Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint

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Summary

During long-distance flights, birds catabolize not only fat but also protein. Because there is no storage form of protein, protein catabolism entails a structural or functional loss. In this study, we investigated which organs were most reduced in lean mass during different phases of fat store loss and whether protein loss can be regarded as adaptive or as a constraint. Body and organ composition were analysed both during the autumn migration over continental Europe (sample from Switzerland) and after a long-distance flight over the Sahara and the Mediterranean Sea in spring (sample from Ventotene, Italy) in four species of passerine bird: pied flycatcher *Ficedula hypoleuca*, willow warbler *Phylloscopus trochilus*, garden warbler *Sylvia borin* and barn swallow *Hirundo rustica*. Large variations in protein mass occurred when long non-stop flights were performed. After a long-distance flight, birds showed a marked increase in net protein loss when fat stores were nearing depletion (analogous to the late phase of endurance fasting when the rate of protein catabolism is increased). When fat reserves were above approximately 5–10 %, protein was derived from all organs, but particularly from the breast muscles. When fat stores diminished further and protein catabolism increased, the mass of the digestive organs was reduced fastest. When the decrease in breast muscle mass during flight was regarded in terms of potential flight performance, it appeared that the use of breast muscle protein with decreasing body mass can be regarded as adaptive as long as fat stores did not reach a critical level. Below approximately 5–10 % body fat, however, protein loss reduced flight performance. This demonstrates that the phase of fasting (the size of the remaining fat stores) is an important condition for understanding the occurrence and effects of protein loss during endurance flights.

Key words: migration, protein catabolism, passerine, mass loss, flight.

Introduction

It is common knowledge that the migratory flights of birds are fuelled largely by previously accumulated fat stores. However, protein is also catabolized during flight (for reviews, see Jenni and Jenni-Eiermann, 1998; Bauchinger and Biebach, 1998), and some of it is stored before flight bouts (McLandress and Raveling, 1981; Marsh, 1984; Piersma and Jukema, 1990; Gauthier et al., 1992; Lindström and Piersma, 1993). Because there is no storage form of protein (apart from a small amino acid pool), a change in the amount of body protein entails a structural or functional change. Variation in organ mass (which affects mainly its protein mass) is considered to be an expression of phenotypic flexibility (Piersma, 1998). Such flexibility is regarded as an adaptation that allows animals to cope successfully with a wide range of conditions and life-cycle events (Piersma and Lindström, 1997).

In migrating birds, protein loss during long-distance flight affects predominantly the breast muscles, the digestive organs including the liver, and the skin (Åkesson et al., 1992; Biebach, 1998; Battley et al., 2000). Before long flight bouts, several species have been shown to enlarge their pectoral muscles (e.g. Fry et al., 1972; McLandress and Raveling, 1981; Marsh, 1984; Davidson and Evans, 1988; Driedzic et al., 1993; Lundgren et al., 1995; Dietz et al., 1999). In waders migrating non-stop over very long distances and in the eared grebe *Podiceps nigricollis*, changes in the few days before take-off are more profound: the heart increases in size, whereas the stomach, intestine, leg muscles and liver become much smaller (Piersma and Jukema, 1990; Jehl, 1997; Piersma and Gill, 1998; Piersma, 1998; Piersma et al., 1999).

With regard to the decrease in lean mass of passerine birds during migratory flights, several questions remain regarding (i) the pattern of variation in lean mass among the organs affected and (ii) its functional role and consequences.

With regard to the pattern of lean mass variation, it is important to realise that the rate of protein catabolism during

fasting increases dramatically when current fat stores drop below a critical level (phase III of fasting) (see Cherel et al., 1988; Jenni and Jenni-Eiermann, 1998; Cherel and Groscolas, 1999) and that a similar pattern is very likely to occur during endurance flight (Jenni et al., 2000). Previous studies on migrating birds, however, did not examine variation in the lean mass of organs with respect to the size of the fat stores, i.e. the phase of fasting; for studies of free-living birds both before and after a flight bout, see Gauthier et al. (1992), Biebach (1998) and Battley et al. (2000); for laboratory studies, see Hume and Biebach (1996) and Karasov and Pinshow (1998).

With regard to the functional roles and consequences of variation in lean organ mass, the following point needs clarification. If protein catabolism occurs predominantly in certain organs, it remains to be determined whether the reduction in organ size is an advantage (in terms of reducing the size of energetically costly organs that are not needed during flight) (Piersma and Lindström, 1997) or a means of minimising overall damage (in terms of obtaining protein from temporarily less important organs). Regarding the breast muscles, hypertrophy prior to migration may represent a compensation of the flight engine to cope with the larger body mass due to fat stores (see Pennycuick, 1975, 1978; Marsh, 1984; Lundgren et al., 1995; Piersma and Gill, 1998) and (at the same time) an adaptation to the expected protein loss during endurance flight or a protein reserve carried to the breeding grounds e.g. in arctic breeders (e.g. Davidson and Evans, 1988). Flight capacity in non-moulting birds is determined mainly by total body mass and breast muscle size (Pennycuick, 1975). Body mass decreases continuously during flight, so birds could minimise their total energy expenditure by reducing their breast muscle tissue. Experiments with red knots flying in a wind tunnel indeed showed a close relationship between body mass and pectoral muscle thickness (Lindström et al., 2000).

