Strategic size changes of internal organs and muscle tissue in the Bar-tailed Godwit during fat storage on a spring stopover site

M. M. LANDYS-CIANNELLI,*† T. PIERSMA‡ and J. JUKEMA§

*Department of Zoology, University of Washington, Box 351800, Seattle, WA 98195, USA, ‡Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, the Netherlands, and Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, the Netherlands, and §Haerdawei 44, 8854 AC Oosterbierum, the Netherlands

Summary

1. In several migrant bird species it has now been established that refuelling is typified not only by fat deposition, but also by changes in lean body protein. The body composition of a long-distance migratory shorebird, the Bar-tailed Godwit Limosa lapponica taymyrensis, was examined on a stopover site in spring to evaluate changes in lean dry mass of organ and muscle tissue over the period of refuelling.

2. It was hypothesized that tissues which support long-distance flight would hypertrophy and reach peak size towards the end of refuelling, i.e. immediately before the 4500-km bout of flight to Arctic breeding grounds. In contrast, it was hypothesized that tissues involved in digestion would initially hypertrophy, but then atrophy during the final stages of stopover, so that wing loading during long-distance flight would be minimized.

3. Flight muscles of godwits steadily increased in lean dry mass during refuelling and reached peak size in birds preparing to depart the stopover site – possibly in an effort to maximize power output.

4. In contrast, the lean dry mass of stomach, liver, kidneys and intestines of godwits increased only during the early stages of refuelling, indicating a quick initial growth of organs involved in the support of refuelling activities. Additionally, in birds readying for departure, the intestines no longer differed in size from those of recent arrivals, suggesting a reduction in size of digestive tissues prior to flight.

5. Such changes in body composition appear consistent with those found in another shorebird and in a grebe preparing for a bout of migratory flight, suggesting a common pattern in allocation of lean body mass in at least some migratory species during fuelling.

Key-words: Body composition, fattening, lean body mass, migration, refuelling, shorebirds

Introduction

Migrating birds typically experience large fluctuations in body mass as they alternate between the migratory stages of fuelling and flight. Originally, changes in body mass were attributed to differences solely in fat content (Connell et al. 1960; Odum et al. 1964; Hicks 1967; Blem 1980). However, more recent studies have shown that in addition to fat, lean body mass and thus protein content of birds also changes during the migratory period (Fry et al. 1972; Marsh 1984; Davidson & Evans 1988; Lindström & Piersma 1993; Jehl 1997; Karasov & Pinshow 1998; Battley et al. 2000), suggesting a change in the size of organs and/or muscle groups.

Migration is characterized by both flight and fuelling. Changes in lean body mass may occur during either stage. Internal organs and muscles most probably display a different pattern of change during migratory flight than during fuelling because of very different physiological requirements: flight is characterized by high energy expenditure (Alerstam 1990; Butler & Woakes 1990) and fuelling is typified by the quick deposition of energy stores (Ramenofsky 1990; Jenni-Eiermann & Jenni 1991). Therefore, changes in body composition during these two migratory stages should be evaluated separately.
To date, only a few species have been examined for changes in body composition specifically during the refuelling period. Among these is the Red Knot Calidris canutus islandica (Piersma et al. 1999b), which undertakes a 4-week refuelling stop in Iceland. Investigations of the body composition of refuelling Red Knots suggest that digestive tissues enlarge only during the early part of refuelling, and then atrophy before the re-initiation of migratory flight. In contrast, the exercise musculature of these birds attains peak size toward the end of the stopover period (Piersma et al. 1999b). A similar pattern in lean body mass has been observed in staging Eared Grebes Podiceps nigricollis as these birds prepare to initiate migration (Jehl 1997).

Much like the Red Knot, the Bar-tailed Godwit Limosa lapponica taymyrensis (Engelmoer & Roselaar 1998) can be easily examined for changes in body composition during refuelling on its single spring stopover site. Preliminary investigations of refuelling godwits suggest that the mass ratio of digestive organs to flight muscle decreases towards the end of stopover (Piersma & Lindström 1997; Piersma 1998). Therefore, godwits may allocate protein components from the digestive tract into the exercise apparatus before departure. Here we examined the body composition of godwits during the complete period of refuelling to: (1) evaluate changes in organ and muscle size as birds prepare to re-initiate flight, and (2) compare the pattern of change in organ and muscle size between godwits and other birds for which comparable data are available, and thereby test for a common pattern of organ and muscle reorganization (Piersma 1998). Based on the knot model (Piersma et al. 1999b), we hypothesized that in godwits preparing to re-initiate flight, tissues involved in the promotion of migratory flight would hypertrophy, whereas those involved in digestion would atrophy, possibly in an effort to minimize wing loading (Piersma 1998; Piersma & Gill 1998).

