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AVIAN PECTORAL MUSCLE SIZE RAPIDLY TRACKS BODY MASS CHANGES DURING FLIGHT, FASTING AND FUELLING

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Summary

We used ultrasonic imaging to monitor short-term changes in the pectoral muscle size of captive red knots *Calidris canutus*. Pectoral muscle thickness changed rapidly and consistently in parallel with body mass changes caused by flight, fasting and fuelling. Four knots flew repeatedly for 10 h periods in a wind tunnel. Over this period, pectoral muscle thickness decreased in parallel with the decrease in body mass. The change in pectoral muscle thickness during flight was indistinguishable from that during periods of natural and experimental fasting and fuelling. The body-mass-related variation in pectoral muscle thickness between and within individuals was not related to the amount of flight, indicating that changes in avian muscle do not require power-training as in mammals. Our study suggests that it is possible for birds to consume and replace their flight muscles on a time scale short enough to allow these muscles to be used as part of the energy supply for migratory flight. The adaptive significance of the changes in pectoral muscle mass cannot be explained by reproductive needs since our knots were in the early winter phase of their annual cycle. Instead, pectoral muscle mass changes may reflect (i) the breakdown of protein during heavy exercise and its subsequent restoration, (ii) the regulation of flight capacity to maintain optimal flight performance when body mass varies, or (iii) the need for a particular protein:fat ratio in winter survival stores.

Key words: flight, pectoral muscle, migration, phenotypic flexibility, exercise, power training, ultrasonic imaging, wind tunnel, red knot, *Calidris canutus*.

Introduction

Avian flyers can be characterised by their weight-saving construction: hollow bones, a light armour of feathers, and the absence of gut structures enabling foregut digestion of plant material (Norberg, 1990; Stevens and Hume, 1995; Shipman, 1998). The pectoral muscles constitute the largest and most energy-consuming organ of flying birds (Greenewalt, 1962; Butler, 1991). An ability to adjust pectoral muscle mass to required power output would yield important energy savings. For example, adaptive decreases in the size of the pectoral muscles during long flights, tracking body mass changes, would allow birds to extend their flight range considerably (Pennycuick, 1975, 1998).

Several authors have documented that during periods of fuel deposition prior to migratory departure individuals with large fat loads have bigger pectoral muscles than lean birds (Fry et al., 1972; Marsh, 1984; Driedzic et al., 1993). Such comparisons may be biased because an unknown proportion of these correlations could be due to covariation in structural size (Lindström and Piersma, 1993). Nevertheless, there are convincing studies using sequential sampling of individuals (Gaunt et al., 1990; Jehl, 1997; Bishop et al., 1998; Piersma et al., 1999) demonstrating that changes take place over periods of weeks. Measurements of intra-individual adjustments in the pectoral muscles have been hard to obtain because the measurements have required dissection. With new and improved non-invasive techniques, detailed studies of organ size at the level of the individual are now within reach (e.g. Piersma and Lindström, 1997; Starck and Burann, 1998).

We present the first evidence, to our knowledge, for rapid and repeated intra-individual changes in avian pectoral muscle mass. Of particular interest was how pectoral muscle mass may change with body mass during the course of extended flights (see Pennycuick, 1975, 1998). We capitalised on the possibility of having birds 'migrating' in a wind tunnel (Lindström et al., 1999), and measured avian pectoral muscle mass using ultrasound techniques (Sears, 1988; Newton, 1993; Dietz et al., 1999a,b).

Materials and methods

Experimental birds and holding conditions

Red knots *Calidris canutus* L. of both the *canutus* and the *islandica* subspecies were trapped in the Dutch Wadden Sea on various occasions between April and September 1998. The *C. c. islandica* knots breed in Greenland and northeast Canada and spend the winter in the North Sea region. The *C. c. canutus* knots breed in Siberia, winter in Africa, and pass The Netherlands only during the spring and autumn migration (Piersma and Davidson, 1992).

