REVIEW

Reviews provide an opportunity to summarize existing knowledge within ornithological research, especially in areas where rapid and significant advances are occurring. Reviews should be concise and should cite all key references. An abstract is required.

Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective

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Unlike exercising mammals, migratory birds fuel very high intensity exercise (e.g., flight) with fatty acids delivered from the adipose tissue to the working muscles by the circulatory system. Given the primary importance of fatty acids for fueling intense exercise, we discuss the likely limiting steps in lipid transport and oxidation for exercising birds and the ecological factors that affect the quality and quantity of fat stored in wild birds. Most stored lipids in migratory birds are comprised of three fatty acids (16:0, 18:1 and 18:2) even though migratory birds have diverse food habits. Diet selection and selective metabolism of lipids play important roles in determining the fatty acid composition of birds which, in turn, affects energetic performance during intense exercise. As such, migratory birds offer an intriguing model for studying the implications of lipid metabolism and obesity on exercise performance. We conclude with a discussion of the energetic costs of migratory flight and stopover in birds, and its implications for bird migration strategies.

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Migration poses distinct physiological challenges for birds. For example, long-distance migrants rely virtually entirely on stored energy and nutrients to fuel each flight, and then must rapidly restore the necessary energy and nutrients at stopover sites along their migration route. Solutions to the physiological challenges associated with alternating intense exercise without feeding and then intense feeding and refueling at stopover sites are often physiologically incompatible. Determining how birds overcome these physiological challenges requires understanding how the physiological capabilities of exercising and fasting birds relate to the ecological conditions encountered during migration.

This review focuses on the nutritional and physiological ecology of birds during migration. We discuss the current model of how birds fuel the costs of migration and the likely limiting steps in lipid transport and oxidation for exercising birds, and how lipid metabolism in birds is different from that in other vertebrates. We then place these aspects of the biochemistry and metabolism of birds during migration within an ecological context. We review selected aspects of the nutritional ecology of birds during migration with an emphasis on how birds accumulate fat stores, how fatty acid composition of diet influences composition of fat stores, and the effect of fatty acid composition of fat

stores on bird performance. We conclude with a discussion of conventional aerodynamic models of flight, empirically derived models of flight, and their relative accuracy and promise for predicting actual costs of flight.

Biochemical aspects of fuels used during migratory flight

The image of migratory birds furiously in search of food and storing fat in preparation for an overnight or even multi-day flight is familiar to both professional and amateur ornithologists. As a result it is commonly accepted that adipose fat is the primary metabolic fuel for avian migration. In fact, the ability to fuel very high intensity exercise with fatty acids (FA) delivered from the adipose tissue to the working muscles by the circulatory system makes migratory birds exceptional among vertebrate animals.

From biochemical first principles it is clear why fat should be the fuel of choice for migrating birds under most circumstances, as described in previous reviews (Ramenofsky 1990, Jenni and Jenni-Eiermann 1998). The fuel storage criterion of greatest importance for weight-economizing migrants is energy content per unit wet mass, and on this basis fat contains eight to ten times more energy than alternative fuel types. Two factors contribute to the high wet energy content of fat. First, FA are more chemically reduced than either carbohydrates or proteins (amino acids), yielding approximately twice the energy per unit dry mass when oxidized. Second, in living tissue FA are stored in a nearly anhydrous state (5% water) as triacylglycerol (TAG; 3 FA esterified to glycerol), whereas carbohydrate (liver and muscle glycogen) and protein stores have high water content (70–80%). Glycogen stores are quantitatively unimportant to endurance flights, but may be required initially while FA utilization mechanisms are up-regulated (Rothe et al. 1987, Schwilch et al. 1996, Jenni-Eiermann et al. 2001), and to maintain blood glucose concentration stable until gluconeogenic pathways are fully induced (Weber 1988). Protein is stored in large quantity as body structure, but its use entails a loss of functional capacity. Nevertheless, some protein is catabolized during flight to satisfy endogenous protein turnover, to provide gluconeogenic precursors for metabolism in the liver, and to counteract the depletion of Krebs cycle intermediates (anaplerotic flux) during oxidative metabolism of fatty acids (Jenni and Jenni-Eiermann 1998, Klaassen et al. 2000). Protein could also be mobilized to provide water under dehydrating flight conditions (Klaassen 1996), or as an adaptive reduction of flight muscle size in response to declining body mass over a flight (Jenni and Jenni-Eiermann 1998, Pennycuick 1998).

Empirical evidence of three types confirms the primary importance of adipose fat as a metabolic fuel in flying birds, with a variable contribution of protein. First, body composition analyses show that migrants deposit fat loads as high as 50% of total body mass, that adipose fat can be severely depleted by migratory flight, and that body protein is used from muscles and particularly from the digestive organs (Piersma and Jukema 1990, Ramenofsky 1990, Lindström and Piersma 1993, Battley et al. 2000, Battley et al. 2001, Bauchinger and Biebach 2001, McWilliams and Karasov 2001). Fat and protein deposited in preparation for migration is referred to as "stores" whereas endogenous fat and protein used only during times of stress is referred to as "reserves" (after King and Murphy 1985, Lindström and Piersma 1993, Van der Meer and Piersma 1994). Given that a bird during migration may use and rebuild both energy and protein stores and reserves, this heuristic distinction may at times be difficult to recognize in practice. Second, a respiratory exchange ratio (RER $= CO_2$ production/O_2 consumption) near 0.7 has been measured in birds flying under controlled conditions, suggesting FA oxidation (Rothe et al. 1987, Suarez et al. 1990). It is important to recognize that protein oxidation by birds with uric acids as an end product also results in a RER near 0.7 (Walsberg and Wolf 1995), so these studies may demonstrate only that some mixture of fat and protein is oxidized. Additional supporting evidence for a dominant role of fat in flight has come recently from stable isotope analysis of post-flight breath samples of pigeons *Columba livia* (Hatch et al. 2002). Third, plasma metabolite analyses show an increase in lipid mobilization from adipose stores during flight in a variety of bird species (Jenni-Eiermann and Jenni 1991, George and John 1993, Schwilch et al. 1996, Jenni-Eiermann et al. 2001, Jenni-Eiermann and Jenni 2003). In-flight accumulation of uric acid in plasma has been interpreted to reflect increased protein utilization (Jenni-Eiermann and Jenni 1991, George and John 1993, Schwilch et al. 1996, Gannes 2001), although a possible exercise-related decrease in plasma uric acid clearance by the kidney has not yet been properly accounted for (Poortmans and Vanderstraeten 1994). As with respirometry studies, measurement of plasma metabolite concentrations provide only a qualitative picture of fuel metabolism, and detailed kinetic studies will be required before flux through exogenous and endogenous fuel pathways is understood.

The fuel selection model that emerges from consideration of both first principles and the empirical evidence is that during endurance flights migrant birds use mainly exogenous FA with a minor contribution of protein (5–15%) to total energy demand. Moreover, the relative contribution of protein may depend on initial fat load (Jenni and Jenni-Eiermann 1998, Jenni-Eiermann and Jenni 2003). Thus, migratory birds must have the

physiological and biochemical capacity to mobilize from adipose tissue, carry through the circulation, transport into muscle cells, and oxidize FA at rates fast enough to meet the very high energetic demands of flight. In the next section we focus on the standard model of fuel selection during exercise developed for running mammals, its inability to explain exogenous FA use for avian migratory flight, and the potential physiological and biochemical mechanisms birds use to surpass the metabolic limitations seen in mammals.

The mammalian exercise model and avian flapping flight

In contrast to migratory birds, running mammals depend on carbohydrate and fat as the major fuels to power exercise, with only a small contribution from protein, which is often ignored (Weber 1992, Roberts et al. 1996). The mixture of carbohydrate and fat is determined by relative exercise intensity (i.e., the percentage of maximal oxygen consumption or % VO_2max), rather than absolute intensity. Thus, mammal species (and individuals within species) of different absolute aerobic performance capabilities have similar fuel composition at the same relative exercise intensity (Roberts et al. 1996).

During very low intensity exercise (<40% VO_2max), FA oxidation can provide nearly all of the energy for exercise. However, absolute FA oxidation peaks near 40% VO_2max, so that as exercise intensity increases the relative contribution of FA oxidation to energy demand declines. Near VO_2max, FA oxidation provides only 20% or less of the fuel requirement. The deficit between total energy demand and FA oxidation is made up by the oxidation of carbohydrate, mainly glycogen stored within muscle cells (Roberts et al. 1996, Weber et al. 1996a). The heavy reliance on muscle glycogen for high intensity endurance exercise provides a metabolic basis for "carbo loading" by human endurance athletes, and when glycogen stores are exhausted a running mammal will as athletes say "hit the wall", left unable to exercise above the low intensity supported by FA oxidation alone.

A close examination of the sources of FA oxidized during mammalian exercise reveals an even lower threshold for fully exogenous FA powered activity. Isotopic tracer studies show that only 25–50% of the FA oxidized during exercise of various intensities are delivered to muscles via the circulation; most are derived from intramyocyte TAG droplets (Weber et al. 1996b). In addition, training, as well as evolutionary adaptation for endurance, often results in an increase in the size and importance of endogenous TAG stores, a fuel strategy that prolongs high intensity endurance time by sparing endogenous glycogen stores in muscles (Weber 1988).

The upshot is that near VO_2max exogenous FA provide only 10% or less of energy demand. Conversely, to operate strictly on exogenous FA, a cursorial mammal must limit activity to extremely low exercise intensity, such as walking (Roberts et al. 1996).

In terms of energy expended in locomotion, there is no equivalent to walking for birds in flapping flight because they cannot reduce energy cost by traveling more slowly. Theoretical flight power curves are generally U-shaped, so that flight cost is minimized at an intermediate speed (Alerstam and Hedenström 1998). Empirical evidence suggests that power requirements may remain near a minimum over a wide range of speeds due to adjustments to flight morphology in some species, but at the slowest and fastest speeds power requirements increase (Dial et al. 1997, Tobalske et al. 2003). On an absolute scale this minimum cost of flying has been estimated to be greater than twice the VO_2max of a similarly-sized running mammal (Butler and Woakes 1990). Even on a relative scale, birds flying at minimum power speed appear to exercise at 60–85% VO_2max (Guglielmo et al. 2002a). Furthermore, migrants may exercise close to VO_2max because they are heavily burdened with fat, and may fly faster than minimum power speed to maximize distance covered per unit energy cost (Alerstam and Hedenström 1998). Thus, on both an absolute and a relative basis it is clear that migrant birds exercise at very high intensity. At steady-state during long-distance flight most of the fuel must be exogenous FA because birds simply cannot store enough intra-myocyte TAG for an entire flight. The standard mammalian exercise model cannot account for this fuel strategy, indicating that the capacity to utilize exogenous FA may be as much as 20-fold greater in migratory birds.

