

REVIEW

Obese super athletes: fat-fueled migration in birds and bats

Christopher G. Guglielmo*

ABSTRACT

Migratory birds are physiologically specialized to accumulate massive fat stores (up to 50–60% of body mass), and to transport and oxidize fatty acids at very high rates to sustain flight for many hours or days. Target gene, protein and enzyme analyses and recent -omic studies of bird flight muscles confirm that high capacities for fatty acid uptake, cytosolic transport, and oxidation are consistent features that make fat-fueled migration possible. Augmented circulatory transport by lipoproteins is suggested by field data but has not been experimentally verified. Migratory bats have high aerobic capacity and fatty acid oxidation potential; however, endurance flight fueled by adipose-stored fat has not been demonstrated. Patterns of fattening and expression of muscle fatty acid transporters are inconsistent, and bats may partially fuel migratory flight with ingested nutrients. Changes in energy intake, digestive capacity, liver lipid metabolism and body temperature regulation may contribute to migratory fattening. Although control of appetite is similar in birds and mammals, neuroendocrine mechanisms regulating seasonal changes in fuel store set-points in migrants remain poorly understood. Triacylglycerol of birds and bats contains mostly 16 and 18 carbon fatty acids with variable amounts of 18:2n-6 and 18:3n-3 depending on diet. Unsaturation of fat converges near 70% during migration, and unsaturated fatty acids are preferentially mobilized and oxidized, making them good fuel. Twenty and 22 carbon n-3 and n-6 polyunsaturated fatty acids (PUFA) may affect membrane function and peroxisome proliferator-activated receptor signaling. However, evidence for dietary PUFA as doping agents in migratory birds is equivocal and requires further study.

KEY WORDS: Exercise, Flight, Lipids, Metabolism, Polyunsaturated fatty acids, PPAR

Introduction

When spring arrives near my home in Canada, the land erupts with the colors and songs of birds not seen since the previous year. Most will only pass through after fueling up for their remaining journey to the boreal forest. The lakes, coasts and wetlands across the continent become crowded with waterfowl and shorebirds feeding frenetically to beat the clock and reach the forests and tundra of the North in time to breed during the short but bountiful summer. The migratory ‘tree-bats’, only three species but in uncounted numbers, come back too, the females carrying the extra burden of pregnancy while they migrate. The process reverses in the autumn as seasoned and newly born travelers gamble on reaching hospitable wintering areas at lower latitudes. Many, especially the young, do not survive these journeys; culled by predators (wild and domestic), poor weather, unrecoverable navigation errors, disease, collisions (with windows,

vehicles and power generation infrastructure) and other threats (Calvert et al., 2013; Newton, 2008; Sillet and Holmes, 2002). A lack of suitable refueling habitat may also slow or defeat the migration effort (Newton, 2008). Similar stories play out across the Earth every year as billions of birds and bats migrate, often thousands of kilometers, along with countless insects, fish, marine and terrestrial mammals, and other animals (Dingle, 1996). This great flux of energy and biomass moving across the planet is powered mainly by fat (Blem, 1980), a light, energy-dense tissue with the fundamental biological function of storing energy in times of abundance for use in times of need. Although much maligned by modern, health-conscious humans, fat is good for migratory birds and bats.

Many excellent reviews summarize aspects of fat storage and utilization in migratory birds (Bairlein, 2002; Berthold, 1993; Biebach, 1996; Blem, 1976, 1980; Guglielmo, 2010; Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004; Newton, 2008; Pierce and McWilliams, 2014; Price, 2010; Ramenofsky, 1990; Weber, 2009), with occasional information on bats (Blem, 1980; Fleming and Eby, 2003; McGuire and Guglielmo, 2009). The purpose of this Review is to use recent findings to compare the role of fat as a fuel for migration of birds and bats. I will highlight key similarities and differences in the deposition of fat stores, the mechanisms used to support fat-fueled flight, and the effects of the fatty acid composition of fat stores on migration performance.

Both the flight and refueling phases of migration are physiologically demanding for birds. Soaring flight can be very inexpensive (Hedenström, 1993; Videler, 2005) but flapping flight requires an energy expenditure that is nearly 12 times the basal metabolic rate (BMR) of most birds, with aerial feeding specialists (swifts, swallows and martins) flying more cheaply (~5×BMR; Fig. 1). Migrants likely exercise at 70–90% of their maximum rate of oxygen consumption ($\dot{V}_{O_{2,max}}$), which is similar in a relative sense to a human elite marathon runner (Guglielmo et al., 2002a). In absolute terms, a flying bird consumes oxygen at approximately double the $\dot{V}_{O_{2,max}}$ of a running mammal of the same mass (e.g. a sparrow compared with a mouse) because the mechanical costs to produce lift and thrust in flight, even at the minimum power speed, are so great (Butler and Woakes, 1990). Yet, an even more striking feature of avian migration is the length of time that birds can sustain such intense exercise without eating or drinking (Newton, 2008). Some species of shorebirds cross oceans by flying non-stop for several days or even for more than a week (e.g. the bar-tailed godwit, *Limosa lapponica baueri*; Gill et al., 2009). Small songbirds typically fly for many hours overnight when crossing land; however, they are also capable of impressive flights lasting several days. The ~25-g northern wheatear (*Oenanthe oenanthe*) travels 14,500 km each way from the North American tundra to wintering areas in sub-Saharan Africa, including non-stop, multiday flights over the North Atlantic Ocean (Bairlein et al., 2012). Smaller still, the 12-g blackpoll warbler (*Setophaga striata*) can fly for almost three days (62 h) over the open Atlantic Ocean as it flies from Nova Scotia to the West Indies and South America (DeLuca et al.,

Department of Biology, Advanced Facility for Avian Research, University of Western Ontario, London, Ontario, Canada N6A5B7.