The aim of the present study was to investigate two aspects of protein loss during migratory flight. First, we investigated which organs were most prone to a reduction in protein mass during migratory flight using two approaches. To analyse whether different situations of migration affect protein catabolism of various organs differently, we compared the organ masses of birds that had just completed a long-distance flight over the Sahara and the Mediterranean Sea (sample of spring birds from Ventotene Island, Italy) with those of conspecifics which had flown only short distances (sample of autumn birds from Switzerland). To study the pattern of protein loss among organs in relation to current fat stores (phase of fasting) in long-distance migrants, we measured the lean mass of organs in a series of birds with different fat stores in the sample from Ventotene Island.

Second, we focused on protein loss from the breast muscles during long-distance flights. We evaluated whether the reduction in breast muscle mass is a metabolically induced constraint or an adaptation to the decrease in body mass during flight. Again, the phase of fasting was taken into account.

Materials and methods

Study sites and animals

Two groups of accidentally killed garden warblers *Sylvia borin* (Boddaert), pied flycatchers *Ficedula hypoleuca* (Pallas), willow warblers *Phylloscopus trochilus* (L.) and barn swallows *Hirundo rustica* (L.) were examined (for sample sizes, see Table 1).

The first group, collected by the Istituto Nazionale per la Fauna Selvatica, Bologna, Italy, consisted of birds that arrived at Ventotene (40°48′N, 13°25′E), an island off the Tyrrhenian coast of Italy, during the spring migration 1997. Nearly all were killed by woodchat shrikes (*Lanius senator*) when caught in mist nets at the local ringing station. All four species winter in sub-Saharan Africa and need to cross the Sahara desert and the Mediterranean Sea, a distance of approximately 2000 km. Arrival directions and patterns at Ventotene indicate that these birds had completed a 14–16 h non-stop flight and crossed the Mediterranean Sea, covering a distance of at least 500 km (Pilastro et al., 1995). The very low number of recaptures at Ventotene indicates that migrants generally stay on the island for less than a day and had landed at most a few hours before capture (F. Spina, personal observation). Therefore, this first group is regarded as having incurred a large energy loss during a previous non-stop flight.

Birds reach Ventotene Island with very different fat stores. Among species, fat stores of arriving birds are associated with the distance from the northernmost adequate sub-Saharan stopover habitat (Pilastro and Spina, 1997). Within species, birds arriving later during the day (particularly against headwinds) generally had lower fat stores than individuals arriving early (Pilastro et al., 1995). This indicates that the size of the remaining fat stores at Ventotene is most closely related to the energy expended during the migration from sub-Saharan stopover sites and for the non-stop flight across the Mediterranean Sea and is not due to variability in energy stores at the onset of flight and possibly acquired during refuelling in North Africa. Therefore, we used the variation in remaining fat stores at Ventotene to investigate the reduction in protein mass during various stages of energy loss.

The second group, collected by the Swiss Ornithological Institute, consisted of birds found dead during the autumn migration season in Switzerland in 1996 and 1997. Most of them were killed by owls at the Alpine ringing site Col de Bretolet (46°09′N, 6°47′E) when caught in mist nets during night migration. The others originated from different sources and were killed by cats or collided with cars or windows. There were no significant differences in any measurements between birds from the ringing site and those from other localities. Garden warblers, pied flycatchers and willow warblers are typical night migrants over continental Europe (Winkler, 1999) and are known to migrate through Switzerland in short hops, with non-stop flights not exceeding one night. In fact, most probably migrate non-stop for a few hours only, as demonstrated by greatly decreasing numbers of night migrants observed aloft by radar after midnight (Bruderer and Liechti,

1998). Half the birds at Col de Bretolet were caught around midnight. The barn swallow migrates predominantly during the day and is capable of feeding during migration. Therefore, this second group consisted of birds on migration that had experienced a flight of only a few hours and that were generally able to reconstitute energy losses after each short flight bout. This group was regarded as having incurred only small energy losses during flight.

Composition of organs

Dead birds were weighed (fresh mass) and then stored frozen until analysis. After measuring the length of primary 8 as a measure of size (Jenni and Winkler, 1989), they were dissected into the following parts: breast muscles (m. pectoralis and m. supracoracoideus), leg muscles of the tarso-metatarsus, heart, liver, gizzard, intestine (without colon), kidneys and the rest of body including feathers. Paired organs were pooled. Fat was extracted for 24 h from the dried parts in a Soxhlet apparatus with petroleum ether (boiling temperature 40 °C to 60 °C) as a solvent. After extraction of fat, the organs were dried again until the mass was stable, and lean dry mass was measured to the nearest milligram. Variation in lean dry mass was assumed to reflect variation in protein mass. Fat mass could not be calculated for one garden warbler and one willow warbler from Ventotene (missing data), which explains why the sample size in these species was reduced by one for some analyses.

