swanson and Olmstead 1999). Pectoralis FABPc protein expression also responded most strongly to medium-term temperature variables in house sparrows, but the increased expression occurred under warmer temperatures, and this pattern was not evident for FABPpm, where significant correlations only occurred in response to long-term average temperatures. These data suggest a prominent role for pectoralis fatty acid-binding proteins, particularly FABPc, in mediating within-winter metabolic flexibility in juncos, but not house sparrows.

Among the key regulators of lipid transport and catabolism, FABPc may show the most consistent variation with the increasing energy demands associated with migration or cold winters. Pectoralis FABPc mRNA or protein expression (or both) increases during migration in barnacle geese (*Branta leucopsis*; Pelsers et al. 1999), western sandpipers (Guglielmo et al. 2002), white-throated sparrows (McFarlan et al. 2009), and during either spring or fall migration in warbling vireos (*Vireo gilvus*), and yellow (*Setophaga petechia*) and yellow-rumped (*Setophaga coronata*) warblers (Zhang et al. 2015a). Exposure to migratory photoperiods also increased FABPc mRNA and protein expression in pectoralis of white-throated sparrows (Zajac et al. 2011). However, white-crowned sparrows (*Zonotrichia leucophrys*) did not show significant variation in pectoralis FABPc mRNA in response to exposure to migratory photoperiod, although values were higher than those for birds on winter photoperiods (Price et al. 2010). Pectoralis FABPc levels were also higher in winter than in summer for black-capped chickadee and white-breasted nuthatch, but not for house sparrow (Liknes et al. 2014), and winter FABPc protein expression was higher in black-capped chickadee and American goldfinch, although the difference was significant only for goldfinches (Zhang et al. 2015b). House sparrows also showed significant increases in pectoralis FABPc protein expression with both exercise and cold training (Zhang et al. 2015c). Finally, pectoralis FABPc protein expression varied with exposure of dark-eyed juncos to cold and day length, with elevated values under cold/long-day photoperiod and warm/short-day photoperiods, suggesting an interaction between temperature and photoperiod in the regulation of FABPc expression in pectoralis (Zhang et al. 2018). Thus, the finding in the present study that pectoralis FABPc in dark-eyed juncos was the most consistently related to medium-term temperature variation fits the general pattern of upregulation of pectoralis FABPc with increasing energy demands in birds.

Variation in the sarcolemmal lipid transporters FABPpm and FAT/CD36 in pectoralis in response to increasing energy demands in birds is less consistent than that for the intramyocyte lipid transporter FABPc. Increases in protein or mRNA expression of one or both pectoralis sarcolemmal lipid transporters occur during migration, or with exposure to migratory photoperiods, in a number of species (McFarlan et al. 2009; Zajac et al. 2011; Zhang et al. 2015a), but not in others (Price et al. 2010; Zhang et al. 2015a). Pectoralis FABPpm, but not FAT/CD36, protein expression increased in winter relative to summer for black-capped chickadees, but American goldfinches showed winter increases in pectoralis protein expression for FAT/CD36, but not FABPpm (Zhang et al. 2015b). Exercise and cold training in house sparrows resulted in significant increases in pectoralis FAT/CD36, but not FABPpm, protein expression (Zhang et al. 2015c). Finally, exposure to cold and short days elevated FAT/CD36 protein expression in pectoralis of dark-eyed juncos, but pectoralis FABPpm protein expression was highest on cold/long days and warm/short days, so the two sacrolemmal lipid transporters showed differing responses to temperature and photoperiod for juncos (Zhang et al. 2018). The varied responses of the pectoralis sarcolemmal lipid transporters to within-winter temperature variation in the two species in the present study is thus consistent with the variable responses of these transporters to changing energy demands in birds generally, and suggest that these transporters are not uniform targets for driving within-winter metabolic flexibility across passerine birds.

Further potential limiting steps to lipid catabolic capacities and organismal summit metabolic rates include transport of fatty acids across the mitochondrial membranes by carnitine acyl transferases and catabolism of fatty acids within the mitochondrion via the β-oxidation pathway (Guglielmo 2010). Neither CPT nor HOAD (proxies for mitochondrial membrane transport and β-oxidation capacities) activities in pectoralis and heart were significantly negatively associated with temperature variables, as we initially hypothesized, for the two species in present study. In addition, activities for both of these enzymes were significantly higher or tended to be higher during the warmer winter of 2011–2012 than the colder winter of 2010–2011, which is also inconsistent with our hypothesis. The absence of a clear negative relationship with temperature variables for CPT and HOAD suggests that these enzymes are not regulators of within-winter

pectoralis and heart lipid catabolic capacity or organismal metabolic capacity. Such a conclusion adds to a growing body of evidence suggesting varied and inconsistent roles for these enzymes in promoting increases in lipid catabolic capacity with increasing energy demands in birds.

