Uncoupling Basal and Summit Metabolic Rates in White-Throated Sparrows: Digestive Demand Drives Maintenance Costs, but Changes in Muscle Mass Are Not Needed to Improve Thermogenic Capacity*

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ABSTRACT

Avian basal metabolic rate (BMR) and summit metabolic rate (M_{sum}) vary in parallel during cold acclimation and acclimatization, which implies a functional link between these variables. However, evidence suggests that these parameters may reflect different physiological systems acting independently. We tested this hypothesis in white-throated sparrows (*Zonotrichia albicollis*) acclimated to two temperatures (−8° and 28°C) and two diets (0% and 30% cellulose). We expected to find an uncoupling of M_{sum} and BMR where M_{sum}, a measure of maximal shivering heat production, would reflect muscle and heart mass variation and would respond only to temperature, while BMR would reflect changes in digestive and excretory organs in response to daily food intake, responding to both temperature and diet. We found that the gizzard, liver, kidneys, and intestines responded to treatments through a positive relationship with food intake. BMR was 15% higher in cold-acclimated birds and, as expected, varied with food intake and the mass of digestive and excretory organs. In contrast, although M_{sum} was 19% higher in cold-acclimated birds, only heart mass responded to temperature (+18% in the cold). Pectoral muscles did not change in mass with temperature but were 8.2% lighter on the cellulose diet. Nevertheless, M_{sum} varied positively with the mass of heart and skeletal muscles but only in cold-acclimated birds. Our results therefore suggest that an upregulation of muscle metabolic intensity is required for cold acclimation. This study increases support for the hypothesis that BMR and M_{sum} reflect different physiological systems responding in parallel to constraints associated with cold environments.

Keywords: metabolic performance, body composition, temperature, diet, basal metabolic rate, summit metabolic rate, *Zonotrichia albicollis*.

Introduction

Studies of birds wintering in cold environments commonly use two indicators of individual performance that are also markers of phenotypic flexibility (McKechine 2008; Swanson and Vézina 2015). Basal metabolic rate (BMR) is thought to reflect physiological maintenance costs (McKechnie 2008), while summit metabolic rate (M_{sum}) is a measure of maximal thermogenic capacity and cold endurance (Dutenhoffer and Swanson 1996; Swanson 2001). Both natural exposure and experimental exposure to cold trigger increases in BMR (McKechnie 2008; Zheng et al. 2008; Barceló et al. 2009) and M_{sum} (Liknes et al. 2002; Vézina et al. 2006), and these parameters are known to correlate at the interspecific level (Dutenhoffer and Swanson 1996; Rezende et al. 2002). These relationships have led to the hypothesis that sustaining high maximal metabolic rates should induce elevated BMR (Bennett and Ruben 1979; Hayes and Garland 1995; Boily 2002; Sadowska et al. 2005). However, the relationship between BMR and M_{sum} loses strength or tends to disappear at the intraspecific level (Vézina et al. 2006; Swanson et al. 2012), and recent evidence suggests that these variables may be functionally independent (Vézina et al. 2006, 2011; Swanson et al. 2012; Petit et al. 2013). Though they often show similar changes in individuals facing a cold environment, variation in BMR and M_{sum} would in fact reflect adjustments in different physiological systems responding to different constraints acting more or less in parallel (Vézina et al. 2006, 2011; Swanson et al. 2012; Petit et al. 2013).

Given that M_{sum} is a measure of metabolism during active shivering, it is influenced by changes in the mass and activity of

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muscles, such as the flight muscles (Cooper 2002; Vézina et al. 2007; McKechnie and Swanson 2010; Swanson et al. 2013; Petit and Vézina 2014; Swanson and Vézina 2015; Zhang et al. 2015), as well as by changes in the mass of the heart (Petit et al. 2014; Zhang et al. 2015). In contrast, variation in BMR appears to be much more influenced by changes in overall lean body mass (e.g., Vézina et al. 2011), with specific body components that significantly affect BMR being context specific and dependent on the birds' current life-history stage (Vézina et al. 2009). In fact, changes in BMR have been associated with adjustments in several body components, such as the reproductive organs (Vézina and Williams 2003), heart, kidney (Daan et al. 1990), muscles, liver, and brain (Chappell et al. 1999; Konarzewski et al. 2000). In the context of cold acclimation and acclimatization specifically, a higher BMR is often associated with larger organs involved in energy acquisition, such as the digestive and excretory organs (Williams and Tieleman 2000; Liu and Li 2006; Zheng et al. 2008, 2014*b*; Maldonado et al. 2009), and BMR has been directly related to food consumption in at least one case (Vézina et al. 2011). Birds challenged by a cold environment increase their daily food consumption, but when energetic needs are above the immediate spare capacity of the digestive system, this leads to the development of larger digestive organs (McWilliams and Karasov 2014). The maintenance of these larger organs would in turn explain the increase in BMR observed in cold-acclimated and cold-acclimatized birds (Williams and Tieleman 2000; Zheng et al. 2008; but see Liknes and Swanson 2011; Petit et al. 2014).

In this experiment, we investigated the causes for variation in BMR and M_{sum} in the context of thermal acclimation. Using a manipulation of temperature and diet quality, we hypothesized that there would be an uncoupling of these metabolic parameters. To test these ideas, we exposed white-throated sparrows (*Zonotrichia albicollis*) to a combination of two temperatures (−8° and 28°C) and diet treatments (powdered food containing 0% or 30% cellulose). We expected birds living at −8°C to develop larger muscles and hearts than individuals kept at 28°C as a response to cold and that the mass of these organs would not be affected by diet. We also predicted that these birds would have a higher M_{sum} as a consequence of their enlarged skeletal and cardiac muscles. We further expected that food intake would be higher in the cold and cellulose treatments, leading to larger digestive and excretory organs. Since these organs are thought to explain variation in BMR in the context of cold acclimatization (Williams and Tieleman 2000; Zheng et al. 2008; Maldonado et al. 2009), we predicted that BMR would vary with both temperature and diet, being higher in the cold and higher on the cellulose diet for a given temperature as a result of larger internal organs in these treatments.

