Hormonal Correlates and Thermoregulatory Consequences of Molting on Metabolic Rate in a Northerly Wintering Shorebird

Vezina, Francois; Gustowska, Anna; Jalvingh, Kirsten M.; Chastel, Olivier; Piersma, Theunis

Published in:
Physiological and Biochemical Zoology

DOI:
10.1086/596512

Hormonal Correlates and Thermoregulatory Consequences of Molting on Metabolic Rate in a Northerly Wintering Shorebird

François Vézina[1,*]
Anna Gustowska[2]
Kirsten M. Jalvingh[1]
Olivier Chastel[3]
Theunis Piersma[1,4]
[1]Department of Marine Ecology and Evolution, Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands; [2]Avian Ecophysiology Unit, Department of Vertebrate Ecology and Zoology, University of Gdansk, Aleja Legionów 9, 80-441 Gdansk, Poland; [3]Centre National de la Recherche Scientifique, Unité Propre de Recherche 1934, Centre d'Études Biologiques de Chizé, 79360 Villiers-en-Bois, France; [4]Animal Ecology Group, Center for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

Accepted 6/20/2008; Electronically Published 1/27/2009

ABSTRACT

Even though molt involves both endocrine and energetic changes in bird bodies, this study is among the first to combine assessments of energy costs together with thyroid hormone variations in molting birds. Individual shorebirds (red knots *Calidris canutus islandica*) were measured while in full summer and winter plumage as well as during peak of molt. Molt was associated with a 9.8% increase in average mass-independent basal metabolic rate (BMR) above nonmolting levels. Individual plasma levels of thyroxine (T_4) were correlated with individual rate of body feather renewal, confirming that T_4 is related to body molt but also showing that it is potentially regulating its rate. Across seasons, mass-independent average heat loss measured as conductance gradually declined with conductance during molt falling between measured values for summer and winter. During the molting period, however, body molting rate was positively correlated with thermal conductance, indicating that for a given ambient temperature below thermoneutrality, the fastest molting birds were losing more body heat. Across seasons, triiodothyronine (T_3), a hormone typically upregulated in response to a cold stimulus, was correlated with individual thermal conductance and BMR. We suggest that the increased heat loss of fast-molting birds leads to a cold-acclimatization response that may be partly responsible for the elevated BMR measured during molt. This could be mediated through a stimulatory effect of T_3 on BMR in response to increased heat loss. Our interpretation is supported by a positive relationship between the individual changes in conductance and the change in BMR from summer to the molting period.

Introduction

Long-distance avian migrants such as shorebirds face an array of physiological challenges throughout the year (Piersma and Morrison 1994; Wiersma and Piersma 1994; Morrison et al. 1997, 2005; Piersma et al. 2003). One potentially demanding event is feather molt, a life-history stage that has been studied in terms of both its energetic implications and its endocrine regulation.

Energy costs added to the daily energy budget during the period of molt may come from many sources, one being the biosynthetic cost of producing new feathers. This cost has been measured previously by comparing levels of basal metabolic rate (BMR; energy consumption of animals in a resting, postabsorptive state within a temperature eliciting no active thermogenic response; Commission for Thermal Biology 2003) in individuals actively molting with levels in individuals that are not molting (Lustick 1970; Dietz et al. 1992; Lindström et al. 1993; Klaassen 1995; Schieltz and Murphy 1995, 1997; Brown and Bryant 1996; Buttemer et al. 2003). The difference between the two BMR values is then interpreted as the energy investment in molt. A wide range of variation in this BMR difference has been reported for various species, from no significant changes (Brown and Bryant 1996) to increases as high as 111% (Lindström et al. 1993). Because the efficiency of turning energy intake into new feather material is typically low (2%–29%, depending on species mass; Murphy 1996), the main interpretation for the elevation of BMR is that it reflects physiological changes associated with feather renewal but that the daily amount of energy deposited in new integument represents only a small part of the total expenditure (Dietz et al. 1992; Murphy and King 1992; Lindström et al. 1993; Murphy 1996; Schieltz and Murphy 1997; Buttemer et al. 2003). Physiological adjustments associated with molt that are likely to contribute to the increase in BMR range from elevated body protein turnover (Murphy and Taruscio 1995; Murphy 1996), skeleton restoration (Murphy et al. 1992), and elevated total body water and water turnover (Newton 1968; Chilgren 1977; Piersma 1988;

* Corresponding author. Present address: Département de Biologie, Chimie et Géographie, Université du Québec à Rimouski, 300 allée des Ursulines, Rimouski, Québec G5L 3A1, Canada; e-mail: francois_vezina@uqar.qc.ca.

Physiological and Biochemical Zoology 82(2):129–142. 2009. 1522-2152/2009/8202-8040$15.00
DOI: 10.1086/596512

Murphy 1996) to increased blood volume (Chilgren and deGraw 1977) and spleen mass (Silverin et al. 1999).

Another type of energy cost that may be associated with avian molt results from active heat production. This happens in response to increased heat loss due to molt-related changes in plumage insulation, elevated evaporative heat loss, and increased vascularization (Chilgren and deGraw 1977; Dolnik and Gavrilov 1979; Murphy 1996; Schieltz and Murphy 1997). Accordingly, conductance (heat loss of an endothermic animal per unit decrease in ambient temperature [T_a] below thermoneutrality; Scholander et al. 1950; Herreid and Kessel 1967) has been reported to increase during molt in several bird species (Lustick 1970; Dietz et al. 1992; Piersma et al. 1995; Schieltz and Murphy 1997; Buttemer et al. 2003).

The endocrine aspect of avian molt has also attracted the attention of functional ecologists, but usually independent of its relation to energy costs. Avian molt is assumed to be at least partly under the influence of thyroid hormone. Indeed, thyroid gland activity (Davis and Davis 1954; Wilson and Farner 1960) as well as plasma levels of thyroxine (T_4) have been reported to peak during molt in several bird species (Smith 1982; Groscolas and Leloup 1986; Silverin et al. 1989; Groscolas and Cherel 1992; Pant and Chandola-Saklani 1995; McNabb 2000; Jenni-Eiermann et al. 2002; Cherel et al. 2004). However, to our knowledge, studies reporting natural changes in T_4 levels in relation to natural molting events have shown population averages with no or little information on individual variability (but see Pant and Chandola-Saklani 1995). If T_4 levels reflect an important hormonal mechanism underlying molt, then one would expect relationships between natural molting rate and natural circulating levels of T_4 at the individual level.

Triiodothyronine (T_3) is a hormone derived from T_4 and is known to be involved in several metabolic functions (see Hulbert 2000 for an extensive review on thyroid hormones and function). Although avian molt is not considered to be under the influence of T_3 (Smith 1982; Groscolas and Leloup 1986; Reinert and Wilson 1997; Jenni-Eiermann et al. 2002; Cherel et al. 2004; but see Pant and Chandola-Saklani 1995), birds and mammals exposed to cold conditions typically exhibit increases in T_3 levels, often in association with elevations of metabolic rate (Bobek et al. 1980; Brigmon et al. 1992; Hulbert 2000; Jenni-Eiermann et al. 2002; Cherel et al. 2004; Duriez et al. 2004). In fact, levels of T_3 in birds (Bobek et al. 1977; Chastel et al. 2003) and T_4 turnover in mammals (Hulbert 2000) have been reported to correlate positively with BMR. Furthermore, independent of any temperature stimuli, experimental T_3 administration leads to short-term increases in metabolic rate (Hulbert 2000). Therefore, in light of the potential thermostatic costs associated with molt and reported changes in BMR, T_3 variations during molt deserve closer scrutiny.

For red knots (*Calidris canutus*), shorebirds that spend most of their lives on widely exposed and windy mudflats (Piersma 2002, 2007), thermostatic costs associated with molt may be an important ecological constraint. We therefore studied thermoregulatory consequences of molt in captive red knots held outdoors by quantifying changes in conductance of individual birds over time. We repeatedly measured the same individuals in winter and summer plumage as well as during the peak of molt intensity. We also measured BMR and T_4 and T_3 plasma levels at all stages to obtain an estimate of the energy involved in the processes underlying molt and to document concurrent individual hormonal variation. Furthermore, given the effect of molt on blood parameters (Chilgren and deGraw 1977; Murphy 1996) and because immunity may be compromised by molt (e.g., Martin 2005), we also measured hematocrit and leucocrit. Specifically, we asked (1) whether red knots experienced a peak of high conductance during the peak of molt compared with during summer and winter conditions, (2) whether individual molting rates were related to natural circulating T_4 plasma levels, (3) whether plasma levels of T_3 were associated with individual changes in body conductance, and (4) whether this variation was linked to changes in BMR.

Methods

Experimental Animals

This experiment complied with the Dutch Law on Experimental Welfare and the animal welfare guidelines of the Royal Netherlands Academy of Art and Sciences (permit 2004.03). Twenty adult red knots (subspecies *islandica*) were used for the experiment (9 females and 11 males; PCR sexing; Baker and Piersma 1999). These birds were captured in the Dutch Wadden Sea between 1995 and 2000 and were kept captive at the Royal Netherlands Institute for Sea Research shorebird facility. Red knots kept under our experimental conditions maintain their seasonal cycles of molt and migratory fattening, which remain in synchrony with those of free-living individuals (Piersma et al. 1995, 2000; Piersma 2002). The birds were fed in excess with a protein-rich trout food diet (ad lib. access; Trouvit: 45% protein, 8% fat, 12% fibers, 3% cellulose, 11% water) during the complete period of captivity, including this experiment, and had ad lib. access to fresh water. Knots were maintained in open outdoor aviaries (4.5 m × 1.5 m × 2.3 m, length × width × height) and experienced natural temperature and photoperiod while being protected from wind and rain. These cages were equipped with an artificial mud flat flooded with running salt water to allow the birds to probe the sediments. The floor of the cage was also flooded with running salt water to prevent health problems caused by dry feet. For this experiment, the birds were maintained in two separate cages containing 10 randomly chosen individuals. During the experiment, the birds were routinely checked (once a week) for health condition and molt scoring and were weighed. We did not measure T_a in the cages but rather extracted the data from a database provided by the Royal Netherlands Meteorological Institute, Den Helder station, less than 1 km away.