*Author for correspondence (cguglie2@uwo.ca)

 C.G.G., 0000-0001-5857-3301

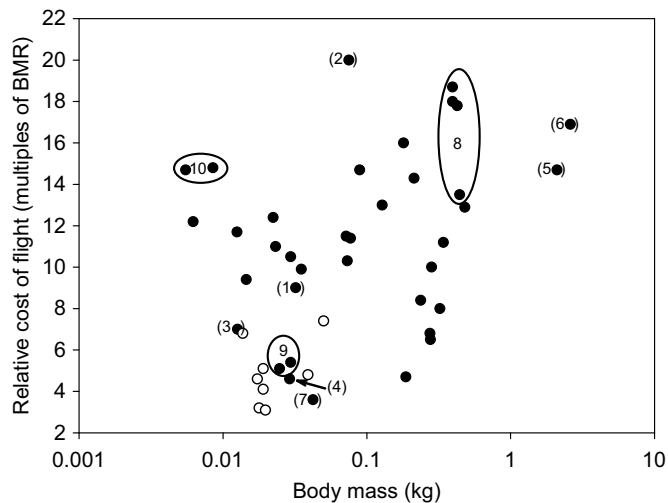


Fig. 1. Relative energy costs of flapping flight for birds expressed as the fold difference from basal metabolic rate (BMR). Data are replotted from table 9.2 in Videler (2005) with soaring birds excluded. Additional data are included: (1) Swainson's thrush, *Catharus ustulatus* (Gerson and Guglielmo, 2011), (2) American robin, *Turdus migratorius* (Gerson and Guglielmo, 2013; BMR estimated from Reynolds and Lee, 1996), (3) yellow-rumped warbler, *Setophaga coronata* (Guglielmo et al., 2017) and (4) western sandpiper, *Calidris mauri* (Maggini et al., 2017a,b). Aerial insectivores (swifts, martins and swallows) have relatively low flight costs and are indicated by open symbols. Other notable observations include: (5) barnacle goose, *Branta leucopsis*, (6) bar-headed goose, *Anser indicus*, (7) Wilson's storm petrel, *Oceanites oceanicus*, (8) rock dove, *Columba livia*, (9) thrush nightingale, *Luscinia luscinia* and (10) hummingbirds.

2015). Trans-Saharan/Mediterranean migrants have similar endurance capabilities when weather conditions permit (Bairlein, 1992; Klaassen et al., 2011; Schmaljohann et al., 2007). Refueling at stopovers between flights is also physiologically demanding, with the combined energy costs of intense foraging and thermoregulation summing to as much as 70–90% of the entire cost of migration (Wikelski et al., 2003). Some birds may expend up to half of their annual energy budget migrating (Drent and Piersma, 1990).

Bats evolved flight independently from birds and, hence, bats offer a complementary model to birds for understanding physiological aspects of flight and migration. Compared with birds, long-distance migratory bats are elusive and have been poorly studied. Generally, the maximum migration distances of bats appear to be much shorter (<2000 km) than those made by birds (Fleming and Eby, 2003). Some bats are thought to fly continuously for 7–9 h to cross barriers such as the Baltic Sea, but they are not generally believed to make multiday non-stop flights (Fleming and Eby, 2003). Some evidence suggests that when flying over land, bats may interrupt flight periodically to rest, drink or feed (Voigt et al., 2012). Aside from scant information on seasonal changes in fat stores (Blem, 1980; Fleming and Eby, 2003), we knew very little about bat migratory behavior and physiology, until recently. Interest in migratory bats has been heightened owing to their disproportionate vulnerability to wind turbines (Kunz et al., 2007), and this has led to recent progress in understanding their migratory biology. In North America, three species of so called 'tree-bats' [hoary bat (*Lasiurus cinereus*), silver-haired bat (*Lasionycteris noctivagans*) and red bat (*Lasiurus borealis*)] as well as a few subtropical species (*Leptonycteris curasoae*, *Leptonycteris nivalis*, *Tadarida brasiliensis* and *Choeronycteris mexicana*) migrate thousands of kilometers (Cryan, 2003; Fleming and Eby, 2003). Several European bat species, such as

Nyctalus noctula and *Pipistrellus nathusii*, migrate similar distances and overwinter within temperate latitudes (Fleming and Eby, 2003). Undoubtedly, many long-distance migratory movements by bats remain to be described and fully studied, and even species that are considered to be high-latitude hibernators make directed migratory movements of up to hundreds of kilometers to traditional hibernacula (Baker, 1978; Fleming and Eby, 2003).

Flight is as energetically costly for bats as it is for birds, although bats are less aerodynamically efficient (Muijres et al., 2012) and, owing to their lower speeds, bats may have greater costs of transport (the energy to move a unit mass a unit distance; Videler, 2005). Thus, bats should be subject to similar natural selective pressures related to the flight phase of migration. Stopovers may be quite different from those experienced by birds because whereas most migratory birds (excepting species such as owls and nightjars) feed during the day and travel at night, bats are inherently constrained by their strict nocturnality to both refuel and/or travel during the night. Migrating bats use torpor to reduce energy expenditure during daytime roosting periods at stopovers (Cryan and Wolf, 2003; Jonasson, 2017; McGuire et al., 2014), whereas migratory birds, apart from a few exceptions (e.g. hummingbirds and nightjars), cannot use torpor. The similarities and differences between bats and birds during flight and refueling increase the potential for a wide variety of physiological solutions to the challenges of migration.

Migratory birds are supreme endurance athletes, as Piersma and van Gils (2011) illustrated using the familiar relationship between rate of energy expenditure and time (Fig. 2). Generally, animals can expend energy at extremely high rates (mostly anaerobically and

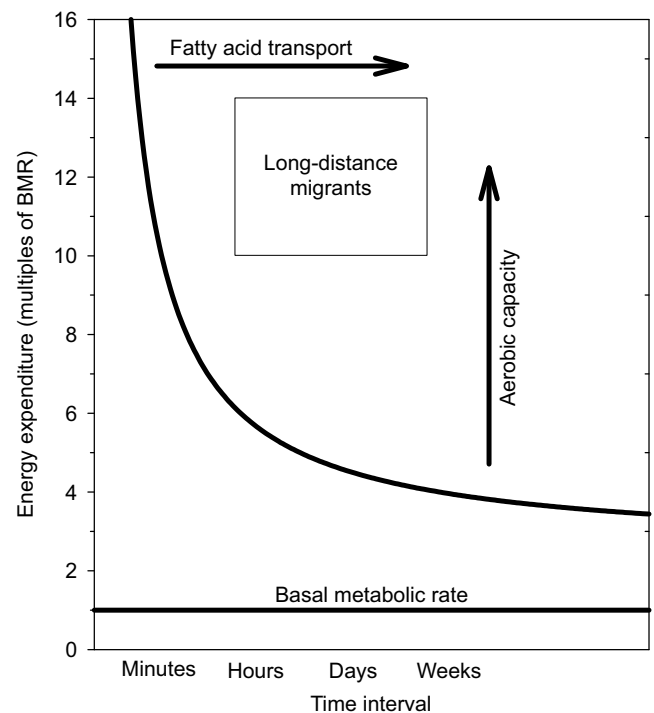


Fig. 2. The generalized relationship between rate of energy expenditure expressed as multiples of basal metabolic rate (BMR) and duration of activity is shown. Very high power output can be maintained for very short periods of seconds to minutes, whereas maximum sustained metabolic rates of ~4–5×BMR can be maintained for weeks. Long-distance migratory birds, and perhaps bats, can exercise at 10–14×BMR for periods ranging from hours to more than a week. This requires increases in both muscle aerobic capacity and fatty acid transport. Redrawn from fig. 35 in Piersma and van Gils, 2011.

