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Alterations in tissue aerobic capacity may play a role in premigratory fattening in shorebirds

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Migratory shorebirds show regulated seasonal increases in body mass (BM) even in captivity, consisting primarily, but not exclusively, of fat. We examined whether captive red knot (*Calidris canutus*) exhibited seasonal alterations in mitochondrial volume (liver, pectoral muscle) and/or succinate dehydrogenase (SDH) activity (liver, pectoral muscle, heart, small intestine) during three distinct life-cycle stages: stable BM, spring peak in BM, and as BM rapidly declined after the spring peak. Mitochondrial volume in liver and pectoral muscle and SDH activity in liver and heart did not alter with life-cycle stage. However, red knot undergoing premigratory fattening exhibited significantly lower pectoral muscle SDH activity in concert with significantly elevated activity in the small intestine compared with the other two time-points, suggesting that tissue metabolic rate alters with life-cycle stage. The increased intestinal SDH activity may indicate an elevation in energy assimilation at a time when intestine hypertrophy occurs, thus maximizing BM increase prior to putative migration. The concomitant decrease in pectoral muscle activity may act to reduce overall metabolic rate, or at least help counter the elevation in intestinal mass-specific metabolic rate. Both tissues hypertrophy prior to migration in wild red knot, but hypertrophy of the intestine precedes that of pectoral muscle. Indeed, it appears that the intestinal mass undergoes atrophy by the time pectoral muscle hypertrophy occurs in wild red knot. Thus, physiological adjustments in tissue metabolism may be an important factor in the life-history strategies of migrating shorebirds.

Keywords: migration; fat deposition; mitochondria; metabolism

1. INTRODUCTION

Many shorebird species undergo significant seasonal increases in body mass (BM) before migration, even in captivity (Piersma *et al.* 1996; Weber & Piersma 1996; Piersma 2002), consisting primarily, but not exclusively, of deposited fat (Lindström & Piersma 1993). This mass increase is associated with hyperphagia (Ramenofsky 1990), although reduced energy expenditure (Stokkan *et al.* 1986), torpor (Hiebert 1993), hypothermia (Butler & Woakes 2001), dietary switches (Bairlein 1990) and enhanced energy uptake in the gut (Bairlein 1990) may be important additional factors. Migratory shorebirds also exhibit 'phenotypic flexibility' (Piersma & Lindström 1997; Piersma 1998), with liver and gut hypertrophy during premigratory fattening optimizing nutrient assimilation, and heart and pectoral muscle hypertrophy immediately prior to migratory flight (Piersma 1998; Piersma *et al.* 1999).

We examined whether different physiological life-cycle stages in captive red knot (*Calidris canutus*) were associated with alterations in tissue aerobic metabolism. Aerobic enzyme activities estimate tissue energy consumption and alter with life-cycle stage in several migratory bird species (Weber & Piersma 1996; Bishop *et al.* 1998). We determined the activity of the mitochondrial enzyme succinate dehydrogenase (SDH), a key enzyme in the electron transport chain (St-Pierre & Boutilier 2001), in pectoralis major muscle, liver, heart and small intestine. Mitochondrial volumes in pectoral muscle and liver were also determined, as this is a reliable estimate of oxygen metabolism.

2. MATERIAL AND METHODS

(a) *Animals and housing*

Twenty-two red knot were captured by cannon-netting (January 1995 or January 1997), under licence from English Nature, and housed at the University of Durham, UK. Fourteen individuals were captured as adults and eight as juveniles (determined by plumage characteristics), and all were maintained in captivity for over 1 year to ensure acclimatization to captivity (Piersma *et al.* 2000). Red knot were kept in groups of 6–10 within indoor aviaries of $1.2\times1.3\times2.4\ m^3$ ($h\times w\times d$), under a day length that followed the natural conditions for UK and temperature close to ambient. Food was provided *ad libitum* consisting of blow-fly larvae (*Calliphora* sp.), trout pellets (Trout Excel 23, Trouw Aquaculture, Nutreco, UK) and an added mineral supplement SA-37 (Intervet UK Ltd, UK). Fresh water flowed through the cages continuously, with baths provided for drinking and bathing.

