Differential catabolism of muscle protein in Garden Warblers (*Sylvia borin*): flight and leg muscle act as a protein source during long-distance migration

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**Abstract** Samples of flight and leg muscle tissue were taken from migratory garden warblers at three different stages of migration: (1) pre-flight: when birds face an extended flight phase within the next few days, (2) post-flight: when they have just completed an extended flight phase, and (3) recovery: when they are at the end of a stop-over period following an extended flight phase. The changes in body mass are closely related to the changes in flight (*P* < 0.001) and leg muscle mass (*P* < 0.001), suggesting that the skeletal muscles are involved in the protein metabolism associated with migratory flight. From pre- to post-flight, the flight and the leg muscle masses decrease by about 22%, but are restored to about 12% above the pre-flight masses during the recovery period. Biochemical analyses show that following flight a selective reduction occurred in the myofibrillar (contractile) component of the flight muscle (*P* < 0.01). As this selective reduction accounts only for a minor part of the muscle mass changes, sarcoplasmic (non-contractile) and myofibrillar proteins of both the flight and leg muscle act as a protein source during long-distance migration. As a loss of leg muscle mass is additionally observed besides the loss in flight muscle mass, mass change seems not to be strictly associated with the mechanical power output requirements during flight. Whereas the specific content of sarcoplasmic proteins in the flight muscle is nearly twice as high as that in the leg muscle (*P* < 0.001), the specific content of myofibrillar proteins differs only slightly (*P* < 0.05), being comparably low in both muscles. The ratio of non-contractile to contractile proteins in the flight muscle is one of the highest observed in muscles of a vertebrate.

**Keywords** Passerine bird · Muscle composition · Sarcoplasmic and myofibrillar protein · Selective protein degradation

**Abbreviations** *HSPB* high salt phosphate buffer · *LSPB* low salt phosphate buffer · *s/m ratio* ratio of the specific content of sarcoplasmic to myofibrillar proteins

**Introduction**

Long-distance migration often includes extensive flight phases, necessary to cover great distances. Flight is the most expensive avian activity per unit of time (Blem 2000) whereby the energetic costs generally have to be covered by endogenous resources. This is reflected by the pronounced variations in body mass during the migratory period (for *Sylvia borin* see summary in Bairlein 1991). In experiments on some long-distance passerine migrants, partially simulating a migratory flight simply by starvation and the following stopover by refeeding, fat accounts for about three-quarters and protein for about one-quarter of the total changes in body mass (Klaassen and Biebach 1994; Klaassen et al. 1997). Similarly, in waders and geese the changes in body mass during migration also involve a change in lean body mass (for review see Lindström and Piersma 1993; Butler et al. 1998; Piersma 1998; Battley et al. 2000). The metabolism of proteins, along with that of fat, during migration in passerine birds has been extensively investigated (for review see Bauchinger and Biebach 1998). Unlike the energy sources fat and glycogen, protein is not located in a special store (Jenni and Jenni-Eiermann 1998) and is drawn from functional organs such as muscles, liver, heart, and digestive tract. The phenotypic flexibility in size of several organs observed during migration (Biebach 1998; Piersma 1998; Battley et al. 2000) clearly documents that the changes in protein content relate to migration, at least during the following three phases: the premigratory period with its general physiological preparation for migration, the migratory
flight phases and the recovery phases. Overall protein metabolism stems to a large extent from the skeletal muscles, mostly from the pectoral muscles, which contribute about 10–25% to the total body mass (Hartman 1961). The pectoral muscle becomes highly variable during many physiological functions (see for review Butler and Bishop 2000), such as egg formation (Jones and Ward 1976; Jones 1991; Houston et al. 1995), preparation for migration (Fry et al. 1972; Marsh 1981, 1984; McLandress and Raveling 1981; Evans 1992; Battley and Piersma 1997; Butler et al. 1998), migration itself (Gaunt et al. 1990; Åkesson et al. 1992; Driedzic et al. 1993; Lindström and Piersma 1993; Jehl 1997; Biebach 1998; Piersma 1998; Battley et al. 2000), moult (Ward 1969; Baggot 1975; Jehl 1997) and starvation (Kendall et al. 1973; Swain 1992).

The skeletal muscles consist of two major functional components. One component comprises the contractile, myofibrillar proteins (e.g. actin and myosin filaments and troponin complexes), which transform biochemical energy into mechanical energy. The other component comprises the non-contractile, sarcoplasmic proteins (glycylcic and mitochondrial enzymes, myoglobin), which generate biochemical energy to power the contraction of the myofibrillar proteins (Stryer 1994). In the present study we focused on how these two components of the skeletal muscle contribute to the protein metabolism during long-distance flights and the preceding recovery phase. We anticipated that in birds, the skeletal muscles may be placed under adaptive pressure during long-distance migration in order to meet the demand of sustained flight locomotion, necessary to cross ecological barriers. We quantified the mass and structural composition of the skeletal muscles of long-distance migratory garden warblers (Sylvia borin), asking the following questions: (I) Does the composition of skeletal muscles change in the course of a long-distance flight? (II) Is a specific muscle the preferred source of protein during long-distance migration? (III) Is one of the two major protein components of a muscle preferentially metabolised? To answer these questions, we took tissue samples during the following phases:

1. The pre-flight phase preceding the onset of an extended migratory flight.
2. The post-flight phase, i.e. the time immediately following the completion of an extended migratory flight.
3. The recovery phase, i.e. at the end of the time during which the birds refuel following an extended migratory flight.