Materials and methods

STUDY ANIMALS

The Bar-tailed Godwits sampled in this study winter along the coastal mudflats of Mauritania and Guinea-Bissau, and have recently been assigned the subspecies status Limosa lapponica taymyrensis (Engelmoer & Roselaar 1998). This subspecies departs from wintering sites in late April (Piersma et al. 1990), and arrives on breeding areas just west and south of the Taymyr Peninsula, Russia (Boere & Smit 1981; Piersma & Jukema 1990) in early June after a month-long refuelling stop in the Wadden Sea of the Netherlands, Germany and Denmark (Drent & Piersma 1990; Piersma & Jukema 1990). Godwits travel the 9000-km distance between wintering sites and breeding grounds in two flight bouts of approximately equal length, each of which takes about 2-6 days to complete (Piersma 1987).

COLLECTION

A total of 92 male and 65 female godwits were accumulated from the Wadden Sea area during 28 April to 1 June, 1988–2000. All birds were casualties of a long-term ringing study during which over 3500 godwits were captured with clap-nets or with the wilsternet (a large wind-driven clap-net; see Jukema et al. 2001). Except for three males which were lured to land in the Castricum dune reserve just 60 km short of the Wadden Sea refuelling site (see Landys-Ciannelli et al. 2002), all birds were collected on one of three refuelling locations along the coast of the Dutch Wadden Sea. Bird carcasses were placed onto ice immediately after collection, and were stored at −20 °C until further analysis.

BODY COMPOSITION ANALYSIS

Specimens were thawed at room temperature one day before dissection. Dissection was performed only by TP and Anne Dekinga to minimize interindividual variability in data collection. Dissection procedures were identical to those described in Piersma et al. (1999b). Briefly, after all feathers from the birds were plucked, the skin was cut open and removed. The tibiotarsis and feet were left attached to the skin. The skin also included subcutaneous fat deposits, as these are more firmly attached to skin than to underlying tissue. The flight muscles – the supracoracoideus and the two pectoralis muscles (major and minor) – were dissected from their attachments to the keel and humerus. The heart, stomach, intestines (which included the large and small intestines, and the caeca), kidneys, liver, lungs, brain and leg muscles were excised. The contents of the stomach and intestines were removed. The fresh wet mass of all body parts was measured to the nearest 0·1 g. Body parts were then dried separately to constant mass at 60 °C. Dried tissues were weighed to the nearest 0·1 g, packed in filter paper and then fat-extracted in a Soxhlet apparatus (Elgebe BV, Leek, The Netherlands) with petroleum-ether as the solvent. Fat extraction was considered done when the solvent no longer showed any yellowish colour for three successive fillings, indicating the complete removal of fat from the tissue. Tissues were once again dried to constant mass, separated from the filter paper, and weighed to the nearest 0·1 g to obtain their fat-free dry mass. Fat content of each tissue was estimated from mass lost during extraction.

DATA ANALYSIS

Because only casualties of trapping were collected, the possibility was examined of a bias in body mass between survivors and casualties across the period of refuelling. Only dates with two or more casualties were considered, and body mass was log_{10}-transformed to meet normality requirements.
Refuelling status for each bird, and thus its readiness to depart the stopover site, was estimated from total fat mass. Because godwits are sexually dimorphic (Piersma & Jukema 1990), total fat mass was corrected for sex-related differences by calculating fat mass residuals for all birds according to the relationship between fat mass and wing length (Bennett 1987; Packard & Boardman 1987, 1988; Landys-Ciannelli et al. 2002). The cube root of fat mass was used to normalize data and establish linearity between fat mass and wing length. Fat mass residuals for the three males lured to land in Castricum were calculated from the slope generated by refuelling birds.

Fat mass residuals were used instead of calendar day to estimate progression of refuelling. Calendar day is not a good indicator of refuelling status if the population does not refuel synchronously or if there is interannual variation in timing of migration. Although Bar-Tailed Godwits display a rather repeatable migratory schedule between years, godwits can migrate as much as 2 weeks apart in any one year (Green et al. 2002).