(b) *Aerobic enzyme activity*

Adult red knot were killed by cervical dislocation during three distinct life-cycle stages (table 1) as previously described (Piersma *et al.* 1996; Piersma 2002). Individuals were sacrificed either outside the migratory period ($n=8$), during the spring premigratory increase in BM ($n=7$) or as mass decreased after the spring maxima ($n=7$). Birds were weighed daily and identified as undergoing premigratory fattening if BM increase was over 10 g per week and mass decrease if greater than 20 g per week (Selman 1998). The right pectoralis major muscle, heart, duodenum (washed in saline) and a portion of liver were dissected and frozen in liquid nitrogen. Enzyme activity was expressed $\mu mol\ min^{-1}\ g^{-1}$ tissue wet tissue (St-Pierre & Boutilier 2001) and the protocol is described fully elsewhere (Selman 1998).

(c) *Mitochondrial volume*

Eight (approximately 100 mg each) serial transverse sections of liver and dorsal and ventral aspects of the left pectoralis major muscle were dissected, post-fixed in osmium tetroxide and embedded in araldite (Selman 1998). Ten randomly selected micrographs from each tissue of each bird were stereoscopically examined at a magnification of $\times12\,000$. Mean mitochondrial volume was calculated and expressed as a percentage of the total micrograph, with 360 micrographs examined ($n=4$ in each group).

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Table 1. Body mass (BM) of captive red knot, with arrows depicting the life-history strategy at time of death. (↑: spring increase in BM (BM immediately before increase [start] and at killing [end]); ↓: spring decrease in BM (BM at spring peak [start] and at death [end]); ↔: outside the migratory period (BM at death [end]). All birds were killed as adults and maintained in captivity for over 1 year, but individuals denoted by asterisks were captured as juveniles.)

id	life-cycle stage	BM (g) start	BM (g) end
1	BM↑	125	150
2	BM↑	147	168
3	BM↑	135	170
4*	BM↑	155	193
5*	BM↑	125	150
6*	BM↑	130	165
7	BM↑	110	140
8	BM↓	184	152
9	BM↓	162	127
10	BM↓	192	161
11*	BM↓	155	128
12*	BM↓	244	158
13*	BM↓	165	152
14*	BM↓	220	180
15*	BM↔	—	100
16	BM↔	—	135
17	BM↔	—	120
18	BM↔	—	150
19	BM↔	—	132
20	BM↔	—	123
21	BM↔	—	118
22	BM↔	—	135

3. RESULTS

Mean activity (per gram tissue wet weight) of SDH in the liver and heart (figure 1*a*,*b*; ANOVA $F_{2,20}=0.242$, $p>0.05$ and $F_{2,20}=1.039$, $p>0.05$ respectively, post hoc Student–Neuman–Keuls $p>0.05$) did not alter with life-cycle stage. However, SDH activity was significantly lower (−60%) in the pectoralis major muscle (figure 1*c*; $F_{2,20}=5.058$, $p<0.05$) and significantly higher (+80%) in the small intestine (figure 1*d*; $F_{2,20}=3.799$, $p<0.05$) during the period of BM increase in spring, than outside this period.

Life-cycle stage (table 2) had no effect on mean percentage volume of mitochondria in the liver or in the dorsal and ventral aspects of the pectoralis major muscle (arcsine-transformed Mann–Whitney *U*-test, $p>0.05$). Mitochondrial volume did not differ between the dorsal and ventral aspects of the pectoral muscle within the same individual (arcsine-transformed paired *t*-test, $t_{11}=1.796$, $p>0.05$).

4. DISCUSSION

In anticipation of migration, birds increase BM (fat and protein) primarily through hyperphagia (Ramenofsky 1990). The mass of organs involved in digestion and food assimilation also increase at this time (Piersma & Lindström 1997; Piersma 1998; Piersma *et al.* 1999). We show that captive red knot, which follow BM changes of wild conspecifics (Piersma *et al.* 1996; Weber & Piersma 1996), exhibited significantly higher SDH activity in their small intestine during premigratory fattening, suggesting elevated tissue-specific metabolism. This, in conjunction with the gut hypertrophy observed in