Mechanisms of fatty acid utilization during exercise

Lipid metabolism is broadly similar in birds and mammals. Therefore, one way to determine the mechanistic basis for the superior utilization of exogenous FA by migrating birds is to identify key components of the system of FA mobilization, transport, and oxidation that constrain fuel selection in exercising mammals. Unlike hydrophilic fuel substrates (e.g., glucose, amino acids), the extremely low (nanomolar) aqueous solubility of FA (Vork et al. 1993) requires the action of soluble protein carriers at every step of FA transport (Fig. 1). During exercise in the post-absorptive state, exogenous FA enter the circulation from adipocytes and the liver. Adipocyte TAG is hydrolyzed to non-esterified FA (NEFA) and glycerol through the action of hormone sensitive lipase (HSL), mainly under the control of catecholamines (Ramenofsky 1990, Raclot 2003). NEFA are bound by plasma albumin, which has several binding sites (Weber
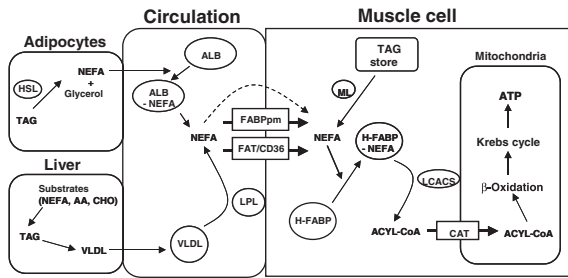
Fig. 1. A model of lipid transport and oxidation in a bird during exercise while post-absorptive. Abbreviations: AA, amino acid; ACYL-CoA, acyl-coenzyme A; ALB, albumin; ATP, adenosine triphosphate; CAT, carnitine acyl transferase; CHO, carbohydrate; FABPpm, plasma membrane fatty acid binding protein; FAT/CD36, fatty acid translocase; H-FABP, heart type fatty acid binding protein; HSL, hormone-sensitive lipase; LCACS, long chain acyl-CoA synthetase; LPL, muscle lipoprotein lipase; ML, muscle lipase, NEFA, non-esterified fatty acid; TAG, triacylglycerol; VLDL, very low density lipoprotein. The dashed arrow indicates passive diffusion of NEFA across the sarcolemma. Based on Weber (1992), Luiken et al. (1999), and McArthur et al. (1999).

1988, Luiken et al. 1999), and carried to the muscles. The liver can synthesize FA *de novo* or uptake NEFA from plasma, and by esterification to glycerol, produce TAG that is packaged into very low density lipoprotein (VLDL) for release to the circulation (Havel 1986, Ramenofsky 1990, Jenni-Eiermann and Jenni 1992). The use of plasma VLDL as a source of FA for working muscles requires hydrolysis by lipoprotein lipase (LPL) at the capillary endothelium (Ramenofsky 1990, McArthur et al. 1999). Generally, plasma VLDL levels fall while NEFA rises during exercise in mammals, making plasma NEFA the primary route of exogenous FA delivery (Jenni-Eiermann and Jenni 1992). At the muscle capillaries NEFA diffuse to the sarcolemmal surface with the aid of interstitial albumin that interacts with a membrane albumin binding protein (Luiken et al. 1999).

Translocation across the sarcolemmal membrane was long thought to take place by simple diffusion of lipophilic FA through the lipid bilayer, with the limiting step being the flip-flop from the outer to inner leaflet (Luiken et al. 1999). More recent studies demonstrate that as much as 80% of muscle FA uptake may be facilitated by superficial and integral membrane transport proteins. Two of the most important FA transporters identified in red muscle are plasma membrane fatty acid binding protein (FABPpm), and fatty acid translocase (FAT/CD36, Luiken et al. 1999, McArthur et al. 1999). During exercise additional FAT/CD36 is translocated to the membrane from intracellular storage sites (Bonen et al. 2000).

Uptake of FA across the sarcolemma is extremely limited without an intracellular FA carrier and subsequent rapid disposal by conversion to acyl-CoA

(McArthur et al. 1999). In the muscle cytosol a small, soluble heart-type fatty acid binding protein (H-FABP) binds and transports NEFA and some acyl-CoA derivatives (McArthur et al. 1999). H-FABP has a single FA binding site, and acts as a "cytoplasmic sink" for FA entering the myocyte, vastly increasing intracellular FA solubility, the rate of removal from the inner sarcolemmal surface, and the rate of intracellular diffusion (McArthur et al. 1999). At this stage FA from intramyocyte TAG droplets may be hydrolyzed by a muscle lipase, and enter the stream of transport towards mitochondrial oxidation. This step circumvents any rate limitations imposed by sarcolemmal uptake processes. Intracellular NEFA are converted to acyl-CoA by long chain acyl-CoA synthetase (LCACS) before being reincorporated into TAG for storage, or more importantly for exercise, before entering the mitochondria for oxidation (McArthur et al. 1999). Long chain acyl-CoA cannot diffuse across the inner mitochondrial membrane, and so it must first be converted by carnitine acyl transferase (CAT) to acyl-carnitine (Weber 1992). After traversing the inner membrane, a second CAT reconverts acyl-carnitine to acyl-CoA, which then enters the β-oxidation pathway. The resulting acetyl-CoA enters the Krebs cycle, leading to the aerobic production of CO_2, H_2O, and ATP.

Analysis of the entire system of exogenous FA transport and oxidation in exercising mammals reveals that overall flux is not limited by adipocyte mobilization or by mitochondrial oxidation capacity. For example, a significant fraction of NEFA liberated by HSL are re-esterified to TAG within the adipocyte (Weber 1992). Rather, the major limitations on flux appear to occur during circulatory transport, and particularly during sarcolemmal uptake (Vock et al. 1996). Thus, when considering migratory birds it is likely that the greatest differences from mammals should be in circulatory routes, and especially in the mechanisms involved in the uptake of FA across the sarcolemma.

Enhanced utilization of exogenous fatty acids in birds

There are many possible ways migratory birds could augment exogenous FA utilization capacity, and although not all are likely to be major determinants of flux, metabolic control theory suggests that several may contribute significantly (Hochachka and Somero 2002). For convenience, mechanisms can be divided into those relating to adipose sources, circulatory pathways, myocyte uptake, and intracellular disposal (esterification and oxidation).

Modulation of fat quality

Besides increasing the amount of fat storage, migrant birds may be able to facilitate FA mobilization by changing the physical properties of fat. Most of the FA stored by vertebrates are 14–24 carbons in length with zero to six double bonds (Raclot 2003). Mammalian adipocytes release FA preferentially based on chain length and unsaturation level. At the same number of carbons, FA with more double bonds are preferentially released, and conversely, at the same number of double bonds, shorter chain lengths are preferred (Raclot 2003). Unsaturated FA may also be preferentially transported and oxidized (Leyton et al. 1987). Although such preferential mobilization and oxidation of unsaturated FA has not been demonstrated in birds, recent empirical studies suggest that fatty acid composition of birds affects performance during migration in birds (see below).

Augmentation of circulatory transport

Improving circulatory transport capacity could be an important adaptive mechanism for exogenous FA transport. Heart size has been shown to increase during migration in some species, probably increasing cardiac output (Piersma et al. 1996, 1999, Guglielmo and Williams 2003). This, however, has been suggested to be related to elevated hematocrit in migrants (Piersma et al. 1996, Landys-Ciannelli et al. 2002). Curiously, high hematocrit reduces the relative amount of plasma available for fuel substrate transport, suggesting that oxygen transport may constrain blood composition more than fuel transport. High capillary density reduces diffusion distances, and could facilitate muscle uptake of FA. Muscle capillarity has been found to increase with migratory distance in passerine bird species, probably as a consequence of the smaller fiber diameters of red muscles of migrants (Lundgren and Kiessling 1988). In any case, even in hummingbird flight muscle with an extremely high capillary density (Suarez 1992), only about one third of the required increase in exogenous FA uptake can be explained by reduced diffusion distances (Guglielmo et al. 2002a).

Circulatory transport capacity could be elevated by increasing plasma albumin concentration or the number of albumin NEFA binding sites. Large increases in plasma albumin concentration are unlikely due to the critical role of albumin in maintaining plasma osmotic pressure (Jenni-Eiermann and Jenni 1992). Plasma albumin decreased during flight in pigeons (George and John 1993). Measurement of the number and affinities of albumin NEFA binding sites is difficult, and there are many discrepancies in the literature (Luiken et al. 1999). Neither of these possibilities has been thoroughly investigated in birds.

A very interesting supplementary mode of circulatory delivery of exogenous FA to muscles was proposed by Jenni-Eiermann and Jenni (1992) based on their studies of migratory passerines. Plasma TAG and VLDL levels were elevated in migrants captured in mid-flight, compared to birds fasted 60 minutes or overnight. They suggested that the high lipid uptake and processing capacity of the liver allows it to act as an alternative sink for exogenous FA originating from adipose tissue, thus freeing plasma albumin to transport more FA per unit time. Fatty acids taken up by the liver would be re-esterified and released back to the plasma in VLDL. This pathway could provide large amounts of FA to muscle without the osmotic effects of increased plasma albumin. Moreover, the hydrolysis of TAG by LPL at the capillary endothelium can create very high local concentrations of NEFA to drive FA influx into myocytes (McArthur et al. 1999). However, elevated plasma TAG during flight has not been further confirmed in controlled field or laboratory studies with other species. Plasma TAG concentration declined in flying pigeons (Bordel and Haase 1993, Schwilch et al. 1996), and declined initially and stabilized in red knots *Calidris canutus* flying for up to ten hours in a wind tunnel (Jenni-Eiermann et al. 2001). Plasma TAG also declined during flight wheel exercise in red-eyed vireos *Vireo olivaceus*, a passerine species (Pierce et al. 2005). Regardless of these negative findings, it is important to recognize that plasma concentrations do not always reflect flux, and that even when plasma TAG levels declined in these studies (e.g., red knots) the absolute concentrations of FA in TAG were similar to or greater than in plasma NEFA (1 mM TAG = 3 mM NEFA).

If circulatory FA transport by VLDL is an adaptation for migratory flight, it might also be expected that muscle LPL activity will be greater in migratory species, or increased during migratory activity. In fact, muscle LPL activity did not change between winter and premigration in rosy pastors *Sturnus roseus*, or between winter and spring migration in dark-eyed juncos *Junco hyemalis* (George and Vallyathan 1964, Ramenofsky et al. 1999). Muscle LPL activity also decreased in dark-eyed juncos over a night of migratory restlessness (Savard et al. 1991). More thorough investigation of the dynamics of the lipoprotein circulatory pathway during flight, and the seasonal modulation of muscle LPL is needed.

Induction of sarcolemmal uptake mechanisms

The mechanisms of sarcolemmal FA transport may be the most important determinants of exogenous FA flux, but they are very poorly understood in migratory birds. Muscle membrane transporters have not yet been identified or systematically studied in birds. In a

preliminary investigation using antibodies to various mammalian proteins, a protein cross-reacting with anti-FABPpm was found in pectoralis muscle membranes of western sandpipers *Calidris mauri* (C. G. Guglielmo and A. Bonen, unpubl. data). A western blot analysis with a small sample (n = 7–11 per group) indicated that FABPpm levels did not differ between non-migratory and migratory sandpipers. FAT/CD36 was not detected using three different antibodies. Characterization and study of the migration-related changes in sarcolemmal membrane transporters should be a priority for future research in avian FA metabolism.