The birds were kept in the experimental shorebird facilities at NIOZ, Texel, The Netherlands, for periods of 1 week to 7 months, before they were transferred to Lund, Sweden. The following morphometric measurements were taken: wing area and wing span, and the length of the bill and tarsus. None of the birds moulted during the study period. The birds were transferred to and from Lund in small groups throughout the autumn so that only 5–10 birds were kept in Lund at any time.

In Lund, the birds were kept in an aviary ($3\,m\times1.5\,m\times2\,m$) in the wind tunnel building. The floor was covered with a plastic carpet ('artificial turf') soaked with salt water. Fresh water was supplied in drinking bowls and in a $1\,m\times1.5\,m$ basin. The aviary was cleaned and new water was supplied on a daily basis. The birds were fed mealworms and commercial trout pellets. Temperature in the wind tunnel hall was not regulated, and therefore dropped from around $+20\,°C$ in early September to $+7\,°C$ in December. The birds were kept on a 12h:12h L:D photoperiod.

The size of the aviary did not allow normal forward flight, but the birds were occasionally seen hovering for a few seconds. This meant that the only flight exercise that the birds received was obtained in the wind tunnel.

Experimental flight, fasting and fuelling

Four of the birds repeatedly flew for 10h at $15\,m\,s^{-1}$ in the wind tunnel (for a description of the wind tunnel, see Pennycuick et al., 1997). Flights started at 09:00h and ended at 19:00h. We measured pectoral muscle thickness at least once before flight, either in the evening before the flight (when food was removed) or just before the flight started. At first, we measured muscle thickness only the evening before the flight to minimise any disturbance of flight performance. We later realised that such worries were unnecessary and measured the birds just before flight started.

We do not know whether, and how much, the avian pectoral muscles swell as a result of hard work (as does, for example, the human biceps after hard exercise). We therefore waited 1h after the end of flight before making the post-flight measurement to allow the potentially blood-filled muscle return to a relaxed state. A second post-flight measurement was taken the following morning (the bird had no food during night). For logistic reasons, sometimes only one of these two post-flight measurements was taken. Hence, for each long flight, we obtained an estimate of the change in pectoral muscle

thickness, but in six cases this included a period of fasting in addition to flight.

Body mass varied in response to flights, to spontaneous fasts and from subsequent refuelling. There was a general increase in body mass in late autumn, possibly reflecting the increase in body mass found in winter in free-living conspecifics (Piersma, 1994). In addition, three birds that were stable in mass for a long period were given submaintenance levels of food for 6 days and maintenance levels for another 3 days. We stopped the partial fasting when the birds reached 110 g, a mass at which red knots are still well above critical minimal levels (Piersma, 1994).

Ultrasound

We used a Pie 200 ultrasound apparatus with a 7.5MHz linear probe and printed images on a Mitsubishi video copy processor, model P90E (Pie Medical Benelux BV, Maastricht, The Netherlands; for further details, see Dietz et al., 1999a). To obtain comparable results within and between individuals, it is crucial to measure the pectoral muscle at the same spot and angle each time. A protocol was designed to try to achieve this. The basic technique is described by Dietz et al. (1999a; see their Figs 1A, 2A).

The bird was placed horizontally on its back, leaning slightly to its right, with its feet towards the observer. Muscle thickness was measured at the proximal and anterior part of the left pectoral muscle. An improvement to the technique of Dietz et al. (1999a) was that the observer's left index finger was bent at its inner joint 'around' the anterior tip of the keel, with the outer two-thirds of the finger kept along the furcula. This grip was easy to repeat, and the index finger created a 'wall' against which the probe could be positioned in a standardised manner. Pectoral muscle thickness was measured to the nearest 0.1 mm as the distance between the upper edge of the coracoid bone and the skin.