Data analysis

Differences in organ mass composition (lean dry organ mass expressed as a proportion of the total lean dry mass of the bird) between the two groups were tested for significance with compositional analysis (Aebischer et al., 1993), which accounts for the fact that the proportions are constrained to sum to 100 %.

Organs were assigned to two groups, 'exercise' and 'nutritional' organs which, in waders, generally show statistical independence (Piersma et al., 1996). This presumes isometry between the different organ groups. This assumption may not be fulfilled in the gizzard, since the mass of this organ is very dependent on diet (Piersma et al., 1993; Starck, 1999; Dekinga et al., 2001). We therefore excluded the gizzard and defined 'nutritional' organs as intestine+liver and 'exercise' organs as breast muscles+heart. Leg muscles were not included in the 'exercise' organs since they are not used during flight.

To test whether breast muscle mass is adapted to the bird's current body mass, we used a measure of the power margin, i.e. the maximum potential vertical flight speed that a bird of a given body mass is able to add to flying horizontally with minimum power speed. This potential vertical flight speed V_z can be regarded as a safety margin with respect to manoeuvrability and predator escape and is computed as:

$$V_z = (P_m - P_{ae})/m\mathbf{g},$$

where P_m is the maximum power output of the muscles, P_{ae} is the minimum aerodynamic power required for horizontal flapping flight, m is body mass and $\mathbf{g}$ is the acceleration due to gravity. P_m and P_{ae} were calculated with the program of Pennycuick (1989) using the individual breast muscle and total body masses and species-specific measures for wing span and wing area. For all other variables, default values as set by the program were used. V_z is overestimated by a factor that relates to the fraction of non-contractile protein within total muscle mass (Pennycuick, 1975). However, this does not change the shape of the relationships provided that the fraction of non-contractile protein remains constant during flight.

Results

Differences in organ mass between the samples from Switzerland and Ventotene

In all species, birds sampled on Ventotene had a significantly lower body mass after their long non-stop flight than birds sampled in Switzerland that had migrated only in short hops (Table 1). The amount of fat remaining in birds on Ventotene was significantly lower than in birds from Switzerland for pied flycatchers and barn swallows. In the garden warbler, this difference was not significant because of the large variation within the Swiss sample. The willow warbler arrived on Ventotene with fat stores similar to or larger than those during the autumn migration in Switzerland. Lean dry mass was significantly lower in birds on Ventotene than in those from Switzerland, except for the garden warbler. A significant difference in the length of primary 8 was found only in the garden warbler (Table 1).

The lower lean dry mass of birds from Ventotene compared with those from Switzerland was reflected in all organs of all species, but was especially large for liver and intestine (47–68 % of the Swiss value; Fig. 1). When examining proportional organ composition (in terms of lean dry mass) by compositional analysis, the liver and intestine of the Ventotene birds took up a significantly smaller proportion than in the Swiss sample in the pied flycatcher, willow warbler and barn swallow. In the willow warbler, the gizzard was also significantly smaller in birds from Ventotene than in birds from Switzerland.

Relationship between lean dry mass and fat stores

In birds that had accomplished a long-distance flight (the Ventotene sample), there was a significant positive relationship between remaining fat stores and total lean dry mass except in the garden warbler, for which the sample size was small (Fig. 2). The relationship between lean dry body mass and relative fat content conformed better to a logarithmic function than to a linear function (difference in Akaike's Information Criterion between the two models 7.73 for pied flycatcher, 2.03 for willow warbler and 2.95 for barn swallow; a difference of 2 is usually regarded as significant) (Burnham and Anderson, 1998). This indicates that lean dry mass decreased progressively when fat stores were nearing depletion. In the willow warbler, the length of primary 8, as a measure of size, also explained some of the variation in lean dry body mass (P=0.04).

Table 1. *Body measurements of birds from Switzerland and Ventotene, including* t-*tests for differences between the two groups*

	Switzerland		Ventotene		t-test	
	Mean	N	Mean	N	F	Significance
Pied flycatcher						
Body mass fresh (g)	10.98±0.78	21	9.53±1.12	10	17.82	***
Total fat (g)	1.48±0.68	21	0.57±0.67	10	12.31	***
Total lean dry mass (g)	3.60±0.20	21	3.17±0.32	10	20.94	***
Primary 8 length (mm)	61.07±1.74	21	61.35±1.80	10	0.17	NS
Willow warbler						
Body mass fresh (g)	7.80±0.71	9	6.66±0.38	18	30.29	***
Total fat (g)	1.17±1.19	9	1.28±0.82	18	0.08	NS
Total lean dry mass (g)	2.54±0.28	9	2.26±0.14	18	12.29	***
Primary 8 length (mm)	50.44±2.59	9	50.25±2.13	18	0.04	NS
Garden warbler						
Body mass fresh (g)	15.97±1.57	4	14.01±1.10	7	6.01	*
Total fat (g)	2.18±2.29	4	0.78±0.43	7	2.70	NS
Total lean dry mass (g)	4.74±0.44	4	4.38±0.26	7	2.95	NS
Primary 8 length (mm)	58.75±1.32	4	62.43±1.59	7	15.15	***
Barn swallow						
Body mass fresh (g)	16.97±1.09	4	13.41±1.16	9	26.97	***
Total fat (g)	3.66±1.04	4	0.46±0.75	9	40.19	***
Total lean dry mass (g)	5.23±0.37	4	4.53±0.37	9	10.13	***
Primary 8 length (mm)	93.25±3.93	4	96.11±3.31	9	1.86	NS