Material and Methods

Capture and Handling

The birds used in this experiment ($n = 32$) included individuals from our captive population at the avian facilities of the Université du Québec à Rimouski (captured in the spring of 2013), to which we added 18 individuals captured around Rimouski, Québec, Canada, during the summer and autumn of 2014. Birds were kept in individual cages (39 cm × 43 cm × 31 cm) with ad lib. access to water and food (Mazuri Small Bird Maintenance Diet; extruded food pellets) and were exposed to a 12L:12D photoperiod and a temperature of 10°C for at least 10 d before beginning the acclimation protocol. After this preacclimation period, all parameters (see below) were measured a first time to confirm the lack of difference between treatment groups before acclimation. The birds were then randomly separated into four groups containing our two crossed treatments: 28°C and a low-fiber diet ($n = 7$), 28°C and a high-fiber diet ($n = 8$), −8°C and a low-fiber diet ($n = 8$), and −8°C and a high-fiber diet ($n = 9$). Samples were slightly unbalanced because of unexpected mortality after the beginning of acclimation. The diets were prepared by grinding the extruded pellets into a powder, which was offered either as is (low fiber; control treatment) or as the same powder mixed with 30% of α-cellulose (Sigma-Aldrich, St. Louis; cellulose treatment). The cellulose diet had the same homogenous appearance as the control diet and was consumed by birds in its entirety. All birds were acclimated to these conditions for 30 d before being measured a second time. This interval is sufficient to induce phenotypic changes in all studied traits (i.e., BMR, gut size, M_{sum}, and pectoral muscle mass; Barceló et al. 2009; McWilliams and Karasov 2014; Zhang et al. 2015). Measurements taken every second day confirmed the stability of body mass after 6 d of acclimation in all treatments (data not shown).

Pre- and postacclimation data included morning (1000 hours) measurement of total body mass as well as lean mass and fat mass. Lean and fat components of body mass were measured noninvasively using quantitative magnetic resonance (EchoMRI; Guglielmo et al. 2011). Daily food intake was also estimated as the difference in mass between the food offered to a given bird at 1000 hours and the food remaining in the tray the following day at the same hour (food spillage was negligible). Pre- and postacclimation food intake were measured over 4 d for each bird, and averaged values of these 4 d were used in further analyses. Food intake was calculated as total (i.e., total amount of food consumed) and actual (i.e., intake recalculated for birds on the cellulose diet to correct for the cellulose component), and both of these variables were considered in analyses (see "Statistical Analyses"). Pre- and postacclimation measurements also included measures of BMR and M_{sum}. Birds were euthanized after the second series of measures for the determination of organ masses (see below).

Respirometry

M_{sum} and BMR were measured using a Servomex oxygen analyzer (model 4100; Boston). M_{sum} trials started at around 1200 hours by measuring two birds, followed by a second trial beginning before 1500 hours. Birds were first weighed (±0.1 g) and body temperature was measured using a copper-constantan thermocouple inserted approximately 10 mm into the cloaca (thermocouple connected to a Sable Systems [Las Vegas] TC-2000). Birds were then placed in a 2.1-L stainless steel metabolic chamber fit-

ted with a perch and were exposed to a 21% oxygen, 79% helium environment (Helox) using an average flow rate of 900 mL min^{-1} controlled by mass-flow valves (Side-Trak; Sierra Instruments, Monterey, CA) previously calibrated for Helox with a Bubble-O-Meter (Dublin, OH). We recorded oxygen consumption for each bird using a sliding cold exposure protocol (Swanson et al. 1996) with a decrease in ambient temperature of 3°C every 20 min, starting at −9°C. We ended trials when the birds became hypothermic, which was identifiable in real time as a steady decline in oxygen consumption for several minutes. Body temperature was measured again immediately after taking the birds out of their chambers. We assumed a bird had reached its M_{sum} when body temperature after a trial was ≤38°C (Cooper and Gessaman 2005), which was the case for all measurements. Birds were weighed a second time after measurements, and the average body mass was used for M_{sum} analyses. Birds were then put back in their cage with food and water ad lib. until BMR measurement started at around 1900 hours that same day. BMR was measured simultaneously in four birds overnight (from 1900 to 0800 hours). Individuals were maintained at 29°C throughout the trial (within the thermoneutral zone; Canterbury 2002) and received a constant 550-mL min^{-1} flow of dry CO_2-free air. As for M_{sum}, birds were weighed before and after measurements, and the average mass was used in BMR analyses.

Metabolic rates were calculated using ExpeData software, version 1.2.6 (Sable Systems), using equation (10.1) of Lighton (2008). M_{sum} and BMR calculations were based on the highest and lowest averaged 10 min of oxygen consumption per measurement, respectively. Active shivering is fueled by lipids in birds (Vaillancourt et al. 2005; Vaillancourt and Weber 2007), and the duration of BMR trials insured that birds were postabsorptive at the time they reached BMR (after 4–6 h of measurements). We therefore estimated energy consumption in watts using a constant equivalent of 19.8 kJ L^{-1} O_2 (Gessaman and Nagy 1988). After BMR measurements, birds were placed back in their cage with access to food and water until being euthanized 1–3 d later.

Organ Mass

Birds were anesthetized using an injection of ketamine-xylasine (ketamine 0.25 mg g^{-1}: Bioniche, Belleville, Ontario; xylasine 0.05 mg g^{-1}: Bayer, Mississauga, Ontario) in the right pectoral muscle and were euthanized by exsanguination. We then dissected the birds to collect and weigh the pectoralis muscles (combined right and left side), liver, heart, emptied intestines, gizzard, lungs, brain, kidneys (combined mass), skin (no plumage), and carcass (remaining muscles and bones). The combined length of the large and small intestines was also measured using a digital caliper. The organs were then kept at −20°C until further processing. Samples were later freeze-dried (FreeZone 2.5; Labconco, Kansas City) for 2 d to obtain constant dry mass (less than 1% mass loss per 24 h). Dry carcasses were burned in a furnace overnight at 450°C to determine ash-free dry carcass mass, which mainly contains the remaining skeletal muscles (dry avian muscles typically contain <10% ash; Santoso 2001; Castellini et al. 2002; Holcman et al. 2003). All bird manipulations were approved by the Université du Québec à Rimouski Animal Care Committee and have been conducted under scientific and banding permits from Environment and Climate Change Canada–Canadian Wildlife Service.