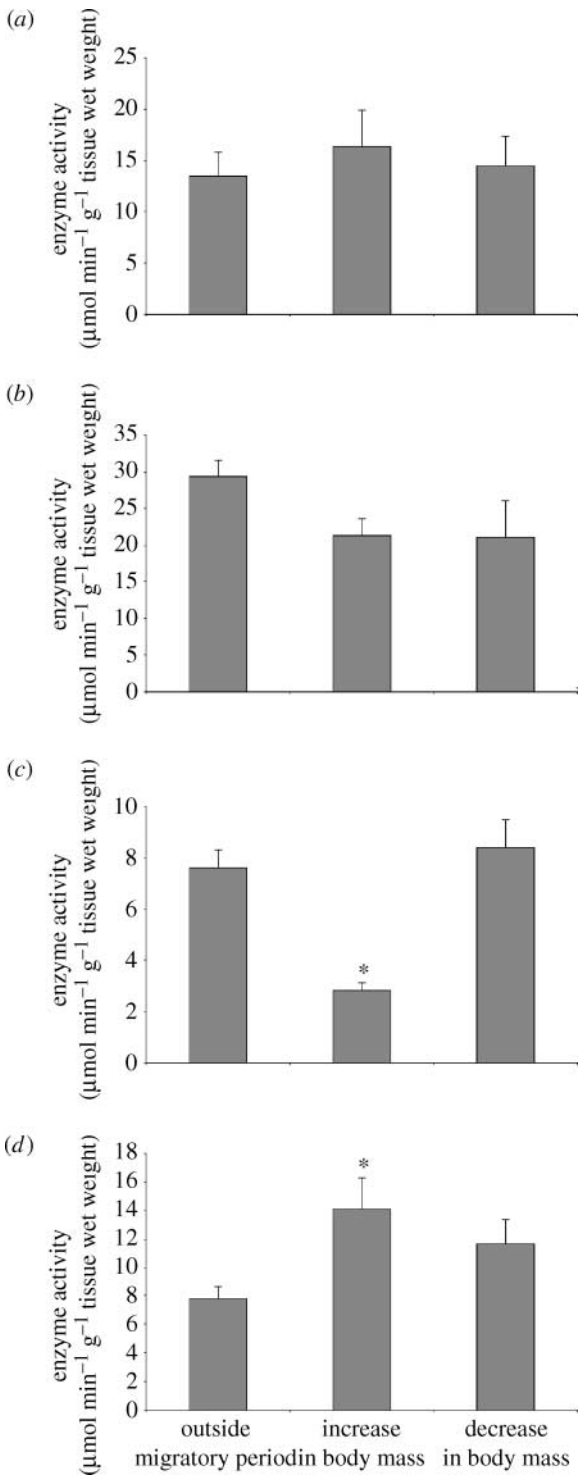

Figure 1. (*a–d*) Mean(+ s.e.) activity (μmol min^{-1} g^{-1} tissue wet tissue) of succinate dehydrogenase (SDH) in liver ($p>0.05$), (*b*) heart ($p>0.05$), (*c*) pectoral muscle and (*d*) small intestine in captive red Knot during three distinct life-cycle stages. Significantly lower SDH activity was observed in pectoral muscle (*a*; $F_{2,20}=5.058$, $p<0.05$) as body mass increase compared with the other two periods, but SDH activity in the small intestine was significantly higher at this same period compared with the other two life-cycle stages (*b*; $F_{2,20}=3.799$, $p<0.05$). $n=8$ outside migratory period; $n=7$ as body mass increased or decreased.

Table 2. Mean ± s.e. mitochondrial volume in liver, dorsal and ventral aspect of pectoralis major (PM) muscle of captive red knot during three distinct life-cycle stages. ($n=4$ in each. No significant differences were observed between groups in any tissue ($p>0.05$).)

	outside migratory period	increase in BM	decrease in BM
liver	21 ± 0.90	22 ± 1.63	23 ± 2.50
dorsal PM	24 ± 1.70	22 ± 1.22	24 ± 1.03
ventral PM	24 ± 1.14	25 ± 0.90	25 ± 1.11

wild red knot (Piersma *et al.* 1999), but not measured in this study, may increase assimilation rate in hyperphagic individuals, thereby maximizing BM increase prior to migration. We suggest that a metabolically active (figure 2*a*) and hypertrophied small intestine may be 'metabolically countered' by the observed reduction in pectoral muscle SDH activity (figure 2*b*) at this time. This may help to minimize any metabolic 'penalty' (Speakman & McQueenie 1996) associated with a large and metabolically active gut. Pectoral muscle also hypertrophies immediately prior to migratory flight, but at a time when the intestinal mass is undergoing atrophy (Piersma *et al.* 1999).