***Significant at $P<0.01$; *significant at $P<0.05$; NS, not significant.
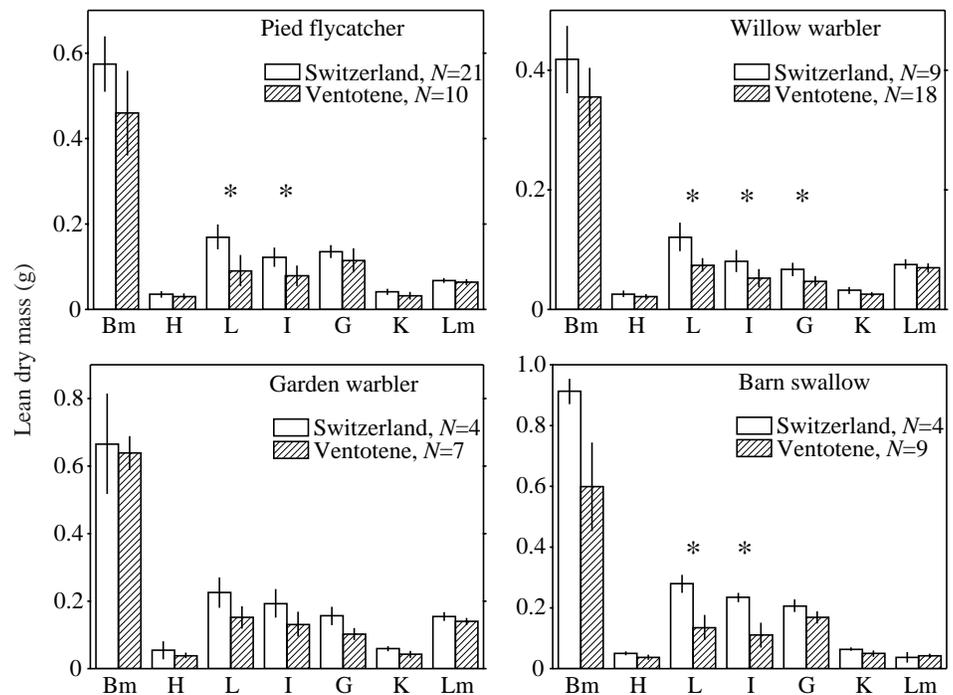Values are means ± S.D.

Fig. 1. Mean lean dry mass (± S.D.) of different organs in birds from Ventotene, after a long-distance flight, and from Switzerland, after only short migratory movements. Bm, breast muscles; H, heart; L, liver; I, intestine; G, gizzard; K, kidney; Lm, leg muscles. Organs that take up a significantly ($P<0.05$) smaller proportion of total lean dry mass in Ventotene birds than in Swiss birds are marked with an asterisk (compositional analysis).

Changes in the proportions of organs

Which parts of the body contribute to the increasing reduction in lean dry mass as the fat stores are being exhausted? We investigated whether the nutritional organs (intestine and liver) vary more in size than the exercise organs (breast muscles and heart) and whether the ratio of active skeletal muscle mass (breast muscles) to passive skeletal muscle mass (leg) changes with total lean dry