Statistical Analyses

Our goal in this experiment was to determine whether the response of BMR and M_{sum} to temperature results from different body components responding to different but parallel constraints associated with thermal challenges (food consumption and temperature). To determine the effect of treatments on food intake, body mass, fat mass, and lean mass, as well as dry body components and metabolic variables, we used ANCOVA models where the effects of temperature and diet and their interaction were tested on each variable. Despite being highly flexible (Piersma 1998; Liknes and Swanson 2011; McWilliams and Karasov 2014), organs may vary in mass with structural body size. To account for this, we used a measure of body size as a covariate in all models examining changes in body organs. Structural body size was computed using a principal component analysis where the first principal component, combining length measurements of head plus beak and tarsus, was used as our measure of size (Rising and Somers 1989; Senar and Pascual 1997). Given that metabolic rates reflect the amount of metabolically active tissue in an organism (Scott and Evans 1992), analyses on BMR and M_{sum} were first performed using uncorrected data and then including lean body mass as a covariate in separate analyses. We also expected that the effect of ambient temperature on body organs and metabolic rate could manifest itself through the influence of daily food consumption (Lindström and Kvist 1995; Vézina et al. 2011; McWilliams and Karasov 2014). To account for this, we also included actual food intake as a covariate in models (the same analyses using total food intake yielded similar qualitative results and are not shown). Covariates were removed from models when their effects were $P \geq 0.1$. The influence of food consumption on body composition was further investigated visually through separate regression analyses testing for relationships between the mass of individual organs and total and actual food intake across treatments. Sex (determined during dissections) was removed in further analyses, as it had no effect on food intake ($P = 0.2$), body mass ($P = 0.5$), dry organ mass ($P > 0.2$ in eight organs; $P > 0.07$ in two organs), BMR, or M_{sum} ($P > 0.9$ in both cases).

To test for the influence of specific organs on BMR and M_{sum}, we first investigated intercorrelations among dry body components. Organs showing correlations with $r > 0.60$ were combined into functional groups to avoid collinearity in analyses. Combined organs were the heart, pectoral muscles, and ash-free carcass (i.e., remaining body muscles), herein referred to as heart and muscles, and the liver, kidney, and intestines, which were labeled as digestive and excretory organs. The lungs, gizzard, and brain were considered individually. Skin mass was not used in these analyses because skin was dissected and dried with the attached subcutaneous fat layer, which could lead to biased results due to

the low metabolic activity of lipid tissues (Scott and Evans 1992; Petit et al. 2010). Most of the variation in skin mass was, in fact, due to the state of the bird's adipose reserves (relationship between dry skin mass and body fat mass measured by quantitative magnetic resonance: $r^2 = 0.80$, $n = 32$, $P < 0.0001$). We then investigated the influence of organs and organ groups on BMR and M_{sum} both within and across temperature treatments using a model selection approach based on second-order Akaike information criteria. Relationships between metabolic rates and the identified organs were further investigated with regression analyses.

Results

Food Intake, Fat Mass, Lean Mass, and Body Mass

All birds had similar food consumption, body mass, fat mass, and lean mass before they were separated in their respective diet and temperature treatments (no differences between birds forming the future experimental groups; $P > 0.1$ in all cases). After being exposed to a combination of temperatures and diets for 30 d, all these variables had changed significantly.

Birds living in the cold ate on average 101.5% more food (12.0 ± 0.5 g d^{-1}) than those living at 28°C (5.9 ± 0.5 g d^{-1}; $F_{1,31} = 77.1$, $P < 0.0001$), while birds fed the cellulose diet ate 39.5% more food (10.4 ± 0.5 g d^{-1}) than those kept on the control diet (7.5 ± 0.5 g d^{-1}; $F_{1,31} = 18.5$, $P < 0.0005$; no significant interaction). When testing treatment effects on actual food intake, however, only temperature influenced food consumption, with birds at −8°C eating 102.7% more food (9.9 ± 0.4 g d^{-1}) than those kept at 28°C (4.9 ± 0.4 g d^{-1}; $F_{1,31} = 86.1$, $P < 0.0001$).

When considering only the effects of temperature and diet, birds living at −8°C were 10.1% heavier (29.9 ± 0.7 g) than those kept at 28°C (27.2 ± 0.7 g; $F_{1,31} = 7.6$, $P < 0.01$; no influence of diet or interaction). However, adding actual food intake to the model revealed that body mass varied positively with body size ($F_{1,31} = 4.9$, $P < 0.05$) and food consumption ($F_{1,31} = 17.7$, $P < 0.0005$; no effect of diet; fig. 1*A* shows residual body mass controlling for body size). Controlling for these effects, birds tended to be 9.9% lighter at −8° than at 28°C ($F_{1,31} = 3.6$, $P = 0.069$).

A model including only temperature and diet showed that the fat component of body mass did not differ between treatments ($P > 0.2$ in both cases). However, including actual food intake as a covariate revealed a different pattern. While body fat varied positively with the amount of food consumed per day ($F_{1,31} = 14.7$, $P < 0.001$) and was affected by temperature ($F_{1,31} = 9.6$, $P < 0.005$), birds on the cellulose diet at −8°C tended to have less fat reserves per unit of food consumed than birds in all other treatment combinations (interaction term: $F_{1,31} = 3.1$, $P = 0.089$; no diet effect; fig. 1*B*).

Lean body mass did not vary with actual food intake ($P = 0.8$), but when controlling for the positive influence of structural body size ($F_{1,31} = 8.1$, $P < 0.01$), it varied significantly with both temperature ($F_{1,31} = 22.4$, $P < 0.001$) and diet, although this latter effect was visible only through its interaction with temperature ($F_{1,31} = 5.1$, $P < 0.05$; diet alone: $P = 0.2$). Birds on the cellulose diet had 15.2% more lean mass when kept at −8° than at 28°C, while birds consuming the control diet showed a nonsignificant (5.2%) difference in lean mass between temperature treatments (fig. 1*C*).

Metabolic Rates

Basal metabolic rate and M_{sum} did not differ among birds before being assigned to their experimental groups ($P > 0.2$ in all cases). After 30 d of exposure to the diet and temperature treatments, both parameters had changed significantly. BMR and M_{sum} were also positively correlated ($r = 0.59$, $n = 29$, $P < 0.001$).

Whole-body BMR was on average 14.6% higher in birds acclimated to −8°C (0.39 ± 0.01 W) than in individuals living at 28°C (0.34 ± 0.01 W; $F_{1,30} = 7.9$, $P < 0.01$; no effect of diet or interaction). However, including covariates in the model showed that this effect was in fact driven by lean body mass ($F_{1,30} = 4.7$, $P < 0.05$; fig. 2*A* shows residual BMR controlling for lean body mass) and actual food intake ($F_{1,30} = 4.6$, $P < 0.05$).