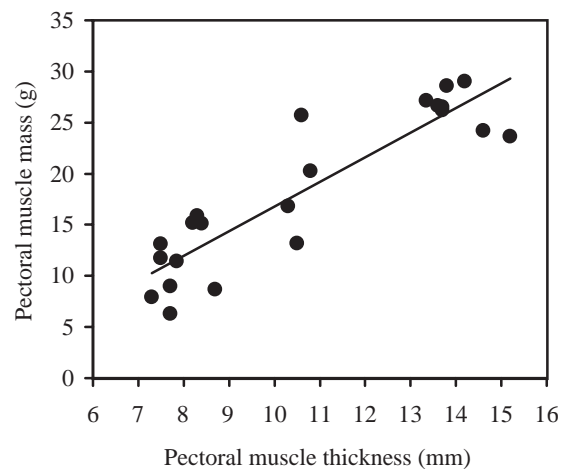A calibration exercise was carried out between 30 July and

Fig. 1. The relationship between pectoral muscle mass (wet mass of the left and right pectoral muscle combined) and pectoral muscle thickness as measured by ultrasound in 22 dead red knots. See text for equation.
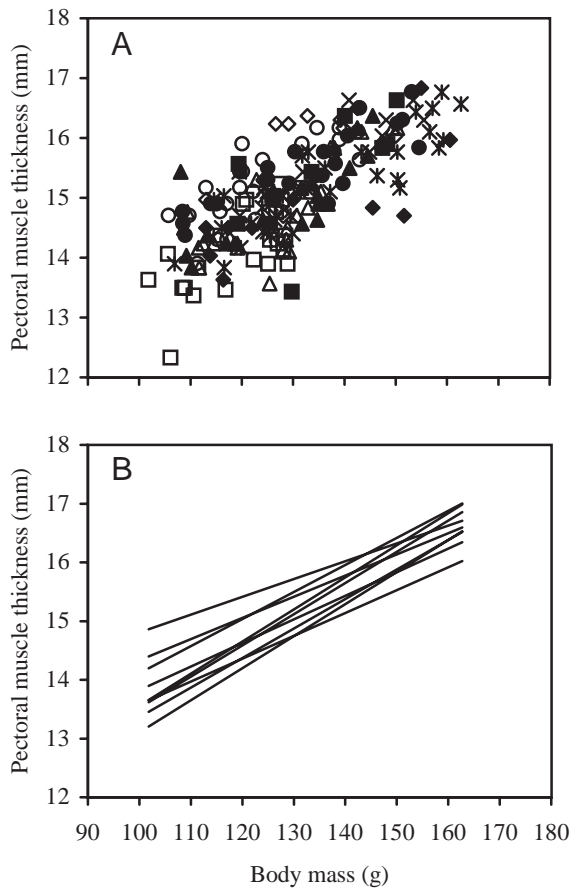
Fig. 2. Variation in pectoral muscle thickness, as measured by ultrasound, in relation to body mass of 10 red knots. (A) Each symbol denotes data for one individual. (B) Individual-specific regression lines for the data presented in A. In two cases, there is complete overlap of two lines; hence, only eight lines are visible. For further information about the regression lines, see Table 1.

1 August 1998 using 22 dead red knots. The measurements reported here were all made by Å.L. The birds belonged to three different cohorts, each from conditions known to involve pectoral muscles of significantly different sizes. After being thawed, each bird was measured twice in a blind and random fashion (following the procedure of Dietz et al., 1999a). One measurement was made each time, and the average of the two measurements for each individual was used. The birds were later dissected, and the combined wet mass of the left and right pectoral muscle was measured.

Ultrasonic measurements were made by Å.L. on the captive red knots in Lund between 9 September and 18 December 1998. Since the observer would normally know the current treatment status of each individual, we aimed at a 'blind' procedure to avoid measuring bias. Therefore, the part of the screen showing the pectoral muscle thickness measurement was covered. The measurement was visible on the printed paper image, but these images were not seen by Å.L. until after the end of the study. Thus, the observer did not see any of the measurements made during the 3 months of data collection. Three measurements were taken on each occasion, from which

we calculated an average value. Individual birds were measured only once per day, except for the birds flying for 10 h, which were sometimes measured twice.