Refuelling birds were divided into three categories according to their fat mass residuals. Birds with the 33% lowest fat mass residuals were categorized as early refuelling birds; birds with the middle 33% fat mass residuals were categorized as mid-refuelling birds; and birds with the 33% highest fat mass residuals were categorized as late refuelling birds. Sample sizes for early, middle and late refuelling males were 33, 29 and 30, respectively. Sample sizes for early, middle and late refuelling females were 19, 24 and 22, respectively. Although three females in the early refuelling group were collected at the end of May and thus might be examples of non-refuelling birds (see Fig. 1), we nevertheless include these individuals in subsequent analyses because: (1) these females might have been late arrivals that were nevertheless attempting to refuel, and (2) body fat and the mass of lean body components were not different between these females and the early refuelling females captured in the first half of May.

Fat content as a percentage of body mass was determined for each individual by calculating the ratio of total fat mass to total body mass. Differences in fat content between males and females were examined for the early, middle and late refuelling periods. Fat content was arcsin square root transformed for use in analyses.

Differences in fat-free dry mass of the pectoralis muscles (major and minor), the supracoracoideus and the leg muscles, as well as the fat-free dry mass of the heart, stomach, intestines, liver, kidneys, lung and brain were examined between males and females over the three refuelling stages. Data were log_{10}-transformed for purposes of normality. P-values for Tukey post-hoc tests are reported. Lean dry mass of the supracoracoideus, the stomach and the liver did not satisfy normality and/or constant variance requirements even after transformation. Nevertheless, results generated by parametric tests are reported for the supracoracoideus and the liver, because results were not different from those generated by non-parametric tests conducted on males and females separately. Non-parametric tests were used to examine patterns in stomach data.

Relationships between all organs except for the lungs and brain were explored with a biplot that was generated from a principal components analysis (Gabriel 1971). In a biplot, the length of each vector is proportional to the variance explained by a principal components model, while the correlation between different variables is indicated by the angle between vectors. Vectors that point in the same direction indicate positively correlated variables; vectors that point in opposite directions indicate negatively correlated variables; and orthogonal vectors indicate the absence of a correlation. For a more detailed explanation, refer to Piersma et al. (1999b).

Results

Body Fat

We found that body mass increased with calendar day (ANCOVA: F_{1,1106} = 275·751, P < 0·001) and was similar between survivors and casualties of trapping (ANCOVA: F_{1,1106} = 1·880, P = 0·171). The average body mass (± SE) of survivors and casualties was 343·3 ± 6·2 g and 334·6 ± 2·0 g, respectively. Thus, we concluded that analysis of the casualties of the two capture techniques employed – clap nets and the wilsternet – did not introduce a bias into our results.

In the sample of godwit casualties examined here (Fig. 1), total fat mass significantly increased with calendar day on the stopover site (ANCOVA: F_{1,151} = 57·193, P < 0·001). Also, fat mass was significantly
higher in female godwits than in male birds (ANOVA: $F_{1,151} = 12.277, P = 0.001$). The average fat mass ($\pm$ SE) of female and male godwits was $80.2 \pm 4.8$ g and $52.4 \pm 3.4$ g, respectively.

Fat content as a percentage of body mass (as compared to total fat mass) was also significantly higher in female godwits than in male birds (two-way ANOVA: $F_{1,151} = 23.988, P < 0.001$; Fig. 2). Average fat content ($\pm$ SE) of early refuelling females and males was $11.2 \pm 1.1\%$ and $7.4 \pm 0.5\%$, respectively. Average fat content ($\pm$ SE) of late refuelling females and males was $27.8 \pm 0.6\%$ and $25.2 \pm 0.8\%$, respectively. As expected (because of the way in which birds were categorized), fat content differed between the periods of early, middle and late refuelling (two-way ANOVA: $F_{2,151} = 263.584, P < 0.001$), with early and late refuelling birds displaying the lowest and highest fat content, $8.8 \pm 0.6\%$ and $26.3 \pm 0.6\%$, respectively (Tukey post-hoc test: $P < 0.05$). The interaction between sex and migratory stage was not significant (two-way ANOVA: $F_{2,151} = 1.421, P = 0.245$).