Pectoralis CPT activity increases with migratory condition in some species (Driedzic et al. 1993; Guglielmo et al. 2002; McFarlan et al. 2009; Zajac et al. 2011), but not in other species (Price et al. 2010; Zhang et al. 2015a). Price et al. (2010) documented lower pectoralis CPT activity in exercised birds in winter than in birds stimulated to migratory condition by long days, but non-exercised winter birds showed intermediate levels, not significantly different from other groups, suggesting that exercise actually reduced CPT activity in winter. Moreover, Zhang et al. (2015b) detected no summer to winter increase in pectoralis or heart CPT activity for black-capped chickadees and American goldfinches, with a tendency toward lower values in winter for both tissues for goldfinches. In addition, neither cold nor exercise training significantly influenced pectoralis or heart CPT activities in house sparrows (Zhang et al. 2015c). The inconsistent patterns of pectoralis and heart CPT variation with increasing energy demands in birds, coupled with the absence of negative associations with temperature in the present study, suggest that an increase in CPT activity is not a regular component of the winter phenotype in passerine birds.

Patterns of pectoralis HOAD variation with increasing energy demands in birds are also varied, with increases with migratory condition or winter/cold exposure in some species but not in others (see Dawson et al. 1983; Swanson 2010; Guglielmo 2010 for reviews). More recent studies indicate similarly varied patterns in pectoralis and heart HOAD with increasing energy demands in birds (Price et al. 2010; Zajac et al. 2011; Liknes and Swanson 2011; Zhang et al. 2015a, b, c). In this sense, the absence of an increase in pectoralis and heart HOAD activity with colder temperatures in the present study fits the overall picture of variation in HOAD responses to energy demands in birds. Collectively, these data suggest that increases in pectoralis HOAD activity, like CPT activity, may function to promote increased lipid catabolic capacity in some species under some conditions, but such increases are far from a uniform response for all birds.

The data in the present study suggest little role for variation in pectoralis or heart lipid transport and catabolism, outside of pectoralis fatty acid-binding proteins in juncos, in mediating within-winter variation in organismal metabolic capacity in passerine birds. The finding of inconsistent patterns of variation among key regulators of lipid transport and catabolism in response to temperature does not support the concept of symmorphosis (Weibel 2000) in this pathway, at least with reference to within-winter variation in response to temperature. Pectoralis transcriptomic analyses examining pathway-level regulation of fatty acid metabolism with changing energy demands in birds also show differing results among species and conditions (Stager et al. 2015; Cheviron and Swanson 2017). For example, winter-collected dark-eyed juncos showed concerted expression changes (27% of genes in the pathway) for pectoralis fatty acid metabolism in response to cold acclimation and when exposed to long days stimulating development of migratory condition (Stager et al. 2015). However, for specific enzymes, CPT mRNA expression was upregulated by cold exposure, but HOAD mRNA expression was not, and HOAD mRNA expression was upregulated by long days, but CPT was not (Stager et al. 2015). Moreover, CPT expression in the Stager et al. (2015) study was not correlated with enzyme activity measured from the same birds, but HOAD expression was positively correlated with enzyme activity. These data emphasize the point that studies of pathway-level regulation may not give identical results to studies of individual key regulatory steps within the pathway (Stager et al. 2015). Seasonal pathway-level patterns of mRNA expression may also differ among species with reference to changes in energy demands. For example, concerted winter upregulation for fatty acid oxidation in pectoralis occurred for black-capped chickadees, but not for American goldfinches (Cheviron and Swanson 2017). The data in the present study are consistent with the general trends from pectoralis transcriptomic analyses of variability in expression among different pathway steps and between-species variability in patterns of change among pathways.

Of the regulators of lipid transport and catabolism in pectoralis measured in this study, the only regulators showing consistent upregulation with short- or medium-term cold temperatures were the fatty acid-binding proteins FABPpm and FABPc in juncos. The strongest effect on medium-term temperature variables on levels of these transporters fits with the similar time frame for within-winter variation in metabolic capacity in this species (Swanson and Olmstead 1999). Combined with the absence of response of pectoralis muscle size and cellular metabolic intensity with short- to medium-term temperatures in this species (Swanson et al. 2017), this suggests that the fatty acid-binding proteins might be prominent regulators of within-winter metabolic capacity in juncos. Similar results did not occur for house sparrows, so the junco pattern does not apply uniformly for passerine birds. The lesser response of fat transport and catabolism to temperature in house sparrows might be associated with their 37% larger body size in the present study (mean ± SE body masses: house sparrow = 28.3 ± 0.3 g; dark-eyed junco = 20.6 ± 0.2 g) and lower surface area to volume ratios, or with their occupation of human-associated microhabitats that might serve to moderate exposure to cold temperatures during the winter (Anderson 2006; Lowther and Cink 2006).