fueled by carbohydrates) for a few seconds to minutes (e.g. sprinting); however, as the duration for which activity must be maintained increases, energy expenditure decreases and metabolism must be fully aerobic. A metabolic ceiling of approximately four to five times BMR seems to exist for activities such as chick provisioning or lactation that are maintained for weeks and require continuous self-feeding (Piersma and van Gils, 2011). Migratory birds in flight clearly lie outside of the stereotypical pattern because very high rates of energy expenditure are maintained for exceptionally long periods without feeding. Although migratory bats and birds exercise at a similar intensity, how long bats are capable of continuous flight and to what extent they may feed on the wing are unknown (see below). For an animal to increase its aerobic performance (i.e. move upward on the y-dimension of the relationship in Fig. 2), the aerobic capacity of muscles and supporting systems must increase. This can be achieved in migratory birds by increasing flight muscle mass (Bauchinger et al., 2005; Guglielmo and Williams, 2003; Marsh, 1984; Piersma and van Gils, 2011), capillarity (Lundgren and Kiessling, 1988), mitochondrial volume (Evans et al., 1992), heart size (Bauchinger et al., 2005; Guglielmo and Williams, 2003; Piersma and van Gils, 2011), hematocrit (Piersma and Everaarts, 1996; Wingfield et al., 1990) and activities of the citric acid cycle and electron transport chain enzymes, such as citrate synthase (CS), malate dehydrogenase (MDH) or cytochrome oxidase (COX; Banerjee and Chaturvedi, 2016; DeMoranville, 2015; Dick, 2017; Driedzic et al., 1993; Guglielmo et al., 2002a; Lundgren and Kiessling, 1985, 1986; Maillet and Weber, 2007; Marsh, 1981; McFarlan et al., 2009; Zajac et al., 2011), and by decreasing anaerobic potential as indicated by muscle lactate dehydrogenase (LDH) activity (Banerjee and Chaturvedi, 2016; Dick, 2017; McFarlan et al., 2009; Zajac et al., 2011). Similarly, hoary bats were found to increase pectoralis muscle CS activity, shift lean body composition toward exercise-related organs and have significantly larger lungs during migration (McGuire et al., 2013a,b). However, for animals to move in the x-dimension in Fig. 2 toward long-duration activity requires a change in fuel supply, and this is where stored fat becomes most important. Using fat to fuel high-intensity exercise requires a suite of changes to pathways of fatty acid transport and oxidation, which appear to be characteristic of long-distance migrants (Guglielmo, 2010; see below).

Fat is the preferred fuel for migration because the highly reduced nature and hydrophobicity of fatty acids result in fat providing approximately eight times more energy per unit wet mass ($\sim 37 \text{ kJ g}^{-1}$) than carbohydrates or protein ($\sim 4\text{--}5 \text{ kJ g}^{-1}$; Blem, 1990; Jenni and Jenni-Eiermann, 1998). Although glucose from muscle and liver glycogen is important for burst flight to escape predators and may be important early in migratory flight while fatty acid mobilization is being induced (Gerson and Guglielmo, 2013), glycogen stores of migrant birds (ranging from $\sim 3\text{--}20 \text{ mg g}^{-1}$ wet depending on the time of day) are too small to meaningfully contribute to multi-hour or multi-day flights (Banerjee and Chaturvedi, 2016; Blem, 1990; Driedzic et al., 1993; Marsh, 1983). For example, using a total pectoralis glycogen content of 34 mg ($\sim 600 \text{ J}$) of departure-ready semipalmated sandpipers (*Calidris pusilla*; Driedzic et al., 1993) and a measured flight cost of $\sim 2.6 \text{ W}$ for similarly sized western sandpipers (*Calidris mauri*; Maggini et al., 2017b) we can calculate that muscle glycogen would power flight for less than five minutes. Marsh (1983) reported up to threefold greater pectoralis glycogen concentrations in gray catbird (*Dumetella carolinensis*) than in sandpipers, and high liver glycogen levels, yet these stores would still only meet flight

energy demands for a short period. Amino acids from muscle and organ proteins are used as gluconeogenic and anaplerotic precursors for the maintenance of blood glucose and metabolites crucial to the citric acid cycle (Jenni and Jenni-Eiermann, 1998). The low energy density and high water content of protein also make it a crucial source of supplemental water under dehydrating flight conditions when metabolic water from fatty acid oxidation is insufficient to balance evaporative water losses (Gerson and Guglielmo, 2011; Jenni and Jenni-Eiermann, 1998; Klaassen, 1996). As fat stores decline over a long flight, a reduction of flight muscle mass to match the reduced power requirement could extend the flight distance (Pennycuik, 1998). Although there is empirical support for such an adjustment of muscle mass with changing body mass (Lindström et al., 2000; Schwilch et al., 2002), most of the lean mass catabolized in flight is usually derived from digestive and other organs, which have inherently greater protein turnover rates than muscle (Battley et al., 2000; Bauchinger and McWilliams, 2009; Bauchinger et al., 2005), suggesting that the maintenance of metabolic and osmotic homeostasis are the primary causes of lean mass catabolism in flight. Empirical measurements made in the field and laboratory by body composition analysis, respirometry and stable isotopic analysis show that fat usually provides $\sim 90\%$ and protein $\sim 10\%$ of the energy for long-distance flights of migratory birds (Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004).

