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A SPORT-PHYSIOLOGICAL PERSPECTIVE ON BIRD MIGRATION: EVIDENCE FOR FLIGHT-INDUCED MUSCLE DAMAGE

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Summary

Exercise-induced muscle damage is a well-described consequence of strenuous exercise, but its potential importance in the evolution of animal activity patterns is unknown. We used plasma creatine kinase (CK) activity as an indicator of muscle damage to investigate whether the high intensity, long-duration flights of two migratory shorebird species cause muscle damage that must be repaired during stopover. In two years of study, plasma CK activity was significantly higher in migrating western sandpipers (a non-synchronous, short-hop migrant), than in non-migrants. Similarly, in the bar-tailed godwit (a synchronous, long-jump migrant), plasma CK activity was highest immediately after arrival from a 4000–5000 km flight from West Africa to The Netherlands, and declined before departure for the arctic breeding areas. Late-arriving godwits had higher plasma CK activity than birds that had been at the stopover site longer. Juvenile western sandpipers making their first southward migration had higher plasma CK activity than adults. These results indicate that muscle damage occurs during migration, and that it is exacerbated in young, relatively untrained birds. However, the magnitude of the increases in plasma CK activity associated with migratory flight were relatively small, suggesting that the level of muscle damage is moderate. Migrants may avoid damage behaviourally, or have efficient biochemical and physiological defences against muscle injury.

Key words: bird, capture stress, creatine kinase, exercise, flight, migration, muscle damage, settling time, *Calidris mauri*, *Limosa lapponica.*

Introduction

Avian migratory flight is exceptional among examples of vertebrate endurance exercise because it combines very high rates of aerobic energy metabolism with prolonged periods of activity and fasting. Flying birds consume oxygen at more than twice the aerobic limit (V_{O_2max}) of similarly sized running mammals, and migratory flights can last 50 or even 100 h (Alerstam, 1990; Butler and Woakes, 1990; Butler, 1991; Berthold, 1996). How birds budget the major resources needed for flight (particularly fat, protein and water) to migrate successfully has been the subject of much empirical and theoretical study (Piersma, 1987; Alerstam and Lindström, 1990; Piersma and Jukema, 1990; Carmi et al., 1992; Klaassen, 1996; Klaassen and Lindström, 1996; Alerstam and Hedenström, 1998; Kvist et al., 1998). Less consideration has been given to how other, non-resource-based effects of endurance flight may influence migration strategies or alter behaviour and refuelling performance at stopover sites.

In addition to refuelling, birds may undergo processes of recovery and repair at stopover sites, implying that migratory flight has costs other than the depletion of metabolic fuels and water (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Piersma, 1997; Biebach, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000). Frequently, birds do not gain mass in the first days after arrival at stopover sites (Alerstam and Lindström, 1990; Klaassen and Biebach, 1994; Gannes, 1999). This ‘settling time’ may represent the time required to locate good feeding areas (energy-rich and safe) or to obtain feeding territories (Rappole and Warner, 1976; Alerstam and Lindström, 1990), but settling time could also have a physiological basis (Langslow, 1976; Klaassen and Biebach, 1994; Karasov and Pinshow, 2000). For example, catabolism of the digestive system in flight may constrain hyperphagia and mass deposition after arrival (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Biebach, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000; Lindström et al., 1999; Battley et al., 2000).