Documentation of the impact of microclimates on metabolic variation and its mechanistic underpinnings in small birds will require further study.

Acknowledgements We thank Ken Renner, Sol Redlin and Kathie Rasmussen for access to their property for collection of house sparrows. We thank Jianqiu Zou and Yi-Fan Li for their advice on western blots. Two anonymous reviewers provided valuable and constructive comments on an earlier version of this manuscript and we thank them for their efforts. The research reported in the manuscript complies with the guidelines and rules for animal care and use for scientific research in the USA, and was conducted under approved protocol number 79-01-11-14C from the University of South Dakota Institutional Animal Care and Use Committee. Birds were collected under active state (10-2, 11-7, and 12-2) and federal (MB758442) collecting permits. This research was funded by NSF IOS-1021218 to DLS.

References

Anderson T (2006) Biology of the ubiquitous house sparrow: from genes to populations. Oxford University Press, Oxford

Arens JR, Cooper SJ (2005) Seasonal and diurnal variation in metabolism and ventilation in house sparrows. Condor 107:433–444

Carey C, Marsh RL, Bekoff A, Johnston RM, Olin AM (1989) Enzyme activities in muscles of seasonally acclimatized house finches. In: Bech C, Reinertsen RE (eds) Physiology of cold adaptation in birds. Plenum, New York, pp 95–104

Cheviron ZA, Swanson DL (2017) Comparative transcriptomics of seasonal phenotypic flexibility in two species of North American songbirds. Integr Comp Biol 57:1040–1054

Dawson WR, Marsh RL, Yacoe ME (1983) Metabolic adjustments of small passerine birds for migration and cold. Am J Physiol 245:R755–R767

Driedzic WR, Crowe HL, Hicklin PW, Sephton DH (1993) Adaptations in pectoralis muscle, heart mass, and energy metabolism during premigratory fattening in Semipalmated Sandpipers (*Calidris pusilla*). Can J Zool 71:1602–1608

Dubois K, Hallot F, Vézina F (2016) Basal and maximal metabolic rates differ in their response to rapid temperature change among avian species. J Comp Physiol B 186:919–935

Guglielmo CG (2010) Move that fatty acid: fuel selection and transport in migratory birds and bats. Integr Comp Biol 50:336–345

Guglielmo CG, Haunerland NH, Hochachka PW, Williams TD (2002) Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. Am J Physiol 282:R1405–R1413

Hohtola E (1982) Thermal and electromyographic correlates of shivering thermogenesis in the pigeon. Comp Biochem Physiol 73A:159–166

Latimer CE, Cooper SJ, Karasov WH, Zuckerberg B (2018) Does habitat fragmentation promote climate-resilient phenotypes? Oikos 127:1069–1080

Lee S-J (2004) Regulation of muscle mass by myostatin. Annu Rev Cell Dev Biol 20:61–86

Liknes ET, Swanson DL (2011) Phenotypic flexibility in passerine birds: seasonal variation of aerobic enzyme activities in skeletal muscle. J Therm Biol 36:430–436

Liknes ET, Guglielmo CG, Swanson DL (2014) Phenotypic flexibility in passerine birds: seasonal variation in fuel storage, mobilization and transport. Comp Biochem Physiol A 174:1–10

Lowther PE, Cink CL (2006). House sparrow (*Passer domesticus*). In: Poole AF (ed) The birds of North America. Cornell Laboratory of Ornithology, Ithaca. https://doi.org/10.2173/bna.12

Marsh RL, Dawson WR (1982) Substrate metabolism in seasonally acclimatized American goldfinches. Am J Physiol 242:R563–R569

Marsh RL, Dawson WR (1989) Avian adjustments to cold. In: Wang LCH (ed) Advances in comparative and environmental physiology 4: animal adaptation to cold. Springer, New York, pp 205–253

McFarlan JT, Bonen A, Guglielmo CG (2009) Seasonal upregulation of fatty acid transporters in flight muscles of migratory white-throated sparrows (*Zonotrichia albicollis*) sparrows. J Exp Biol 212:2934–2940

McWilliams SR, Guglielmo CG, Pierce B, Klaassen M (2004) Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35:377–393

Milbergue MS, Blier PU, Vézina F (2018) Large muscles are beneficial but not required for improving thermogenic capacity in small birds. Sci Rep 8:14009

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100:403–405

Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecol 15:1044–1045

O'Connor TP (1995) Seasonal acclimatization of lipid mobilization and catabolism in House Finches (*Carpodacus mexicanus*). Physiol Zool 68:985–1005

Pelsers MMAL, Butler PJ, Bishop CM, Glatz JFC (1999) Fatty acid binding protein in heart and skeletal muscles of the migratory barnacle goose throughout development. Am J Physiol 276:R637–R643