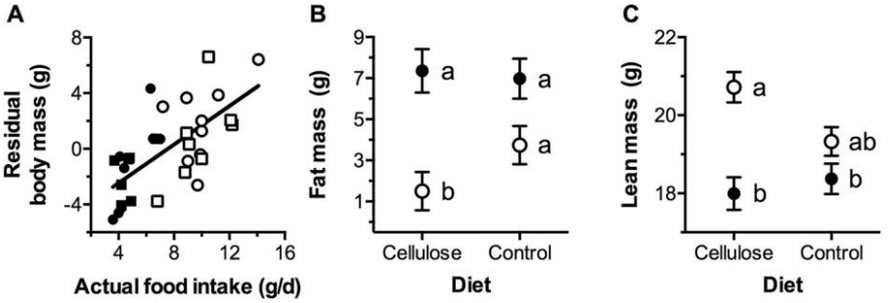

Figure 1. Effects of diet and actual food intake on body mass, fat mass, and lean mass in white-throated sparrows. In *A*, squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C. In *B* and *C*, open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C. Values in *A* are residuals controlling for the effect of structural body size. Values in *B* are least square means controlling for the effect of actual food intake. Values in *C* are least square means controlling for the effect of structural body size. See the main text for details. Different lowercase letters indicate significant differences between temperature and diet treatments.

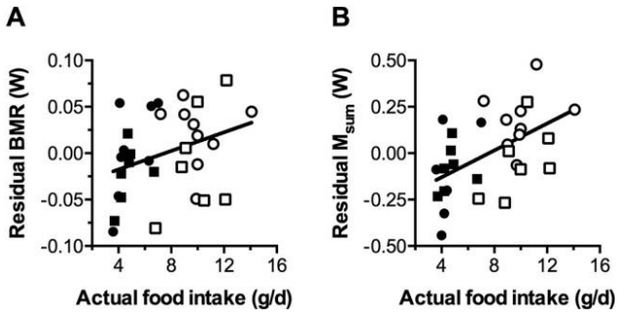

Figure 2. Effect of actual food intake on basal metabolic rate (BMR; *A*) and summit metabolic rate (M_{sum}; *B*) in white-throated sparrows. Residuals control for the effects of lean body mass (see the main text for details). Squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C.

In other words, for a given lean body mass and level of food consumption, BMR did not differ between temperatures and diets.

A similar pattern was found for M_{sum}. Whole-body M_{sum} was 18.7% higher in cold-acclimated birds than in birds kept at 28°C ($F_{1,28} = 31.5$, $P < 0.0001$; no effect of diet or interaction); however, as with BMR, including covariates in the model indicated that M_{sum} changed only with lean body mass ($F_{1,28} = 7.8$, $P < 0.01$; fig. 2*B* shows residual M_{sum} controlling for lean body mass) and actual food intake ($F_{1,28} = 14.9$, $P < 0.001$). Thus, for a given lean body mass and level of food intake, M_{sum} did not vary with temperature and diet.

Body Organs

Lungs and skin mass were not affected by the manipulations of temperature and diet and were independent of food intake ($P > 0.1$ in all cases). Size-independent brain mass (size: $F_{1,31} = 6.0$, $P < 0.05$) tended to vary positively with actual food intake ($F_{1,31} = 4.1$, $P = 0.053$) but was not affected by diet and temperature.

Digestive and Excretory Organs. Dry liver mass was 25% higher in birds living in the cold than in birds living at 28°C (temperature: $F_{1,31} = 4.4$, $P < 0.05$; no significant effect of diet or interaction). However, this effect was due to higher food consumption at −8° than at 28°C, since including actual food intake as a covariate led to a final model where only actual food intake was related to liver mass ($r^2 = 0.17$, $n = 32$, $P < 0.05$; fig. 3*A*).

Birds acclimated to −8°C also had 41.4% heavier kidneys than individuals kept at 28°C ($F_{1,31} = 29.2$, $P < 0.0001$; no significant effect of diet), but here again, the effect was driven by food consumption. Adding actual food intake as a covariate led to a final model where only this variable was related to kidney mass ($r^2 = 0.50$, $n = 32$, $P < 0.0001$; fig. 3*B*).

Gizzard mass was affected by both temperature (+27.9% in the cold; $F_{1,31} = 35.7$, $P < 0.0001$) and diet (+14.9% on cellulose; $F_{1,31} = 11.4$, $P < 0.005$). However, these variables also interacted to affect gizzard mass ($F_{1,31} = 5.9$, $P < 0.05$), and post hoc analyses indicated that although birds from both diets had larger gizzards at −8° than at 28°C (control: +17.2%; cellulose: +38.5%), this difference was significant only for birds fed cellulose (fig. 3*C*). Here again, the underlying effect of food consumption appeared to play an important role. Food intake was not significant as a covariate in the model, but inspecting the distribution of points for each treatment in a regression analysis indicated that the treatment effects were driven by the relationship between gizzard mass and food intake. This was particularly evident for the relationship with total food consumption ($r^2 = 0.56$, $n = 32$, $P < 0.0001$; fig. 3*D*).

The intestines of birds living in the cold were also 13.9% longer than those of birds acclimated to warm conditions ($F_{1,31} = 22.1$, $P < 0.0001$; no effect of diet or food intake; no interaction; fig. 4*A*). However, examining the relationship between intestine length and food intake across treatments also revealed that intestinal length increased with actual ($r^2 = 0.39$, $n = 32$, $P < 0.0001$; fig. 4*B*) and total food consumption. Interestingly, the latter relationship was curvilinear ($r^2 = 0.42$, $n = 32$, $P < 0.0005$; fig. 4*C*). Intestinal length increased with total food intake in birds living at 28°C but began to plateau in birds eating approximately 10 g of food per day (i.e., the lowest amount consumed by cold-acclimated birds). Intestinal mass did not vary with temperature or diet but in-

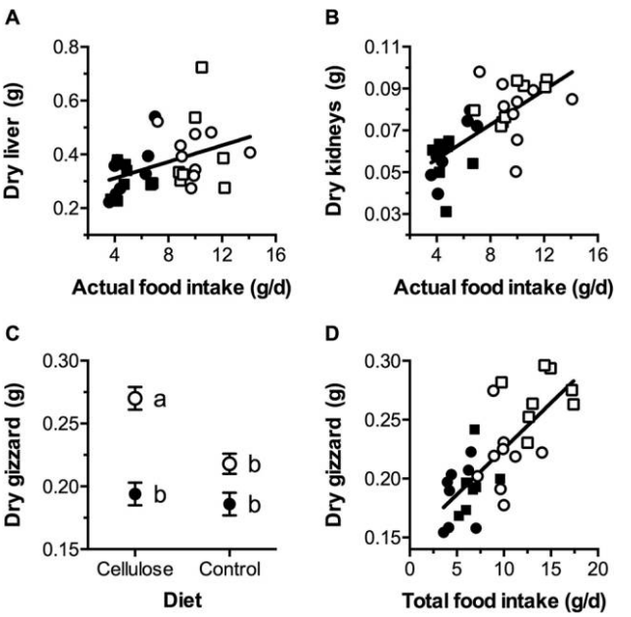

Figure 3. Effects of food intake, temperature, and diet on dry liver mass (*A*), dry kidney mass (*B*), and dry gizzard mass (*C*, *D*) in white-throated sparrows. In *A*, *B*, and *D*, squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C. In *C*, open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C. Different lowercase letters show significant differences between temperature and diet treatments.