Intra-myocyte FA binding capacity is crucial to muscle FA uptake, and there are two reasons to predict *a priori* that muscle H-FABP is important for migratory flight in birds. The first is a positive correlation between muscle FA oxidation capacity and H-FABP content across a variety of animal species (Haunerland 1994). The second reason is the intriguing pattern of H-FABP expression in flight muscles of desert locusts *Schistocerca gregaria*, which like birds rely on FA oxidation for migratory flight (Haunerland 1994). During the flightless nymph stages no H-FABP is expressed in locust flight muscle. Transcription of the H-FABP gene is initiated dramatically soon after the final ecdysis to the winged adult, and continues until H-FABP becomes the most abundant cytosolic protein in locust flight muscle (18% cytosolic protein). Locusts cannot support endurance flight until high levels of H-FABP are present, and flight training further increases H-FABP expression. Although the presence of FA binding by cytosolic protein fractions of chicken *Gallus domesticus* muscle was reported some time ago (Collins and Hargis 1989), the first positive identification of H-FABP in a bird was made only recently in pectoralis muscle of migratory western sandpipers (Guglielmo et al. 1998). As predicted, H-FABP was several fold more abundant in sandpiper flight muscle than in any mammalian skeletal muscles studied (Guglielmo et al. 1998, Guglielmo et al. 2002a). Furthermore, H-FABP levels increased by approximately 70% during migration (Fig. 2a, Guglielmo et al. 2002a). Pectoralis H-FABP concentration was also found to increase during migration in barnacle geese *Branta leucopsis* (Pelsers et al. 1999). As noted above, the available evidence indicates that even birds in a non-migratory state can utilize FA as the primary fuel for flight (at least for a short time), probably by relying on intra-myocyte TAG. Thus, the rise in pectoralis H-FABP in migrants appears to be a response to the specific requirement for exogenous FA, rather than a mechanism simply to increase total FA oxidation capacity. Additional study of the endocrine and metabolic regulation of H-FABP in flight muscles of migrants would be informative, especially in light of an apparent requirement for endurance flight training to stimulate protein expression (Pelsers et al. 1999, Guglielmo et al. 2002a).
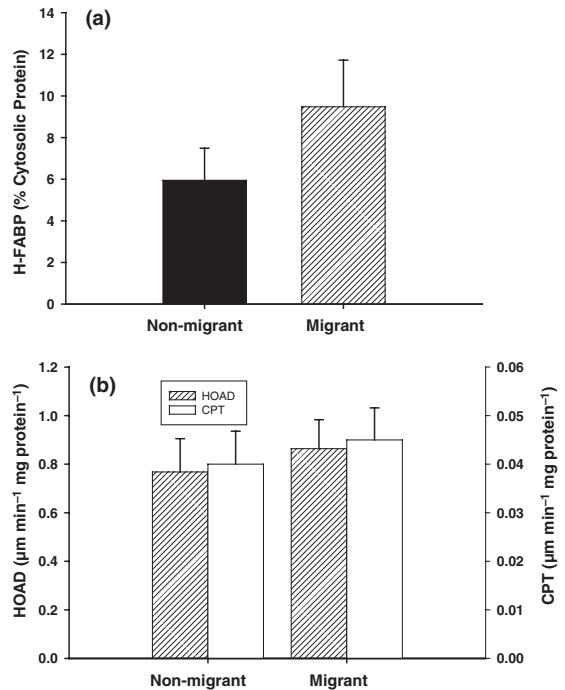

Fig. 2. Migration related increases in pectoralis muscle (a) heart type fatty acid binding protein (H-FABP) concentration ($F_{1,105}$ = 92.7, P = 0.0001), and (b) carnitine palmitoyl transferase (CPT; $F_{1,103}$ = 17.3, P = 0.0001) and hydroxyacyl-CoA-dehydrogenase (HOAD; $F_{1,103}$ = 14.6, P = 0.0002) activities of female western sandpipers (adult and juvenile combined, n = 30 for both non-migrants and migrants). Values are means ± S.D.

Enhancement of intracellular disposal and oxidation

The extracellular to intracellular gradient that drives NEFA uptake by myocytes during exercise can be maintained only if NEFA are rapidly processed into other biomolecules and subsequently oxidized (McArthur et al. 1999). The first step in this process, the conversion to acyl-CoA by LCACS, has not been studied in migratory birds. However, migration-related changes in activities of mitochondrial enzymes involved in FA oxidation have been measured in a number of studies (George and Vallyathan 1964, Marsh 1981, Lundgren and Kiessling 1985, 1986, Driedzic et al. 1993, Guglielmo et al. 2002a). Migration is often associated with increased muscle mitochondrial volume densities (Evans et al. 1992), and higher activities of CAT, β-oxidation enzymes (e.g., hydroxyacyl-CoA-dehydrogenase, HOAD), Krebs cycle enzymes (e.g., citrate synthase), and enzymes of the mitochondrial electron transport chain (e.g., cytochrome oxidase). In western sandpipers carnitine palmitoyl transferase (CPT) and HOAD activity increased in pectoralis muscle by only 12–13% during migration (Guglielmo et al. 2002a; Fig. 2b), much less than the relative increase in muscle H-FABP (Fig. 2a). This again suggests that muscle FA

oxidation capacity in the non-migration state is probably sufficient to support flight exercise, but that endurance time may be limited by the intra-myocyte TAG supply.

Physiological constraints and rate of fat storage in birds

In previous sections, we have described how migratory birds fuel very high intensity exercise during flight with fatty acids delivered from the adipose tissue to the working muscles by the circulatory system. Given the primary importance of fatty acids for fueling intense exercise, migratory birds must store enough fat in adipose and other tissues to successfully accomplish a migratory flight. The rate at which fat stores are deposited affects the overall tempo of migration (Lindström 1991, 2003). We now focus on physiological constraints that influence the rate of fat storage in wild birds. In the next section we focus on the process of fat storage and what determines the fatty acid composition of body fat in migratory birds.

Birds during migration alternate between fasting while flying and then feeding and refueling upon arrival at migratory stopover sites in preparation for subsequent migratory flight(s). These rapid changes in feeding rate and food quality over short periods of time (i.e., hours to days) affect the structure and function of the digestive system in migratory birds that in turn influences the tempo of bird migration. For example, fasting during a migratory flight reduces the size of the digestive system in birds (Battley et al. 2000), when fasted birds are provided *ad libitum* food their food intake is reduced for one or several days until their digestive system is rebuilt (Klaassen and Biebach 1994, Hume and Biebach 1996, Klaassen et al. 1997, Karasov and Pinshow 1998, Karasov and Pinshow 2000, Pierce and McWilliams 2004). Thus, features of the gut (e.g., size, nutrient uptake rates, digestive enzyme activity) of migratory birds are modulated in response to changes in diet quality and quantity (see reviews by McWilliams and Karasov 2001, and Piersma 2002), and these digestive adjustments are likely important for permitting the high feeding rate of migratory birds. However, these digestive adjustments can also constrain the rate of energy intake and diets eaten by birds during migration because of the time-lag associated with rebuilding gut capacity (Piersma 2002, Karasov and McWilliams 2004, McWilliams and Karasov 2004). Other recent, comprehensive reviews of phenotypic flexibility in the digestive system of birds and mammals provide more discussion of this important topic (Karasov 1996, Piersma and Lindström 1997, Starck 1999, McWilliams and Karasov 2001, Karasov and McWilliams 2004, McWilliams and Karasov 2004).

Although we focus on the dynamics of storage and use of fat in birds during migration, lean mass of birds is also quite dynamic over even short time scales (e.g., hours and days) (Piersma 1990, Piersma and Jukema 1990, Lindström and Piersma 1993, Bauchinger and Biebach 2001). The increase in muscle capacity in migratory birds is necessary to satisfy the physiological demands of long-distance flight (reviewed by Butler and Bishop 2000). The nutrient-limitation hypothesis suggests that the initially slow rate of mass gain observed in migrating birds at stopover sites (Rappole and Warner 1976, Biebach et al. 1986, Moore and Kerlinger 1987, Gannes 2002) occurs because birds utilize protein during migration, and recovery of these protein reserves must occur first, and is slow (Klaassen and Biebach 1994, Klaassen et al. 1997, McWilliams and Karasov 2001). The gut-limitation hypothesis described above and the nutrient-limitation hypothesis may not be mutually exclusive if protein in the gut is used as a primary source of reserve protein as suggested by recent studies (Karasov and Pinshow 1998, Piersma 1998, Pierce and McWilliams 2004).

The process of fattening and the fatty acid composition of body fat

If fat is the prime fuel, how do birds fill up their fuel tank?

Birds are like other vertebrates in that they get fatter by increasing energy intake, decreasing energy expenditure, and/or decreasing excreted energy (i.e., fecal and urinary energy). Migratory birds accumulate fat stores primarily by eating more (i.e., hyperphagia) and by selecting diets based in part on total lipid content (Blem 1990, Stiles 1993, Bairlein and Gwinner 1994, Biebach 1996).

Diet composition affects both rate of fattening and the fatty acid composition of the fat deposited. For example, diets with high protein-to-calorie ratios minimize fat storage and enhance muscle building in chickens because excess protein increases levels of insulin-like growth factor-I which inhibits fat deposition. In contrast, diets with low protein-to-calorie ratios maximize fat storage (Rosebrough and McMurtry 1993, Klasing 1998). Chickens fed isocaloric diets composed of mostly fat or carbohydrates accumulated body fat at similar rates as long as the ratio of dietary protein to metaboliz-able energy in their food was similar (Laurin et al. 1985). Although fat deposition in these birds was similar, fatty acid composition of the fat stores of birds fed the high-fat diet was more similar to that of dietary fats whereas that of birds fed the high-carbohydrate diet was often different because the dietary carbohydrates were used by the liver to synthesize fatty acids, a process called *de novo* synthesis (Klasing 1998).

There are few comparable studies conducted using birds other than domestic poultry. In one of the few such

studies, Bairlein (1998) found that high-protein diets inhibited fattening rate in garden warblers *Sylvia borin*, a long-distance migratory songbird. However, unlike chickens, fattening rates in garden warblers were affected by the relative amounts of dietary fat and carbohydrates (Bairlein 1998). Such studies are particularly relevant to the many songbirds that switch from feeding on insects (high protein-to-calorie ratio) to fruits (low protein-to-calorie ratio) during their migration (Bairlein 1991, Bairlein and Gwinner 1994, Parrish 1997). More studies are needed that address how diet composition affects fat deposition rates in other wild birds.

What determines the fatty acid composition of body fat?

Dietary fats provide a pool of fatty acids used to satisfy immediate requirements for energy and other essential functions (e.g., pigments, cellular transport, membrane structure) and to store fat in adipose and other tissues in birds. Lipids are the only dietary component that is deposited intact into tissues (Klasing 1998). Consequently, fatty acid composition of the diet can primarily determine the fatty acid composition of fat stores in birds (West and Meng 1968, Thomas and George 1975, West and Peyton 1980), although some conversion of dietary fatty acids occurs and this selective metabolism can create differences between fatty acid composition of diet and body fat stores (Blem 1990, Klasing 1998). The extent to which dietary fatty acids are converted after intestinal absorption is potentially much greater in birds compared to mammals. Dietary fats in the intestine of birds are absorbed as portomicrons into the portal blood and then go directly to the liver where they may be metabolized (Klasing 1998). In contrast, dietary fatty acids in the intestine of mammals are collected together with phospholipids and cholesterol into droplets called chylomicrons that are then conveyed in lymph directly to the circulatory system and so bypass the liver (Randall et al. 2002).