Fig. 2. Relationship between total lean dry mass and fat content in birds after a long-distance flight (Ventotene sample). Fat content was calculated as a percentage of total dry body mass. Regressions were significant in all species except the garden warbler ($N=6$). In the willow warbler, the length of primary 8 also explained some variability in lean dry body mass. Regression lines and sample sizes are as follows: pied flycatcher, $y=2.476+0.326\ln x_1$ ($r^2_{adj}=0.86$, $P<0.001$, $N=9$); willow warbler, $y=0.641+0.082\ln x_1+0.027x_2$ ($r^2_{adj}=0.52$, $P=0.002$, $N=17$); barn swallow, $y=4.030+0.320\ln x_1$ ($r^2_{adj}=0.56$, $P=0.013$, $N=9$), where y is lean dry body mass (g), x_1 is the amount of fat as a percentage of total dry mass and x_2 is the length of primary 8 (mm).
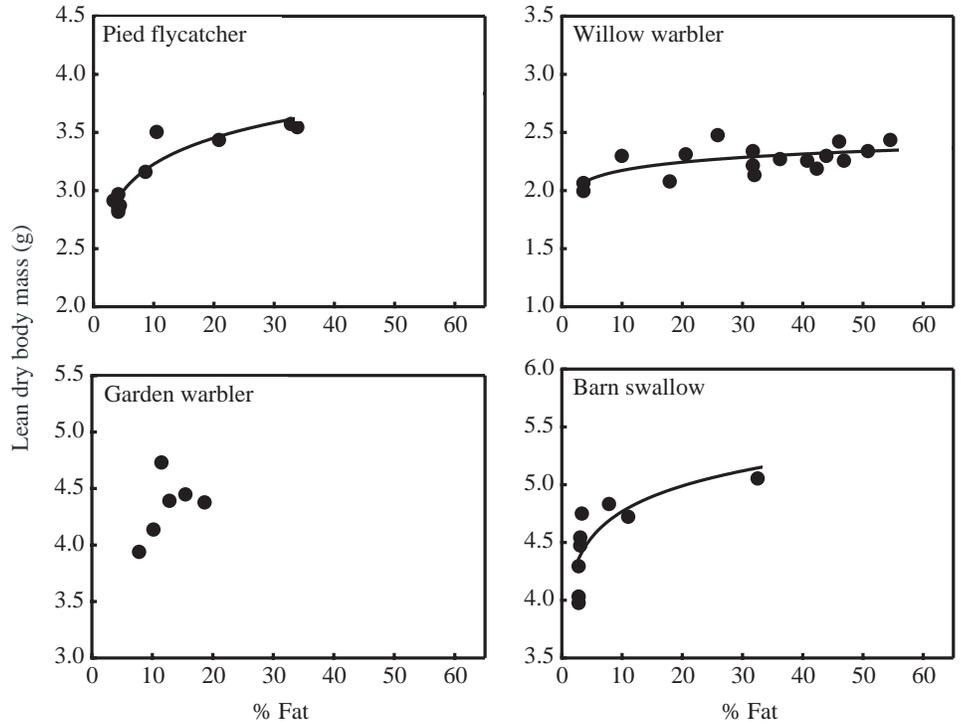

Fig. 3. Relationship between the ratios of the masses of different organs and total lean dry mass in pied flycatchers ($N=10$) and willow warblers ($N=18$) after a long-distance flight. Nutritional organs/exercise organs=lean dry mass of intestine and liver divided by lean dry mass of breast muscles and heart. In the pied flycatchers, the length of primary 8 also explained some of the variability in the ratio breast muscles/leg muscles. Regression lines are as follows: (A) $y_1=-0.059+0.126x_1$ ($r^2_{adj}=0.335$, $P=0.046$); (B) $y_2=8.400+2.899x_1-0.170x_2$ ($r^2_{adj}=0.898$, $P=0.001$); (C) $y_1=1.130-0.349x_1$ ($r^2_{adj}=0.382$, $P=0.003$); (D) $y_2=1.333+1.671x_1$ ($r^2_{adj}=0.301$, $P=0.011$), where y_1 is nutritional organs/exercise organs, y_2 is breast muscles/leg muscles, x_1 is the total lean dry mass (g) and x_2 is the length of primary 8 (mm).
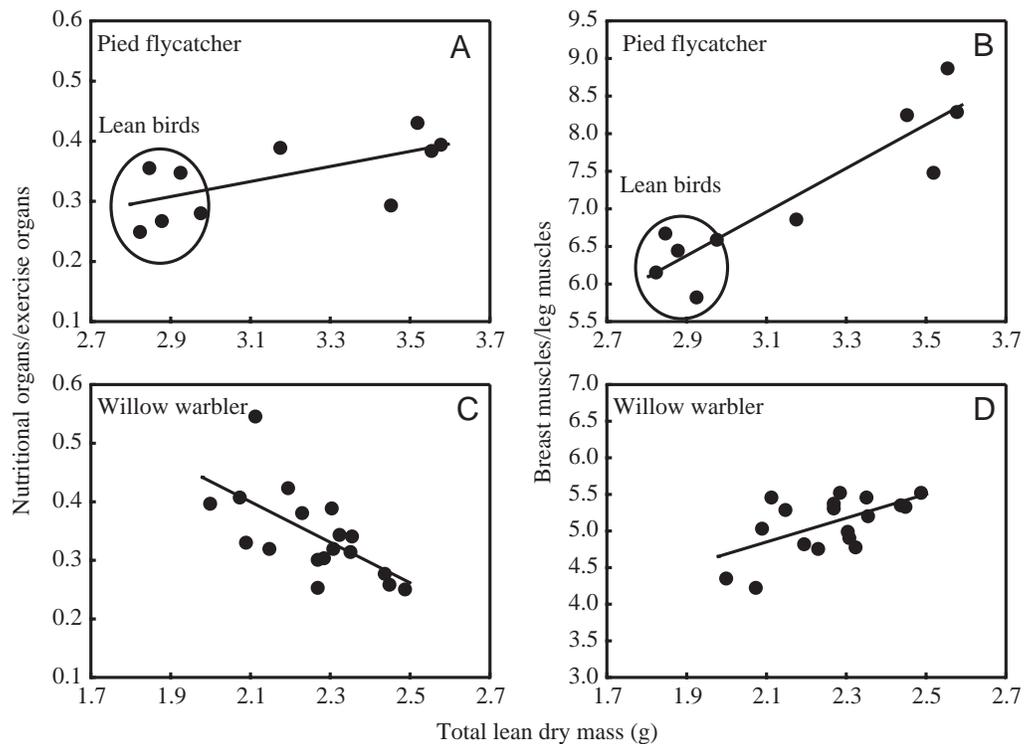
mass. This was performed for birds from Ventotene and was restricted to pied flycatchers and willow warblers, the only species with an appreciable variation in fat mass (see Fig. 2).