### Materials and methods

The study was carried out on long-distance migratory garden warblers (S. borin), which cross the Sahara desert and the Mediterranean sea when migrating. During this crossing, birds have no opportunity to feed and drink. However, their flight strategy varies, some birds fly non-stop while others fly at night and rest during the day (Biebach 1990; Biebach et al. 2001). Hence, depending on their strategy, they require 2–4 days, including extended flight phases, to cover the approximately 2,500-km-wide inhospitable ecological barrier (Moreau 1961).

Garden warblers were caught in mist nets during autumn migration in southern Turkey (36°40’N; 33°5’E), close to the Mediterranean coast during their pre-flight phase, when they were facing the challenge of crossing the Mediterranean sea and the Sahara desert. These pre-flight birds were caught between August 30 and September 5 1996, within the 1st h 2 after dawn, whilst feeding on figs. The post-flight birds were caught during spring migration at an inhospitable area in northern Sinai (Egyptian Mediterranean coast, 33°08’N; 33°25’E), between May 5 and May 16 1996, within the 1st 4 h after dawn. The post-flight status of these birds was confirmed by the observation that none of the total of 330 garden warblers caught during the spring of 1996 and 1998 were observed whilst feeding or retracted. Therefore, these birds are considered to have interrupted their migratory flight for 1 day, before continuing migration in the evening. These birds are also considered to have crossed the Sahara desert, a distance of about 1800 km, in the preceding nights. Ten birds of the post-flight group were kept individually in cages (75 × 36 × 33 cm) with food and water ad libitum (mealworms, Tenebrio molitor). They were kept for 7 days under the natural light and temperature conditions of northern Sinai. Towards the end of this simulated stopover the increase in body mass stopped and the birds reached a stable body mass. These warblers represent birds at the end of a simulated stop-over period which followed immediately after the flight phase (recovery birds).

All birds were weighed within 30 min after capture to the nearest 0.1 g (Sartorius 1002 MP). Wing length, the length of the eighth primary and tarsus length were measured. Twelve ‘pre-flight’ and ten ‘post-flight’ birds were killed by decapitation on the morning of capture, and ten ‘recovery’ birds were similarly killed in the morning of the 7th day of refeeding. Within 8 min, the carcass was frozen in liquid nitrogen. After 3 weeks it was transferred to a −70°C freezer and kept there until analysis.

### Protein fraction

Sarcoplasmic and myofibrillar protein contents were determined in the muscle tissues obtained from the left flight (Musculus pectoralis and supracoracoideus) and leg muscles (Musculus fibularis longus, Musculus gastrocnemius, Musculus tibialis, Musculus extensor) using a method originally developed by Helderland (1957) and later modified by Swain (1992). The whole muscles of the left lower leg (see above) weighing a minimum of 250 mg and the rostral half of the left flight muscle weighing a minimum of 500 mg were removed within 5 min after the carcass was removed from the freezer and immediately minced with scissors and then homogenised while being cooled in an ice bath (Janke and Kunkel, Ultra-turax TP 18/10) in 1:10 W/V LSPB (low-salt-phosphate-buffer: 0.03 potassium phosphate, pH 7.4). After 3 h incubation under gentle shaking at 4 °C, the homogenates were centrifuged for 20 min at 1,400 g (Beckman centrifuge J-6) and the supernatant decanted and stored as the first LSPB-fraction at 4 °C. The pellet was suspended in another ten volumes LSPB, vortexed, incubated and centrifuged as described above. The supernatant was decanted and stored as the second LSPB-fraction. The pellet was resuspended, but this time in ten volumes of HSPB (high-salt-phosphate-buffer: 0.1 potassium phosphate, pH 7.4 and 1.1 M potassium iodide) for extracting the myofibrillar proteins. After incubation for 15 h, the suspension was centrifuged and the supernatant was collected as the first HSPB-fraction. The second HSPB-fraction was collected in the same way as the second LSPB-fraction. Thus, the two LSPB-fractions contained the sarcoplasmic proteins and the two HSPB-fractions contained the myofibrillar proteins. The protein content of each sample was determined by a modification of the Lowry (Lowry et al. 1951) procedure, using Sigma-kid no. P-5565 (Peterson 1983). The pellet after the second HSPB fraction, containing the connective tissue, tendons and salt