Comparable data on fuel use in flight for migratory bats are generally lacking. Fasted bats are capable of fueling flight with fat, at least for short periods (Welch et al., 2008). However, whether the fat used under these conditions is entirely supplied from adipose, as is required for long migratory flights, or involves intramuscular fat stores is unknown. Migratory bats may instead use a mixed fuel strategy where flight is fueled by stored fat and ingested nutrients (Jonasson, 2017; Voigt et al., 2012). Bats normally feed at night on insects, fruit or nectar, making it possible for them to feed before initiating a migratory flight or to pause to feed as they travel (Jonasson, 2017). Although there is not enough information to make firm conclusions about the fuel mixture of migratory bats in flight, stored fat appears to play a significant role.

Fat: how much and what kind?

By human standards, migratory birds reach obese levels of fatness (with fat sometimes comprising $>50\%$ of their body mass). Birds store fat in as many as 16 bodily locations, with the majority of the fat stored in sub-cutaneous adipose depots (Berthold, 1993; Blem, 1976; Maillet and Weber, 2006; Pond, 1978). Fat is also stored in mesenteries and connective tissue in the abdominal cavity, sometimes enveloping the intestines (Blem, 1976; Pond, 1978; C.G.G., personal observation). The enlargement of adipocytes rather than hyperplasia is thought to mainly explain increases in adipose storage (Blem, 1976; Ramenofsky et al., 1999). Triacylglycerol deposits within bird flight muscles increase non-linearly as birds fatten to a maximum of $\sim 60\text{--}80 \text{ mg g}^{-1}$ wet tissue (DeMoranville, 2015; Maillet and Weber, 2006; Marsh, 1984; Napolitano and Ackman, 1990). However, even in the fattest pre-departure migrants, pectoralis fat stores comprise $<5\%$ of the total body fat stores (Maillet and Weber, 2006). Liver lipid contents in fat migratory sandpipers were shown to be low (35 mg g^{-1} wet tissue) and did not change during fattening (Maillet and Weber, 2006; Napolitano and Ackman, 1990). Despite large changes in body fat, the liver lipid content of gray catbirds was found to remain near $60\text{--}80 \text{ mg g}^{-1}$ wet mass through the wintering, breeding and migratory seasons (Corder et al., 2016).

The body composition of migratory birds has usually been measured by chemical analysis of the carcass. Although fat mass, lean mass and water content can be accurately quantified using this method, it is also lethal and precludes the repeated measurement of individuals. Semi-quantitative visual fat scoring of small birds with translucent skin is used widely by migration-monitoring stations and researchers. Visual fat score categories range from no visible fat (i.e. a score of 0) to all subcutaneous areas deeply covered in fat (i.e. a score of 8; Kaiser, 1993). Abdominal profiling schemes may be used for larger birds such as waterfowl (Owen, 1981) and shorebirds (Wiersma and Piersma, 1995). In some cases, fat or fuel load is calculated from the size-corrected difference between body mass and the mass of a standardized lean bird with a fat score of zero (Alerstam and Lindström, 1990). This may not accurately measure fat because refueling birds may deposit significant amounts of lean mass (Lindström and Piersma, 1993; Newton, 2008), although some studies have attempted to account for this (Salewski et al., 2009). Non-invasive body composition analysis methods such as heavy water dilution (Karasov and Pinshow, 1998; McWilliams and Whitman, 2013), total body electrical conductivity (McWilliams and Whitman, 2013), dual X-ray absorptiometry (Korine et al., 2004), magnetic resonance imaging (Wirestam et al., 2008) and quantitative magnetic resonance (QMR; Guglielmo et al., 2011) can be used to non-lethally measure birds and to repeatedly sample individuals through time as they refuel. Studies employing these technologies confirm that lean mass may comprise 10–50% or more of the mass gained at stopovers (Karasov and Pinshow, 1998; Kennedy, 2012; Seewagen and Guglielmo, 2011) and, in some cases, suggest that in the fattest migrants, lean mass may be gained to an upper threshold, after which only fat is deposited (Wojciechowski et al., 2014). These findings confirm earlier patterns demonstrated by chemical extraction (Carpenter et al., 1993; Lindström and Piersma, 1993).

How much fat is stored?

The size of the fat stores of birds has been variously expressed as an index (g g^{-1} lean dry mass), as percentages of fat to wet lean mass or total body mass, or as a ratio of the total body mass to wet lean mass (Alerstam and Lindström, 1990; Blem, 1976; Klaassen, 1996). The rationale for correcting fat content to lean body mass was originally based on the idea that lean mass was constant in migratory birds, and such correction would thus control for variation in structural size and metabolic rate (Blem, 1976). Given that lean body components may vary within individuals across seasons or during refueling, the justification for this way of evaluating fatness is weakened. However, there is validity in quantifying the amount of fat relative to the non-fat portion of the body to avoid part-whole correlation (Christians, 1999). Dry indices of migrant birds have been reported to range from 0.2 to >4.0 (Blem, 1976, 1980). These dry indices of fat translate to a range of ~ 7 –150% of wet lean mass or 7–60% of the total body mass by assuming a ratio of water to lean dry mass of 1.78 (or 64% water in lean mass; averaged from table 5.2 in Newton, 2008). Fat stores expressed as percentages or proportions relative to wet lean mass are most commonly used in the ecological and migration modeling literature (Alerstam and Lindström, 1990; Klaassen, 1996), but have been derived from both chemical analysis and by comparing total body mass to the mass of lean birds assuming all extra mass or a standard proportion is fat. Absolute amounts of fat in the body, or fat expressed as a proportion of wet lean mass (if it is measured, not assumed) or total body mass are probably of the greatest utility for studying how the size of fat stores affects metabolism and other physiological

processes. With the advent of non-invasive technologies, such as QMR, it would be a very worthwhile exercise to systematically re-examine the storage of fat and lean mass across a wide range of migratory birds in different locales.