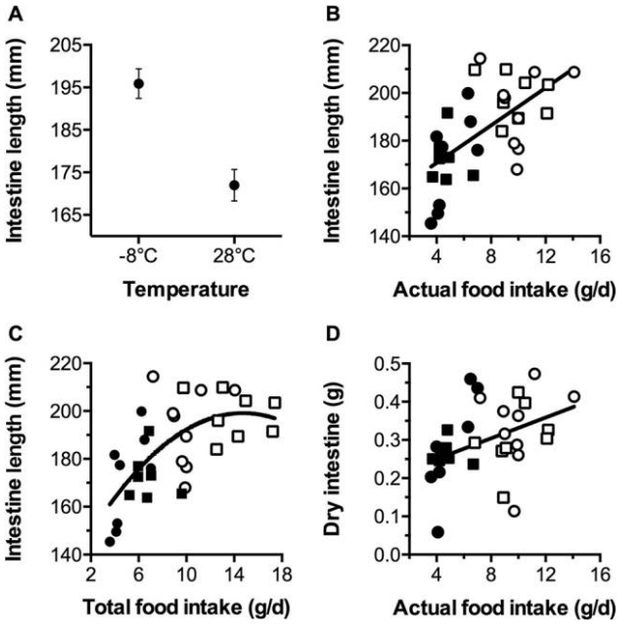

Figure 4. Effect of temperature and actual food intake on intestine length (*A–C*) and dry intestine mass (*D*) in white-throated sparrows. In *B–D*, squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C.

creased linearly with actual food intake ($r^2 = 0.17, n = 32, P < 0.05$; fig. 4*D*).

Muscles. Dry hearts were 18.3% heavier in cold-acclimated birds than in those living at 28°C ($F_{1,31} = 10.3, P < 0.005$; fig. 5*A*) and tended to be 9.6% lighter in birds fed the cellulose diet compared to control individuals ($F_{1,31} = 3.6, P = 0.067$; no significant interaction). In a regression analysis, heart mass was also related to actual food intake across treatments ($r^2 = 0.19$, $n = 32$, $P < 0.05$).

Dry pectoral muscles were affected only by diet ($F_{1,31} = 4.3$, $P < 0.05$), with birds eating the diluted cellulose food having pectoral muscles 8.2% lighter on average than birds forming the control group (fig. 5*B*). Food intake did not influence pectoral muscle mass when considered either as a covariate or in a separate regression analysis.

Ash-free dry carcass mass did not vary with diet but was related to structural body size ($F_{1,31} = 11.24, P < 0.005$) and actual food intake ($F_{1,31} = 5.5, P < 0.05$). Considering these effects, carcass mass tended to be 11.9% lighter in the cold ($F_{1,31} = 3.7, P = 0.064$). The same pattern was found when combining dry pectoral muscles with ash-free dry carcass mass (size: $F_{1,31} = 9.8, P < 0.005$; actual food intake: $F_{1,31} = 5.6, P < 0.05$; temperature: $F_{1,31} = 3.6, P = 0.068$; −11.7% in at −8°C). Therefore, for a given body size, birds eating more food had larger muscles on average but apparently less so in the cold treatment.

Influence of Body Composition on Basal and Summit Metabolic Rates

The best model explaining variation in BMR across treatments highlighted the expected effect of organs involved in energy acquisition. Indeed, only the digestive and excretory organs were identified as potential contributors to variation in BMR ($r^2 = 0.16, n = 31, P < 0.05$; fig. 6*A*). Conducting analyses within temperature treatments indicated that this effect remained significant in birds maintained at 28°C ($F_{1,14} = 8.3, P < 0.05, \beta = 0.51$; regression $r^2 = 0.37, n = 15, P < 0.05$) and that a comparable portion of the variation in BMR was also explained by the combined mass of the heart and muscles ($F_{1,14} = 8.3, P < 0.05, \beta = 0.52$; regression $r^2 = 0.37, n = 15, P < 0.05$). No organs significantly influenced BMR in birds kept at −8°C.

Across treatments, variation in M_{sum} was not explained by muscle mass as anticipated. Instead, the organs with the most influence on M_{sum} were the digestive and excretory organs ($F_{1,28} = 11.0, P < 0.001, \beta = 0.47$), the brain ($F_{1,28} = 5.6, P < 0.05, \beta = 0.33$), and the lungs ($F_{1,28} = 3.5, P < 0.07, \beta = 0.26$; total model $r^2 = 0.57, F_{3,28} = 11.1, P < 0.0001$; fig. 6*B–6D*). Analyses within temperature treatments highlighted a significant effect of the heart and muscles on M_{sum}, although it also indicated that this effect was significant only in cold-acclimated birds ($r^2 = 0.59$, $n = 16, P < 0.001$), with no other organs related to M_{sum} in either temperature treatment (fig. 7).

Discussion

Food Intake, Fat Mass, Lean Mass, and Body Mass

Birds living in the cold had elevated energy expenditure, as demonstrated by the >100% increase in food consumption at −8° relative to 28°C. Similarly, diluting the food with 30% indigestible cellulose led to birds consuming 40% more food on average across thermal treatments compared to individuals fed the control diet. Therefore, our experimental approach succeeded in manipulating energy expenditure and daily food processing.

Birds kept in the cold maintained a 10% heavier body mass than those living at 28°C. This difference is comparable to seasonal and experimental mass variation reported for small

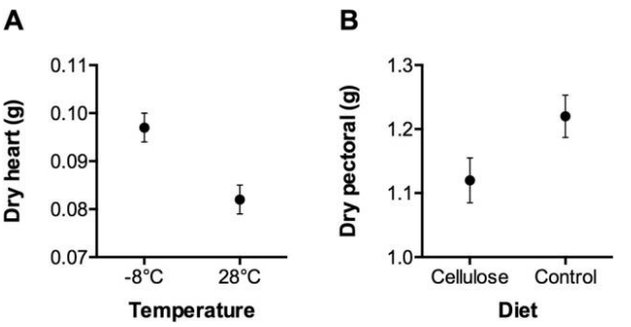

Figure 5. Effect of temperature on dry heart mass (*A*) and diet on dry pectoral mass (*B*) in white-throated sparrows.