Timing of Measurements

One of the aims of the experiment was to measure individual birds repeatedly while in full alternate plumage (period herein called summer, average $T_a \pm SE = 16.8° \pm 0.4°C$), then during

a period as close as possible to each individual's peak of molting intensity (herein called molt, average $T_a = 15.8° \pm 0.4°C$), and finally in full basic plumage (herein called winter, average $T_a = 5.0° \pm 0.4°C$). Our respirometry setup limited us to measuring two birds per day, and thus we needed 10 d to collect BMR and thermal conductance data on each individual. We therefore obtained three measurements of BMR and conductance per individual, one for each of the three seasons. Thyroid hormone levels and blood parameters (hematocrit, leucocrit) were measured six times in total: two measures per season, one before and one after BMR and conductance measurements. Two birds did not molt at all during the whole experiment, and one individual developed a breast wound creating a patch of open feathers on the chest. Data for these individuals were discarded. The sequence of data collection went according to the schedule described below.

Summer and Winter. We began the experiment on June 25, 2005, when the birds were in full alternate plumage, and terminated the summer measurements on July 12, 2005. In red knots, the time window between the end of migratory fattening and the beginning of intense molting is rather short (about a month; see Piersma et al. 2000). We therefore timed the beginning of our summer measurements with the end of the captive fattening event, when most birds were thought to have resumed a nonmigratory body mass. Winter measurements took place between November 21 and December 7, 2005, a time during which the birds exhibited full basic plumage. Measurements obtained for these two seasons followed the same protocol.

Individual nutritional state may affect T_3 and T_4 plasma levels (May 1978; Chandola and Pathak 1980; Klandorf et al. 1981; Klandorf and Harvey 1985; Darras et al. 1995; Hulbert 2000; Burger and Denver 2002; Cherel et al. 2004). To avoid this confounding effect on individual thyroid hormone levels, we fasted the birds by removing food from the cages at 1700 hours the day preceding blood sampling. The following day, all birds were bled at 1430 hours, with the time of entering a cage and bleeding recorded for each individual (average interval from entering the cage to bleeding: 6 min, 42 s). Food access for free-living knots is related to the tide cycle, and thus they may routinely fast for up to 7 h twice per day (van Gils et al. 2005, 2006) and for up to several days, depending on tides and weather conditions (Zwarts et al. 1996). Therefore, the length of the imposed fasting period for this experiment would likely not impair the birds' health. We entered the cage only once and gathered the birds in plastic holding boxes (32 cm × 40 cm × 69 cm, height × width × length). We then bled the birds by puncturing the brachial vein with a 30-gauge needle and collecting blood in four heparinized microhematocrit tubes (80 μL), which were centrifuged at 12,000 rpm for 12 min to separate the blood cells from the plasma. After bleeding, the birds were molt scored and released back into their cage with ad lib. access to food. Plasma samples were transferred into sealable plastic tubes (Eppendorf 1.5 mL) and maintained on ice until storage at −20°C. For each individual, we measured hematocrit and leucocrit (ratios of packed red and white cells, respectively, on total sample volume) on one of the sample tubes by measuring the length of each component with a digital caliper under a microscope.

The birds were given a full day to recover from the fasting and bleeding procedure before beginning the metabolic rate protocol. The next day, and every day for the next 10 d, two randomly chosen birds were isolated from food access, but not from water, at 0900 hours by putting them in a holding box kept in the cage. Isolated birds could therefore communicate with other birds of their group but could not feed. To minimize disturbance, the birds were collected from the same cage, so each cage was visited every other day. That same day, we began the BMR protocol at 1900 hours. The birds were first weighed and had their natural daytime body temperature measured by inserting a thermocouple (calibrated against a standard mercury thermometer) into the rectum 2.5 cm deep. The first stable temperature reading maintained for at least 2 s was recorded. This was always obtained very shortly (seconds) after inserting the thermocouple. We then measured BMR (see method below) until the following morning. At 0900 hours, the birds were taken out of the chambers and weighed and had their body temperature measured again. The birds were then allowed to relax by keeping them in a holding box for approximately 30 min (time to clean the metabolic chambers and reset the system). After this period, we put the birds back in the respirometer and proceeded with the conductance measurements until 1730 hours, after which the birds were weighed and had their body temperature measured for a last time before being released into their cage. It should be noted here that knots do not exhibit diurnal variation in resting levels of metabolic rate (F. Vézina and T. Piersma, unpublished data), as reported for other bird species (Aschoff and Pohl 1970). Because knots are molluskivores and feed at low tide, daily activity is synchronized with food availability and therefore with the tide cycle, which can occur at any time of day. We therefore consider our daytime measurements of conductance to be independent of diurnal variations in metabolic rate.

After the last BMR and conductance measurement, the birds were given one resting day without disturbance (so the last two measured birds could recover from the metabolic procedure). The following day, we removed the food from the cages at 1700 hours and repeated the bleeding and scoring procedure, as described above, the next morning.

Molt. During molt, data collection followed the same approach as for summer and winter except that we bled the birds two at a time before and after the metabolic measurements. Each day, two birds were isolated in a holding box at 1700 hours. The next day, these individuals were bled and scored for molt according to the protocol described above and released back into their cage for 1 d of recovery. The following day, the same birds were isolated again at 0900 hours until 1900 hours, when we began the BMR and conductance protocol. At the end of the conductance measurements, the birds were released back in the cage for a resting day before being isolated, bled, and

molt scored one last time. The order of sampling for each individual was based on its molting rate, which was determined during both the experimental and the routine molt scoring. We attempted to make physiological measurements on each bird as close as possible to its period of maximal molting intensity.

Molt Scoring

Each bird was scored once a week during the routine health check and every time it was involved in an experimental procedure. We scored the body plumage according to its color and feather growth (e.g., Piersma et al. 1995) from 1 = full gray winter plumage, 2 = trace of breeding plumage, 3 = one-quarter breeding plumage, 4 = one-half breeding plumage, up to 7 = full red breeding plumage. The growth of each primary feather was scored on a scale of 0 to 5, with 0 = old feather, 1 = feather fallen or growing pin, up to 5 = full-length new feather (maximal score for both wings = 100). We also scored the amount of growing body feathers from 0 = none, 1 = light (at least one pin visible), 2 = medium, and 3 = heavy (more than half of the body surface covered with pins). To obtain an estimate of the individual rate of molt in body and primary plumage for the three seasons, we calculated the difference between two individual scores distant in time by at least 2 wk (including a series of metabolic and hormonal measurements) and divided it by the number of days separating them (average 15.8 d, range 10–25 d). These scores therefore represent the daily change in body and primary molt score in normal scaling units.

Respirometry

BMR and conductance measurements were obtained by respirometry, using the same setup and technique as that described by Piersma et al. (2004) and Vézina et al. (2006, 2007) for BMR. Briefly, fasted birds were weighed to the nearest 0.1 g before being placed in a metabolic chamber for overnight BMR measurements. During measurements, the birds were maintained in the dark at 21°C (within the zone of thermoneutrality; Wiersma and Piersma 1994; Piersma et al. 1995) and received a flow of dry air at 50 L/h. Birds were reweighed at the end of the measurement session, and body mass was calculated as an average of first and second mass measured. We calculated $\dot{V}O_2$ and $\dot{V}CO_2$, taking into account the presence of CO_2 in reference air, as described by Piersma et al. (2004). We evaluated BMR as the lowest consecutive 10-min recording of $\dot{V}O_2$, which was recorded at 30-s intervals. Average respiratory quotient ± SE over all the trials was 0.70 ± 0.003, indicating that the birds were using fat as an energy source during the experiments. Therefore, energy consumption was estimated using a thermal equivalent of 19.8 kJ/L O_2 and then converted to watts (Gessaman and Nagy 1988; Piersma et al. 1995, 1996, 2004; Weber and Piersma 1996). Calculations were performed with Warthog Systems LABANALYST X (Riverside, CA). O_2 and CO_2 analyzers were calibrated on a daily basis, and calculating $\dot{V}O_2$ and $\dot{V}CO_2$ from burning a known mass of pure alcohol in the chamber revealed that the measurements were accurate to 4% (F. Vézina and K. M. Jalvingh, unpublished data).

During the conductance trial, we increased the airflow to the metabolic chamber to 100 L/h and exposed the birds to four values of T_a below thermoneutrality. We programmed the temperature-controlled cabinet (Weiss Enet model HETK 3057.S) to remain at set temperatures of 15°, 5°, −5°, and −10°C for 2 h per temperature before changing to the next set point in the direction of warm to cold. These cabinet temperatures led to average chamber temperatures ± SE of 15.5° ± 0.1°, 4.6° ± 0.9°, −4.6° ± 0.1°, and −9.3° ± 0.1°C, respectively. As for BMR, we calculated $\dot{V}O_2$ using the lowest 10 min of measurements. We used only the second hour of recording for each T_a. By this time, both temperature in the chamber and $\dot{V}O_2$ had stabilized. The slope of the relationship between metabolic rate and T_a below thermoneutrality did not predict the expected normothermic body temperature (T_b) of 42.0° ± 0.2°C (mean ± SE; our measured daytime value). Instead, the slope equation predicted a T_b at metabolic rate zero of 56.7°C (e.g., McNab 1980; Piersma et al. 1995). We therefore used the same approach as Piersma et al. (1995) and calculated minimal thermal conductance for each test temperature according to $C = MR/(T_b - T_a)$, where C is thermal conductance, MR is metabolic rate, T_b is body temperature (based on the average of the values measured before BMR and after conductance; average difference ± SE = 2.5° ± 0.2°C), and T_a is ambient temperature measured in the chamber. For this analysis, we considered the lowest conductance measured per bird as minimal conductance, which always occurred at one of the two coldest T_a's tested. Because we did not determine evaporative water loss, our measurements of minimal thermal conductance reflect overall heat loss below thermoneutrality and not heat transfer through the plumage alone (i.e., wet thermal conductance; Schleucher and Withers 2001). Nevertheless, evaporative heat losses are usually a small component of total heat loss at these low temperatures (Schleucher and Withers 2001). Consequently, conductance values we report will reflect mainly insulative properties of the plumage.