Fat mass of specific organs and muscle groups changed between the periods of early and late refuelling. A substantial deposition of fat in both females and males occurred in the subcutaneous fat deposits of the skin ($66.9 \pm 3.5$ g and $51.8 \pm 2.2$ g, respectively) and in the abdominal fat pad ($8.1 \pm 0.5$ g and $5.4 \pm 0.3$ g, respectively). In contrast, only minimal amounts of fat were deposited in and around the muscles of the leg, the flight muscles, the heart, stomach, intestines, liver, kidneys, lung and brain (Fig. 3).

**FAT-FREE DRY MASS**

The fat-free dry mass of most investigated organs and muscle groups was significantly different among birds in early, middle and late states of refuelling, and fat-free dry mass of all muscles and organs was greater in female godwits than in male birds (Tables 1 and 2).

Among the tissues associated with locomotion, the pectoralis flight muscles consistently increased in lean dry mass between successive refuelling periods (post-hoc test: $P < 0.05$; Fig. 4), and reached peak size in late refuelling birds. The supracoracoideus and leg muscles, and the heart and the lungs, were significantly larger in middle and in late refuelling godwits than in early refuelling birds (post-hoc test: $P < 0.05$; Fig. 4), but did not increase in size between the middle and late refuelling periods (post-hoc test: $P > 0.05$; Fig. 4).

Organs associated with digestion and/or water balance, i.e. stomach (but only in males; Kruskal–Wallis one-way ANOVA on ranks: $H = 14.990, df = 2, P < 0.001$), liver and kidneys, increased in lean dry mass during the early phases of refuelling (post-hoc test: $P < 0.05$), but then did not further increase in size from middle to late refuelling (post-hoc test: $P > 0.05$; Fig. 5). In females, the stomach only displayed a trend to increase during the early part of refuelling (Kruskal–Wallis one-way ANOVA on ranks: $H = 5.469, df = 2, P = 0.065$; Fig. 5).

Even though lean dry mass of the intestines increased during the initial period of refuelling (post-hoc test: $P < 0.05$), the intestines of late refuelling birds were identical in lean dry mass to those of early
refuelling birds (post-hoc test: \( P > 0.05 \); Fig. 5), suggesting intestinal atrophy in godwits preparing to re-initiate flight.

In comparison with organs and muscles associated with exercise and digestion, the structural size of the brain did not exhibit changes over the period of refuelling.

The biplot graphs for males and females (Fig. 6) summarize relationships between the above-mentioned tissues in a succinct way and establish the precise nature of correlations. Based on the similar projection angles of their representative vectors, the flight and leg muscle groups, the heart and the stomach were closely correlated, as were the kidneys and the liver. On the other hand, the almost orthogonal projection of the vector representing the intestines with respect to most other tissues in both males and females indicates that this structure had a unique pattern of change over the refuelling period.

**Discussion**

This study examined the body composition of Bar-Tailed Godwits during their refuelling period on the Wadden Sea coast. We found that godwits dramatically increased in total fat content during the course of refuelling. All birds arrived on the stopover site with depleted fat stores and most showed increasingly greater fat mass as time to departure for the last leg of migration approached. Both males and females stored the majority of fat in subcutaneous fat deposits and in the abdominal fat pad.

As expected because of their larger body size (Piersma & Jukema 1990), female godwits displayed a higher total fat mass than male birds. Fat content as a percentage of total body mass was also significantly higher in females than in males, suggesting that relative to their body mass, females deposit disproportionately greater fat stores.

Analysis of the lean body components of godwits refuelling in the Wadden Sea area suggests that refuelling is characterized not only by changes in total fat mass, but also by changes in the lean body mass of internal organs and muscle groups. We found that the flight muscles of godwits steadily increased in size during refuelling to reach peak mass in birds preparing to depart the stopover site. In contrast, the size of the heart, the leg muscles, kidneys, stomach, liver and intestines of godwits increased only during the early part of refuelling. Interestingly, the intestines of birds about to depart the stopover site did not differ in size from those of recent arrivals, suggesting that organs unnecessary for flight may atrophy before departure.
Lean dry mass of investigated muscles and organs was greater in female godwits than in male birds. However, we found no differences in the pattern of tissue change between sexes, suggesting that both males and females similarly allocate protein among body components during refuelling.