Peña-Villalobos I, Nuñez-Villegas M, Bozinovic F, Sabat P (2014) Metabolic enzymes in seasonally acclimatized and cold acclimated rufous-collared sparrows inhabiting a Chilean Mediterranean environment. Curr Zool 60:338–350

Petit M, Lewden A, Vézina F (2013) Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and thermogenic capacity. PLoS One 8:e68292

Petit M, Lewden A, Vézina F (2014) How does flexibility in body composition relate to seasonal changes in metabolic performance in a small passerine wintering at a northern latitude? Physiol Biochem Zool 87:539–549

Petit M, Clavijo-Baquet S, Vézina F (2017) Increasing winter maximal metabolic rate improves intrawinter survival in small birds. Physiol Biochem Zool 90:166–177

Price ER, McFarlan JT, Guglielmo CG (2010) Preparing for migration? The effects of photoperiod and exercise on muscle oxidative enzymes, lipid transporters, and phospholipids in white-crowned sparrows. Physiol Biochem Zool 83:252–262

Rogers BD, Garikipati DK (2008) Clinical, agricultural, and evolutionary biology of myostatin: a comparative review. Endocr Rev 29:513–534

Stager M, Swanson DL, Cheviron Z (2015) Regulatory mechanisms of metabolic flexibility in the dark-eyed junco (*Junco hyemalis*). J Exp Biol 218:767–777

Swanson DL (1990) Seasonal variation in cold hardiness and peak rates of cold-induced thermogenesis in the dark-eyed junco, *Junco hyemalis*. Auk 107:561–566

Swanson DL (1991) Substrate metabolism under cold stress in seasonally acclimatized dark-eyed juncos. Physiol Zool 64:1578–1592

Swanson DL (2001) Are summit metabolism and thermogenic endurance correlated in winter acclimatized passerine birds? J Comp Physiol B 171:475–481

Swanson DL (2010) Seasonal metabolic variation in birds: functional and mechanistic correlates. Curr Ornithol 17:75–129

Swanson DL, Liknes ET (2006) A comparative analysis of thermogenic capacity and cold tolerance in small birds. J Exp Biol 209:466–474

Swanson DL, Olmstead KL (1999) Evidence for a proximate influence of winter temperature on metabolism in passerine birds. Physiol Biochem Zool 72:566–575

Swanson DL, Vézina F (2015) Environmental, ecological and mechanistic drivers of avian seasonal metabolic flexibility in response to cold winters. J Ornithol 156(Suppl 1):S377–S388

Swanson DL, King MO, Culver W, Zhang Y (2017) Within-winter flexibility in muscle masses, myostatin and cellular metabolic intensity in passerine birds. Physiol Biochem Zool 90:210–222

Vaillancourt E, Weber J-M (2007) Lipid mobilization of long-distance migrant birds in vivo: the high lipolytic rate of ruff sandpipers is not stimulated during shivering. J Exp Biol 210:1161–1169

Vaillancourt E, Prud'Homme S, Haman F, Guglielmo CG, Weber J-M (2005) Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running. J Exp Biol 208:317–325

Weibel EW (2000) Symmorphosis: on form and function in shaping life. Harvard University Press, Cambridge

Yacoe ME, Dawson WR (1983) Seasonal acclimatization in American goldfinches: the role of the pectoralis muscle. Am J Physiol 245:R265–R271

Zajac DM, Cerasale DJ, Landman S, Guglielmo CG (2011) Behavioral and physiological effects of photoperiod-induced migratory state and leptin on *Zonotrichia albicollis*: II. Effects on fatty acid metabolism. Gen Comp Endocrinol 174:269–275

Zhang Y, King MO, Harmon E, Eyster K, Swanson DL (2015a) Migration-induced variation of fatty acid transporters and cellular metabolic intensity in passerine birds. J Comp Physiol B 185:787–810

Zhang Y, King MO, Harmon E, Swanson DL (2015b) Summer-to-winter phenotypic flexibility of fatty acid transport and catabolism in skeletal muscle and heart of small birds. Physiol Biochem Zool 88:535–549

Zhang Y, Carter T, Eyster K, Swanson DL (2015c) Cross-training in birds: acute cold and exercise training increase similar aspects of fatty acid transport and catabolism in house sparrows, *Passer domesticus*. J Exp Biol 218:3885–3893

Zhang Y, Eyster K, Swanson DL (2018) Context-dependent regulation of pectoralis myostatin and lipid transporters by temperature and photoperiod in dark-eyed juncos. Curr Zool 64:23–31

Zhou L-M, Xia S-S, Chen Q, Wang R-M, Zheng W-H, Liu J-S (2016) Phenotypic flexibility of thermogenesis in hwamei (*Garrulax canorus*): responses to cold acclimation. Am J Physiol Regul Integr Comp Physiol 310:R330–R336