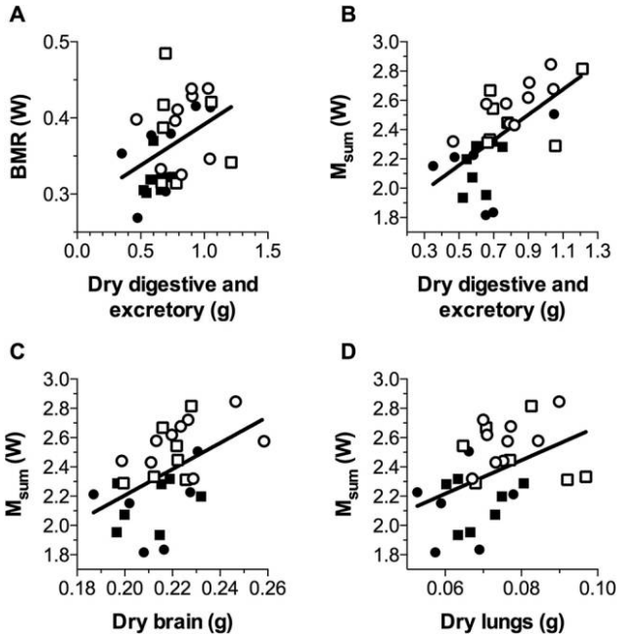

Figure 6. Relationships across treatments between the combined dry mass of digestive and excretory organs and basal metabolic rate (BMR; *A*) and summit metabolic rate (M_{sum}; *B*), and relationships between the dry mass of the brain (*C*) and the lungs (*D*) and M_{sum} in white-throated sparrows. Squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C.

birds during cold exposure (McKechnie et al. 2015) and is known to be part of seasonal acclimatization in passerines (Lehikoinen 1987). Unsurprisingly, when considering the effect of structural body size, this mass difference was driven by food intake, with birds eating more at −8°C also being heavier than at 28°C (fig. 1*A*). In theory, this could partly be due to differences in the amount of food contained in the gizzard and gut, but we believe that this influence is, at most, minimal. Cold-acclimated birds ate 6.1 g more food on average per day than birds living at 28°C. This is 0.5 g h^{-1}, based on a 12-h day. Birds living in the cold being on average 2.7 g heavier than those living at 28°C, for the difference in body mass to be caused mostly by food contained in the gizzard and gut, these organs would need to contain 5.4 times the amount of food the birds could eat per hour. Our data also showed that for each gram of food consumed, birds fed the cellulose diet at −8°C were leaner than birds in any other treatments (fig. 1*B*). This was not the result of a negative energy budget since body masses were stable in all treatments. Instead, our results suggest that for each gram of food consumed, these birds were converting less of that food into fat reserves when living in the cold, a pattern that contrasts with that observed for lean body mass, where lean mass was not affected by food intake but varied with structural body size. In fact, for a given size, birds fed the cellulose diet maintained 7% more lean tissue when kept at −8°C than when kept at 28°C, while control individuals showed no significant lean mass change in the cold. Therefore, it appears that birds on the cellulose diet not only were eating food containing 30% less usable nutrients per unit mass but also had to invest more than control birds in building and maintaining metabolically active tissues when living in a cold environment, a situation that apparently limited their capacity to convert their nutritional intake into fat reserves.

Organ Mass and Function in the Context of Cold Acclimation

Digestive and Excretory Organs. We found larger livers (+25%), kidneys (+41%), and gizzards (+28%) and longer intestines (+14%) in birds maintained at −8°C compared to birds kept at 28°C. This is consistent with our prediction of larger digestive and excretory organs in birds living in the cold. However, we also expected larger digestive and excretory organs in birds fed the cellulose diet within both temperature treatments, and this was found only for the gizzard. Nevertheless, the treatment effects generally appeared to be driven by food consumption. For example, the liver and kidneys responded only to food intake, while the two remaining organs appeared to respond to temperature and diet via a linear or curvilinear relationship with food intake (figs. 3, 4).

Livers were 25% heavier in cold-acclimated birds. Although this observation may not be generalizable (Liknes and Swanson 2011; Petit et al. 2014), it is consistent with several reports on body composition of captive cold-acclimated birds (Williams and Tieleman 2000; Cavieres and Sabat 2008; Zheng et al. 2008; Maldonado et al. 2009; McWilliams and Karasov 2014) and free-living birds wintering at northern latitudes (Liu and Li 2006; Zheng et al. 2008; Liknes and Swanson 2011; Ruthrauff et al. 2013). Since the liver has high oxidative capacity (Webster 1981; Cañas et al. 1982; Scott and Evans 1992), it has been suggested that this organ could play an active role in thermogenesis (Baconnier et al. 1979; Bobyleva et al. 2000; Dewasmes et al. 2003; Villarin et al. 2003; Vézina et al. 2006; Zheng et al. 2008, 2014*a*). Functionally speaking, increasing liver size in

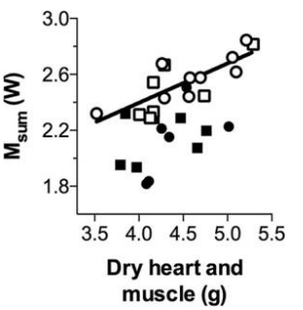

Figure 7. Relationship within thermal treatments between the combined dry mass of the heart and skeletal muscles and summit metabolic rate (M_{sum}) in white-throated sparrows. The effect was detected only in birds acclimated to −8°C. Squares represent birds on the cellulose diet and circles represent birds on the control diet, while open symbols show birds kept at −8°C and filled symbols show birds kept at 28°C.

wintering or cold conditions could contribute to internal heat production. However, this organ plays an important role in digestion, and its size also varies with the rate of food consumption (Ankney and Scott 1988; Yahav et al. 1998; Geluso and Hayes 1999; Williams and Tieleman 2000; this study). Our results showed that the effect of food intake on dry liver mass was greater than the effect of temperature or diet quality. Therefore, the typical increase in liver mass observed in association with cold acclimation or acclimatization in birds is likely a response to the requirements for processing larger amounts of nutrients rather than a direct response to the need for thermoregulation. Thus, the metabolic heat produced by such a large internal organ may be only an indirect benefit for routine thermoregulation in birds living in the cold.