Thyroid Hormone Assay

Total (free and bound) plasma levels of triiodo-L-thyronine (T_3) and thyroxin (T_4) were measured using a radioimmunoassay as detailed by Chastel et al. (2003) and Duriez et al. (2004) for T_3 and by Cherel et al. (2004) for T_4. Intra-assay coefficients of variation were 3.8% and 8.8% for T_3 and T_4, respectively ($n = 4$ replicates for T_3 and $n = 7$ replicates for T_4). Two assays were performed for T_3 and one for T_4. The inter-assay coefficient of variation for T_3 was 3.2% ($n = 3$ duplicates). Pooled plasma of different red knots produced a dose-response curve that paralleled the T_3 and T_4 standard curves. The lowest concentration detectable was 0.26 ng/mL for T_3 and 0.87 ng/mL for T_4 (lowest concentrations measured: 1.02 and 1.14 ng/mL for T_3 and T_4, respectively). We controlled for the potential effects of diel variations in T_3 and T_4 (Klandorf et al. 1978) by

collecting all blood samples at the same hour. Hormone plasma levels may be affected by handling time before blood sampling (Gratto-Trevor et al. 1991). However, preliminary analyses showed that handling time did not affect T_3 and T_4 plasma levels in this study (mixed general linear model [GLM] analysis controlling for season, measure within season, group composition, and individual variation; handling time T_3: $F_{1,84} = 3.0$, $P = 0.08$; T_4: $F_{1,84} = 0.002$, $P = 0.96$). Furthermore, none of the outcomes were changed by inclusion of handling time in our analyses.

Molt Costs and Efficiency

We calculated molt costs and efficiency according to Klaassen (1995). Synthesis and associated costs were calculated as the average difference in BMR between peak-molting and nonmolting stages reported on the total duration of molt. Total molt cost was considered to be synthesis costs added to the total energy content of the new plumage. Molt efficiency was calculated by dividing the plumage energy content by total molt costs. These calculations assume constant daily feather production and feather energy content of 22 kJ/g dry mass (Murphy and King 1982). Birds in this experiment molted into their basic plumage. We therefore used a total plumage dry mass of 7.19 g. This value was obtained from dissection data of 244 red knots collected in the Wadden Sea between 1984 and 1994 from October to December (M. W. Dietz et al., unpublished data). All these birds were in full basic plumage and showed no signs of molt. The average number of days necessary to complete molt in our birds was calculated from the routine molt score data.

Statistical Analysis

We used repeated-measures ANOVA and ANCOVA (mixed GLM) to test for the effect of seasons on the various parameters. In all cases, we included cage and bird ID (nested in cage) as random independent variables. We kept these variables in the models even when they were not significant. To control for the effect of body mass on BMR and conductance, we also included mass in the models as a covariate together with its interaction with seasons (discarded when nonsignificant) and thus report season effects as least squares means. For all parameters other than BMR and conductance, we had two measures per season. Therefore, analyses on these parameters also included measure sequence (nested in seasons).

We were interested in the relationships between body and primary feather molting rate and BMR, conductance, and thyroid hormones. We were also interested in potential relationships between our metabolic variables and T_3 and T_4 plasma levels, as well as hematocrit and leucocrit. Furthermore, because of the known effect of T_a on thyroid hormones, we wanted to consider this potential factor in our analyses. Technically, testing these effects would require stepwise regressions to identify which independent variables were potentially important in our model. However, our nested, repeated experimental design prevented us from using stepwise regressions. To circumvent this problem, we first extracted residuals from the repeated-measure analyses (GLM). That approach provided variables that were independent of seasons, group composition, and individual effects, as well as body mass in the case of BMR and conductance. We then used stepwise regressions as our model selection tool to determine which independent variables should be considered in a final multiple mixed GLM using the original data.

We used this approach to first analyze general effects across the three seasons. We then restrained our analysis to the period of molt in order to highlight relationships that would be specifically related to molt. In this case, extracted residuals were controlling for group composition (metabolic variables) or group composition and measurement sequence (blood parameters). We were not interested in the specific effects of individual birds, group composition, or measurement sequence on our dependent variables. Rather, we simply wished to control for these confounding factors while testing for relationships with molting rates, hematocrit, leucocrit, and T_a. We therefore report only the statistical results that are related to the latter effects. Data are presented as mean ± SE. When comparing thyroid hormone data (two measures per season) with BMR and conductance (one measure per season), we used the T_3 and T_4 values that were closest in time to the particular metabolic measurement. For hematocrit and leucocrit, averages were used.

Note on Body Mass Variation and Molting Rate in Summer

Red knots in alternate plumage and resuming a nonmigratory body mass offer only a very short time window for measurements before significant changes appear in plumage composition. Despite our efforts to time the summer measurements between the end of fattening and the beginning of molt, at the first summer measurements, the birds were still relatively heavy and losing mass (Fig. 1*A*; average mass loss 29.9 ± 5.3 g, range 0.7–75.7 g; see "Results"). Furthermore, body molt started during the period of mass loss (see Fig. 1*C*; "Results"). Therefore, we could not obtain a summer sample free of significant body mass variation and molting activity. However, we believe that our summer sampling was the closest in time to a full alternate plumage phenotype that excludes the confounding effects of physiological changes associated with migratory fattening (Piersma et al. 1996, 1999). To minimize the potential problems associated with changes in body mass, we chose the order of measurements for the metabolic variables by giving priority to individuals that had attained or were near a stable nonmigratory body mass. Furthermore, the differences in individual nutritional state were standardized by fasting the birds before bleeding. We also considered variation due to season, group composition, sampling sequence, and individual in our statistical analysis. If anything, the onset of molt in summer increased the range of individual variation in molting rate data, which, in turn, improved our chances of detecting effects related to molting activity across seasons.

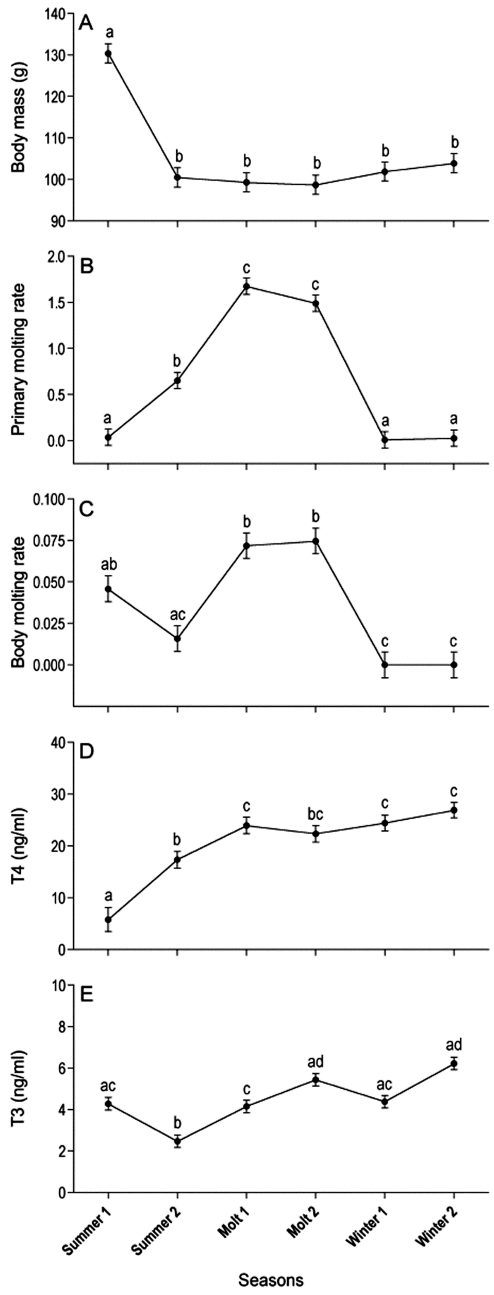

Figure 1. Variations in body mass (*A*), primary feather molt rate (*B*), body feather molt rate (*C*), plasma levels of thyroxine (T_4; *D*), and plasma levels of triiodothyronine (T_3; *E*) over all sampling seasons. Each season is made of two sampling sessions numbered 1 and 2. Molting rates represent the daily change in molt scores in normal scoring units. Data are least squares means controlling for the random effects of group composition and individual. Different letters indicate significant differences as tested by a post hoc Tukey test.

Results

Body Mass

Body mass varied significantly with season ($F_{2,107} = 27.5$, $P < 0.0001$). This difference mainly reflected the influence of the first measure of the summer period (measure[season] $F_{3,107} = 27.9$, $P < 0.0001$; Fig. 1*A*). From the second measurement of summer until the last measurement of winter, however, post hoc analysis (Tukey HSD) revealed no significant differences. Body mass associated with BMR and conductance trials was not affected by seasons ($P = 0.1$; summer: 107.8 ± 2.4 g; molt: 101.2 ± 2.3 g; winter: 105.7 ± 2.3 g).