In this study we explore the possibility that flight-induced

muscle damage is a mechanism by which the physiological side-effects of endurance flight influence migration strategies and stopover behaviour. Recent studies demonstrate that the pectoralis muscles are catabolized during endurance flights (Battley et al., 2000; Lindstrom et al., 2000). The utilization of muscle protein for metabolic fuel occurs through an alteration in the balance between the rates of protein synthesis and degradation (Goldspink, 1991). In contrast, muscle damage is a pathological consequence of strenuous exercise, especially apparent in untrained individuals (Armstrong et al., 1991; Clarkson and Sayers, 1999). The most severe damage is caused by mechanical stresses acting on muscles during eccentric exercise (stretching while active, e.g. lowering a weight; Armstrong et al., 1991; Fridén and Lieber, 1992). Prolonged, high intensity concentric exercise (e.g. cycling or running) can also lead to muscle damage, possibly as a result of metabolic factors (reactive oxygen species, elevated temperature, lowered pH, ionic shifts; Armstrong et al., 1991; Byrd, 1992; Virtanen et al., 1993; Rama et al., 1994; Havas et al., 1997; Koller et al., 1998; Fallon et al., 1999; Margaritis et al., 1999). Exercise-induced muscle damage is characterized by ultrastructural disruption (Z-line streaming), elevation of muscle-specific proteins in plasma (e.g. creatine kinase, myoglobin) and immune system responses (e.g. neutrophil infiltration; Armstrong et al., 1991; Byrd, 1992; Lieber et al., 1996; Clarkson and Sayers, 1999). Acute damage causes loss of strength, reduced mobility, pain, edema and, in extreme cases, rhabdomyolysis and kidney failure (Clarkson et al., 1992; Kuipers, 1994). Chronic damage may contribute to the immunosuppression characteristic of overtraining (Shephard and Shek, 1998). During flight, the avian pectoralis performs mainly concentric work (Biewener et al., 1998), but the high intensity and long duration of migratory flights could result in significant muscle damage. Disruption of flight muscle ultrastructure in Canada geese *Branta canadensis* upon arrival at the bre96wng flgh aromteinalsosssom strebre9oconcealsoof strT* 0.1424 Tw (muscltion str-assoly)tuscle damage is chn birde.g.Geors cl.,

from plumage (Wilson, 1994; P. D. O'Hara unpublished data). Protocols conformed with the Canadian Committee for Animal Care guidelines.

Bar-tailed godwits

Bar-tailed godwits were studied while en route from West Africa to breeding areas on the Taimyr Peninsula, Russia during their 1 month long stopover in the Dutch Wadden Sea (Piersma and Jukema, 1990). Early arriving godwits were captured in 1998 (29 April – 6 May) on the dunes near Castricum (52 °32′N, 04 °37′E) on the Dutch mainland, as they completed a long-distance flight from Africa (Piersma and Jukema, 1990). These birds were attracted with recorded calls and decoys, and captured in clap nets (Landys et al., 2000). In 1997 (N=142) and 1998 (N=24), refuelling godwits were captured on the island of Texel (53 °03′N, 04 °48′E) in wilsternets (Koopman and Hulscher, 1979) between 13 and 29 May. Godwits were sexed based on body size (Piersma and Jukema, 1990). At Texel, relatively late-arriving birds were identified based on the absence of body moult (Piersma and Jukema, 1993; Piersma et al., 1996). At both sites, blood sampling was timed from the moment of capture. Blood was taken by puncturing the brachial vein (23-gauge needle) and collecting the blood into heparinized capillary tubes. Samples were stored on ice, centrifuged within 10 h of collection at 6900 ***g*** for 10 min and stored at –80 °C. Plasma was transported

migratory stage and age combination ($P>0.10$), nor did age have any effect in wintering or pre-migratory birds ($P>0.27$); however, in fall at Boundary Bay, juvenile migrants had significantly higher plasma CK activities than adults ($P=0.04$). We also tested for an age difference with plasma collected in fall 1995 at Boundary Bay, and although all samples were stored for 9 months at −20 °C prior to analysis, juveniles again had higher plasma CK activities than adults ($P=0.02$). Plasma CK activity varied among migratory stages ($F=5.0_{1,275}$, $P=0.0001$; Fig. 2) with no stage-by-mass interaction ($P=0.49$). In 1996, pre-migrants had lower plasma CK activity than fall adult migrants ($P=0.01$), and tended to be lower than spring migrants ($P=0.03$). In 1997, wintering birds had lower plasma CK activity than spring migrants ($P=0.003$). When considered together, non-migrants had significantly lower plasma CK activity than all migrants combined (linear contrast $P=0.0001$). There was no difference in plasma CK activity between juveniles stopping at Sidney Island and Boundary Bay ($P=0.08$), or between spring and fall migrant adults ($P=0.69$).