Although kidney mass did not differ between diets, sparrows had 41% larger kidneys when acclimated to cold, a feature typical of birds wintering in cold environments and of captive cold-acclimated birds (Williams and Tieleman 2000; Tieleman et al. 2003; Maldonado et al. 2009; Zheng et al. 2014*b*). Nonetheless, several studies have shown little or no seasonal variation in this organ (Casotti 2001; Liu and Li 2006; Cavieres and Sabat 2008; Zheng et al. 2008; Liknes and Swanson 2011; Petit et al. 2014). As such, the underlying causes for variation in kidney mass seem complex with regard to the effects of temperature. Larger kidneys are associated with elevated food consumption in birds and mammals as a result of the increase in protein intake (Hammond et al. 1994; Konarzewsky and Diamond 1994; Hammond and Janes 1998; Sabat et al. 2004). In this experiment, we found that kidney mass varied only with actual food intake across treatments, with cold-acclimated birds eating more and having larger kidneys than warm-acclimated individuals (fig. 3*B*). Since the pellets used to prepare our diets contain 14.5% crude protein and since kidney mass varied with actual rather than total food intake, birds on both diets consumed the same amount of protein per actual unit of food consumed. Therefore, our data suggest that larger kidneys in cold-acclimated birds may simply be a consequence of higher food consumption and protein intake rather than a response to temperature.

The gizzard is well known to vary in size with both the quantity and the quality of food consumed (Geluso and Hayes 1999; Stark 1999; Dekinga et al. 2001; Piersma and Drent 2003; Piersma and van Gils 2010). The changes we observed in gizzard mass were therefore consistent with prior studies and our predictions. Cold-acclimated birds had 28% heavier gizzards compared to those kept at 28°C, while birds fed the cellulose diet had 15% larger gizzards than those eating the control diet. Within treatments, this effect culminated in cellulose diet birds having gizzards nearly 40% heavier at −8° than at 28°C (fig. 3*C*). Large gizzards are commonly found in free-living and captive birds experiencing cold environments (Williams and Tieleman 2000; Tieleman et al. 2003; Liu and Li 2006; Zheng et al. 2008, 2014*b*; Liknes and Swanson 2011; Petit et al. 2014). This likely reflects their high daily food intake.

Despite elevated total food consumption in the cold and cellulose treatments, white-throated sparrows did not develop heavier intestines in these treatments, a finding that contrasts with previous reports (Geluso and Hayes 1999; Williams and Tieleman 2000; Tieleman et al. 2003; Liu and Li 2006; Zheng et al. 2008, 2014*b*; Liknes and Swanson 2011; McWilliams and Karasov 2014; Petit et al. 2014). Intestine length did, however, change with ambient temperature, with birds living in the cold having 14% longer intestines than birds living at 28°C, irrespective of diet quality. Intestines often increase in length or mass in association with situations requiring elevated rates of food intake (Dykstra and Karasov 1992; Hammond et al. 1994; Geluso and Hayes 1999; Selman et al. 2001; McWilliams and Karasov 2014). Accordingly, we found that intestine length and mass both varied linearly with actual food intake, while intestine length followed a curvilinear relationship with total food consumption (fig. 4*C*, 4*D*). For total food consumption between 2.5 and 10 g d^{-1} (found mostly in warm-acclimated birds), intestine length increased with food intake. However, for consumption rates between 10 and 18 g d^{-1} (found in cold-acclimated birds), intestine length was relatively stable. This suggests that cold-acclimated birds had attained an upper limit to the length of their intestine near 200 mm (fig. 4*C*). Interestingly, this is very close to the 191 mm (combining small and large intestine as in this study) found by McWilliams and Karasov (2014) in white-throated sparrows acclimated to −20°C on a similar powder diet. If these birds had to increase the length of their intestine to meet their energy requirements while the internal body cavity limited the maximal length of that organ, then this constraint could explain why cold-acclimated birds that were fed the cellulose diet (i.e., those at the far right in fig. 4*C*) were much less efficient in converting their food intake into fat reserves (fig. 1*B*). The relationship between intestine mass and actual food intake (fig. 4*D*) suggests that this limitation could have been partially compensated by an increase in the surface of absorption.

Muscles. We expected larger skeletal muscles and hearts in the cold treatment, and we predicted that diet would not influence the mass of these muscles. These predictions were only partially supported. In fact, only the heart showed the expected effect of temperature, and contrary to our expectations, both the heart and pectoral muscles were smaller in the cellulose treatment.

Finding a large heart in cold-acclimated individuals matches results from recent studies on captive and free-living birds (Liu and Li 2006; Maldonado et al. 2009; Liknes and Swanson 2011; Petit et al. 2014; see Swanson 2010; Swanson and Vézina 2015 for reviews). Avian cold acclimatization and acclimation are associated with an increase in the proportion of erythrocytes in the blood (hematocrit; Swanson 1990; Buehler et al. 2012; Petit and Vézina 2014), and this is thought to improve M_{sum} and cold endurance via an increase in overall oxygen-carrying capacity (Swanson 1990; Petit and Vézina 2014). However, an increase in hematocrit also presumably increases blood viscosity, which in turn may require a larger heart (Schuler et al. 2010; Williams et al. 2012; Petit and Vézina 2014).

The lack of temperature effect on the mass of the pectoral muscles was a surprising result given the measured increase in whole-body M_{sum} in cold-acclimated birds. Other cases of un-

altered skeletal muscles after exposure to cold have been reported (e.g., Williams and Tieleman 2000; Tieleman et al. 2003; Peña-Villalobos et al. 2014), but these studies used relatively mild temperatures as their cold treatment (e.g., 15°C) and did not include M_{sum} measurements. Nonetheless, similar to our study, American goldfinches (*Spinus tristis*) showed an increase in M_{sum} (Dawson and Carey 1976) with no associated changes in pectoral muscles (Carey et al. 1978), and captive dark-eyed juncos (*Junco hyemalis*) developed a larger heart and increased their M_{sum} in response to cold without a change in the size of their pectoral muscles (Swanson et al. 2014*c*). Yet, other studies in both species revealed the expected changes in muscle size in association with an increase in M_{sum} (Swanson 1991; Liknes et al. 2002; Swanson et al. 2014*a*; but see Swanson 2014*b*). These studies and our findings therefore suggest that although an increase in the mass of skeletal muscles may be beneficial, it may not always be required for improving thermogenic capacity and cold endurance (Vézina et al. 2007; Petit and Vézina 2014; Swanson et al. 2014*b*, 2014*c*; Swanson and Vézina 2015; this study). Whether this is commonly found in white-throated sparrows will require further study.