Molting Rate

The rate of growth in body and primary feather changed over the three seasons (body $F_{2,107} = 45.4$, $P < 0.0001$; primary $F_{2,107} = 143.8$, $P < 0.0001$; Fig. 1*B*, 1*C*). Feather growth rate also varied between measurements within season, although this effect was marginally nonsignificant for body feathers (measure[season] primary $F_{3,107} = 7.2$, $P < 0.0005$; body $F_{3,107} = 2.5$, $P = 0.06$). By the time our second summer sampling session took place, primary molt had begun, although at a relatively slow rate. Except for summer, post hoc Tukey analysis revealed no significant differences between measurements within seasons in primary molt rate (Fig. 1*B*). Surprisingly, birds in full alternate plumage showed molt of body feathers, indicating that some of the birds were already progressing toward a basic plumage phenotype when our summer measurements were taken. The rates of molt in body and primary feathers were not correlated across seasons ($F_{1,107} = 2.8$, $P = 0.1$) or when considering the molting period only ($F_{1,35} = 3.0$, $P = 0.1$). We therefore considered these variables independent of each other and tested their effects in parallel in subsequent analyses.

Hematocrit and Leucocrit

Hematocrit increased from molt to winter (season $F_{2,105} = 29.2$, $P < 0.0001$; summer: 42.7 ± 0.4; molt: 44.8 ± 0.4; winter: 47.8 ± 0.4; post hoc Tukey showed no significant difference between summer and molt) while leucocrit decreased mainly between summer and molt (season $F_{2,98} = 4.0$, $P < 0.05$; summer: 0.43 ± 0.02; molt: 0.36 ± 0.02; winter: 0.37 ± 0.02; post hoc Tukey identified a significant difference between summer and molt). Both parameters varied with the measurement sequence within seasons (measure[season] hematocrit $F_{3,105} = 4.7$, $P < 0.01$; leucocrit $F_{3,98} = 2.7$, $P < 0.05$). However, post hoc Tukey analysis revealed no significant within-season differences. We therefore used per season averages for comparisons with BMR and conductance. Stepwise regression analysis on residual hematocrit highlighted a potential effect of residual body mass as well as residual body and primary molting rates across seasons. However, none of these variables turned out to be statistically distinct in a mixed GLM. When the analysis was restricted to the molting period, stepwise multiple regression suggested an effect of T_a, residual body mass, and residual body molting rate on hematocrit. Mixed GLM confirmed only the effect of T_a ($F_{1,35} = 5.6$, $P < 0.05$), with residual hematocrit being negatively correlated to T_a during the period of molt. However, this relationship was marginally significant ($r = -0.33$, $n = 36$, $P = 0.05$).

Stepwise regression on residual leucocrit suggested no specific effects of any independent variables across seasons. During the period of molt, however, it highlighted a potential effect of T_a on residual leucocrit. Mixed GLM confirmed this temperature effect ($F_{1,35} = 7.6$, $P < 0.01$), with residual leucocrit being significantly and positively correlated to T_a ($r = 0.39$, $n = 36$, $P < 0.05$). Hematocrit and leucocrit were not correlated across seasons ($F_{1,97} = 0.02$, $P = 0.9$) and were therefore considered unrelated variables.

Basal Metabolic Rate

BMR changed with season ($F_{2,51} = 8.8$, $P < 0.005$) and, as expected, was under the influence of variation in body mass ($F_{1,51} = 55.1$, $P < 0.0001$). During molt, least squares mean BMR, controlling for the effect of body mass, was 9.8% higher than values during both summer and winter, with the latter two not differing significantly (summer: 0.92 ± 0.02 W; molt: 1.01 ± 0.02 W; winter: 0.92 ± 0.02 W; Fig. 2*A*). Stepwise regression on residual BMR suggested a potential relationship with residual T_3 and T_4 plasma levels as well as residual average hematocrit and leucocrit. However, mixed GLM including body mass as covariate showed that BMR was related only to T_3 ($F_{1,50} = 7.9$, $P < 0.01$). Indeed, birds showing high residual T_3 plasma levels also showed high residual BMR values ($r = 0.28$, $n = 52$, $P < 0.05$) across seasons (Fig. 3*A*). When concentrating the analysis on the molting period, stepwise regression suggested an effect of the residual rate of molting body feathers on residual BMR variations. However, this effect was weak; the analysis relating residual BMR to residual body molting rate revealed a nonsignificant positive relationship ($r = 0.35$, $n = 18$, $P = 0.15$). Therefore, BMR increased by nearly 10% during molt in comparison to summer and winter average levels, and birds with high levels of T_3 across season also had high BMR.

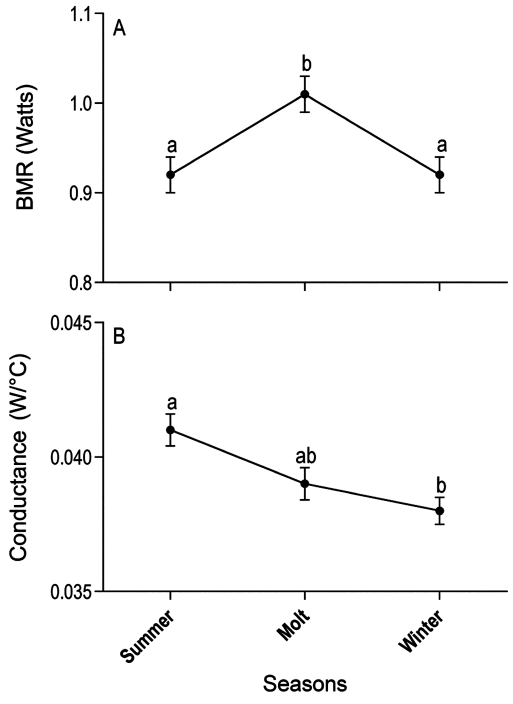

Figure 2. Seasonal differences in basal metabolic rate (BMR; *A*) and conductance (*B*). Data are least squares means controlling for body mass as well as the random effects of group composition and individual. Different letters indicate significant differences as tested by a post hoc Tukey test.

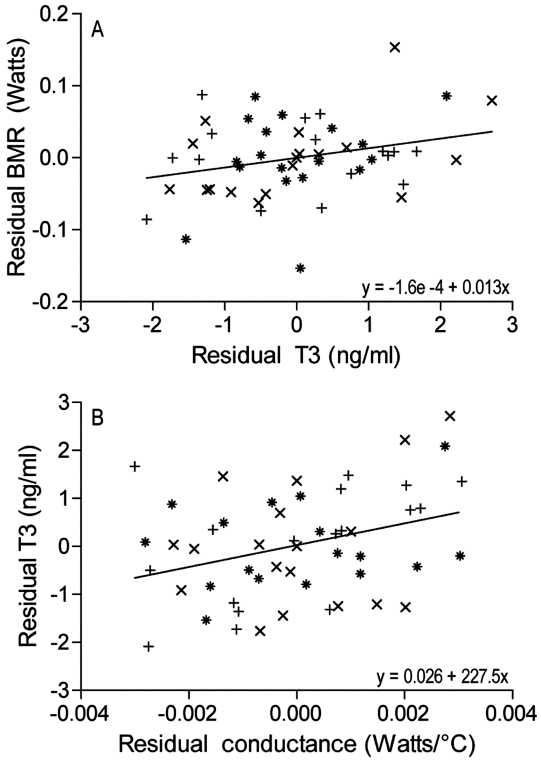

Figure 3. Relationships between residual triiodothyronine (T_3) plasma levels and residual basal metabolic rate (BMR; *A*) and residual conductance (*B*). Residuals correct for the effect of seasons, random effects of group composition and individual, and, in the case of BMR and conductance, the effect of body mass. Each bird is represented three times, once per season. *Plus signs*, summer; *asterisks*, molt; *crosses*, winter.

Minimal Thermal Conductance

Thermal conductance changed over time ($F_{2,51} = 7.9$, $P < 0.005$), with birds in summer showing a 7.9% higher average conductance than during winter (summer: 0.041 ± 0.001 W/°C; molt: 0.039 ± 0.001 W/°C; winter: 0.038 ± 0.001 W/°C; Fig. 2*B*). Although the largest difference in conductance appeared between summer and molting periods (4.9% decrease), post hoc Tukey analysis revealed that conductance differed significantly only between summer and winter values (Fig. 2*B*). Therefore, the change in conductance was gradual and did not spike during molt. Stepwise multiple regression on residual conductance highlighted a potential effect of residual T_3, residual leucocrit, and residual body molting rate. Including these variables

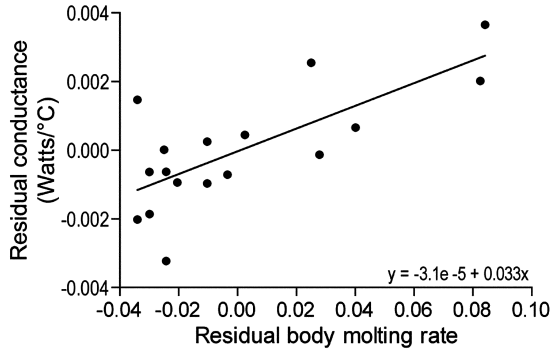

Figure 4. Relationship between residual body molting rate and residual conductance during the molting period. Residuals correct for the random effect of group composition and, in the case of conductance, the effect of body mass.

in a mixed model resulted in T_3 being the only significant variable ($F_{1,50} = 5.7$, $P < 0.05$). Therefore, for a given ambient temperature, individuals with higher heat loss had higher T_3 plasma levels across seasons (residual conductance vs. residual T_3; $r = 0.33$, $n = 52$, $P < 0.05$; Fig. 3*B*). Restraining the analysis to the molting period revealed a very clear effect of body molting rate on conductance. Indeed, stepwise regression highlighted residual body molting rate as the only possible variable having an effect on residual conductance, and mixed GLM supported this finding ($F_{1,16} = 15.3$, $P < 0.005$). Accordingly, residual conductance was highly correlated to residual body molting rate ($r = 0.73$, $n = 17$, $P < 0.001$; Fig. 4). Therefore, although average conductance during molt was between the values for summer and winter plumage, during peak of molt, individuals exhibiting the fastest rate of body feather growth also experienced higher rates of heat loss than slower-molting birds.