Although few studies document fatty acid composition of fat stores in birds during migration (Table 1), some patterns are evident. Most lipids in migrating birds are comprised of 16- or 18-carbon fatty acids, and unsaturated fatty acids (mostly 16:1, 18:1 and 18:2) usually predominate over saturated fatty acids (mostly 16:0 and 18:0)(Blem 1976, Blem 1980). Three fatty acids (16:0, 18:1 and 18:2) usually comprise at least 75% of the fatty acids in body fat of birds during migration (Table 1). Songbirds may store more 18:0 during fall compared to spring migration (Table 1), although the few studies conducted since Walker (1964) do not support this claim. Discerning patterns in the relative abundance of these more common fatty acids in migrating birds is complicated because older studies analyzed the whole carcass so that structural and storage lipids were not separated whereas more recent studies focus on stored subcutaneous fat (Table 1).

Blem (1990) noted no consistent pattern in fatty acid composition of body fat between nonmigratory birds, birds during their migration, and migratory birds during their non-migratory periods, except that the body fat of nonmigratory birds had proportionately less 18:1 compared to 18:2 whereas body fat of migratory birds had proportionately more 18:1 compared to 18:2. A few recent studies have directly compared fatty acid composition of fat depot just before and during migration in the same bird species, an approach recommended by Blem (1976). Proportion of 16:1 and 18:1 increased between winter and spring migration in western sandpipers (Egeler and Williams 2000), and proportion of 18:1 increased just prior to migration in wood thrush *Hylocichla mustelina* (Conway et al. 1994). Similarly, proportion of 16:1 increased between breeding period (2%) and fall migration (28%) in red-eyed vireos (Pierce 2003). Thus, birds during migration may increase the proportion of monounsaturated 16- and 18-carbon fatty acids although this trend requires confirmation in many more migratory birds.

The predominance of 16- and 18-carbon fatty acids in migrating birds is surprising given the diverse food habits of those bird species studied, including frugivores, nectarivores, insectivores, granivores, and omnivores. If diet primarily determines the fatty acid composition of birds, then the foods eaten by this diversity of birds must contain primarily these three fatty acids. Alternatively, selective metabolism may play a very important role in determining the fatty acid composition of fat stores in birds during migration.

Behavioral preferences of birds for diets with specific fatty acids

Migratory birds prefer diets with specific fatty acids and this may allow birds with quite different food habits to select those foods with certain fatty acid compositions. For example, red-eyed vireos preferred diets with long-chain unsaturated fatty acids (18:1) over diets with long-chain saturated fatty acids (18:0), and they ate more when offered diets with mostly unsaturated fat than when offered diets with more saturated fat (Pierce et al. 2004). Garden warblers (Bairlein 1991) and yellow-rumped warblers *Dendroica coronata* (McWilliams et al. 2002) also preferred diets with 18:1 over diets with 18:0. Although wood thrushes preferred diets with primarily 18:1 (olive oil) over diets with 18:2 and 18:3 (soy oil, Zurovchak 1997), red-eyed vireos did not have a specific preference for diets with 18:1 over diets with 18:2 (safflower oil, Pierce et al. 2004). When given choices of diets with only saturated fatty acids, red-eyed vireos

Table 1. Fatty acid composition (%) of subcutaneous fat or whole carcass for birds captured during fall or spring migration, or during migration (fall and spring combined). Fatty acid nomenclature is number of carbon atoms to number of double bonds (e.g., 18:0 has 18 carbon atoms and no double bonds).

Species	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Tissue	Location	Source
Fall Migration												
Red-eyed vireo				12.00	5.00	21.00	17.00	9.00		Subcutaneous fat	Northern Florida	Walker 1964[a]
Red-eyed vireo				17.53	27.99	2.50	32.83	16.13	1.89	Subcutaneous fat	Rhode Island	Pierce 2003
Bobolink				13.00	8.00	26.00	19.00	6.00		Subcutaneous fat	Northern Florida	Walker 1964[a]
Magnolia warbler				12.00	7.00	24.00	17.00	23.00		Subcutaneous fat	Northern Florida	Walker 1964[a]
Tennessee warbler		3.00		11.00	6.00	23.00	18.00	20.00		Subcutaneous fat	Northern Florida	Walker 1964[a]
Western sandpiper		3.00		37.00	14.00	10.00	25.00	1.00	2.00	Subcutaneous fat	Boundary Bay B.C.	Egeler and Williams 2000[a]
Wood thrush				15.20	2.40	7.60	61.60	7.60	1.30	Whole animal	Rhode Island	Conway et al. 1994[b]
Spring Migration												
Canada geese		0.37		24.75	4.18	4.91	45.79	19.15	0.86	Subcutaneous fat	Missouri	Thomas and George 1975
Lapland longspurs		1.10		22.30	5.20	6.70	39.70	19.90		Whole animal	Yukon Alaska	West and Peyton 1980[b]
Mallard		1.02		17.12	2.87	5.95	51.90	19.07	2.05	Peritoneal Fat	Eastern Missouri	Heitmeyer and Fredrickson 1990
Slate-colored junco	0.13	0.67		18.17	3.14	7.19	30.37	28.93	1.00	Whole animal	Central Pennsylvania	Bower and Helms 1968[c]
Sora				10.00	7.00	28.00	11.00			Subcutaneous fat	Northern Florida	Walker 1964[a]
Western sandpiper		4.00		34.00	13.00	10.00	27.00	2.00	1.00	Subcutaneous fat	Boundary Bay B.C.	Egeler and Williams 2000[a]
White-crowned sparrow	2.00	31.80	2.50	16.60	4.20	5.80	24.40	10.60	1.60	Whole animal	Southern California	Morton and Liebman 1974[d]
Fall/Spring Migration												
American redstart		1.50		19.30	15.10	2.70	38.20	21.30	1.90	Whole animal	Central Michigan	Caldwell 1973[c]
Bay-breasted warbler	1.20	3.60		20.90	6.50	6.70	35.80	18.00	6.40	Whole animal	Central Michigan	Caldwell 1973[c]
Black and white warbler		2.30		19.90	9.20	2.50	41.80	14.70	8.10	Whole animal	Central Michigan	Caldwell 1973[c]
Blackburnian warbler		2.60		20.30	6.80	2.60	40.90	17.30	9.30	Whole animal	Central Michigan	Caldwell 1973[c]
Blackpoll warbler		3.60		25.80	6.80	6.40	39.10	8.70	8.80	Whole animal	Central Michigan	Caldwell 1973[c]
Black-throated blue warbler		2.60		22.40	8.10	4.40	35.90	18.60	8.00	Whole animal	Central Michigan	Caldwell 1973[c]
Black-throated green warbler		1.30		19.90	6.40	3.10	37.70	18.40	12.30	Whole animal	Central Michigan	Caldwell 1973[c]
Cape May warbler	3.10	27.40	2.20	17.90	8.10	2.70	27.80	9.10	1.70	Whole animal	Central Michigan	Caldwell 1973[c]
Chestnut-sided warbler				19.90	6.60	3.20	38.20	18.10	13.00	Whole animal	Central Michigan	Caldwell 1973[c]
Connecticut warbler		1.90		22.50	7.50	4.80	39.40	12.20	13.00	Whole animal	Central Michigan	Caldwell 1973[c]
Gray-cheeked thrush				30.20	8.40	3.60	53.20	3.20	10.70	Whole animal	Central Michigan	Caldwell 1973[c]
Indigo bunting	2.20		2.00	23.20	4.50	6.70	41.00	23.50		Whole animal	Central Michigan	Caldwell 1973[c]
Nashville warbler		19.90		18.10	7.80	2.60	31.00	12.00	3.80	Whole animal	Central Michigan	Caldwell 1973[c]
Northern waterthrush		1.40		25.60	14.70	5.50	37.60	10.50	3.70	Whole animal	Central Michigan	Caldwell 1973[c]
Ovenbird		1.00		23.50	6.50	7.30	40.10	14.40	6.00	Whole animal	Central Michigan	Caldwell 1973[c]
Philadelphia vireo		1.00		21.70	5.70	2.60	38.50	13.90	16.60	Whole animal	Central Michigan	Caldwell 1973[c]
Red-eyed vireo				22.40	4.70	3.50	34.10	19.50	14.50	Whole animal	Central Michigan	Caldwell 1973[c]
Rose-breasted grosbeak	2.60	3.90		21.70	2.30	9.70	34.30	15.80	3.80	Whole animal	Central Michigan	Caldwell 1973[c]
Tennessee warbler		5.80		22.80	8.30	3.60	37.40	7.80	12.40	Whole animal	Central Michigan	Caldwell 1973[c]
Yellow warbler		4.50		19.80	6.40	5.10	40.50	15.60	8.00	Whole animal	Central Michigan	Caldwell 1973[c]

[a] Estimated from bar graphs.
[b] Gizzard and gut contents removed.
[c] Gizzard and gut contents not removed.
[d] Samples taken 23 April.

(Pierce et al. 2004) and garden warblers (Bairlein 1991) ate more of the diet(s) containing shorter-chained fatty acids. For example, garden warblers preferred diets with 16:0 over diets with 18:0, and vireos ate more of diets with 14:0 than diets with 16:0. Thus, migratory birds prefer diets with certain fatty acids. These diet preferences for specific fatty acids may ensure that a bird's fat stores are composed of certain fatty acids prior to some energetically demanding event (i.e., migration, egg-laying, living in the cold).

Selective metabolism and the fatty acid composition of migrating birds

Our understanding of fatty acid metabolism in birds is based almost entirely on studies of the domestic chicken *Gallus gallus*. Given that most elongation and desaturation of fatty acids is mediated by specific enzymes, and there is much variation among other vertebrates in the activity and capacity of these specific enzymes (Klasing 1998), we expect future studies to reveal that bird taxa differ in their ability to metabolize dietary fatty acids and that these differences have important ecological significance.

Chickens have the enzymatic capacity to desaturate linoleic acid (18:2n-6), an essential fatty acid, to γ-linolenic acid (18:3n-6) which may be elongated by two carbons and desaturated again to arachidonic acid (20:4n-6; Klasing 1998). Recent work by Pierce (2003) suggests that red-eyed vireos, a long-distance migratory songbird, also have the enzymatic capacity to desaturate linoleic acid. Additional evidence for selective metabolism of fatty acids in vireos included: (a) their ability to preferentially store 16:0 as indicated by its proportion in the diet (10.9%) compared to that in the depot fat of vireos fed this diet for six months (24.9%), and (b) a gradual increase from 11% to 22% in the proportion of 18:2n-6 in the fat of vireos while they were fed a semi-synthetic diet with 28% 18:2n-6 for >1 year (Pierce et al. 2005). Selective metabolism plays an important role in determining the fatty composition of body fat in vireos and other birds (reviewed by Blem 1976, 1980), although fatty acid composition of diet more strongly influenced fatty composition of vireos. Clearly, as noted above, more studies are needed that ascertain the capacity for selective metabolism of dietary fatty acids in a diversity of migratory bird taxa.