Statistical analyses

Statistical analyses were carried out using SYSTAT 7.0.1 and Microsoft Excel Analysis Tool Pack. Non-significant interaction terms were excluded from analyses of covariance (ANCOVAs). Repeatabilities were calculated according to Lessells and Boag (1987).

The calibration equations relating pectoral muscle mass to ultrasonic measurements and body mass were calculated using linear regression. Regressions based on the cube and logarithm of the ultrasonic measurements did not produce a better fit. To test the predictive value of the equations, five of the 22 calibration individuals were excluded at random. The linear regression equation estimated from the remaining 17 individuals was used to predict pectoral muscle mass for excluded individuals. When testing the equation predicting muscle mass from body mass, three of the nine cases were excluded. Predicted pectoral muscle masses (y_{pred}) were compared with observed masses (y_{obs}), and the absolute ($|y_{pred}-y_{obs}|$) and relative $[|(y_{pred}/y_{obs})-1|]$ deviations were calculated. This was repeated for 10 000 runs. The discrepancy values presented are the average and relative deviations from this bootstrap procedure (see Dietz et al., 1999a).

Results

Calibration

Repeatability between the two measurements in the calibration exercise was 0.94 (analysis of variance, ANOVA, $F_{21,22}=30.5$, $P<0.001$). Linear regression of wet pectoral muscle mass M_m (g) on pectoral muscle thickness T (mm), measured by ultrasound, produced the calibration equation: $M_m=2.4T-7.344$ ($F_{1,20}=83.8$, $P<0.001$, Fig. 1). The bootstrap procedure showed an average discrepancy between predicted and observed values of 3.0 g or 21 %.

Total body mass M_b (g), which was available for nine of the 22 calibration individuals, was an even better predictor of pectoral muscle mass than the ultrasound measurements: $M_m=0.264M_b-10.5$ ($F_{1,8}=96.3$, $P<0.001$). The discrepancy when using the body mass equation was 2.2 g or 16 %.

Intra-individual variation in pectoral muscle thickness

In total, 216 values of pectoral muscle thickness were collected (each made up of the average of three measurements) from 18 individuals. Repeatability was 0.87 for the three measurements on which each pectoral muscle thickness estimate was based (ANOVA, $F_{215,432}=20.6$, $P<0.001$). Since our aim was to study within-individual changes, we restricted our analysis to the 10 red knots that were measured at least 10 times (Table 1). Of these birds, one was *C. c. canutus* (individual DB) and nine were *C. c. islandica*.

Body mass varied between 102 and 163 g. At body masses above 150 g, the birds sometimes had a very thin layer of subcutaneous fat deposited on top of the pectoral muscles,

Table 1. *Summary statistics for 10 red knots studied in the Lund wind tunnel during autumn 1998*

Individual	Wing span (m)	Wing area (m²)	Bill length (mm)	Tarsus length (mm)	Flight time (h)	Mass range (g)	Muscle thickness range (mm)	Slope (mm g⁻¹)	r^2	P	Exp	N
AN	0.517	0.0318	32.5	30.7	42	113–150	14.9–16.5	0.030	0.29	0.068	0.26	12
DA	0.509	0.0328	31.5	30.1	16	109–150	13.6–16.2	0.050	0.59	<0.001	0.42	20
DB	0.505	0.0299	32.6	34.3	50	102–133	12.3–15.2	0.055	0.50	<0.001	0.46	24
DG	0.538	0.0332	33.5	31.2	81	107–163	13.8–16.8	0.040	0.71	<0.001	0.36	35
FI	–	–	38.1	32.8	1	108–155	14.4–16.8	0.036	0.71	<0.001	0.30	22
LB	0.502	0.0301	32.6	29.6	16	113–161	13.6–16.8	0.039	0.62	0.007	0.34	10
LG	–	–	33.8	32.6	1	120–156	14.4–16.6	0.055	0.74	<0.001	0.48	13
RE	0.491	0.0289	31.7	31.0	4	119–150	13.4–16.6	0.053	0.43	0.038	0.46	10
SK	0.521	0.0331	33.0	30.1	55	106–143	13.9–16.2	0.046	0.60	<0.001	0.38	24
SW	–	–	30.5	30.5	5	108–146	13.8–16.4	0.040	0.55	<0.001	0.33	21

All birds belonged to the *Calidris canutus islandica* subspecies apart from DB, which was *C. c. canutus*.