These data collected on the body composition of refuelling godwits are similar in pattern to data previously collected from another refuelling shorebird, the Red Knot (Piersma et al. 1999b). In both godwits and
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Knots, organs and muscle groups involved in activity increase in size during successive stages of refuelling, possibly in an effort to maximize power output before re-initiation of migratory flight. Godwits and knots also show parallel changes in size of most digestive organs (an initial increase followed by a plateau toward middle of refuelling), indicating a quick initial hypertrophy of organs involved in the support of intense feeding activities. However, components of the digestive system seem to decrease in size toward end of stopover in both species, suggesting a reduction of excess tissue immediately before flight (Pietersma 1998). Grebes preparing to depart staging areas in fall display changes in body composition that are similar to those noted in refuelling shorebirds: an increase in pectoral muscle mass coupled with a dramatic decrease in mass of the intestine, liver and stomach (Jehl 1997).

Because godwits were not examined for body composition after departure from the Wadden Sea stopover site, it is not clear whether further reduction of digestive machinery occurs during flight. However, as a consequence of a 5400-km trans-oceanic trip, Great Knots Calidris tenuirostris do show a large reduction in various internal organs, as well as in flight muscle (Battley et al. 2000). In addition, the investigation of Garden Warblers Sylvia borin before and after a long desert crossing suggests a large loss of tissue proteins from the digestive tract (Biebach 1998). Migrating Pied Flycatchers Ficedula hypoleuca, Willow Warblers Phylloscopus trochilus and Barn Swallows Hirundo rustica also show decreased lean dry mass of digestive tissues in conjunction with long-distance flight (Schwilch et al. 2002).

Even though allocation of protein reserves from the digestive tract to the flight apparatus towards the end of refuelling may minimize energy expenditure during long-distance flight, it may also limit dietary intake upon arrival at destination sites. For example, research has shown that godwits with light digestive organs prefer softer prey (Pietersma et al. 1993, 1999a). If limited dietary choice is the only consequence of a reduced digestive tract, then food intake of shorebirds on breeding sites may not be adversely affected: most shorebirds subsist upon soft-bodied invertebrates in the Arctic (Holmes 1966; Tulp et al. 1998). However, if similar changes in the digestive tract occur prior to southward migration, then the diet of fall migrants employing the strategy of digestive tissue reduction may be constrained.

A decrease in size of the digestive system may limit not only dietary choice, but also digestive efficiency and therefore assimilation of the food matter that is ingested. For example, reduced gastrointestinal mass resulting from a 2-day fast in migrant songbirds is associated with lower assimilation rates once feeding is re-initiated (Hume & Biebach 1996; Karasov & Pinshow 2000). However, the specific physiological and ecological implications of a reduced digestive tract in godwits remains to be examined.

Pietersma et al. (1993, 1999b) hypothesized that migration-associated changes in the size of organs and muscle groups may result not only as a direct consequence of flight or fuelling, but may also occur independently of migratory events through neuronal or hormonal regulation, so that migrants may dissociate organ size from a current physiological optimum in order to anticipate future physiological requirements. Data collected on godwits and knots support this hypothesis. During preparations for departure, digestive tissue in both species is reduced in spite of continued feeding activities.
The glucocorticosteroid corticosterone may be involved in the regulation of this observed reduction in organ size. Corticosterone affects the distribution of body fat and body protein (Gray et al. 1990; Hadley 1996), and plasma concentrations of this hormone rise toward end of refuelling both in godwits (Landys-Ciannelli et al. 2002) and in knots (Piersma et al. 2000). On the other hand, resorption of digestive organs towards the end of refuelling may simply be a consequence of a dietary shift toward softer prey. For example, captive knots decrease stomach size when hard-shelled prey are not provided (Piersma et al. 1993). Nevertheless, a shift in dietary preferences in free-living shorebirds may be endogenously triggered.

In summary, godwits show changes in the lean body mass of various internal organs and muscle groups during successive stages of refuelling. In general, flight muscle hypertrophy increases in size as refuelling nears completion, while digestive organs grow only during the early part of refuelling. Additionally, although the size of the intestines initially increases they are similar in size between early refuelling godwits and godwits preparing to depart the stopover site, suggesting a reduction of excess tissue before migratory flight. These changes in organs and muscle groups mirror the changes previously noted in Red Knots and in Eared Grebes, suggesting that at least some migrants exhibit a common pattern of organ and muscle reorganization during preparations for long-distance flight.

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