Fatty acid composition of birds affects their performance during exercise

Thus far we have discussed how birds acquire fat stores and what determines the fatty acid composition of their body fat. These mechanisms may explain why the fat stores of birds during migration are comprised of certain 16- and 18-carbon fatty acids (Table 1). An equally intriguing question is whether the fatty acid composition of migrating birds has any functional significance. Fatty acid composition of fat stores affects exercise performance in rats and fish in part because unsaturated fatty acids are preferentially used during metabolism over saturated fatty acids (Leyton et al. 1987, Raclot and Groscolas 1995, McKenzie et al. 1997, McKenzie et al. 1998). In rats and humans, high levels of essential n-6 polyunsaturated FA in muscle membrane phospholipids have been associated with improved endurance capacity, and n-6 FA appear to be depleted from membranes by repetitive exercise (Ayre and Hulbert 1996, 1997, Andersson et al. 1998). Adriatic sturgeon fed a diet rich in highly unsaturated fatty acids had lower resting oxygen consumption rates, and performed better in hypoxic conditions, than did sturgeon fed a diet rich in saturated fatty acids (McKenzie et al. 1997).

No previous study of birds has documented whether fatty acid composition of fat stores has performance consequences for birds. We fed red-eyed vireos, a long-distance migratory songbird, semisynthetic diets with either 82% unsaturated fat (82%U) or 58% unsaturated fats (58%U). We then measured the vireo's resting metabolic rate using standard procedures and their peak metabolic rate using a hover-flight wheel (see Chappell et al. 1999, Pierce et al. 2005). As expected given the importance of dietary fat in determining fat composition of birds, vireos fed the 82%U diet had fat stores comprised of mostly (77%) unsaturated fatty acids, and vireos fed the 58% U diet had fat stores comprised of less (66%) unsaturated fatty acids. Resting metabolic rate of vireos fed the 58%U diet was similar to that of vireos fed the 82%U diet (2.75 ± 0.32 mlO$_2$/g/h and 2.30 ± 0.30 mlO$_2$/g/h, respectively; $F_{1,8} = 1.09$, P = 0.33). However, peak metabolic rate of vireos fed the 58%U diet was significantly higher than that of vireos fed the 82%U diet ($F_{1,8} = 9.45$, P = 0.015; Fig. 3).
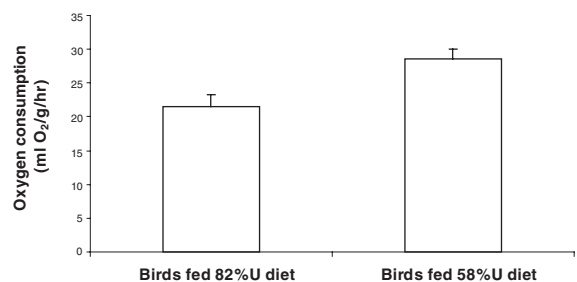

Fig. 3. Mass-specific peak metabolic rate (MR$_{peak}$) for two groups (n = 5 per group) of red-eyed vireos fed diets with different fatty acid composition. MR$_{peak}$ is the maximum consecutive 1-min O$_2$ consumption of red-eyed vireos forced to hover and hop for ca 40 minutes in a modified enclosed running wheel. MR$_{peak}$ was significantly different at P <0.05 between diet groups.

This recent work demonstrates that fatty acid composition of a migratory bird affects energetic performance during intense exercise. Such whole-animal effects of fat composition of birds suggest that birds during migration would benefit from selecting foods with certain fatty acids. However, the results from the vireo study are intriguing in that vireos composed of less long-chain unsaturated fatty acids had higher peak metabolic rates even though vireos chose diets with more long-chain unsaturated foods during behavioral preference experiments. Perhaps there is some ''optimum'' fatty composition of migratory birds that maximizes the performance of birds during intense exercise, although this hypothesis has yet to be tested. For example, muscle phospholipids were more monounsaturated during migration in western sandpipers, and n-6 fatty acids decreased from premigration through migration seasons (Guglielmo et al. 2002b). Whether the very high constitutive levels of arachidonic acid (20:4n-6) measured in muscle phospholipids of premigrant sandpipers, or other changes in membrane FA profiles improve FA utilization is an interesting question for future study.

Energetic costs of migratory flight

In previous sections we have emphasized the importance of fat stores for fueling the energetic costs of migration, and the physiological processes and ecological conditions that influence the quality and quantity of fat stored by birds during migration. Although many associate migration with migratory flight, it is important to recognize that approximately 90% of the migration period is spent stationary at successive stopover sites (Hedenström and Alerstam 1998). Birds at stopover sites spend their time resting and foraging as they rebuild protein and energy stores in preparation for their next migratory flight. Recent estimates of energy expenditure of songbirds over their entire migration suggest that birds expend twice as much energy during stopovers than during migratory flight (Hedenström and Alerstam 1998, Wikelski et al. 2003). Thus, a complete energy budget of birds during migration must consider both the energy gains and losses incurred during stopovers as well as during migratory flight. We conclude this review with a summary of how the costs of migratory flight have been estimated, their accuracy relative to measured flight costs, and the application of these estimates of flight cost for predicting flight range, optimal departure fuel load, and size-dependency in breeding strategy of birds.

Flight costs can be estimated using aerodynamic theory (e.g., Pennycuick 1989) and allometric equations (e.g., Masman and Klaassen 1987). Aerodynamic models are often preferred because they can account for how variation in flight altitude, body mass, morphometry, air density, and other factors affect flight costs. But, how

well do estimates from aerodynamic models fit empirical flight cost estimates? In Fig. 4, we provide an overview of empirical flight cost estimates in unrestrained birds during prolonged flight and compared these with two predictions from aerodynamic theory: one from Pennycuick's original 1989 model (from now on referred to as Pen89) and one with the same model but using a much lower body drag coefficient (Pennycuick et al. 1996; referred to as Pen96). Although the match on the depicted log-log scale may look impressive, predictions according to aerodynamic theory were significantly different from empirical findings: on average 13% (range: 50 to 99%) and -28% (range: 66 to 51%) for Pen89 and Pen96 predictions, respectively (Repeated measures ANCOVA: $F_{2,30} = 3.33$, $P < 0.05$). In the remainder of
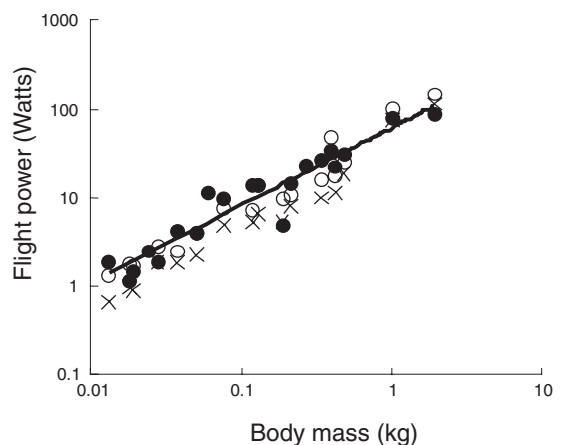

Fig. 4. Empirically estimated power requirements for flight for 20 species of birds in relation to body mass (black dots; $Y = 10^{1.780 \pm 0.073} X^{0.868 \pm 0.065}$, $R^2 = 0.908$; error terms are standard errors). In the same graph predicted power requirements according to Pennycuick (1989); circles; $Y = 10^{1.790 \pm 0.060} X^{0.914 \pm 0.055}$, $R^2 = 0.948$, N = 17) and Pennycuick et al. (1996); crosses; $Y = 10^{1.644 \pm 0.060} X^{0.975 \pm 0.055}$, $R^2 = 0.954$, N = 17) are depicted. Predictions from aerodynamic models were based on birds flying at sea level and with the species-specific wing characteristics and flight speeds. Most empirical flight cost studies provided for these parameters, but, data on wing morphometrics were also obtained from Robbins et al. (1966), Masman and Klaassen (1987), Tobalske and Dial (1994), Pennycuick et al. (1996), Butler et al. (1998), Bruderer and Boldt (2001), and from some internet sources. Additional flight speed data came from Bruderer and Boldt (2001). Studies included are on *Anas rubripes* (Berger et al. 1970), *Branta leucopsis* (Butler et al. 2000), *Calidris canutus* (Kvist et al. 2001), *Columba livia* (LeFebvre 1964, Butler et al. 1977, Rothe et al. 1987, Gessaman and Nagy 1988), *Corvus cryptoleucus* (Hudson and Bernstein 1983), *Delichon urbica* (Hails 1979, Westerterp and Bryant 1984), *Falco sparverius* (Gessaman 1980), *Falco tinnunculus* (Masman and Klaassen 1987), *Hirundo rustica* (Hails 1979, Turner 1982a,b), *Larus atricilla* (Tucker 1972), *Larus delawarensis* (Berger et al. 1970), *Luscinia luscinia* (Klaassen et al. 2000), *Melopsittacus undulatus* (Tucker 1966), *Progne subis* (Utter and Levebre 1970), *Riparia riparia* (Turner 1982a,b, Westerterp and Bryant 1984), *Sterna fuscata* (Flint and Nagy 1984), *Sturnus vulgaris* (Torre-Bueno and LaRochelle 1978, Westerterp and Drent 1985, Ward et al. 2001). Only the least square fit through the empirical data points is depicted.

this section we show that predicted flight range, optimal departure fuel load, and size-dependency in breeding strategy differ depending on the choice of model used.

Maximum flight range estimates

The maximum coverable distance is an important parameter in birds that have to migrate across inhospitable areas such as oceans and deserts, and for birds that make use of discontinuously distributed food resources and are bound to specific habitats for stopover. Aerodynamic theory predicts that flight range of birds sharply decreases with size (Klaassen 1996). However, if we estimate maximum flight range (m) by multiplying maximum fuel load of a bird (J) by flight speed (m/s) and then dividing this by the power requirements for flight (Watt or J/s) estimated from the empirical flight cost model, this results in a far smaller change in flight range with body size than predicted using aerodynamic theory (Klaassen 2003, also see Jenni-Eiermann and Jenni 2003).

Size-dependent breeding strategy in arctic breeding birds

"Capital" and "income" breeding in birds are two alternative breeding strategies characterized by the extent to which the nutritional and energy costs of reproduction are satisfied by endogenous resources versus local exogenous food resources. Klaassen (2003) investigated whether capital breeding in arctic-breeding birds was size-dependent by estimating the costs of migration and reproduction for birds of different sizes. If Pennycuick's predictions for power requirements of migratory flight model were used, then the capital breeding strategy was predicted to be extremely difficult for large birds given their short flight range and long refuelling times. However, if the empirical flight cost model was used, capital breeding was predicted to be a more likely breeding strategy for large birds. Support for the empirical flight cost model comes from various sources indicating the use of a capital breeding strategy in large but not small arctic breeding birds (Klaassen et al. 2001, Klaassen 2003).