T_4

Plasma levels of T_4 varied with season ($F_{2,107} = 32.8$, $P < 0.0001$; Fig. 1*D*). Indeed, post hoc Tukey analysis revealed that T_4 increased by 99.1% between summer and molt and then remained high during winter, with only a slight and nonsignificant increase (11.3% higher than molt; summer: 11.6 ± 1.3 ng/mL; molt: 23.1 ± 1.2 ng/mL; winter: 25.7 ± 1.1 ng/mL). T_4 also varied within seasons (measure[season] $F_{3,107} = 5.7$, $P < 0.005$), mainly because of increased levels during the second measure of summer (Fig. 1*D*). T_4 plasma levels during the two measures of the molt and winter season did not differ significantly. Stepwise regression highlighted a potential effect of residual body molting rate, residual body mass, and residual leucocrit on residual T_4 levels across seasons. Of these variables, only leucocrit was highlighted as significant in a mixed GLM. Therefore, across seasons, birds with low levels of T_4 also had high leucocrit (residual T_4 vs. residual leucocrit; $r = -0.24$, $n = 98$, $P < 0.05$). When analysis was concentrated to the molt period, stepwise regression suggested a relationship of residual body mass, residual body molting rate, and residual hematocrit and leucocrit with residual T_4. Mixed GLM demonstrated that only the effect of body molting rate was significant ($F_{1,35} = 4.0$, $P = 0.05$). Therefore, birds showing high levels of plasma T_4 during molt also had high rates of body feather renewal (residual T_4 vs. residual body molting rate; $r = 0.47$, $n = 36$, $P < 0.005$, Fig. 5).

T_3

Plasma levels of T_3 varied with season ($F_{2,107} = 22.2$, $P < 0.0001$). Post hoc Tukey analysis revealed an increase in average T_3 of 42.4% between summer and molt (summer: 11.6 ± 1.3 ng/mL; molt: 23.1 ± 1.2 ng/mL), with a nonsignificant further 10.4% increase from molt to winter (winter: 25.7 ± 1.1 ng/mL). T_3 plasma levels also changed within seasons ($F_{3,107} = 15.4$, $P < 0.0001$) with significant differences within summer and molt seasons (Fig. 1*E*). Interestingly, variations in T_3 were not related to variations in T_a; stepwise regression analysis on residual T_3 suggested a potential relationship only with residual body molting rate and residual leucocrit. The relationship with body molting rate was confirmed by a mixed GLM ($F_{1,97} = 4.8$, $P < 0.05$). Therefore, birds with high rates of body feather growth across seasons had higher levels of plasma T_3 (residual body molting rate vs. residual T_3; $r = 0.26$, $n = 108$, $P < 0.01$). When the analysis was performed with only data collected during molt, stepwise multiple regression highlighted potential effects of residual body and primary molting rates, residual body mass, and T_a on residual T_3 plasma levels. Mixed GLM, however, highlighted only a weak effect of body mass ($F_{1,35} = 4.2$, $P = 0.049$), and the correlation between residual T_3 levels and residual body mass was not significant ($P = 0.1$).

Molt Costs and Efficiency

The time between the beginning and completion of molt (from first sight of new feathers to end of growth of the last wing

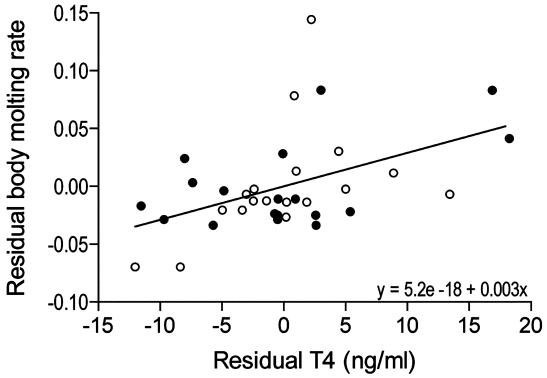

Figure 5. Relationship between residual body molting rate and residual thyroxine (T_4) plasma levels during molt. Residuals correct for measure sequence and the random effects of group composition. Each bird is represented twice because they were sampled two times for blood parameters during molt. *Filled symbols*, first measure of molt; *open symbols*, second measure of molt.

feather) lasted on average 101.4 d (range 96–125 d). We estimated the energy content of the plumage as 158.18 kJ, leading to an estimated total molt cost of 970.41 kJ and a molt efficiency of 16.3%.

Discussion

Hematocrit and Leucocrit

Our data suggest that molt has undetectable or no effect on hematocrit level in red knots. Hematocrit did not change between the summer and molting periods, nor was it related to our measures of molting rate. This is in contrast with previous studies on molting white-crowned sparrows (*Zonotrichia leucophrys*) where hematocrit has been reported to increase by 14%, despite a 20% increase in blood volume (Chilgren and deGraw 1977). In winter, however, the knots' hematocrit levels were higher than during both summer and molt. Elevated hematocrit in winter has been reported for other species (Swanson 1990; O'Connor 1996; reviewed in Fair et al. 2007) and has been interpreted as an adaptation to improve oxygen carrying capacity in cold-acclimatized birds (Swanson 1990).

Interestingly, in their study of six captive red knots kept in outdoor conditions and followed on a monthly basis, Piersma et al. (2000) could not detect the seasonal pattern in hematocrit that we report here. However, because their birds were subjected to repeated experimental bleeding and because hematocrit levels were found to be consistently lower than known values for free-living individuals, the authors called for confirmatory observations of undisturbed captive individuals. Average hematocrit level in knots caught in the Dutch Wadden Sea during the months of July to August is 0.49 ± 0.01 (M. W. Dietz, unpublished data). With our summer values being 0.44 ± 0.41, our measurements confirm that long-term captive knots tend to have low hematocrit levels when kept in outdoor conditions. Our data also confirm that the seasonal pattern reported for other species (Swanson 1990; O'Connor 1996; Fair et al. 2007) stands out. Captive knots do have higher hematocrit levels in winter than in summer, and this seasonal effect seems to begin during molt; we found a negative relationship between hematocrit and T_a during that period.

White blood cell count as measured by leucocrit decreased between summer and winter and was already at winter levels during the peak of molt. This pattern is in agreement with data presented by Piersma et al. (2000), where a seasonal decline from summer to winter was also highlighted in outdoor captive knots. Although this measurement is only a crude estimate of the amount of white cells present in the blood (Ots et al. 1998), a decrease in leucocrit during molt suggests a reduction in immunity compared to summer levels. Consistent with this interpretation, Martin (2005) highlighted a trade-off between feather growth and cutaneous immune activity to phytohemagglutinin in the house sparrow (*Passer domesticus*). However, we found no relationship between leucocrit and body or primary feather molting rate at the individual level. Therefore, the reported variation could also simply reflect a seasonal change in leucocrit (e.g., Piersma et al. 2000) independent of molt.

Molt and Metabolic Parameters

The energy investments in the various processes associated with molt lead to a 10% increase in mass-corrected BMR in comparison to summer and winter levels. This figure is consistent with changes in BMR reported for other species (e.g., Lustick 1970; Klaassen 1995; Buttemer et al. 2003), but it is only part of a wide range of interspecific variation; changes from 0% to 111% have been reported (Lindström et al. 1993; Brown and Bryant 1996). The actual amount of energy sequestrated in new integument is widely considered to be only a small proportion of the total energy cost associated with molt (Dietz et al. 1992; Murphy and King 1992; Lindström et al. 1993; Murphy 1996; Schieltz and Murphy 1997; Buttemer et al. 2003), and red knots are no exception to this rule; we calculated 16.3% feather production efficiency. This value, for a molting bird with an average mass of 99 ± 1.6 g, falls in line with data reported by Murphy (1996) for birds ranging in mass from 0.013 to 2.9 kg. However, because our summer birds were already involved in some molting activity when we measured BMR, this could potentially lead to an underestimation of molt efficiency. We therefore suggest caution with these values.

Mass-corrected conductance did not increase significantly during molt compared to summer and winter. Instead, we found a smooth transition in conductance across seasons. Although prebasic molt occurs at a relatively warm time of the year (July/August), during this period, free-living knots are exposed to T_a's below thermoneutrality (15.8° ± 1.6°C during our molt measurements) as well as to rain and wind, both factors known to significantly increase heat loss (Lustick and Adams 1977; Webster and Weathers 1988; Kelly et al. 2002). A gradual decline in average conductance likely reflects a seasonal improvement in insulation. Accordingly, Piersma et al. (1995) presented the dry mass of body feathers collected from 72 wild red knots and spanning the complete range of molt scores (from 1 to 7). Their data indicated a gradual increase in feather material and possibly insulation as the bird progresses from full summer to full winter phenotype, and this is consistent with our findings.

Average conductance values during molt were found to lie between average values for birds in summer and winter plumage. However, within the molting period, individual variability in conductance was partly explained by body molting rate. Individual birds exhibiting the fastest body molt experienced higher heat loss relative to individuals molting at a slower rate. Suggestions formulated to explain elevated conductance in molting birds range from increased heat loss through evaporative heat transfer to disruption in plumage properties (Murphy 1996). In the present case, our data do not allow for pinpointing the exact source of heat loss in fast-molting individuals. However, it remains that the molting condition was not enough to generate a spike of high average conductance relative to summer and winter stages in our birds.