In the willow warbler, in which most individuals arrived at Ventotene with substantial fat stores (see Fig. 2), the exercise organs were relatively more reduced than the nutritional organs with decreasing total lean dry mass (Fig. 3C). Pied flycatchers, however, showed a relatively larger reduction in nutritional organs than exercise organs with decreasing total lean dry mass (Fig. 3A). This is due to the low ratios of the very lean individuals. Both species showed a larger reduction in the mass

Fig. 4. Breast muscle mass (lean dry mass) *versus* total body mass (fresh mass) in birds after a long-distance flight. Pied flycatcher, $y=-0.102+0.056x$ ($r^2_{adj}=0.880$, $P<0.001$, $N=10$); willow warbler, $y=0.058+0.038x$ ($r^2_{adj}=0.630$, $P<0.001$, $N=17$); garden warbler, $y=0.108+0.037x$ ($r^2_{adj}=0.724$, $P=0.020$, $N=6$); barn swallow, $y=-0.538+0.084x$ ($r^2_{adj}=0.806$, $P<0.001$, $N=9$), where x is total body mass (g) and y is lean dry breast muscle mass (g). The length of primary 8 had no significant effect on the relationships between breast muscle mass and total body mass.
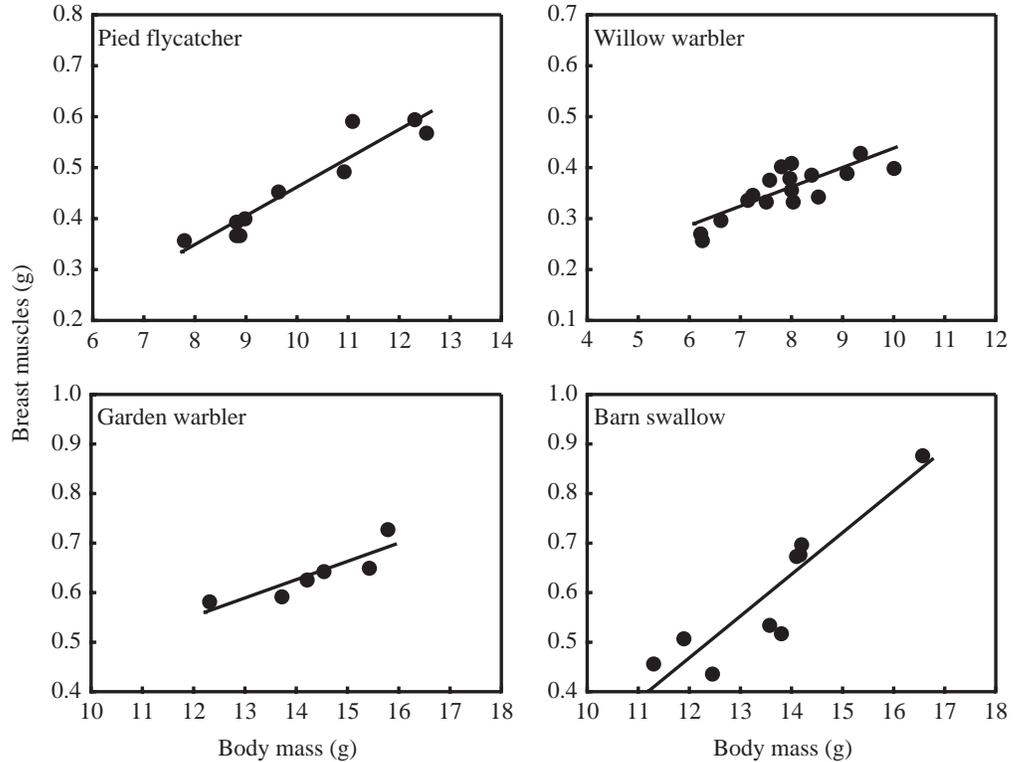
of active breast muscles with decreasing total lean dry mass than in the mass of passive leg muscles (Fig. 3B,D).

Breast muscle mass and flight performance

If birds fattening for migration keep their flight capacity at a constant level, we would expect lean dry breast muscle mass to increase with increasing total body mass. During migratory flight, breast muscle mass could shrink in parallel with decreasing fuel load (to spare costly muscle tissue for unnecessary extra flight capacity) (Pennycuick, 1975) and/or as a result of protein breakdown necessary for metabolic reasons (Jenni and Jenni-Eiermann, 1998). Fig. 4 shows that breast muscle mass was positively related to total body mass in birds after a long-distance flight. This was due mainly to lean birds having small breast muscles, but it was also apparent in willow and garden warblers, which had substantial fat stores.