Total flight time is the time the bird spent flying in the wind tunnel during the study period.

Slope, r^2 and P refer to the individual-specific linear regressions of pectoral muscle thickness on body mass (see Fig. 2), Exp is the slope of the log–log regression for the same variables, and N is the number of measurements made of pectoral muscle thickness.

which was difficult to correct for. Since we estimate this layer to have been less than 0.5 mm thick (visual inspection), and few birds heavier than 150 g were measured, the influence on the overall results should be negligible. Muscle thickness was on average 15.1±0.8 mm (mean ± S.D, range 12.3–16.8 mm). Thus, all measurements were within a range of 4.5 mm.

There was a significant effect of both body mass and individual on pectoral muscle thickness (ANCOVA, effect of body mass, $F_{1,180}$=252.6, P<0.001; effect of individual, $F_{9,180}$=8.7, P<0.001; Fig. 2). Hence, although the variation in pectoral muscle thickness in relation to body mass was similar for all birds, some red knots had thicker pectoral muscles than others at a given body mass. The pooled linear regression slope was 0.043 mm g⁻¹ (95 % CI; 0.037–0.048 mm g⁻¹). The variation between individuals, as expressed in adjusted least-square means, was not significantly correlated with any of the morphological measurements (Pearson correlation, P>0.1 in all cases; Table 1). Neither did the adjusted least-square means differ between the four birds that regularly flew in the wind tunnel (individuals DB, DG, SK and AN; 15.2±0.5 mm, mean ± S.D.) and those that did not (15.1±0.3 mm; two-sample *t*-test, t_8=0.50, P=0.63). The former group flew on average a total of 58±17 h during the study period (mean ± S.D, range 42–81 h), which is significantly more than the 'non-regular' flyers (8±7 h, range 1–16 h; Table 1; two-sample *t*-test, t_8=6.59, P<0.001).

In total, 13 flights of 10 h duration were carried out by four different knots (DB made three flights, DG made four, SK made three and AN made three). On seven occasions, pectoral muscle thickness was measured both just before and just after the flights (Fig. 3). Mean body mass loss was 12.3 g during these flights. During six of the flights, pectoral muscle thickness decreased. The average decrease of 0.23±0.3 mm (mean ± S.D.) was not significantly different from zero (one-sample *t*-test, t_6=−2.00, P=0.093).

For all the flights combined, where some of the estimates of pectoral muscle thickness also include non-flight periods

(referred to as 'flight/fast sessions' in contrast to 'flights'), the pattern was similar (Fig. 3). In 10 of the 13 cases, pectoral muscle thickness decreased. For all flight/fast sessions and flights combined, incurring an average body mass loss of 17.0 g, the decrease in pectoral muscle thickness was significantly different from zero at the 5 % level (one-sample *t*-test, t_{12}=−2.51, P=0.028). When using individual averages as independent data points, the difference was not significant (one-sample *t*-test, t_3=−2.05, P=0.13).