Departure fuel load

Aerodynamic theory predicts that flight costs for an individual bird on migration will decrease as fuel stores are catabolised. These predictions have recently been challenged by Kvist et al. (2001) studying red knots flown in a windtunnel. In Fig. 5, Kvist and colleagues' findings for the red knot are plotted together with the Pen89 and Pen96 predictions. The empirical data suggest
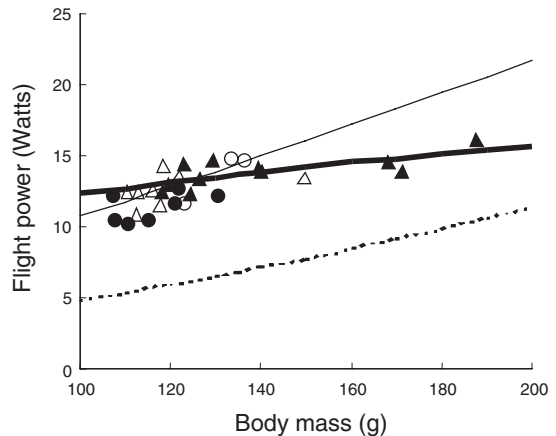

Fig. 5. Flight costs in relation to body mass for individual red knots (different symbols indicate different individuals) according to measurements by Kvist et al. 2001 (heavy line) and predictions by aerodynamic models (Pennycuick 1989, thin line; Pennycuick et al. 1996, dashed line). For the aerodynamic models we used the default settings of Pennycuick's (1989) program. For the model calculations, the red knot was assumed to have a lean mass of 100 g, with a wing span of 0.512 m, an aspect ratio of 8.35, an energy density of its fuel stores of 30 kJ/g, and to fly in air with an air density of 1.25 g/l.

that flight costs decrease at a much slower rate with a decrease in fuel load than indicated by the aerodynamic models. These differences between empirical findings and predictions of the aerodynamic model have important implications because aerodynamic models have been extensively used to predict aspects of stopover behavior of migrating birds such as optimal staging time, optimal
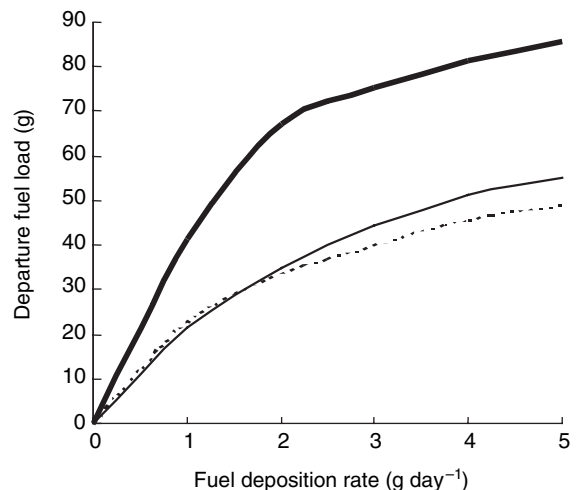

Fig. 6. Optimal departure fuel load in relation to fuel deposition rate (or site quality) for red knots exhibiting the three different flight cost curves depicted in Fig. 5: empirical flight cost model according to Kvist et al. 2001 (heavy line) and Pennycuick's 1989 (thin line) and Pennycuick et al. 1996 (dashed line) aerodynamic flight cost models. Optimal departure fuel loads were calculated according to Alerstam and Lindström 1990 assuming a search/settling time of 2 days.

range and optimal fuel load (e.g., Alerstam and Lindström 1990, Klaassen and Lindström 1996, Weber and Houston 1997, Weber et al. 1998). As an example, the empirical flight cost model predicts much higher optimal departure fuel loads than Pennycuick's models for a given fuel deposition rate (or site quality; Fig. 6).

In summary, measuring flight costs of a focal species is seldom feasible, forcing researchers to use empirical or theoretical models to generate predictions. The analyses presented by Weber and Houston (1997), Klaassen (2003) and here indicate that the choice of models may strongly affect the predictions, so that sensitivity analyses should be standard procedure in studies involving flight cost estimates.

Conclusions

The evolution of long distance migration in birds required a suite of behavioral, morphological, and physiological adaptations. Primary among these was the ability to sustain very high intensity exercise for very long periods of time. Although the predictions from the various energetic flight cost models show considerable error they all highlight the substantial power requirements for flight. The extreme endurance times achieved by migrants could therefore only be accomplished by the oxidation of FA originating in extramuscular adipose tissue. Ironically, the hydrophobic properties of fat that make it an ideal fuel for storage (high energy to wet mass ratio), also make it extremely difficult to transport and oxidize during intense exercise. Soluble protein carriers are needed at every step of FA transport. Major physiological adjustments in FA transport have likely occurred in pathways of circulatory transport, and especially in mechanisms of myocyte uptake. Progress has been made in the study of lipid metabolism of migratory birds, but there is much yet to be discovered at both the biochemical and physiological level. Future research should focus on the development of methods to measure the kinetics of endogenous and exogenous FA utilization (e.g., tracer infusions), the resolution of the issue of VLDL as a FA transporter in avian exercise, the identification of membrane transporters and their seasonal cycles, and the endocrine and metabolic regulation of FA transport and oxidation pathways. Migratory birds are superbly adapted to store and use fat. Throughout their lives they repeatedly become "morbidly obese", exercise at levels that far surpass elite athletes, and then cure themselves. Thus, they offer an intriguing model for study of lipid metabolism, exercise, and obesity.

References

Alerstam, T. and Hedenström, A. 1998. The development of bird migration theory. – J. Avian Biol. 29: 343–369.

Alerstam, T. and Lindström, Å. 1990. Optimal bird migration: the relative importance of time, energy, and safety. – In: Gwinner, E. (ed.). Bird migration. Springer, Berlin, pp. 331–351.

Andersson, A., Sjödin, A., Olsson, R. and Vessby, B. 1998. Effects of physical exercise of phospholipid fatty acid composition in skeletal muscle. – Am. J. Physiol. (Endocrin. Metab.) 274: E432–E438.

Ayre, K. J. and Hulbert, A. J. 1996. Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. – J. Nutrit. 126: 653–662.

Ayre, K. J. and Hulbert, A. J. 1997. Dietary fatty acid profile affects endurance in rats. – Lipids 32: 1265–1270.

Bairlein, F. 1991. Nutritional adaptations to fat deposition in the long-distance migratory garden warbler (*Sylvia borin*). – Acta Congr. Intern. Ornithol. 20: 2149–2158.

Bairlein, F. 1998. The effect of diet composition on migratory fuelling in garden warblers *Sylvia borin*. – J. Avian Biol. 29: 546–551.

Bairlein, F. and Gwinner, E. 1994. Nutritional mechanisms and temporal control of migratory energy accumulation in birds. – Ann. Rev. Nutr. 14: 187–215.

Battley, P. F., Dietz, M. W., Piersma, T., Tang, S., Dekinga, A. and Hulsman, K. 2000. Empirical evidence for differential organ reductions during trans-oceanic bird flight. – Proc. R. Soc. London 267B: 191–196.

Battley, P. F., Dietz, M. W., Piersma, T., Dekinga, A., Tang, S. and Hulsman, K. 2001. Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. – Physiol. Biochem. Zool. 74: 435–449.

Bauchinger, U. and Biebach, H. 2001. Differential catabolism of muscle protein in garden warblers (*Sylvia borin*): flight and leg muscle act as a protein source during long-distance migration. – J. Comp. Physiol. 171B: 293–301.

Berger, M., Hart, J. S. and Roy, O. Z. 1970. Respiration, oxygen consumption and heart rate in some birds during rest and flight. – Z. vergl. Physiologie 66: 201–214.

Biebach, H. 1996. Energetics of winter and migratory fattening. – In: Carey, C. (ed.). Avian energetics and nutritional ecology. Chapman and Hall, New York, pp. 280–323.

Biebach, H., Friedrich, W. and Heine., G. 1986. Interaction of body mass, fat, foraging and stopover period in trans-Sahara migrating passerine birds. – Oecologia 69: 370–379.

Blem, C. R. 1976. Patterns of lipid storage and utilization in birds. – Am. Zool. 16: 671–684.

Blem, C. R. 1980. The energetics of migration. – In: Gauthreaux, S. A. Jr. (ed.). Animal migration, orientation, and navigation. Academic Press, New York, pp. 175–224.

Blem, C. R. 1990. Avian energy storage. – In: Power., M. (ed.). Curr. Ornithology. Plenum Press, New York, pp. 59–113.

Bonen, A., Luiken, J. J. F. P., Arumugam, Y., Glatz, J. F. C. and Tandon, N. N. 2000. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. – J. Biol. Chem. 275: 14501–14508.

Bordel, R. and Haase, E. 1993. Effects of flight on blood parameters in homing pigeons. – J. Comp. Physiol. B 163: 219–224.

Bower, E. B. and Helms, C. W. 1968. Seasonal variation in fatty acids of the slate-colored junco (*Junco hyemalis*). – Physiol. Zool. 21: 157–168.

Bruderer, B. and Boldt, A. 2001. Flight characteristics of birds: I. radar measurements of speeds. – Ibis 143: 178–204.

Butler, P. J. and Bishop, C. M. 2000. Flight. – In: Whittow, G. C. (ed.). Sturkie's avian physiology. Academic Press, New York, pp. 391–435.

Butler, P. J. and Woakes, A. J. 1990. The physiology of bird flight. – In: Gwinner, E. (ed.). Bird migration: physiology and ecophysiology. Springer-Verlag, New York, pp. 300–318.

Butler, P. J., West, N. H. and Jones, D. R. 1977. Respiratory and cardiovascular responses of the pigeon to sustained level flight in a wind-tunnel. – J. Exp. Biol. 71: 7–26.

Butler, P. J., Woakes, A. J., Bevan, R. M. and Stephenson, R. 2000. Heart rate and rate of oxygen consumption during flight of the barnacle goose, *Branta leucopsis*. – Comp. Biochem. Physiol. 126A: 379–385.

Butler, P. J., Woakes, A. J. and Bishop, C. M. 1998. Behaviour and physiology of Svalbard barnacle geese *Branta leucopsis* during their autumn migration. – J. Avian Biol. 29: 536–545.

Caldwell, L. D. 1973. Fatty acids of migrating birds. – Comp. Biochem. Physiol. 44B: 493–497.

Chappell, M. A., Bech, C. and Buttermer, W. A. 1999. The relationship of central and peripheral organ masses to aerobic performance. – J. Exp. Biol. 202: 2269–2279.

Collins, D. M. and Hargis, P. S. 1989. Distributions of fatty acid binding proteins in tissues and plasma of *Gallus domesticus*. – Comp. Biochem. Physiol. 92B: 283–289.

Conway, C. J., Eddleman, W. R. and Simpson, K. L. 1994. Seasonal changes in fatty acid composition of the wood thrush. – Condor 96: 791–794.

Dial, K. P., Biewener, A. A., Tobalske, B. W. and Warrick, D. R. 1997. Mechanical power output of bird flight. – Nature 390: 67–70.

Driedzic, W. R., Crowe, H. L., Hicklin, P. W. and Sephton, D. H. 1993. Adaptations in pectoralis muscle, heart mass, and energy metabolism during premigratory fattening in semi-palmated sandpipers (*Calidris pusilla*). – Can. J. Zool. 71: 1602–1608.

Egeler, O. and Williams, T. D. 2000. Seasonal, age, and sex-related variation in fatty-acid composition of depot fat in relation to migration in western sandpipers. – Auk 117: 110–119.