Bar-tailed godwits

Plasma CK activity was negatively correlated with sample date in both years for this synchronous migrant (1997: $r=-0.14$, $P=0.045$; 1998: $r=-0.31$, $P=0.035$). Data from 1997 and 1998 were combined since we could detect no difference in CK activity between years ($P=0.87$), nor any interactions between year and bleed-time, body mass or date ($0.09<P<0.39$). Plasma CK activity did not differ between sexes, controlling for bleed-time and mass ($P=0.31$). Blood samples were obtained very quickly from godwits (median 2 min), yet a nearly significant positive effect of bleed-time on CK activity was still apparent ($F=3.0_{1,175}$, $r^2=0.02$, $P=0.09$). Controlling for bleed-time, there was no relationship between CK activity and body mass ($F=2.4_{1,174}$, $P=0.13$). Godwits captured at Castricum and birds showing evidence of body moult at Texel were classed as ‘early arrivals’, while birds not moulting at Texel were classed as ‘late arrivals’. Controlling for bleed-time and arrival status, plasma CK activity declined with date ($F=4.4_{1,173}$, $P=0.018$). There was no interaction between date and arrival status ($P=0.85$), and taking into account the effect of date, late arrivals had higher plasma CK activity than early arrivals ($F=3.1_{1,173}$, $P=0.04$, Fig. 3).

Discussion

We found evidence of flight-induced muscle damage during migration in both western sandpipers and bar-tailed godwits, using a different investigative approach for each species. Our results indicate that, in addition to replenishing fuel stores (fat and protein), birds at migratory stopovers undergo some degree of repair to damaged flight muscles. Migrating western sandpipers had higher plasma CK activity than non-migrants in each year of study. In bar-tailed godwits, plasma CK activity was highest soon after arrival at the Wadden Sea and declined as birds prepared for the next leg of their journey. Also in agreement with our predictions, late-arriving godwits had higher mean plasma CK activity on a given day than birds that had arrived earlier. Late-arriving godwits also have lower body mass and reduced hematocrit (Piersma et al., 1996). It now seems apparent that behind-schedule godwits also may have less time to repair flight muscles before leaving the Wadden Sea for the breeding grounds.

a greater index of muscle damage than spring migrants, which make short flights along a coastal route (Iverson et al., 1996; Butler et al., 1996). In contrast, body composition analysis indicated that migrants were more likely to deposit lean mass (including flight muscle) in the fall than in spring (Guglielmo, 1999), illustrating that utilization of muscle protein for energy is fundamentally different from muscle damage. Similarly, in herring gulls *Larus argentatus*, plasma CK, lactate dehydrogenase (LDH) and aspartate transaminase (AST) activities were unaffected by fasting, but increased on handling (Totzke et al., 1999). Hence, protein can be mobilized from the pectoralis without increasing the susceptibility to muscle damage and its associated functional impairment.

Migration may be especially challenging for young birds undertaking their first journey. There is good evidence that juvenile birds differ behaviourally from adults in ways that could reduce migration performance (e.g. inefficient foraging ability, low social status; Groves, 1978; Burger and Gochfeld, 1986; Hockey et al., 1998), but it is less clear if physiological factors are important. In the Arctic, shorebird chicks must grow quickly, and the pectoralis muscles grow most rapidly just prior to fledging (Hohtola and Visser, 1998). High growth rate is thought to be incompatible with the development of functional maturity of skeletal muscle (Ricklefs et al., 1998), and this could be particularly important for physiological systems that require training to achieve maximum performance (e.g. Pelsers et al., 1999). Like adults, juvenile western sandpipers must make an over-water flight to British Columbia as one of their first migratory flights. Juveniles at fall stopover (1995 and 1996) did not differ from adults in structural size, body mass, fat load, pectoralis muscle mass or pectoralis water fraction (an index of functional maturity; Guglielmo, 1999; C. G. Guglielmo, unpublished data). However, elevated plasma CK activity in juveniles in both years suggests that their relatively untrained state may put them at greater risk of