The average body-mass-specific change in pectoral muscle thickness for each individual during the flight/fast sessions (mean 0.032 mm g⁻¹, range 0.014–0.074 mm g⁻¹, N=4) was not
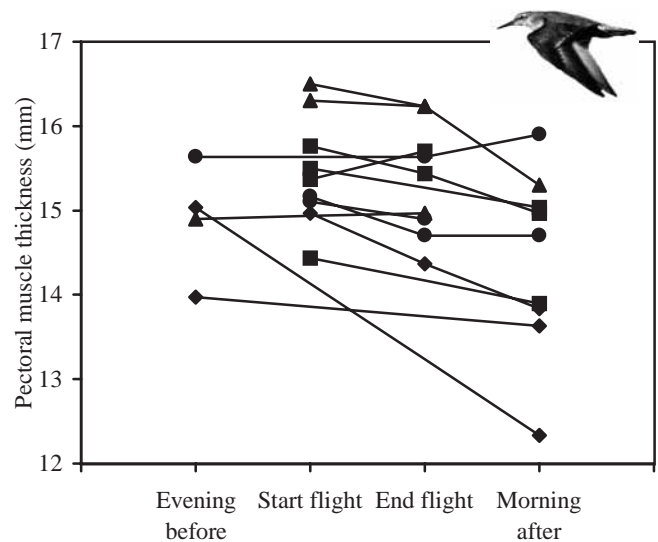

Fig. 3. Changes in pectoral muscle thickness, as measured by ultrasound, of four birds flying for 10 h in a wind tunnel. Different individuals are denoted by different symbols, and the lines connect measurements made during the same flight session. The birds were measured the evening before the flight, just before the flight, 1 h after the flight and/or in the morning following the flight.

significantly different from the overall regression slope of the same individual, after excluding the flight session data (mean 0.040 mm g^{-1}, range 0.008–0.060 mm g^{-1}, N=4; paired t-test, t_3=0.487, P=0.66).

The three birds given limited food rations lost 25–30 g. When given food *ad libitum* again, they increased in mass by 35–45 g within a week. Pectoral muscle thickness during this fast and fuelling varied closely in parallel with the changes in body mass (Fig. 4). The mean body-mass-specific variation in pectoral muscle thickness for each individual over the fasting and refuelling phase, 20 October to 5 November (mean 0.048 mm g^{-1}, range 0.040–0.056 mm g^{-1}, N=3), was not significantly different from the overall regression slope of the same individual, after

excluding the fast-refuelling data (0.057 mm g^{-1}, range 0.033–0.078 mm g^{-1}, N=3; paired t-test, t_2=1.06, P=0.40).

Discussion

The necessity and usefulness of a non-invasive technique

The fact that a bird cannot be killed more than once makes it very difficult to determine the relationships between changes in the mean mass of a particular organ with changes in body mass in individual birds (Lindström and Piersma, 1993). Accordingly, rapid intra-individual changes in organ mass can be studied only by non-invasive techniques such as the ultrasound method applied here. All non-invasive techniques have to be calibrated. In our calibration exercise, body mass was a better predictor of pectoral muscle mass than the ultrasonic measurements (average error 16 and 21 %, respectively). One may therefore argue that the ultrasound technique is an expensive way of obtaining less accurate data. This is not true for the following reasons.

First, a calibration curve based on body mass variation between individuals is likely to be partly unreliable since variation in structural size will contribute to the variation in mass between individuals, but not within individuals (Lindström and Piersma, 1993; Van der Meer and Piersma, 1994). All else being equal, this will cause the slope of the calibration equation to be higher than that for the individual. Second, even if differences in structural size can be taken into account, the individual bird may not follow the between-individual calibration equation. Third, the size of individual organs, such as the stomach and pectoral muscle, may change in relation to ecological context (Piersma and Lindström, 1997), even in the direction opposite to body mass changes (Piersma et al., 1993, 1999; Jehl, 1997).