In captive red knot switched from soft trout chow to a hard mussel (*Mytilus edulis*) diet, a significant increase was observed in BM, lean mass and gizzard mass (Piersma *et al.* 2004), accompanied by a reduction in basal metabolic rate. It was suggested that this reduction in the metabolic intensity of certain lean tissues may occur in response to stressful or energy-limiting situations (Piersma *et al.* 2004). We propose that alterations in intestinal and flight muscle metabolic intensity are an energetically prudent strategy to maintain metabolically expensive digestive machinery, while minimizing the impact of other major contributors to overall metabolism (e.g. flight muscle) at a time when the primary objective is to maximize energy storage. The converse should be true immediately prior to migration, where pectoral muscle should hypertrophy and increase in metabolic intensity, combined with an atrophied and relatively metabolically inactive gut (Piersma 1998; Piersma *et al.* 1999). It is likely that individuals were sampled during the middle 10-day period of intestinal and liver hypertrophy (Piersma *et al.* 1999) rather than immediately before 'migratory flight', which is characterized by pectoral muscle and cardiac hypertrophy. It seems improbable that the power requirements of migratory flight would be associated with any reduction in muscle aerobic capacity. The absence of any correlated effect in pectoral muscle mitochondrial volume may be a result of the small sample size ($n=4$) and/or because it may be energetically more economical to alter enzyme activity rather than mitochondrial number or volume.

These findings provide insights into what underpins seasonal variations in BM and metabolic rate, and suggest that different metabolic strategies may be adopted at the tissue level, depending on the physiological requirements of that individual. Our data complement the suggestions of Piersma *et al.* (2004), who postulated that birds may employ energy-saving mechanisms during times of 'stress' by decreasing organ tissue metabolism. Seasonal variations in both organ mass and, as we report, tissue metabolic rate may represent an 'evolutionary compromise' dependent on the life-history strategy adopted (Piersma 1998). The explicit test of these results will be an examination of these various parameters under field conditions.

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Bairlein, F. 1990 Nutrition and food selection in migratory birds. In *Bird migration: physiology and ecophysiology* (ed. E. Gwinner), pp. 198–213. Berlin: Springer.

Bishop, C. M., Butler, P. J., El Haj, A. J. & Eggington, S. 1998 Comparative development in captive and migratory populations of the barnacle goose. *Physiol. Zool.* **71**, 198–207.

Butler, P. J. & Woakes, A. J. 2001 Seasonal hypothermia in a large migrating bird: saving energy for fat deposition? *J. Exp. Biol.* **204**, 1361–1367.

Hiebert, S. 1993 Seasonal changes in body-mass and use of torpor in migratory hummingbirds. *Auk* **10**, 787–797.

Lindström, Å. & Piersma, T. 1993 Mass changes in migrating birds: the evidence for fat and protein storage re-examined. *Ibis* **135**, 70–78.

Piersma, T. 1998 Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight. *J. Avian Biol.* **29**, 511–520.

Piersma, T. 2002 When a year takes 18 months: evidence for a strong circannual clock in a shorebird. *Naturwissenschaften* **89**, 278–279.

Piersma, T. & Lindström, Å. 1997 Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol. Evol.* **12**, 134–138.

Piersma, T., Bruinzeel, L., Drent, R., Kersten, M., Van der Meer, J. & Wiersma, P. 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., Gudmundsson, G. A. & Lilliendahl, K. 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., Reneerkens, J. & Ramenofsky, M. 2000 Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions. *Gen. Comp. Endocrinol.* **120**, 118–126.

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

Ramenofsky, M. 1990 Fat storage and fat metabolism in relation to migration. In *Bird migration: physiology and ecophysiology* (ed. E. Gwinner), pp. 214–231. Berlin: Springer.

Selman, C. 1998 The causes of individual and seasonal variation in the metabolic rate of Knot *Calidris canutus*. PhD thesis, University of Durham, Durham, UK.

Speakman, J. R. & McQueenie, J. 1996 Limits to sustained metabolic rate: the link between food intake, basal metabolic rate and morphology in reproducing mice, *Mus musculus*. *Physiol. Zool.* **69**, 746–769.

Stokkan, K. A., Mortensen, A. & Blix, A. S. 1986 Food intake, feeding rhythm and body mass regulation in the Svalbard rock ptarmigan. *Am. J. Physiol.* **251**, R264–R271.

St-Pierre, J. & Boutilier, R. G. 2001 Aerobic capacity of frog skeletal muscle during hibernation. *Physiol. Biochem. Zool.* **74**, 390–397.

Weber, T. P. & Piersma, T. 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.