To investigate whether breast muscle mass is adjusted to keep flight capacity at a constant level, we assumed that potential vertical flight speed V_z should be kept constant. The fat content (percentage of total dry mass) was taken as a measure of the remaining fuel load. Potential vertical flight speed showed a curvilinear relationship with fat stores for pied flycatchers and willow warblers (Fig. 5, Table 2). With decreasing fat stores, potential maximum vertical flight speed first increases, but then decreases as the fat stores fall below approximately 20 % (Fig. 5).

Discussion
Protein mass in samples from Ventotene and Switzerland

The comparison of the samples from Ventotene and Switzerland was not planned to study changes in body composition before and after a flight (e.g. Biebach, 1998;
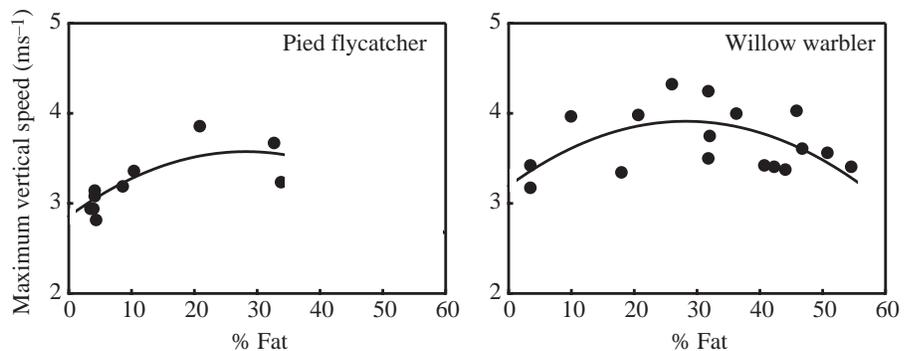

Fig. 5. Relationship between maximum potential vertical flight speed and relative fat load in pied flycatchers ($N=10$) and willow warblers ($N=17$) after a long-distance flight. For further explanation and statistics, see Materials and methods and Table 2.

Table 2. *Results of analysis of covariance (type III) with maximum potential vertical flight speed as a dependent variable*

Variable	Estimate	S.E.M.	P
Intercept	3.193	0.162	<0.001
Species	–0.337	0.130	0.016
% Fat	0.051	0.013	<0.001
(% Fat)^2	–0.001	0.0002	<0.001

The independent variables were species, % fat, (% fat)^2, species × % fat and species × (% fat)^2.

The interaction terms were not significant (*P*>0.17) and were therefore excluded from the model.

Codes for species were as follows: 0, willow warbler; 1, pied flycatcher. r^2_{adj}=0.573; *N*=27.

Bauchinger and Biebach, 1998; Battley et al., 2000), but showed that protein mass varied considerably among conspecifics in different situations of migration. The example of the willow warbler demonstrated that birds after a long non-stop flight had a lower protein mass than birds after a short flight, despite similar fat stores. Similarly, robins *Erithacus rubecula*, having crossed the Baltic Sea in autumn, had a lower lean dry mass than, but similar fat stores to, individuals migrating over land in short hops (Åkesson et al., 1992). In both studies, birds that had performed a long non-stop flight continue to migrate after a short stopover of a few hours without (much) refuelling and are thus not at the end of a migration leap.

At the level of the different organs, the nutritional organs (intestine, gizzard and liver) of the Ventotene birds were more reduced compared with the Swiss birds than the exercise organs (breast muscles, heart and leg muscles) (Fig. 1). A similar pattern was also found in red knot *Calidris canutus*, great knot *C. tenuirostris* and garden warbler before and after a long flight (Piersma et al., 1996; Biebach, 1998; Battley et al., 2000). In waders, gut size can be reduced before the onset of very long non-stop migratory flights (Piersma and Gill, 1998; Piersma et al., 1999). Whether this also happens in passerines and contributes to the difference in nutritional organ mass found between birds from Ventotene and Switzerland remains an open question.

Protein loss during flight

As outlined in the Materials and methods section, variation in remaining fat stores among birds on Ventotene was used to investigate protein loss during the course of energy loss under the assumption that differences among individuals with various fat stores reflect changes within the individual. Inferring the variation of an individual from the variation of a sample of birds can cause methodological problems (e.g. Lindström and Piersma, 1993) if the aim is to obtain precise values of body or organ composition or when inter-individual variation in structural size is high relative to the variation in body composition. However, in all analyses discussed below, we did not attempt to give precise values for the composition of body or organs, and variation in body composition relative to the variation of structural size was high. Structural size was taken into account by including the length of primary 8 as a covariate.