Klaassen (1995) suggested that the increase in BMR typically found in molting birds could be related to an associated change in conductance. By definition, birds living in the cold lose more

body heat than in a warmer environment because of a greater body to air temperature gradient. Elevated BMR in response to cold climate, in either experimental or natural conditions, has been observed in several species (Weather and Caccamise 1978; Swanson 1991; Cooper and Swanson 1994; Liknes and Swanson 1996; Williams and Tieleman 2000; Cooper 2002; Klaassen et al. 2004; Arens and Cooper 2005), including captive knots (Vézina et al. 2006). Furthermore, this acclimatization response appears to be rapid and reversible within individuals (McKechnie et al. 2007). Klaassen (1995) suggested that because the molting birds would perceive their environment as colder as a result of increased conductance, higher heat loss could lead to a transient metabolic change similar to the acclimatization response seen in wintering species. We verified this hypothesis at the individual level by testing for a relationship between individual changes in conductance and individual changes in BMR from summer to molt, a time when birds faced constant (changed by only 1°C) average T_a below thermoneutrality. We used residuals, controlling for the change in body mass (no significant group composition effect). From summer to molt, birds experiencing the most pronounced increase in conductance, and therefore having the largest increase in heat loss, did indeed show the largest elevation in BMR ($r = 0.51$, $n = 16$, $P < 0.05$; Fig. 6). This observation provides support for the cold-acclimatization hypothesis.

The cold-acclimatization hypothesis is further supported by an experiment performed by Schieltz and Murphy (1997). The authors measured the energy investment involved in feather production by comparing changes in metabolic rates at various T_a's in individuals that had different amounts of body feathers removed. Birds maintained at thermoneutrality showed no changes in metabolic rate in association with feather regrowth. However, there was an increase in metabolic rate in plucked birds maintained at temperatures below thermoneutrality, with a stronger response after 9–12 d, when heavy feather regrowth was observed. This latter finding was interpreted as increased heat loss due to newly exposed vascularized feather quills. However, one could also argue that the increase in conductance due to feather removal led to a transient acclimatization response associated with a gradual elevation of BMR visible over 9–12 d.

It is therefore possible that an "acclimatization" response to elevated heat loss may take place during molt. This may partly explain the increased BMR typically found in some molting bird species. If this is true, this finding is fundamental because it suggests that the "synthesis and associated" costs as we and others defined it (i.e., increase in BMR between nonmolting and molting stages; e.g., Lindström et al. 1993; Klaassen 1995) is not directly related to feather replacement.

This, of course, raises the question of why did we not detect any difference between summer and winter mass-corrected BMR levels in our birds. Two arguments may explain this discrepancy. First, our summer birds were involved in some level of molting activity, which may potentially have increased their BMR, making it indistinguishable from winter levels. Second, and most importantly, the acclimatization effect on BMR

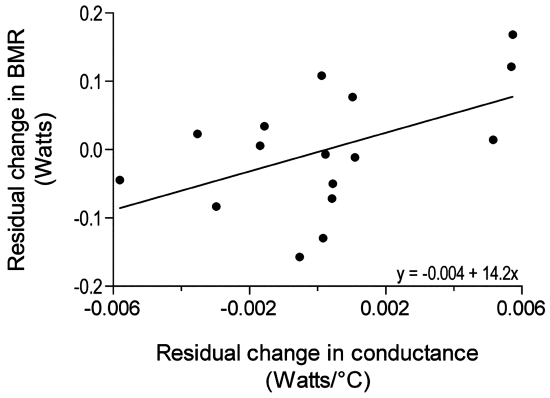

Figure 6. Residual change in basal metabolic rate as a function of the residual change in conductance from summer to the molting period. Residuals correct for the effect of change in body mass.

shown by Vézina et al. (2006) in red knots was found when comparing indoor captive birds acclimatized to cold winterlike (4°C) and thermoneutral conditions (25°C), a 21°C temperature gradient. Our birds experienced T_a's averaging 5.0° ± 0.4° and 16.8° ± 0.4°C in winter and summer, respectively, thus a considerably smaller temperature gradient of 11.8°C. It could be that free living *islandica* knots show little difference in BMR between summer and winter values simply because they experience T_a below thermoneutrality throughout the year.

Lindström et al. (1993) calculated feather production costs in bluethroats (*Luscinia svecica*) and common redpoll (*Carduelis flammea*) by relating individual metabolic rate to the daily amount of new feather produced per individual (i.e., molting rate). The slope of the relationships therefore represented the actual energy cost of producing 1 g of new feather, with higher energy consumption in individuals exhibiting faster molting rates. We showed that high molting rate leads to higher conductance at the individual level. Assuming an associated acclimatization response to elevated heat loss, a higher BMR in individuals producing more feathers on a daily basis could simply be a reflection of an upregulated thermoregulatory machinery. Weathers and Caccamise (1978; also reviewed in Swanson 2009) showed that the interspecific winter to summer BMR ratio is negatively correlated to body mass, with small species showing larger increases in wintering BMR compared to summer levels. Therefore, if the interspecific feather production costs also reflect an acclimatization response to higher heat loss, it is perhaps not surprising to find that smaller species, with high mass-specific conductance (Herreid and Kessel 1967) and high mass-specific BMR, have higher feather production costs, as demonstrated by Lindström et al. (1993, 1998) and Klaassen (1995). This suggests, in contrast to Murphy's (1996) reasoning, that the low energetic efficiency of molt reported here and elsewhere (Lindström et al. 1993; Klaassen 1995; Murphy 1996) is in fact affected by thermostatic costs and therefore underestimates actual feather synthetic efficiencies.

Piersma et al. (1995) studied variations in BMR and body conductance over a complete yearly cycle in three individual

captive red knots in the context of seasonal changes in plumage. Although their study did not have the resolution to ask whether red knots were experiencing additional thermoregulatory costs during intense molt, it did show seasonal changes in the insulative properties of the plumage. Our conductance values differ from those reported by Piersma et al. (1995) in that ours are on average lower and show a smaller difference between seasons. Their values ranged from 0.058 to 0.043 W/°C for summer and winter plumage, respectively. Our values range from 0.041 to 0.038 W/°C for the same time periods. We find the comparison difficult to assess, given that our values are corrected for group composition, individual variation, and, more importantly, body mass. Piersma et al. (1995) reported no body mass correction. Furthermore, although this may have only a trivial effect on the calculations, they assumed body temperature to equal 41°C for each bird when calculating minimal conductance, while we used the actual body temperature data. Despite the differences in experimental approaches, some of their reported conductances for the months of November and December are very close to our winter values, which were obtained at the same time period (i.e., 0.035–0.041 W/°C, estimated from their Fig. 5). Summer values of these two studies, however, are probably not comparable because of differences in the fattening sequence between their and our birds (their high summer conductance values were reported for birds at their peak of migratory fattening) and the fact that our summer measurements may be influenced by molting activity.

Molt and Thyroid Hormones

Our data support the idea that T_4 has a direct influence on molt rate in birds. T_4 levels increased threefold between the first and second summer samples, during which time significant primary molt was first recorded, in addition to body molt, which was already underway. During the molt period, T_4 levels were four times higher than at the first sample of summer, and individual rate of body feather molt was positively correlated to natural T_4 plasma levels. Elevated average levels of circulating T_4 hormones during molting activity in birds have been reported several times in the literature (Smith 1982; Groscolas and Leloup 1986; Silverin et al. 1989; Pant and Chandola-Saklani 1995; McNabb 2000; Cherel et al. 2004), and this includes red knots (Jenni-Eiermann et al. 2002). Furthermore, T_4 is known to have stimulatory effects on protein production (Carter et al. 1971), and injections of T_4 in thyroidectomized, deplumed spotted munia (*Lonchura punctulata*) stimulated feather regrowth in a dose-dependent fashion (Pant and Chandola-Saklani 1995). However, as far as we know, this study is the first report of a relationship between the natural rate of body feather molt and natural T_4 variations at the individual level. We conclude that both the overall molt process and each individual's rate and duration of molt are influenced by plasma levels of T_4. This may have important ecological implications because rate of molt can affect feather quality, leading to fitness consequences (Dawson et al. 2000).

It was a surprise to find high T_4 levels in wintering birds. This finding contrasts with results of an earlier study on red knots, where patterns of T_4 variation showed lower levels in winter than during molt (Jenni-Eiermann et al. 2002). Furthermore, annual T_4 levels are highest during molt in free-living great tits (*Parus major*) and willow tits (*Parus montanus*; Silverin et al. 1989) and in outdoor captive and free-living house sparrows (*Passer domesticus*) and white-crowned sparrows (Smith 1982). In this study, the high T_4 levels recorded in winter cannot be related to molting intensity because these samples were taken in birds that had completed molt. However, thyroid hormones are involved in many physiological functions and may respond to a variety of stimuli (Hulbert 2000). The underlying causes of the discrepancy between ours and other studies remain unclear.

Although T_3 is not believed to play an active role in avian molt (Smith 1982; Groscolas and Leloup 1986; Reinert and Wilson 1997; Jenni-Eiermann et al. 2002; Cherel et al. 2004), our finding of a positive relationship between body molting rate and T_3 plasma levels across seasons suggests that this hormone may nevertheless be functionally implicated. This is consistent with studies of Pant and Chandola-Saklani (1995) that demonstrated that T_3 stimulates feather growth when T_4 is blocked, albeit to a lesser extent than when T_4 is present.