Evans, P. R., Davidson, N. C., Uttley, J. D. and Evans, R. D. 1992. Premigratory hypertrophy of flight muscles: an ultra-structural study. – Ornis Scand. 23: 238–243.

Flint, E. N. and Nagy, K. A. 1984. Flight energetics of free-living sooty terns. – Auk 101: 288–295.

Gannes, L. Z. 2001. Comparative fuel use of migrating passerines: effects of fat stores, migration distance, and diet. – Auk 118: 665–677.

Gannes, L. Z. 2002. Mass change pattern of blackcaps refueling during spring migration: evidence for physiological limitations to food assimilation. – Condor 104: 231–239.

George, J. C. and John, T. M. 1993. Flight effects on certain blood parameters in homing pigeons *Columba livia*. – Comp. Biochem. Physiol. 106A: 707–712.

George, J. C. and Vallyathan, N. V. 1964. Lipase and succinate dehydrogenase activity of the particulate fractions of the breast muscle homogenate of the migratory starling *Sturnus roseus* in the pre-migratory and post-migratory periods. – Can. J. Physiol. Pharm. 42: 447–452.

Gessaman, J. A. 1980. An evaluation of heart rate as an indirect measure of daily energy metabolism of the American kestrel. – Comp. Biochem. Physiol. 65A: 273–289.

Gessaman, J. A. and Nagy, K. A. 1988. Transmitter loads affect the flight speed and metabolism of Homing Pigeons. – Condor 90: 662–668.

Guglielmo, C. G. and Williams, T. D. 2003. Phenotypic flexibility of body composition in relation to migratory state, age, and sex in the western sandpiper (*Calidris mauri*). – Physiol. Biochem. Zool. 76: 84–98.

Guglielmo, C. G., Haunerland, N. H. and Williams, T. D. 1998. Fatty acid binding protein, a major protein in the flight muscle of migrating western sandpipers. – Comp. Biochem. Physiol. 119B: 549–555.

Guglielmo, C. G., Haunerland, N. H., Hochachka, P. W. and Williams, T. D. 2002a. Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a long-distance migrant shorebird. – Am. J. Physiol. 282: R1405–R1413.

Guglielmo, C. G., Williams, T. D., Zwingelstein, G., Brichon, G. and Weber, J-M. 2002b. Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird. – J. Comp. Physiol. B 172: 409–417.

Hails, C. J. 1979. A comparison of flight energetics in hirundines and other birds. – Comp. Biochem. Physiol. 63A: 581–585.

Hatch, K. A., Pinshow, B. and Speakman, J. R. 2002. The analysis of $^{13}C/^{12}C$ ratios in exhaled CO_2: its advantages and potential application to field research to infer diet, changes in diet over time, and substrate metabolism in birds. – Integ. Comp. Biol. 42: 21–33.

Haunerland, N. H. 1994. Fatty acid binding protein in locust and mammalian muscle. Comparison of structure function and regulation. – Comp. Biochem. Physiol. 109B: 199–208.

Havel, R. J. 1986. Lipid transport function of lipoproteins in blood plasma. – Am. J. Physiol. 253: E1–E5.

Hedenström, A. and Alerstam, T. 1998. How fast can birds migrate? – J. Avian Biol. 29: 424–432.

Heitmeyer, M. E. and Fredrickson, L. H. 1990. Fatty acid composition of wintering female mallards in relation to nutrient use. – J. Wildl. Manage. 54: 54–61.

Hochachka, P. W. and Somero, G. N. 2002. Biochemical adaptation – mechanisms and process in physiological evolution, 2nd edition. – Oxford University Press, New York.

Hudson, D. M. and Bernstein, M. H. 1983. Gas exchange and energy cost of flight in the white-necked raven, *Corvus cryptoleucos*. – J. Exp. Biol. 103: 121–130.

Hume, I. and Biebach, H. 1996. Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. – J. Comp. Physiol. 166B: 388–395.

Jenni, L. and Jenni-Eiermann, S. 1998. Fuel supply and metabolic constraints in migrating birds. – J. Avian Biol. 29: 521–528.

Jenni-Eiermann, S. and Jenni, L. 1991. Metabolic responses to flight and fasting in night-migrating passerines. – J. Comp. Physiol. B 161: 465–474.

Jenni-Eiermann, S. and Jenni, L. 1992. High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates? – Physiol. Zool. 65: 112–123.

Jenni-Eiermann, S. and Jenni, L. 2003. Fuel deposition rates in migrating birds: causes, constraints and consequences. – In: Berthold, P., Gwinner, E. and Sonnenschein, E. (eds). Avian migration. Springer-Verlag, New York, pp. 293–306.

Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindström, Å. and Piersma, T. 2001. Fuel use and metabolic response to endurance exercise: a windtunnel study of a long-distance migrant shorebird. – J. Exp. Biol. 205: 2453–2460.

Karasov, W. H. 1996. Digestive plasticity in avian energetics and feeding ecology. – In: Carey, C. (ed.). Avian energetics and nutritional ecology. Chapman and Hall, New York, pp. 61–84.

Karasov, W. H. and McWilliams, S. R. 2004. Digestive constraint in mammalian and avian ecology. – In: Starck, J. M. and Wang, T. (eds). Consequences of feeding in vertebrates. Science Publishers Inc., India.

Karasov, W. H. and Pinshow, B. 1998. Changes in lean mass and in organs of nutrient assimilation in a long-distance migrant at a springtime stopover site. – Physiol. Zool. 71: 435–448.

Karasov, W. H. and Pinshow, B. 2000. Test for physiological limitation to nutrient assimilation in a long-distance passer-

ine migrant at a springtime stopover site. – Physiol. Biochem. Zool. 73: 335–343.

King, J. R. and Murphy, M. 1985. Periods of nutritional stress in the annual cycles of endotherms: fact or fiction? – Am. Zool. 25: 955–964.

Klaassen, M. 1996. Metabolic constraints on long-distance migration in birds. – J. Exp. Biol. 199: 57–64.

Klaassen, M. 2003. Relationships between migration and breeding strategies in arctic breeding birds. – In: Berthold, P., Gwinner, E. and Sonnenschein, E. (eds) Avian migration. Springer-Verlag, Berlin, pp. 237–249.

Klaassen, M. and Biebach, H. 1994. Energetics of fattening and starvation in the long-distance migratory garden warbler, *Sylvia borin*, during the migratory phase. – J. Comp. Physiol B 164: 362–371.

Klaassen, M. and Lindström, Å. 1996. Departure fuel loads in time-minimizing migrating birds can be explained by the energy costs of being heavy. – J. Theor. Biol. 183: 29–34.

Klaassen, M., Lindström, Å. and Zijlstra, R. 1997. Composition of fuel stores and digestive limitations to fuel deposition rate in the long-distance migratory thrush nightingale *Luscinia luscinia*. – Physiol. Zool. 70: 125–133.

Klaassen, M., Kvist, A. and Lindström, Å. 2000. Flight costs and fuel composition of a bird migrating in a wind tunnel. – Condor 102: 444–451.

Klaassen, M., Lindström, Å., Meltofte, H. and Piersma, T. 2001. Ornithology-Arctic waders are not capital breeders. – Nature 413: 794–794.

Klasing, K. C. 1998. Comparative avian nutrition. – CAB International, New York.

Kvist, A., Lindström, Å., Green, M., Piersma, T. and Visser, G. H. 2001. Carrying large fuel loads during sustained bird flight is cheaper than expected. – Nature 413: 730–732.

Landys-Ciannelli, M. M., Jukema, J., Piersma, T. and Castricum Ringing Group. 2002. Blood parameter changes during stopover in a long-distance migratory shorebird, the bar-tailed godwit *Limosa lapponica taymyrensis*. – J. Avian Biol. 33: 451–455.

Laurin, E. E., Touchburn, S. P., Chavez, E. R. and Chan, C. W. 1985. Effect of dietary fat supplementation on the carcass composition of three genetic lines of broilers. – Poultry Science 64: 2131–2135.

LeFebvre, E. A. 1964. The use of $D_2{}^{18}O$ for measuring energy metabolism in *Columba livia* at rest and in flight. – Auk 81: 403–416.

Leyton, J., Drury, P. J. and Crawford, M. A. 1987. Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. – Brit. J. Nutr. 57: 383–393.

Lindström, Å. 1991. Maximum fat deposition rates in migrating birds. – Ornis Scand. 22: 12–19.

Lindström, Å. 2003. Fuel deposition rates in migrating birds: causes, constraints, and consequences. – In: Berthold, P., Gwinner, E. and Sonnenschein, E. (eds). Avian migration. Springer-Verlag, Berlin, pp. 307–320.

Lindström, Å. and Piersma, T. 1993. Mass changes in migrating birds: the evidence for fat and protein storage re-examined. – Ibis 135: 70–78.

Luiken, J. J. F. P., Schaap, F. G., van Nieuwenhoven, F. A., van der Vusse, G. J. and Glatz, J. F. C. 1999. Cellular fatty acid transport in heart and skeletal muscle as facilitated by proteins. – Lipids 34: S169–S175.

Lundgren, B. O. and Kiessling, K.-H. 1985. Seasonal variation in catabolic enzyme activities in breast muscle of some migratory birds. – Oecologia 66: 468–471.

Lundgren, B. O. and Kiessling, K.-H. 1986. Catabolic enzyme activities in the pectoralis muscle of premigratory and migratory juvenile reed warblers *Acrocephalus scirpaceus* (Herm.). – Oecologia 68: 529–532.

Lundgren, B. O. and Kiessling, K.-H. 1988. Comparative aspects of fibre types, areas, and capillary supply in the pectoralis muscle of some passerine birds with differing migratory behaviour. – J. Comp. Physiol. B 158: 165–173.

Marsh, R. L. 1981. Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the gray catbird (*Dumetella carolinensis*). – J. Comp. Physiol. B 141: 417–423.

Masman, D. and Klaassen, M. 1987. Energy expenditure during free flight in trained and free-living Eurasian kestrels (*Falco tinnunculus*). – Auk 104: 603–616.

McArthur, M. J., Atshaves, B. P., Frolov, A., Foxworth, W. D., Kier, A. B. and Schroeder, F. 1999. Cellular uptake and intracellular trafficking of long chain fatty acids. – J. Lipid Res. 40: 1371–1383.

McKenzie, D. J., Higgs, D. A., Dosanjh, B. S., Deacon, G. and Randall, D. J. 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. – Fish Physiol. Biochem. 19: 111–122.

McKenzie, D. J., Piraccini, G., Papini, N., Galli, C., Bronzi, P., Bolis, C. G. and Taylor, E. W. 1997. Oxygen consumption and ventilatory reflex responses are influenced by dietary lipids in sturgeon. – Fish Physiol. Biochem. 16: 365–379.

McWilliams, S. R. and Karasov, W. H. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. – Comp. Biochem. Physiol. 128A: 579–593.

McWilliams, S. R. and Karasov, W. H. 2004. Migration takes guts: digestive physiology of migratory birds and its ecological significance. – In: Marra, P. and Greenberg, R. (eds). Birds of Two Worlds. Johns Hopkins University Press, Baltimore, M. D.