quite high, leading to strong selection for birds to manage flight distances and conditions to minimize damage. For example, muscle strength and mobility can decrease by half following damage (Clarkson et al., 1992), which would decrease flight performance and increase predation risk. Injured birds might need to devote more time to vigilance behaviour, or restrict their activities to the safest habitats. While damage can occur rapidly, the repair process can be prolonged (Clarkson et al., 1992; Clarkson and Sayers, 1999), and the best strategy for migrants may be to avoid muscle injuries in the first place. Third, the flight muscles could be superbly adapted to high intensity exercise, and possess morphological, physiological and biochemical mechanisms to prevent damage (e.g. antioxidants). Indeed this explanation seems likely, given that the exercise performance of migrants such as the bar-tailed godwit is so extreme, relative to activities known to cause significant muscle damage in other model systems (e.g. human marathon runners; Koller et al., 1998).

Even moderate muscle damage associated with migration could have an important influence on bird behaviour and ecology by impairing immune responses to pathogens. It is well known that intense repetitive exercise can cause immunosuppression (Fitzgerald, 1991; Nieman 1994). The repair of chronic, low-level muscle damage may be a contributing factor by constantly taxing the immune system (Shephard and Shek, 1998). Long-distance migrant shorebirds are high-performance exercise machines that can expend up to half of the annual energy budget during migration (Drent and Piersma, 1990). Piersma (1997) proposed that among shorebird species there is a physiological trade-off between migration distance (i.e. exercise performance) and pathogen resistance (i.e. immune function), which limits the longest distance migrants to relatively parasite-free locales (e.g. high arctic tundra and marine intertidal). As one mechanism for the trade-off, he suggested that the repair of muscle damaged by migratory flight impairs the immune defence against pathogens (Piersma, 1997). The detection of muscle damage in bar-tailed godwits and western sandpipers during migration provides partial evidence for these ideas, but more detailed studies will be required to fully test the hypothesis of a trade-off between migration distance and pathogen resistance, and its evolutionary consequences.

A major impetus for our study was a desire to understand how the physiological side effects of migratory flight can feedback to reduce subsequent performance at stopover, particularly by influencing settling time. Recent studies demonstrate that digestive system size is reduced by flight and fasting to such an extent that birds cannot fatten maximally at arrival (Klaassen and Biebach, 1994; Hume and Biebach, 1996; Klaassen et al., 1997; Biebach, 1998; Piersma and Gill, 1998; Karasov and Pinshow, 1998; Karasov and Pinshow, 2000; Lindström et al., 1999), and while our results are difficult to interpret functionally, they suggest that muscle damage is also a factor. Migration optimization models indicate that settling time can strongly influence optimal stopover duration and departure fuel load, but settling time is considered to be constant and unrelated to the duration or conditions of the previous flight (Alerstam and Lindström, 1990; Gudmundsson et al., 1991; Weber et al., 1994; Alerstam and Hedenström, 1998). If settling time is mainly a physiological phenomenon (not behavioural or ecological), its duration could be positively related to the length of the previous flight (i.e. longer flights entail longer fasting and more exercise). The implications of such a dependence of settling time on flight duration for the optimization of flight distances, fuel loads and stopover times has not been explored, but it seems to be a fertile subject for future theoretical and empirical study.

Capture stress

By far the highest plasma CK activities we measured were associated with capture stress. Plasma CK activity increased rapidly following capture in a mist net even though birds were removed as quickly as possible. 1–2 h after capture, plasma CK activity in western sandpipers was often in the range of 2000–5000 units l^{-1}, a tenfold increase from capture (C. G. Guglielmo, unpublished data). Plasma CK and AST activities have been used to assess the risks of capture myopathy in mallards *Anas platyrhynchos* (Bollinger et al., 1989; Dabbert and Powell, 1993). Bollinger et al. found that muscle damage varied among different capture methods, and was highest in enveloyin weoamage
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