Fat stores of birds preparing for migration or at stopovers vary among species and individuals depending on arrival/departure status, and other factors such as body size, age, sex, season (spring or autumn), migration strategy, habitat quality, predation risk and the presence of large ecological barriers (oceans, deserts or mountain ranges) with little or no opportunity for refueling (Newton, 2008). Some birds arriving after long barrier crossings may be emaciated, with essentially no fat stores and greatly reduced muscle and organ sizes (Jenni et al., 2000; Moore and Kerlinger, 1987). Refueling can last from several days to weeks, during which fat and often lean mass will increase. Typical departure fat loads of passerine birds migrating continuously over hospitable areas range from 20–30% of the lean body mass (17–23% of the total body mass), and reach 50–70% and rarely up to 90–150% of the lean body mass in birds preparing to cross barriers (Alerstam and Lindström, 1990; Blem, 1980; Newton, 2008). Departure fat loads of shorebirds are generally larger and more variable (20–70% of the lean body mass or 17–40% of the total body mass), and can also reach levels of more than 100% of the lean body mass at barriers (up to 55% of the total body mass; Alerstam and Lindström, 1990; Piersma and Gill, 1998). Small birds, which have high power margins for flight, have a maximum capacity to carry relatively more fat and to fly absolutely greater non-stop distances than large birds (Hedenström and Alerstam, 1992; Klaassen, 1996). Bird fat stores are often larger in spring than in autumn, possibly as insurance against cold and unpredictable spring weather, or to improve reproduction following arrival at breeding areas (Blem, 1976; Kennedy, 2012). Irruptive and partial migrants appear to store only 10–20% fat relative to lean body mass (Alerstam and Lindström, 1990), and raptors that soar over land may also migrate with similarly low fat loads (Kerlinger, 1989). Optimal migration theory and empirical evidence suggest that site quality can positively influence the departure fat load for time-minimizing migrants (Alerstam and Lindström, 1990; Lindström and Alerstam, 1992; Newton, 2008). Carrying excess fat may be detrimental for birds by increasing flight energy costs or reducing burst flight performance and, hence, increasing predation risk (Burns and Ydenberg, 2002; Witter and Cuthill, 1993). Thus, although fat stores of migrating birds can be very large, their size is influenced by a variety of intrinsic and extrinsic factors.

The size of the fat stores of migratory bats has rarely been documented, and can be difficult to interpret from studies where bats may also be storing fat for impending winter hibernation. In studies of insectivorous bats sampled during autumn migration, fat loads were $\sim 25\%$ of body mass whereas a frugivorous species had $\sim 12\%$ body fat (Blem, 1980; Fleming and Eby, 2003). McGuire et al. (2013a) found that female hoary bats were fatter during spring migration (16% of the total body mass) than during the summer (11%), but that males were lean in both seasons (11–12%). Fat stores of bats cannot be assessed visually owing to their thick fur; however, non-invasive QMR analysis has greatly increased our understanding of their fat storage (McGuire and Guglielmo, 2010). Silver-haired bats migrating through Ontario, Canada, in the autumn average $\sim 19\%$ body fat, with little difference between ages and sexes (McGuire et al., 2012). In the spring, migrating female silver-haired bats are generally fatter than males (10–17% versus 6–11% of the total body mass depending on year), likely reflecting a more conservative energy strategy in females (Jonasson and Guglielmo,

2016). QMR analysis also suggests that ~50% of the mass gained by refueling silver-haired bats is lean tissue (Jonasson, 2017). These findings indicate that tree bats migrate with modest fat stores that are similar to passerine birds migrating over land, and that pregnant females are often fatter than males in spring. Using torpor to minimize fat losses at stopovers and feeding in flight may reduce the dependence of migratory bats on fat storage.

Fatty acid composition of adipose fat stores and flight muscle membranes

The fatty acid composition of fat stores may also be important for migratory birds and bats. The adipose triacylglycerol in non-migratory and migratory birds is predominately composed of 16 and 18 carbon fatty acids that are saturated (no double bonds, 18:0, 16:0) and monounsaturated (MUFA, one double bond with 'n' indicating the position of the first double bond relative to the methyl end, e.g. 16:1n-7 or 18:1n-9; Blem, 1976; McWilliams et al., 2004). The most abundant constituents are usually 16:0 and 18:1n-9, comprising 10–37% and 17–61% of the total adipose fatty acids, respectively, in migratory songbirds and waterbirds (Blem, 1976; Egeler and Williams, 2000; Klaiman et al., 2009; Maillet and Weber, 2006; McWilliams et al., 2004; Napolitano and Ackman, 1990; Pierce and McWilliams, 2005). Although 18:0 and 16:1n-7 are important, they are less abundant than 16:0 and 18:1n-9, each comprising 3–28% of the total fatty acids. The essential 18 carbon polyunsaturated fatty acids (PUFA) linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3) may also be abundant (up to 40% and 17% of the total fatty acids, respectively), particularly in terrestrial passerine species (Blem, 1976; Klaiman et al., 2009; McWilliams et al., 2004). Taken together, the 16 and 18 carbon fatty acids usually make up 75–90% of the lipid stores of migratory birds (Blem, 1976; McWilliams et al., 2004; Pierce and McWilliams, 2014). Birds that eat marine-derived diets rich in long-chain n-3 PUFA, particularly eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), accumulate PUFA in adipose triglycerides, comprising up to ~20% of the total fatty acids during refueling, yet 16 and 18 carbon fatty acids still comprise >75% of the total fatty acids in their fat stores, similar to avian terrestrial migrants (Egeler and Williams, 2000; Maillet and Weber, 2006; Napolitano and Ackman, 1990).

The predominance of unsaturated fatty acids, especially 18:1n-9 and PUFA, in bird fat stores and indications that unsaturation may increase during migration suggest that increased unsaturation may be beneficial to migration. From first principles, long and saturated fatty acids have greater energy content and mitochondrial ATP production potential than shorter and unsaturated counterparts (Price, 2010; Weber, 2009). Yet, the greater mobilization ability from adipose and the greater mitochondrial utilization capacity of short and unsaturated fatty acids (see below) greatly outweighs these energy advantages (Price, 2010). When the effects of carbon chain length and unsaturation on transport processes are accounted for, a 10% reduction in the energy storage of triacylglycerol by storing unsaturated fatty acids may result in as much as an 80% increase in the potential rate of ATP production (Price, 2010). Based on limited evidence, Blem (1976) tentatively concluded that there was no overall tendency for migratory birds to have a greater proportion of unsaturated fatty acids than non-migratory species or for fatty acid unsaturation to increase during migration, but suggested there may be a relative increase in the ratio of 18:1n-9 to 18:2n-6 during migration. Blem (1976) cautioned that direct seasonal comparisons were scarce and more investigation was needed. When considering intraspecific studies where direct comparisons between seasons

were made ($N=7$), the evidence for an increase in total unsaturation or in the ratio of 18:1n-9 to 18:2n-6 (or 16:1n-7+18:1n-9 to 18:2) is equivocal (Klaiman et al., 2009; Pierce and McWilliams, 2005, 2014; Price, 2010). However, a more general pattern (six of seven species) is for the total proportion of unsaturated fatty acids to either remain the same or to increase during migration (Klaiman et al., 2009; Pierce and McWilliams, 2005), appearing to converge on ~70% unsaturated fatty acids in fat stores. Increased saturation in migrants was only evident in the white-crowned sparrow (*Zonotrichia leucophrys*) where captive migrants stored unusually high amounts (32%) of 14:0, a short and labile saturated fatty acid (Morton and Liebman, 1974). In the closely related white-throated sparrow (*Zonotrichia albicollis*), total unsaturation and the fatty acid double-bond index increased significantly in the fat stores of migrants compared with wintering birds, caused mainly by a decline in 16:0 and a substantial increase in 18:2n-6, which comprised up to 35% of the total fatty acids (Klaiman et al., 2009).