For a given temperature, birds on the cellulose diet tended to have a smaller heart and smaller pectoral muscles than birds on the control diet. This suggests that these individuals might have been limited in their capacity to allocate protein to the maintenance of these organs. Birds on the cellulose diet were also converting less of their food intake into fat reserves and maintained a higher lean body mass when living in the cold (fig. 1*B*, 1*C*). Consequently, these birds could have been channelling a large portion of their protein intake toward the maintenance of larger digestive organs, as seen in the gizzard (fig. 3*C*). If correct, the birds on the cellulose diet would have had to make compromises in their allocation of protein to the maintenance of specific organs. Supporting this hypothesis, Geluso and Hayes (1999) reported larger digestive organs and reduced pectoral muscles in European starlings (*Sturnus vulgaris*) fed a high-fiber diet compared to individuals fed a high-quality, low-fiber diet (see also Vézina et al. 2010 for a similar example with shorebirds).

Metabolic Rates and Influence of Body Composition on BMR and M_{sum}

BMR. Whole-body BMR was 15% higher in birds kept at −8°C than in birds kept at 28°C. This difference is within the range of seasonal variation reported for birds wintering in cold environments (McKechnie 2008; Swanson 2010; McKechnie et al. 2015) and is comparable to temperature effects in experimental studies with captive species (Vézina et al. 2006; McKechnie 2008; Barceló et al. 2009). However, BMR also varied with lean body mass and, when considering this effect, remained independent from temperature and increased with food intake, a finding comparable to observations in captive red knots (*Calidris canutus*; Vézina et al. 2011). This suggests that the effect of ambient temperature on mass-independent BMR (e.g., McKechnie et al. 2015) acted mainly through the effect of food consumption. Digestive and excretory organs (liver, gizzard, kidney, and intestines) all increased with food intake, and this organ group was the only one affecting BMR across treatments. Thus, as we predicted, although BMR varied positively with total lean body mass, the components of that lean mass that had the greatest influence on BMR variation across treatments were the digestive and excretory organs. Birds eating more in the cold developed larger digestive and excretory organs, and those in turn contributed to the increase of BMR.

Our results therefore support the energy-demand hypothesis of Williams and Tieleman (2000), which posits that elevated energy expenditure leads to enlarged digestive and excretory organs as a result of higher food intake, ultimately affecting BMR. However, they partially contrast this with recent observations in black-capped chickadees (*Poecile atricapillus*), where BMR varied only with the mass of skeletal muscles and the combined mass of the liver and kidneys across seasons (Petit et al. 2014). The causes of these discrepancies are not obvious, although in chickadees there was a clear and significant increase in skeletal muscle mass in winter relative to summer, while in both our study and that of Williams and Tieleman (2000) on hoopoe-larks (*Alaemon alaudipes*), no significant change in muscle mass was found between thermal treatments. BMR reflects variation in lean body mass (e.g., Vézina et al. 2011), and which body component has the most influence on BMR is context specific (Vézina et al. 2009). Given the proportion of lean body mass that is made up by skeletal muscles in chickadees (73%; M. Petit and F. Vézina, unpublished data), it could well be that the influence of digestive and excretory organs on BMR, as postulated by the energy-demand hypothesis, is visible only when minimal changes are detected in total body musculature. BMR is often higher in cold-acclimatized birds (McKechnie 2008; McKechnie et al. 2015), but studies on metabolic flexibility of wintering species lack food intake data that could elucidate the role of food consumption on winter BMR variation in nature. This question therefore requires further scrutiny.

M_{sum}. As found in most species wintering in cold environments (McKechnie et al. 2015), whole M_{sum} was 18% higher in cold- than in warm-acclimated birds. This is consistent with the predicted influence of temperature on thermogenic capacity. However, M_{sum} did not vary with muscle mass across treatments but instead varied with changes in lean body mass, and in contrast with our expectations, it increased with actual food intake. Therefore, since pectoral muscles did not differ in mass between thermal treatments and since total size-independent body muscles tended to be smaller in cold-acclimated birds, it appears that the observed increase in whole-body M_{sum} in the cold resulted mainly from adjustments at the cellular level (i.e., metabolic intensity; Zheng et al. 2008, 2014*a*, 2014*b*; Peña-Villalobos et al. 2014; but see Zhang et al. 2015). Such contributions of metabolic intensity to M_{sum} and cold endurance have also been suggested for dark-eyed juncos and house sparrows (*Passer domesticus*; Swanson et al. 2014*b*; Stager et al. 2015; see also Buttemer et al. 2008).

The only organs that were correlated with M_{sum} across treatments were the lungs, the brain, and the digestive and excretory organs. An influence of the lungs on M_{sum} is not surprising given the known relationship between oxygen-carrying capacity, measured as hematocrit, and M_{sum} (Petit and Vézina 2014). In fact, a similar influence of the lungs on M_{sum} has been reported in black-capped chickadees (Petit et al. 2014), and lung mass is related to maximum oxygen consumption, measured through physical activity, in house sparrows (Chappell et al. 1999). In contrast, the link between thermogenic capacity and brain mass is not as clear and will require further investigation, as we have no obvious explanation for this relationship. The influence of digestive and excretory organs on M_{sum} is likely due to the parallel effect of food consumption on these variables. Indeed, individuals with a high thermogenic capacity were also those with a high level of food consumption (fig. 2*B*), and the organs included in the digestive and excretory group (liver, kidney, and intestines) all covaried in size with food intake. Alternatively, large digestive and excretory organs could also be beneficial for improving nutritional condition and, consequently, shivering endurance.

White-throated sparrows were able to improve thermogenic capacity without changing muscle size. This, however, does not mean that large muscles are not beneficial for cold endurance. The positive influence of muscle and heart mass on thermogenic capacity has been demonstrated repeatedly in birds (reviewed in Swanson 2010; Swanson and Vézina 2015). In fact, we did find the expected relationship between M_{sum} and the combined mass of muscles and heart but only in cold-acclimated birds (fig. 7). Therefore, for a comparable range of masses in these organs, not only did individuals living at −8°C have a higher thermogenic capacity than individuals living at 28°C but also individuals that had larger muscles and hearts within that group had a higher metabolic performance. Thus, our data suggest that muscle enlargement alone may not be sufficient to improve thermogenic capacity during cold acclimatization (Stager et al. 2015). Birds acclimated to 28°C had muscles as large as individuals living at −8°C, but as they were presumably unprepared to face episodes of acute cold, muscle mass had little influence on shivering heat production (fig. 7).

Overall, our results provide support for the hypothesis that BMR and M_{sum} reflect different physiological systems responding in parallel to a cold environment. As expected, food consumption and digestive organs were the main drivers of variation in BMR across treatments. However, thermogenic capacity increased in the cold independently of changes in muscle mass, which suggests that an upregulation of muscle metabolic intensity is required during the process of cold acclimation and acclimatization (Stager et al. 2015). Our results also suggest that this change in intensity is a prerequisite for birds to benefit from larger muscles when living in the cold.

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