McWilliams, S. R., Kearney, S. and Karasov, W. H. 2002. Dietary preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. – J. Avian Biol. 33: 167–174.

Moore, F. and Kerlinger, P. 1987. Stopover and fat deposition by North American wood-warblers (*Parulinae*) following spring migration over the Gulf of Mexico. – Oecologia 74: 47–54.

Morton, M. L. and Liebman, H. A. 1974. Seasonal variations in fatty acids of a migratory bird with and without a controlled diet. – Comp. Biochem. Physiol. 48A: 329–335.

Parrish, J. D. 1997. Patterns of frugivory and energetic condition in nearctic landbirds during autumn migration. – Condor 99: 681–697.

Pelsers, M. M. A. L., Butler, P. J., Bishop, C. M. and Glatz, J. F. C. 1999. Fatty acid binding protein in heart and skeletal muscles of the migratory barnacle goose throughout development. – Am. J. Physiol. 276: R637–R643.

Pennycuick, C. J. 1989. Bird flight performance: a practical calculation manual. – Oxford University Press.

Pennycuick, C. J. 1998. Computer simulation of fat and muscle burn in long-distance bird migration. – J. Theor. Biol. 191: 47–61.

Pennycuick, C. J., Klaassen, M., Kvist, A. and Lindström, Å. 1996. Wingbeat frequency and the body drag anomaly: wind-tunnel observations on a thrush nightingale (*Luscinia luscinia*) and a teal (*Anas crecca*). – J. Exp. Biol. 199: 2757–2765.

Pierce, B. 2003. The nutritional ecology of songbirds: how food quality, diet preferences, and food limitation influence nutrient storage and use during migration. – Ph.D. dissertation, University of Rhode Island, Kingston, R.I.

Pierce, B. and McWilliams, S. R. 2004. Interactive effects of diet quality and food limitation on the dynamics of body composition and digestive organs in a migratory bird. – Physiol. Biochem. Zool. 77, in press.

Pierce, B. J., McWilliams, S. R., Place, A. R. and Huguenin, M. A. 2004. Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory red-eyed vireos (*Vireo olivaceous*). – Comp. Biochem. Physiol. A, in press.

Pierce, B. J., McWilliams, S. R., O'Connor, T., Place, A. R. and Guglielmo, C. 2005. Effect of dietary fatty acid composition on depot fat and exercise performance in red-eyed vireos:

implications for songbirds during migration. – J. Exp. Biol., in press.

Piersma, T. 1990. Pre-migratory "fattening" usually involves more than the deposition of fat alone. – Ring. Migr. 11: 113–115.

Piersma, T. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight. – J. Avian Biol. 29: 511–520.

Piersma, T. 2002. Energetic bottlenecks and other design constraints in avian annual cycles. – Integ. Comp. Biol. 42: 51–67.

Piersma, T. and Jukema, J. 1990. Budgeting the flight of a long-distance migrant: changes in nutrient reserve levels of bar-tailed godwits at successive spring staging sites. – Ardea 78: 315–337.

Piersma, T. and Lindström, Å. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. – Trends Ecol. Evol. 12: 134–138.

Piersma, T., Everaarts, J. M. and Jukema, J. 1996. Build-up of red blood cells in refuelling bar-tailed godwits in relation to individual migratory quality. – Condor 98: 363–370.

Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. – Physiol. Biochem. Zool. 72: 405–415.

Poortmans, J. R. and Vanderstraeten, J. 1994. Kidney function during exercise in healthy and diseased humans. – Sports Medicine 18: 419–437.

Raclot, T. 2003. Selective mobilization of fatty acids from adipose tissue triacylglycerols. – Prog. Lipid Res. 42: 257–288.

Raclot, T. and Groscolas, R. 1995. Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. – J. Lipid Res. 36: 2164–2173.

Ramenofsky, M. 1990. Fat storage and fat metabolism in relation to migration. – In: Gwinner, E. (ed.). Bird migration: physiology and ecophysiology. Springer-Verlag, New York, pp. 214–231.

Ramenofsky, M., Savard, R. and Greenwood, M. R. C. 1999. Seasonal and diel transitions in physiology and behavior in the migratory dark-eyed junco. – Comp. Biochem. Physiol. 122A: 385–397.

Randall, D., Burggren, W. and French, K. 2002. Eckert animal physiology: mechanisms and adaptations, 5th edition. – W. H. Freeman and Company, New York.

Rappole, J. H. and Warner, D. W. 1976. Relationships between behavior, physiology and weather in avian transients at a migration stopover site. – Oecologia 26: 193–212.

Robbins, C. S., Bruun, B. and Zim, H. S. 1966. Birds of North America. – Golden Press.

Roberts, T. J., Weber, J-M., Hoppeler, H., Weibel, E. R. and Taylor, C. R. 1996. Design of the oxygen and substrate pathways. II. Defining the upper limits of carbohydrate and fat oxidation. – J. Exp. Biol. 199: 1651–1658.

Rosebrough, R. W. and McMurtry, J. P. 1993. Protein and energy relations in the broiler chicken. II. Effects of protein quantity and quality on metabolism. – Brit. J. Nutr. 70: 667–678.

Rothe, H. J., Biesel, W. and Nachtigall, W. 1987. Pigeon flight in a wind tunnel II. Gas exchange and power requirements. – J. Comp. Physiol. 157B: 99–109.

Savard, R., Ramenofsky, M. and Greenwood, M. R. C. 1991. A north temperate migratory bird: a model for the fate of lipids during exercise of long duration. – Can. J. Physiol. Pharm. 69: 1443–1447.

Schwilch, R., Jenni, L. and Jenni-Eiermann, S. 1996. Metabolic responses of homing pigeons to flight and subsequent recovery. – J. Comp. Physiol. B 166: 77–87.

Starck, J. M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates-implications for evolutionary morphology. – Zool. Anzeiger 238: 87–101.

Stiles, E. W. 1993. The influence of pulp lipids on fruit preference by birds. – Vegetatio 107/108: 227–235.

Suarez, R. K. 1992. Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. – Experientia 48: 565–570.

Suarez, R. K., Lighton, J. R. B., Moyes, C. D., Brown, G. S., Gass, C. L. and Hochachka, P. W. 1990. Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. – Proc. Nat. Acad. Sci. 87: 9207–9210.

Thomas, V. G. and George, J. C. 1975. Plasma and depot fat fatty acids in Canada geese in relation to diet, migration, and reproduction. – Physiol. Zool. 48: 157–167.

Tobalske, B. W. and Dial, K. P. 1994. Neuromuscular control and kinematics of intermittent flight in budgerigars (*Melopsittacus-Undulatus*). – J. Exp. Biol. 187: 1–18.

Tobalske, B. W., Hedrick, T. L., Dial, K. P. and Biewener, A. A. 2003. Comparative power curves in bird flight. – Nature 421: 363–366.

Torre-Bueno, J. R. and LaRochelle, J. 1978. The metabolic cost of flight in unrestrained birds. – J. Exp. Biol. 75: 223–229.

Tucker, V. A. 1966. Oxygen consumption of a flying bird. – Science 154: 150–151.

Tucker, V. A. 1972. Metabolism during flight in the laughing gull, *Larus atricilla*. – Am. J. Physiol 222: 237–245.

Turner, A. K. 1982a. Optimal foraging by the swallow (*Hirundo rustica*): prey size selection. – Anim. Behav. 30: 862–872.

Turner, A. K. 1982b. Timing of laying by swallows (*Hirundo rustica*) and sand martins (*Riparia riparia*). – J. Animal Ecol. 51: 29–46.

Utter, J. M. and Levebre, E. A. 1970. Energy expenditure for free-flight by the purple martin (*Progne subis*). – Comp. Biochem. Physiol. 35: 713–719.

Van der Meer, J. and Piersma, T. 1994. Physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. – Physiol. Zool. 67: 305–329.

Vock, R., Weibel, E. R., Hoppeler, H., Ordway, G., Weber, J-M. and Taylor, C. R. 1996. Design of the oxygen substrate pathways. V. Structural basis of vascular substrate supply to muscle cells. – J. Exp. Biol. 199: 1675–1688.

Vork, M. M., Glatz, J. F. C. and van der Vusse, G. J. 1993. On the mechanism of long chain fatty acid transport in cardiomyocytes as facilitated by cytoplasmic fatty acid binding protein. – J. Theor. Biol. 160: 207–222.

Walker, A. T. 1964. Major fatty acids in migratory bird fat. – Physiol. Zool. 37: 57–64.

Walsberg, G. E. and Wolf, B. O. 1995. Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. – J. Exp. Biol. 198: 213–219.

Ward, S., Moller, U., Rayner, J. M. V., Jackson, D. M., Bilo, D., Nachtigall, W. and Speakman, J. R. 2001. Metabolic power, mechanical power and efficiency during wind tunnel flight by the European starling *Sturnus vulgaris*. – J. Exp. Biol. 204: 3311–3322.

Weber, J-M. 1988. Design of exogenous fuel supply systems: adaptive strategies for endurance locomotion. – Can. J. Zool. 66: 1116–1121.

Weber, J-M. 1992. Pathways for oxidative fuel provision to working muscles: Ecological consequences of maximal supply limitations. – Experientia 48: 557–564.

Weber, J-M., Brichon, G., Zwingelstein, G., McClelland, G., Saucedo, C., Weibel, E. R. and Taylor, C. R. 1996b. Design of the oxygen and substrate pathways. IV. Partitioning energy provision from fatty acids. – J. Exp. Biol. 199: 1667–1674.

Weber, J-M., Roberts, T. J., Vock, R., Weibel, E. R. and Taylor, C. R. 1996a. Design of the oxygen and substrate pathways. III. Partitioning energy provision from carbohydrates. – J. Exp. Biol. 199: 1659–1666.

Weber, T. P. and Houston, A. I. 1997. Flight costs, flight range and the stopover ecology of migrating birds. – J. Animal Ecol. 66: 297–306.

Weber, T. P., Ens, B. J. and Houston, A. I. 1998. Optimal avian migration: A dynamic model of fuel stores and site use. – Evol. Ecol. 12: 377–401.

West, G. C. and Meng, M. S. 1968. Seasonal changes in body weight and fat and the relation of fatty acid composition to diet in the Willow Ptarmigan. – Wilson Bull. 80: 426–441.

West, G. C. and Peyton, L. J. 1980. Fatty acids of depot lipids in migrating lapland longspurs. – J. Field Ornithol. 51: 138–143.

Westerterp, K. and Drent, R. 1985. Energetic cost and energy saving mechanisms in parental care of free-living passerine birds as determined by the $D_2{}^{18}O$ method. – Acta Congr. Int. Ornithol. 18: 392–398.

Westerterp, K. R. and Bryant, D. M. 1984. Energetics of free existence in swallows and martins (*Hirundinidae*) during breeding: a comparative study using doubly labeled water. – Oecologia 62: 376–381.

Wikelski, M., Tarlow, E. M., Raim, A., Diehl, R. H., Larkin, R. P. and Visser, G. H. 2003. Costs of migration in free-flying songbirds. – Nature 423: 704.

Zurovchak, J. G. 1997. Nutritional role of high-lipid fruits in the diet of migrant thrushes. – Ph.D. dissertation, Rutgers University, New Brunswick, N. J.