Data regarding the fatty acid composition of fat stores of migratory bats are very limited but show that mostly 16 and 18 carbon fatty acids are present. The total body lipids of three migratory *Myotis* species had low relative amounts of 18:0 (1.5–3.5%) and high levels of 16:0 (12–18%), 16:1n-7 (9–17%), 18:1n-9 (31–49%) and 18:2n-6 (10–19%), with ~5–6% 18:3n-3 (Ewing et al., 1970). McGuire et al. (2013a) analyzed adipose triglycerides of migratory hoary bats and found similarly high levels of 16:0 (~25%) and 18:1n-9 (~57%), but lower levels of 16:1 (~2%), 18:0 (~3%), 18:2n-6 (~8%) and 18:3n-3 (~5%). The fatty acid composition of hoary bat fat stores changed slightly between summer and migration, and sex was a significant factor. Total unsaturation and the double-bond index increased during migration, driven by decreased 16:0 and 16:1n-7, increased 18:2n-6 and 18:3n-3, and 18:1n-9 increasing slightly in females and decreasing in males (McGuire et al., 2013a). Like the fat stores of birds, the fat stores of migratory bats appear to contain ~70% unsaturated fatty acids.

Despite the potential importance for flight performance, even less information is available about the fatty acid composition of the flight muscle membrane phospholipids of free-living migratory birds and bats because most studies only extract lipids from adipose tissue, or from entire animals or muscles without separating lipid classes (McWilliams et al., 2004; Napolitano and Ackman, 1990; Pierce et al., 2005). In birds, 18:0 is noticeably more abundant (25–30% versus 6–13%) whereas 18:1n-9 is reduced (8–14% versus 30–35%) in flight muscle phospholipids compared with adipocyte triacylglycerol of the same animals (Egeler and Williams, 2000; Guglielmo et al., 2002b; Klaiman et al., 2009; Maillet and Weber, 2006). Some PUFA may also be highly abundant in the flight muscle phospholipids of birds (18:2n-6 up to 16%, 20:4n-6 up to 22%, 20:5n-3 up to 17% and 22:6n-3 up to 23%; Guglielmo et al., 2002b; Klaiman et al., 2009; Maillet and Weber, 2006), and likely play a role in maintaining the physical characteristics of membranes and protein function. Although the individual fatty acids (particularly PUFA) in bird flight muscle phospholipids may change dramatically between winter and migration, total unsaturation or the double-bond index tend to decrease slightly, and the ratio of n-6/n-3 PUFA may increase or decrease (Guglielmo et al., 2002b; Klaiman et al., 2009; Price et al., 2010). McGuire et al. (2013a) reported that the fatty acid composition of the flight muscle phospholipids of hoary bats differed substantially from adipose triacylglycerol by having more 18:0 (8% versus 2%), and by the presence and high abundance of 20 and 22 carbon PUFA (up to ~20% 22:6n-3). Migration-related changes were again complicated

by sex differences: 22:6n-3 increased and 18:2n-6 decreased in migrant females, whereas the opposite occurred in males. Although total unsaturation did not change during migration, the double-bond index decreased in males and increased in females, and the n-6/n-3 ratio increased in males and decreased in females (McGuire et al., 2013a). Thus, no general pattern emerges regarding migration-related changes in the fatty acid composition of muscle membranes of free-living migratory birds and bats, except that changes in long-chain n-3 PUFA appear to be offset by opposite changes in n-6 PUFA so that overall unsaturation remains stable (Guglielmo et al., 2002b; Maillet and Weber, 2006; McGuire et al., 2013a). However, drawing conclusions from only a few suitable studies is difficult, and more standardized studies need to be undertaken.

How do birds and bats get fat?

The deposition of fat and the alteration of the fatty acid composition of fat stores of migratory birds are achieved through changes in both physiology and behavior (Bairlein, 2002; Berthold, 1993; Biebach, 1996; Newton, 2008). During the migratory phase, birds become hyperphagic, typically eating 20–40% more food than maintenance levels (Bairlein, 2002) and gaining mass at a rate of ~1–7% of lean body mass per day (Alerstam and Lindström, 1990; Lindström, 1991). However, when food and foraging time are unlimited, migratory birds are capable of increasing food intake by three- to fivefold, reaching a gluttonous energy assimilation rate of up to 10 times BMR and gaining mass at a rate of 15% or more of lean body mass per day (Kvist and Lindström, 2000, 2003). Birds may also alter their food preferences during migration, for example, by including more fruit in the diet when it is available in the autumn (Bairlein, 2002; Berthold, 1993; Parrish, 1997). High dietary energy (carbohydrates and lipids) to protein ratios, such as those found in fruit or seeds, promote fattening (Bairlein, 2002) because carbohydrates can be readily converted to fat, lipids can be directly absorbed and stored, and high food intake rates allow migrating birds to meet protein requirements, even if the protein content of their diet is low (Langlois and McWilliams, 2010; Marshall et al., 2015). Yellow-rumped warblers (*Setophaga coronata*) prefer high-carbohydrate to high-protein diets, adjusting intake seasonally to match changing energy targets (Marshall et al., 2015). Furthermore, yellow-rumped warblers and white-throated sparrows maintain larger fat stores when fed high-carbohydrate diets compared with high-protein diets (Guglielmo et al., 2017; Smith and McWilliams, 2009).