When fat stores dropped below approximately 5–10 %, lean dry mass loss increased rapidly (Fig. 2). This two-phased pattern of protein loss with decreasing fat stores is analogous to phases II and III of fasting in resting birds. In phase II, protein is spared and lipid utilisation is high, while protein utilisation is progressively and greatly increased with the onset of phase III (Cherel et al., 1988). Hence, despite greatly increased energy expenditure, the pattern of fuel use during endurance flight is similar to that of resting fasting birds (see Jenni et al., 2000) and is characterised by low rates of protein loss when fat stores are high (phase II) and dramatically increasing rates of protein loss when fat stores drop below the critical level of approximately 5–10 % of total dry mass (phase III).

During phases II and III of fasting during flight, protein loss seems to affect different organs differently. In the pied flycatcher (half the birds were in phase III of fasting), the nutritional organs were relatively more reduced with decreasing total lean mass than the exercise organs (Fig. 3A). In the willow warbler (most birds were in phase II of fasting), the opposite was true (Fig. 3C). Hence, during phase II, the exercise organs were, in relative terms, more readily catabolized than the nutritional organs, whereas during phase III, when overall protein catabolism markedly increased, the nutritional organs were relatively more reduced than the exercise organs. This suggests that, during phase II, the majority of the protein needed could be derived from the exercise organs, which could apparently be reduced in parallel with the decreasing body mass. The nutritional organs, although also reduced, were saved in relative terms. During this phase, protein utilisation is at a low level, and protein is probably used mainly to supply the citric acid cycle with intermediates and to supply gluconeogenic precursors (Jenni and Jenni-Eiermann, 1998). During phase III, however, protein contributes much more and increasingly to energy expenditure. During this phase, protein utilisation from the exercise organs was increased, but it was increased even more from the nutritional organs (Fig. 3).

The active breast muscles were relatively more reduced with decreasing total lean dry mass than were the passive leg muscles, irrespective of the phase of fasting (Fig. 3B,D). This result coincides with the findings of Battley et al. (2000) that, in great knots, breast muscles were more reduced than leg muscles. The many possible explanations for this phenomenon cannot be narrowed down at this stage.

Breast muscle mass and flight performance

Several authors have proposed that breast muscle mass is finely tuned to total body mass to keep the body aloft (Marsh, 1984; Lundgren et al., 1995; Piersma and Gill, 1998; Lindström et al., 2000). If this is true, breast muscle mass

should be a function of total body mass. This was indeed the case in the birds from Ventotene that had completed a long-distance flight (Fig. 4). In robins, breast muscle mass depended on fat stores only in individuals that had crossed the Baltic Sea, but not in those that were migrating in smaller hops over land (Åkesson et al., 1992). This indicates that breast muscle mass may not be perfectly adjusted to total body mass in all situations, but is correlated with body mass at least during long-distance flights (see also Lindström et al., 2000).

The calculation of the theoretical maximum potential vertical flight speed that can be added when flying horizontally at minimum power speed showed that muscle size was not perfectly adapted to body mass in such a way that the bird's manoeuvrability would always stay the same. For fat stores greater than approximately 20 % of total dry mass, birds with large fat stores were less agile than lean birds (Fig. 5), which agrees with experimental evidence (Witter et al., 1994; Kullberg et al., 1996). During long-distance flights, birds lose mainly fat, become lighter and therefore more manoeuvrable. When fat stores become low, however, increased breast muscle losses apparently offset the advantages of body mass reduction by fat loss, and the birds lose manoeuvring capability (Fig. 5).

During phase II of fasting, maximum potential vertical flight speed increased with decreasing energy stores, but the opposite was true during phase III of fasting (Fig. 5). It is therefore questionable whether the catabolism of breast muscles can be interpreted as a means of saving energy. If this were the case, we would expect maximum vertical flight speed to remain constant. However, these calculations assumed that the fraction of non-contractile muscle protein remained constant, as found in eared grebes (Gaunt et al., 1990); this remains to be investigated in migrating passerines.

Concluding remarks

During long non-stop flights, birds undergo large changes in protein mass, particularly when their fat stores become low. For birds during long flights (as assumed when comparing birds at Ventotene with different fat stores), we found that total protein loss and protein loss from the nutritional and exercise organs were dependent on the phase of fasting. Therefore, measurement of the phase of fasting will be a prerequisite for evaluating and comparing the pattern, amount and sources of protein loss in future studies.

From our findings, it seems that the minimal protein losses during phase II of fasting imposed by metabolic constraints (see Jenni and Jenni-Eiermann, 1998) can be accommodated in an adaptive reduction of mainly the breast muscles. This reduction does not impair flight capability and may even improve it slightly because of the reduction in total mass. The nutritional organs are also reduced, but seem to be spared in relative terms, which would allow a more rapid reconstitution and refuelling after landing.

During phase III of fasting, the greatly increased protein catabolism reduces the protein mass of all organs, particularly the nutritional organs, and flight capability decreases. The accelerated protein utilisation from breast muscles and the gut is probably not adaptive with respect to flight capability (e.g. Pennycuick, 1975) and saving weight and energy (see Hume and Biebach, 1996; Piersma and Lindström, 1997), but is an adaptation with respect to whole-body metabolism when fat stores are nearing depletion (for a review, see Cherel et al., 1988).

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