In the calibration exercise, the repeatability was high, and the average error made when predicting pectoral muscle mass was similar to the 20–25 % found by Dietz et al. (1999a) using the same method. However, our measurements of live birds were more accurate than the calibration exercise suggests. First, following the reasoning in the previous paragraph, changes in pectoral muscle thickness as measured by ultrasound will probably reflect pectoral muscle mass more closely within an individual than between individuals sampled under different ecological conditions. Second, during our experiments, we performed more repeats, had more experience with the technique and used an improved standardisation procedure compared with the calibration exercise. Indeed, in our experimental data, there was a high consistency both between and within measurement sessions. Because of the absence of intra-individual calibration curves, it is difficult to convert ultrasonic measurements to true pectoral muscle mass. Also, too much importance should not be given to single ultrasonic measurements. However, we feel confident that the ultrasound technique is highly reliable for studying changes in pectoral muscle mass on a relative scale.
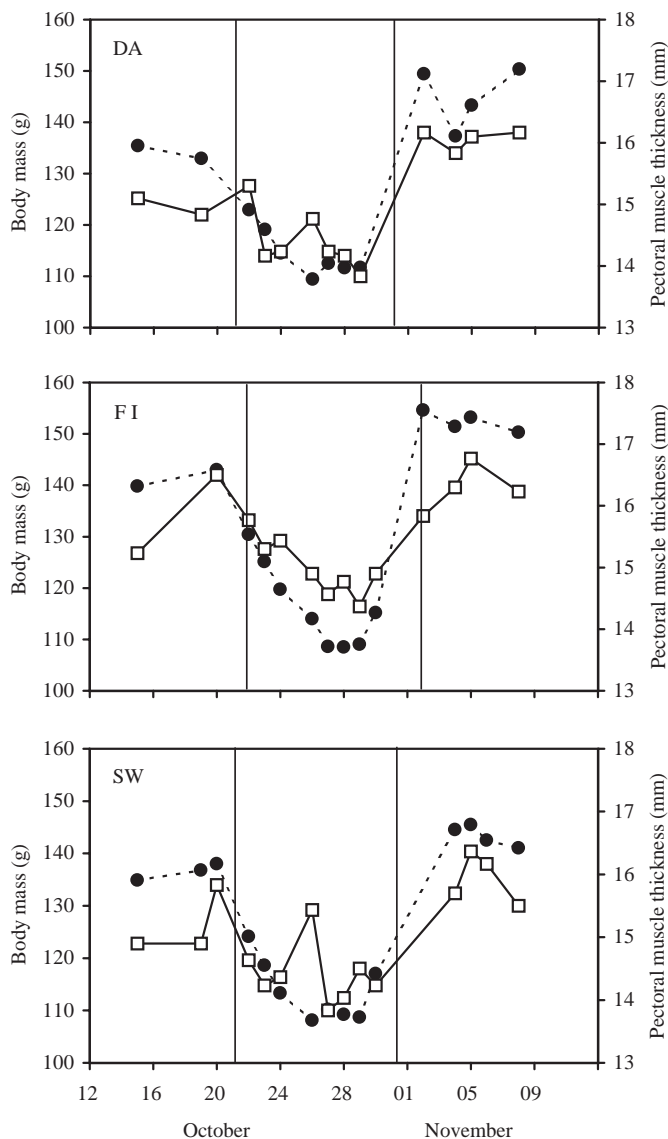

Fig. 4. Variation in body mass (filled circles and dotted lines) and pectoral muscle thickness (open squares and solid lines), as measured by ultrasound, of three red knots during a period of partial fasting (between vertical lines) and during subsequent refuelling (regain of weight). Bird identity is given at the top left of each panel.

Pectoral muscle size tracks changes in body mass

Pectoral muscle thickness showed a strong tendency to

decrease during the experimental flights. Whether the somewhat smaller, but non-significant, mass-specific changes in pectoral thickness during flight compared with fasting reflect true differences between these processes remains to be shown. However, since the magnitude of mass-specific change in muscle thickness during flight was not statistically distinguishable from the overall variation in muscle thickness due to fasting and refuelling, our results suggest that pectoral muscle mass varies in parallel with body mass, irrespective of the direction and cause of mass changes. This contrasts with the finding of Biebach (1998) that the pectoral muscle of garden warblers *Sylvia borin* is partly catabolised during non-stop flights over the Sahara Desert, whereas a mass loss of similar magnitude during non-exercise fasting did not affect pectoral muscle mass.