Migratory birds prefer diets with unsaturated rather than saturated fatty acids and prefer 18:1n-9 to 18:2n-6 (Bairlein, 2002; McWilliams et al., 2004, 2002; Pierce and McWilliams, 2005, 2014); however the extent to which diet selection and selective metabolism in nature are important for seasonal changes in bird tissue fatty acid composition is unclear. The fatty acid composition of bird triacylglycerol stores and membrane phospholipids respond readily to changes in dietary fatty acid composition, especially when diet fat content or food intake is high because dietary fatty acids can be directly incorporated into tissues (Dick, 2017; Egeler et al., 2003; Maillet and Weber, 2006; Pierce and McWilliams, 2005; Price and Guglielmo, 2009). When diet fat content is low, *de novo* fatty acid synthesis from carbohydrate and amino acid precursors will favor a fatty acid composition of mostly 16 and 18 carbon saturated and monounsaturated fatty acids because most animals lack the desaturases needed to produce n-3 and n-6 PUFA (Gurr et al., 2016). Most animals do possess the required elongases and desaturases to produce 20 and 22 carbon PUFA (e.g. 20:4n-6, 20:5n-3 and 22:6n3) from dietary 18:2n-6 and 18:3n-3, so long-

chain PUFA can be abundant in fat stores or membranes given the appropriate diet with these precursors (Gurr et al., 2016). When captive birds are held on constant diets for long periods there do not seem to be any endogenously controlled changes in fatty acid composition during migration seasons (Blem, 1976; Pierce and McWilliams, 2005; Price et al., 2010), suggesting that dietary changes may be required to alter the composition of fat stores. Whereas the fatty acid composition of red-eyed vireos (*Vireo olivaceus*) fed a 30% lipid diet seemed to be mainly driven by dietary fatty acids (Pierce and McWilliams, 2005), western sandpiper fat stores were influenced more by dietary 18:2n-6 content than by 16:0 or 18:1n-9 content when fed 5–10% fat diets, suggesting that both diet and selective metabolism were important (Egeler et al., 2003). Klaiman et al. (2009) concluded that the accumulation of high levels of 18:2n-6 by migrating white-throated sparrows was likely owing to dietary changes. However, despite feeding mainly on *Corophium volutator*, a benthic invertebrate with 45% n-3 PUFA in its body lipids, and becoming enriched themselves in n-3 PUFA as they refuel to high-fat loads (40% of body mass), the fat stores and muscle membranes of wild semipalmated sandpipers remained relatively depleted in PUFA and enriched in 18:1n-9 and 16:0 compared with the diet (Maillet and Weber, 2006). Maillet and Weber (2006) hypothesized that 20 and 22 carbon PUFA were converted to 16:0 and 18:0; however, this would require extensive partial oxidation and resynthesis of fatty acids. Another possible explanation for this pattern is the very low lipid content (1.2% of wet weight) of *C. volutator* (Ackman et al., 1979), which may require sandpipers to use *de novo* synthesis of 16 and 18 carbon fatty acids to fatten rapidly. The resultant fatty acid composition of migratory bird fat stores depends on a combination of factors: the rate of food intake, the lipid content of the diet, the fatty acid composition of dietary lipid, the capacity for *de novo* synthesis of fatty acids from non-lipid dietary components (a function of liver size and enzyme capacity – see below), the activity of liver elongases and desaturases that alter carbon chain length and desaturation of dietary or *de novo* origin fatty acids, and selective storage and oxidation of saturated fatty acids, MUFA and PUFA (summarized in Fig. 3). Muscle membrane fatty acid composition, although influenced by the mix of fatty acids available in the body, is also regulated to maintain fluidity and function (Gurr et al., 2016).

Physiological changes in digestive function, post-absorptive nutrient processing, and energy allocation to maintenance or growth may all contribute to fattening in migratory birds. The increased demands placed on migratory birds to digest food and process nutrients into fat are met by increases in the size and functional capacity of the gut and liver. Hyperphagia causes increases in the size of the alimentary tract, which allows birds to process greater volumes of food without reducing digestive efficiency (McWilliams and Karasov, 2001, 2014). In some species, the gut and liver may nearly double in mass between winter and migration seasons (Guglielmo and Williams, 2003), and they can also increase in size during stopovers coincident with periods of peak food intake and fattening (Piersma et al., 1999; Piersma and van Gils, 2011). Digestive efficiency has been reported to increase in some species but not in others during migration (Bairlein, 2002; McWilliams and Karasov, 2001). Digesta retention time, activities of digestive enzymes (including lipases), and intestinal transporters may be modified in migratory birds to match changes in diet nutrient composition (Afik et al., 1995, 1997a; McWilliams and Karasov, 2001; Stein et al., 2005). Bird intestines have a much greater capacity for passive nutrient absorption than that of mammals (excepting bats – see below), and leakiness may be modulated