We found a positive relationship between plasma levels of T_3 and mass-corrected BMR across seasons. Such a correlation in birds has been demonstrated previously by Bobek et al. (1977), Chastel et al. (2003), and Duriez et al. (2004) and highlights the stimulatory effects of T_3 on tissue metabolic intensity (Carter et al. 1971; Deaton et al. 1997; Hulbert 2000; Short et al. 2001). What, then, drives the changes in T_3 levels? T_3 fluctuations are known to be associated with parameters such as nutritional state, where fasting generally leads to a decrease in T_3 levels (May 1978; Chandola and Pathak 1980; Klandorf et al. 1981; Klandorf and Harvey 1985; Darras et al. 1995), and T_a with cold stimuli resulting in rapid elevation of T_3 circulating levels (Bobek et al. 1980; Brigmon et al. 1992; Hulbert 2000; Jenni-Eiermann et al. 2002; Cherel et al. 2004; Duriez et al. 2004). Just as in this study, Jenni-Eiermann et al. (2002) found higher average levels of plasma T_3 during winter but failed to detect a significant relationship between T_a and T_3 in outdoor captive red knots when comparing data for two successive winters. Our data, however, indicated a positive relationship between individual conductance and T_3 levels across seasons (Fig. 3*B*). This finding suggests that heat loss per se has a larger influence on individual circulating T_3 levels than does T_a. It is reasonable to assume, then, that increased individual heat loss, through its effects on plasma T_3 levels, may cause upregulation of aerobic metabolic intensity, resulting in higher BMR via elevated nonshivering internal heat production (Fig. 3*A*). This may be an underlying cause for the higher BMR typically recorded in cold-acclimatized birds and could also be driving the acclimatization response to molt. Although our T_3 data supports this presumption only when considered across seasons, consistent with this hypothesis is a significant positive relationship between individual change in conductance and individual change in BMR from summer to molt (Fig. 6). We do

not pretend that this is the only cause of BMR elevation during molt but rather that our data highlight a phenomenon likely to mislead the actual interpretation of molt costs in birds. Further experimental studies are needed to verify this hypothesis.

Acknowledgments

We are grateful to the shorebird/benthos group at the Royal Netherlands Institute for Sea Research (NIOZ) for helpful suggestions on the ideas and analyses presented in this article. We also thank Bernard Spaans and Anne Dekinga for catching the birds, as well as Maarten Brugge for bird care and help. We are equally grateful to Carlos César Jesus, Kimberley Mathot, Tanya Compton, Jeroen Reneerkens, and Piet van den Hout for help with bleeding sessions. A special thanks to Maurine W. Dietz for providing comparative hematocrit data and feather mass data for wintering birds. At the Centre d'Études Biologiques de Chizé, we thank André Lacroix and Colette Trouvé for thyroid hormone assays. We also thank Oliver P. Love, who commented on an earlier version of this manuscript. This research was funded through an operating grant from NIOZ to T.P., postdoctoral funding to F.V. from the Natural Science and Engineering Research Council of Canada, and a Veni grant to F.V. from the Netherlands Organization for Scientific Research.

Literature Cited

Arens J.R. and S.J. Cooper. 2005. Metabolic and ventilatory acclimatization to cold stress in house sparrows (*Passer domesticus*). Physiol Biochem Zool 78:579–589.

Aschoff J. and H. Pohl. 1970. Rhythmic variations in energy metabolism. Fed Proc 29:1541–1552.

Baker A.J. and T. Piersma. 1999. Molecular vs. phenotypic sexing in red knots. Condor 101:887–893.

Bobek S., M. Jastrzebski, and M. Pietras. 1977. Age-related changes in oxygen consumption and plasma thyroid hormone concentration in young chicken. Gen Comp Endocrinol 31:169–174.

Bobek S., J. Niezgoda, M. Pietras, M. Kacinska, and Z. Ewy. 1980. Effect of acute cold and warm ambient temperatures on the thyroid hormone concentration in blood plasma, blood supply, and oxygen consumption in Japanese quail. Gen Comp Endocrinol 40:201–210.

Brigmon R.L., E.L. Besch, and F.B. Mather. 1992. Seasonal temperature and its influence on plasma corticosterone, triiodothyronine, thyroxine, plasma protein and packed cell volume in mature male chickens. Comp Biochem Physiol A 102:289–293.

Brown C.R. and D.M. Bryant. 1996. Energy expenditure during molt in dippers (*Cinclus cinclus*): no evidence of elevated costs. Physiol Zool 69:1036–1056.

Burger M.F. and R.J. Denver. 2002. Plasma thyroid hormone concentrations in a wintering passerine bird: their relationship to geographic variation, environmental factors, metabolic rate, and body fat. Physiol Biochem Zool 75:187–199.

Buttemer W.A., S.C. Nicol, and A. Sharman. 2003. Thermoenergetics of pre-moulting and moulting kookaburras (*Dacelo novaeguineae*): they're laughing. J Comp Physiol B 173: 223–230.

Carter W.J., F.H. Faas, and J. Wynn. 1971. Thyroxine stimulation of protein synthesis in vitro in the absence of mitochondria. J Biol Chem 246:4973–4977.

Chandola A. and V.K. Pathak. 1980. Restricted food-intake and annual thyroid cycle of spotted munia. Gen Comp Endocrinol 42:39–46.

Chastel O., A. Lacroix, and M. Kersten. 2003. Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*. J Avian Biol 34:298–306.

Cherel Y., J.M. Durant, and A. Lacroix. 2004. Plasma thyroid hormone pattern in king penguin chicks: a semi-altricial bird with an extended posthatching developmental period. Gen Comp Endocrinol 136:398–405.

Chilgren J.D. 1977. Body composition of captive white-crowned sparrows during post-nuptial molt. Auk 94:677–688.

Chilgren J.D. and W.A. deGraw. 1977. Some blood characteristics of white-crowned sparrows during molt. Auk 94:169–171.

Commission for Thermal Biology. 2003. Glossary of terms for thermal physiology. J Therm Biol 28:75–106.

Cooper S.J. 2002. Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. Physiol Biochem Zool 75:386–395.

Cooper S.J. and D.L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the black-capped chickadee. Condor 96:638–546.

Darras V.M., M. Cokelaere, E. Dewil, S. Arnouts, E. Decuypere, and E.R. Kuhn. 1995. Partial food restriction increases hepatic inner ring deiodinating activity in the chicken and the rat. Gen Comp Endocrinol 100:334–338.

Davis J. and B.S. Davis. 1954. The annual gonad and thyroid cycles of the English sparrow in Southern California. Condor 56:328–345.

Dawson A., S.A. Hinsley, P.N. Ferns, R.H.C. Bonser, and L. Eccleston. 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. Proc R Soc B 267:2093–2098.

Deaton K.E., C.M. Bishop, and P.J. Butler. 1997. The effect of thyroid hormones on the aerobic development of locomotor and cardiac muscles in the barnacle goose. J Comp Physiol B 167:319–327.

Dietz M.W., S. Daan, and D. Masman. 1992. Energy requirements for molt in the kestrel *Falco tinnunculus*. Physiol Zool 65:1217–1235.

Dolnik V.R. and V.M. Gavrilov. 1979. Bioenergetics of molt in the chaffinch (*Fringilla coelebs*). Auk 96:253–264.

Duriez O., L. Pastout-Lucchini, M. Boos, O. Chastel, H. Fritz, Y. Ferrand, and J. Clobert. 2004. Low levels of energy expenditure in a nocturnal, forest-dwelling wader, the Eurasian woodcock *Scolopax rusticola*. Ardea 92:31–42.

Fair J., S. Whitaker, and B. Pearson. 2007. Sources of variation in hematocrit in birds. Ibis 149:535–552.

Gessaman J.A. and K.A. Nagy. 1988. Energy metabolism: errors in gas-exchange conversion factors. Physiol Zool 61:507–513.

Gratto-Trevor C.L., L.W. Oring, and A.J. Fivizzani. 1991. Effects of blood-sampling stress on hormone levels in the semipalmated sandpiper. J Field Ornithol 62:19–27.

Groscolas R. and Y. Cherel. 1992. How to molt while fasting in the cold: the metabolic and hormonal adaptations of emperor and king penguins. Ornis Scand 23:328–334.

Groscolas R. and J. Leloup. 1986. The endocrine control of reproduction and molt in male and female emperor (*Aptenodyte forsteri*) and Adelie (*Pygoscelis adeliae*) penguins. Gen Comp Endocrinol 63:264–274.

Herreid C.F. and B. Kessel. 1967. Thermal conductance in birds and mammals. Comp Biochem Physiol 21:405–414.

Hulbert A.J. 2000. Thyroid hormones and their effects: a new perspective. Biol Rev 75:519–631.

Jenni-Eiermann S., L. Jenni, and T. Piersma. 2002. Temporal uncoupling of thyroid hormones in red knots: T3 peaks in cold weather, T4 during moult. J Ornithol 143:331–340.

Kelly J.P., N. Warnock, G.W. Page, and W.W. Weathers. 2002. Effects of weather on daily body mass regulation in wintering dunlin. J Exp Biol 205:109–120.

Klaassen M. 1995. Molt and basal metabolic costs in males of 2 subspecies of stonechats: the European *Saxicola torquata rubicula* and the east African *Saxicola torquata axillaris*. Oecologia 104:424–432.

Klaassen M., M. Oltrogge, and L. Trost. 2004. Basal metabolic rate, food intake, and body mass in cold- and warm-acclimated garden warblers. Comp Biochem Physiol A 137:639–647.

Klandorf H. and S. Harvey. 1985. Food intake regulation of circulating thyroid hormones in domestic fowl. Gen Comp Endocrinol 60:162–170.

Klandorf H., P.J. Sharp, and I.J.H. Duncan. 1978. Variations in levels of plasma thyroxine and triiodothyronine in juvenile female chickens during 24- and 16-hr lightning cycles. Gen Comp Endocrinol 36:238–243.

Klandorf H., P.J. Sharp, and M.G. Macleod. 1981. The relationship between heat production and concentrations of plasma thyroid hormones in the domestic hen. Gen Comp Endocrinol 45:513–520.