There are two reasons why we believe that the changes observed in the pectoral muscles of the knots do not depend on changes in the hydration status of the muscle. First, in two data sets of dissected red knots, 60 from Iceland and 24 from Delaware Bay, including lean birds arriving after a 2500 km flight, fuelling birds and heavy birds close to departure, 95 % of the birds had a muscle water content of 67–72 % (Piersma et al., 1999; T. Piersma, unpublished data). Hence, pectoral muscle hydration levels seem to be fairly constant, regardless of fuel store levels and migration state. Second, doubly-labelled water studies of the same knots as measured in the present study show that the body water pool did not change over the 10 h flights, indicating that no overall dehydration takes place during flight (A. Kvist, Å. Lindström, T. Piersma and G. H. Visser, unpublished data).

The size of the pectoral muscles of the red knots used in this study appeared to be unaffected by the level of exercise in the wind tunnel. Birds flying repeatedly did not have thicker pectoral muscles than the 'non-fliers'. This is different from the situation in mammals, in which training and immobilisation have strong effects on the size of the skeletal muscles (Goldspink, 1991; Kannus et al., 1992). Yet, it is in accordance with the findings of Bishop et al. (1998) and Dietz et al. (1999b) that, on a seasonal basis, pectoral muscle mass can increase substantially in parallel with fuel storage for migration in non-exercising (caged) birds.

The adaptive significance of rapid changes in muscle size

Several hypotheses have been put forward to explain why pectoral muscle mass in birds should vary in parallel with body mass, especially in the context of long migratory flights (for a review, see Lindström and Piersma, 1993). Changes in pectoral muscle mass may reflect (i) a regulation of flight capacity to maintain optimal flight performance when body mass varies (Pennycuick, 1975, 1998; Marsh, 1984), (ii) protein stores to be used on arrival at the breeding grounds to enhance reproductive performance (e.g. McLandress and Raveling, 1981; Davidson and Evans, 1988), and (iii) a build-up of protein in anticipation of protein loss during long-lasting flights (Piersma, 1990; Jenni and Jenni-Eiermann, 1998).

Nine of our ten birds, and three of the four flyers, belonged to the *C. c. islandica* subspecies. During our experiments, these birds were in the early winter phase of their annual cycle (Cadée et al., 1996). Therefore, the changes in pectoral muscle mass are unlikely to reflect reproductive needs. However, since *C. c. islandica* are known to carry out long-distance movements within their winter range (Davidson and Wilson, 1992), we cannot exclude the possibility that the changes in pectoral muscle thickness relate to increasing needs during long flights.

At least two possible explanations, not mutually exclusive, remain. If a period of fasting always involves a certain amount of protein breakdown (Cherel et al., 1988; Jenni and Jenni-Eiermann, 1998), the increase in pectoral muscle mass of our knots during weight gain may reflect the need to add a certain amount of protein in parallel with fat stores for winter survival. Bar-tailed godwits *Limosa lapponica* wintering in northern England, however, showed no increase in pectoral muscle mass in parallel with the deposition of fat in mid winter (Evans and Smith, 1975).

If a bird wants to maintain a certain level of flight capacity, for endurance exercise (migration) or sprint performance (e.g. predator evasion), one would expect pectoral muscle mass to change in parallel with body mass (Pennycuick, 1975). The inter-individual calibration curve does not allow us to convert our ultrasonic measurements to actual pectoral muscle mass in a reliable way. Therefore, we cannot estimate the quantitative changes in pectoral muscle mass, and we cannot say whether the changes are sufficient to maintain optimal flight capacity when body mass changes. However, the changes are unlikely to be sufficiently large, since caged knots are much easier to catch by hand when heavy than when light (A. Kvist, Å. Lindström and T. Piersma , personal observations), and several studies have documented how take-off ability is hampered at high body mass (Metcalfe and Ure, 1995; Kullberg et al., 1996). What we can say is that changes in pectoral muscle mass can occur rapidly, within days, and that these changes in pectoral muscle mass track increases and decreases in body mass seemingly independently of whether the bird flies, fasts or builds up body stores. It remains to be shown whether the rapid intra-individual changes in pectoral muscle thickness described here for red knots represent a general pattern in birds.

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