Liknes E.T. and D.L. Swanson. 1996. Seasonal variation in cold tolerance, basal metabolic rate, and maximal capacity for thermogenesis in white-breasted nuthatches *Sitta carolinensis* and downy woodpeckers *Picoides pubescens*, two unrelated arboreal temperate residents. J Avian Biol 27:279–288.

Lindström Å., G.H. Visser, and S. Daan. 1993. The energetic cost of feather synthesis is proportional to basal metabolic rate. Physiol Zool 66:490–510.

———. 1998. The energetic cost of feather synthesis is proportional to basal metabolic rate. Erratum to 66:490, 1993. Physiol Zool 71:245.

Lustick S. 1970. Energy requirements of molt in cowbirds. Auk 87:742–746.

Lustick S. and J. Adams. 1977. Seasonal variation in effects of wetting on energetics and survival of starlings (*Sturnus vulgaris*). Comp Biochem Physiol A 56:173–177.

Martin L.B. 2005. Trade-offs between molt and immune activity in two populations of house sparrows (*Passer domesticus*). Can J Zool 83:780–787.

May J.D. 1978. Effect of fasting on T3 and T4 concentrations in chicken serum. Gen Comp Endocrinol 34:323–327.

McKechnie A.E., K. Chetty, and B.G. Lovegrove. 2007. Phenotypic flexibility in the basal metabolic rate of laughing doves: response to short term thermal acclimation. J Exp Biol 210:97–106.

McNab B.K. 1980. On estimating thermal conductance in endotherms. Physiol Zool 53:145–156.

McNabb F.M.A. 2000. Thyroids. Pp. 461–471 in G.C. Whittow, ed. Surkies's Avian Physiology. Academic Press, San Diego, CA.

Morrison R.I.G., N.C. Davidson, and T. Piersma. 1997. Daily energy expenditure and water turnover of shorebirds at Alert, Ellesmere Island, N.W.T. Can Wildl Serv 211:1–8.

———. 2005. Transformations at high latitudes: why do red knots bring body stores to the breeding grounds? Condor 107:449–457.

Murphy M.E. 1996. Energetics and nutrition of molt. Pp. 158–198 in C. Carey, ed. Avian Energetics and Nutritional Ecology. Chapman & Hall, New York.

Murphy M.E. and J.R. King. 1982. Amino acid composition of the plumage of the white-crowned sparrow. Condor 84:435–438.

———. 1992. Energy and nutrient use during molt by white-crowned sparrows *Zonotrichia leucophrys gambelii*. Ornis Scand 23:304–313.

Murphy M.E. and T.G. Taruscio. 1995. Sparrows increase their rates of tissue and whole body protein synthesis during the annual molt. Comp Biochem Physiol A 111:385–396.

Murphy M.E., T.G. Taruscio, J.R. King, and S.G. Truitt. 1992. Do molting birds renovate their skeletons as well as their plumages? osteoporosis during the annual molt in sparrows. Can J Zool 70:1109–1113.

Newton I. 1968. Temperatures weights and body composition of molting bullfinches. Condor 70:323–332.

O'Connor T.P. 1996. Geographic variation in metabolic seasonal acclimatization in house finches. Condor 98:371–381.

Ots I., A. Murumagi, and P. Horak. 1998. Haematological health state indices of reproducing great tits: methodology and sources of natural variation. Funct Ecol 12:700–707.

Pant K. and A. Chandola-Saklani. 1995. T3 fails to mimic certain effects of T4 in munia birds: physiological implications for seasonal timing. Comp Biochem Physiol C 111:157–164.

Piersma T. 1988. Breast muscle atrophy and constraints on foraging during the flightless period of wing moulting great crested grebes. Ardea 76:96–106.

———. 2002. Energetic bottlenecks and other design constraints in avian annual cycles. Integr Comp Biol 42:51–67.

———. 2007. Using the power of comparison to explain hab-

itat use and migration strategies of shorebirds worldwide. J Ornithol 148(suppl. 1):45–59.

Piersma T., L. Bruinzeel, R. Drent, M. Kersten, J. Van der Meer, and P. Wiersma. 1996. Variability in basal metabolic rate of a long-distance migrant shorebird (red knot, *Calidris canutus*) reflects shifts in organ sizes. Physiol Zool 69:191–217.

Piersma T., N. Cadée, and S. Daan. 1995. Seasonality in basal metabolic rate and thermal conductance in a long-distance migrant shorebird, the knot (*Calidris canutus*). J Comp Physiol B 165:37–45.

Piersma T., J.A. Gessaman, A. Dekinga, and G.H. Visser. 2004. Gizzard and other lean mass components increase, yet basal metabolic rates decrease, when red knots *Calidris canutus* are shifted from soft to hard-shelled food. J Avian Biol 35:99–104.

Piersma T., G.A. Gudmundsson, and K. Lilliendahl. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. Physiol Biochem Zool 72:405–415.

Piersma T., A. Koolhaas, A. Dekinga, and E. Gwinner. 2000. Red blood cell and white blood cell counts in sandpipers (*Philomachus pugnax*, *Calidris canutus*): effects of captivity, season, nutritional status, and frequent bleedings. Can J Zool 78:1349–1355.

Piersma T., Å. Lindström, R.H. Drent, I. Tulp, J. Jukema, R.I.G. Morrison, J. Reneerkens, H. Schekkerman, and G.H. Visser. 2003. High daily energy expenditure of incubating shorebirds on high Arctic tundra: a circumpolar study. Funct Ecol 17: 356–362.

Piersma T. and R.I.G. Morrison. 1994. Energy expenditure and water turnover of incubating ruddy turnstones: high costs under high arctic climate conditions. Auk 111:366–376.

Reinert B.D. and F.E. Wilson. 1997. Effects of thyroxine (T-4) or triiodothyronine (T-3) replacement therapy on the programming of seasonal reproduction and postnuptial molt in thyroidectomized male American tree sparrows (*Spizella arborea*) exposed to long days. J Exp Zool 279:367–376.

Schieltz P.C. and M.E. Murphy. 1995. Diurnal variation in oxygen consumption by molting and nonmolting sparrows. Comp Biochem Physiol A 112:265–272.

———. 1997. The contribution of insulation changes to the energy cost of avian molt. Can J Zool 75:396–400.

Schleucher E. and P.C. Withers. 2001. Re-evaluation of the allometry of wet thermal conductance for birds. Comp Biochem Physiol A 129:821–827.

Scholander P.F., V. Walters, R. Hock, and L. Irving. 1950. Body insulation of some arctic and tropical mammals and birds. Biol Bull 99:225–236.

Short K.R., J. Nygren, R. Barazzoni, J. Levine, and K.S. Nair. 2001. T3 increases mitochondrial ATP production in oxidative muscle despite increased expression of UCP2 and -3. Am J Physiol 281:E761–E769.

Silverin B., R. Fange, P.A. Viebke, and J. Westin. 1999. Seasonal changes in mass and histology of the spleen in willow tits *Parus montanus*. J Avian Biol 30:255–262.

Silverin B., P.A. Viebke, J. Westin, and C.G. Scanes. 1989. Seasonal changes in body weight, fat depots, and plasm levels of thyroxine and growth hormone in free living great tits (*Parus major*) and willow tits (*Parus montanus*). Gen Comp Endocrinol 73:404–416.

Smith J.P. 1982. Changes in blood levels of thyroid hormones in 2 species of passerine birds. Condor 84:160–167.

Swanson D.L. 1990. Seasonal variation of vascular oxygen transport in the dark-eyed junco. Condor 92:62–66.

———. 1991. Seasonal adjustments in metabolism and insulation in the dark-eyed junco. Condor 93:538–545.

———. 2009. Seasonal metabolic variation in birds: functional and mechanistic correlates. Curr Ornithol (forthcoming).

van Gils J.A., A. Dekinga, B. Spaans, W.K. Vahl, and T. Piersma. 2005. Digestive bottleneck affects foraging decisions in red knots *Calidris canutus*. II. Patch choice and length of working day. J Anim Ecol 74:120–130.

van Gils J.A., B. Spaans, A. Dekinga, and T. Piersma. 2006. Foraging in a tidally structured environment by red knots (*Calidris canutus*): ideal, but not free. Ecology 87:1189–1202.

Vézina F., K.M. Jalvingh, A. Dekinga, and T. Piersma. 2006. Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size. J Exp Biol 209:3141–3154.

———. 2007. Thermogenic side effects to migratory predisposition in shorebirds. Am J Physiol 292:R1287–R1297.

Weathers W.W. and D.F. Caccamise. 1978. Seasonal acclimation to temperature in monk parakeets. Oecologia 35:173–183.

Weber T.P. and T. Piersma. 1996. Basal metabolic rate and the mass of tissues differing in metabolic scope: migration-related covariation between individual knots *Calidris canutus*. J Avian Biol 27:215–224.

Webster M.D. and W.W. Weathers. 1988. Effect of wind and air temperature on metabolic rate in verdins, *Auriparus flaviceps*. Physiol Zool 61:543–554.

Wiersma P. and T. Piersma. 1994. Effects of microhabitat, flocking, climate and migratory goal on energy expenditure in the annual cycle of red knots. Condor 96:257–279.

Williams J.B. and B.I. Tieleman. 2000. Flexibility in basal metabolic rate and evaporative water loss among hoopoe larks exposed to different environmental temperatures. J Exp Biol 203:3153–3159.

Wilson A.C. and D.S. Farner. 1960. The annual cycle of thyroid activity in white-crowned sparrows of eastern Washington. Condor 62:414–425.

Zwarts L., J.B. Hulscher, K. Koopman, and P.M. Zegers. 1996. Short-term variation in the body weight of oystercatchers *Haematopus ostralegus*: effect of available feeding time by day and night, temperature and wind force. Ardea 84A:357–372.