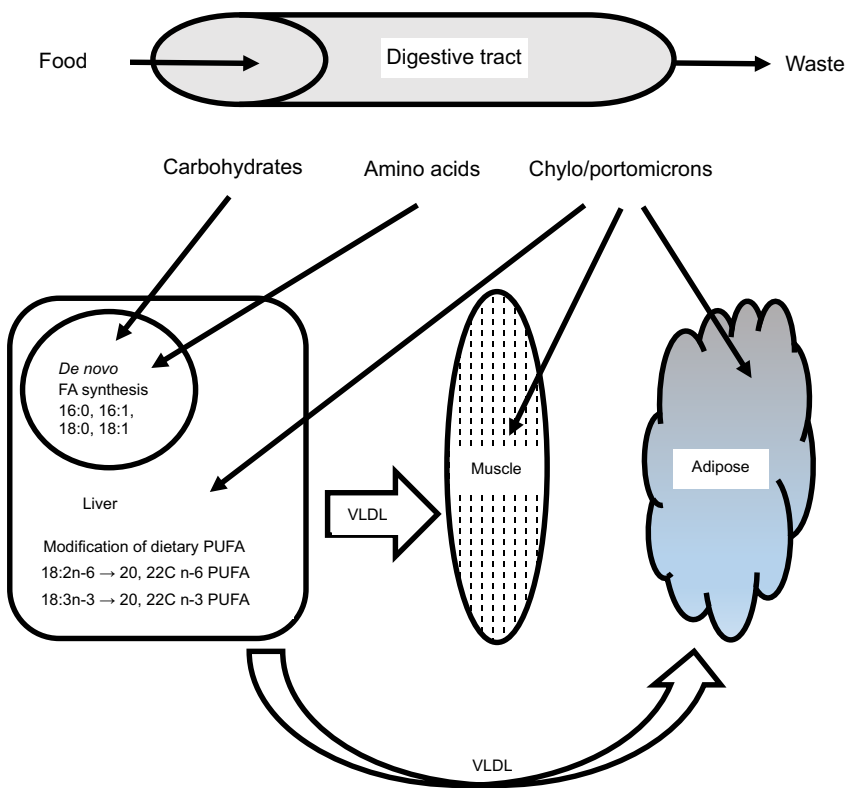


Fig. 3. A schematic representation of the flow of ingested nutrients into fat stores and muscle membrane phospholipids. Ingested fatty acids (FA) and other lipids are packaged into chylomicrons in mammals, which travel through the lymphatic system to the systemic circulation, and can be taken up by adipocytes, muscle or the liver. In birds, digested lipids are packaged into portomicrons, which first move through the hepatic portal system. Data from chickens indicate that owing to their large size and the reduced fenestration of avian portal capillaries, most portomicrons reach the systemic circulation and other tissues before returning as smaller lipoprotein remnants to the liver (Fraser et al., 1986). Dietary n-3 and n-6 fatty acids may be modified by the liver into longer and more unsaturated fatty acids. Surplus amino acids and carbohydrates can be used for *de novo* synthesis of mostly 16 and 18 carbon saturated and monounsaturated fatty acids in the liver. Triacylglycerol, phospholipids, non-esterified fatty acids and other lipids, such as cholesterol, are packaged into very low-density lipoproteins (VLDLs) for transport from the liver to other tissues.

during stopover to promote rapid fueling (Afik et al., 1997b; Caviedes-Vidal et al., 2007; Tracy et al., 2010). Liver glucose-6-phosphate dehydrogenase activity nearly doubled and malic enzyme activity increased fivefold in rosy pastors (*Sturnus roseus*) and white wagtails (*Motacilla alba*) during the pre-migration period (Shah et al., 1978). Liver fatty acid synthase (FAS) and 9-desaturase activities doubled during spring migration in western sandpipers compared with birds wintering and pre-migratory fattening in the tropics, which combined with a doubling of liver mass resulted in a fourfold increase in *de novo* fatty acid production capacity (Egeler et al., 2000; Guglielmo and Williams, 2003). Liver FAS activity also approximately doubled in wild red-headed buntings (*Emberiza bruniceps*) during the pre-migration phase (Banerjee and Chaturvedi, 2016), and in white-throated sparrows during spring and autumn migration compared with birds wintering in Mississippi, USA (1.28 ± 0.66 s.d. versus $0.65 \pm 0.23 \mu\text{mol min}^{-1} \text{g}^{-1} \text{tissue}$; $F_{1,69} = 6.802$, $P = 0.002$; measured using birds collected by McFarlan et al., 2009 and methods described in Zajac et al., 2011; S. Landman, L. P. McGuire, and C.G.G., unpublished data). However, liver FAS decreased relative to winter in captive white-throated sparrows that were photostimulated into a vernal migratory state (Zajac et al., 2011). This result was attributed to the sparrows having unlimited food during shorter daylight hours (8 h) in the winter photoperiod, requiring greater fattening capacity than 'migrants' (16 h) that could not migrate. This could also explain why FAS did not increase in pre-migratory western sandpipers even though they were depositing fat (Egeler et al., 2000). Liver FAS, adipose lipoprotein lipase and muscle lipoprotein lipase did not change from winter to spring migration in dark-eyed juncos (*Junco hyemalis*), suggesting *de novo* synthesis and peripheral lipoprotein hydrolysis capacities are not adjusted for migration in this species that was wintering at a colder northern latitude in New York State, USA (Ramenofsky et al., 1999; Savard et al., 1991). Hummingbirds use nightly torpor to reduce

body temperature and energy expenditure to enhance fat deposition during migration (Carpenter et al., 1993; Hiebert, 1993). Seasonal migratory hypothermia has also been documented in barnacle geese (*Branta leucopsis*), which may serve the function of directing nutrients toward storage rather than maintenance metabolism (Butler and Woakes, 2001). Evidence for seasonal or nightly hypothermia in songbirds and other bird species during migration is scant (Wojciechowski and Pinshow, 2009); however, this mechanism could be used to retain fuel deposited during feeding times.

More is known about the short-term homeostatic regulation of feeding in birds than about mechanisms of rheostatic change of the seasonal body mass or adiposity set points (Cornelius et al., 2013). In birds, as in mammals, homeostatic regulation of appetite and energy status is controlled by neuroendocrine cells of the arcuate nucleus of the hypothalamus; rheostatic changes may be controlled elsewhere in the brain (Boswell, 2005; Boswell and Dunn, 2015). Appetite stimulatory neurons in the arcuate nucleus express transcripts for neuropeptide Y (NPY) and agouti-related protein (AGRP), whereas inhibitory neurons express pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). The expressed peptides then stimulate receptors of the melanocortin system to control feeding. Baseline changes in the expression of AGRP and POMC do not appear to play a role in the hyperphagia of migratory birds (Boswell and Dunn, 2015; Cornelius et al., 2013). The arcuate nucleus is outside the blood-brain barrier, exposing it to circulating energy metabolites, and the regulatory neurons receive input through receptors for insulin, leptin, ghrelin, corticosteroids and cholecystokinin (Boswell and Dunn, 2015). Insulin is elevated in the fed state and is inhibitory to feeding in mammals and birds by stimulating POMC and inhibiting NPY and AGRP (Boswell and Dunn, 2015). In mammals, leptin is produced by adipocytes, signaling fat status, inhibiting feeding, and elevating metabolic rate (Boswell and Dunn, 2015; Rousseau et al.,

2003). In white-throated sparrows, peripheral injections of murine leptin inhibited feeding and reduced body mass in the winter condition, but not in the migratory condition (Cerasale et al., 2011), suggesting that a change in leptin sensitivity could be permissive to excess feeding and fattening in migrants. Although leptin receptors were known to be present in the hypothalamus and other brain and peripheral regions of birds (Boswell and Dunn, 2015; Cerasale et al., 2011), the existence of avian leptin was not definitively shown until recently, and it is not expressed by adipose tissue (Friedman-Einat et al., 2014; Huang et al., 2014). Avian leptin may have autocrine or paracrine functions within the brain and its signaling functions are not understood (Boswell and Dunn, 2015). Thus, leptin may not provide direct peripheral feedback about fat stores to the central appetite and energy regulatory systems of birds, and its role in migration is still unknown. Ghrelin is produced by the stomach and in mammals it stimulates appetite in the energy-deprived state; however, it inhibits feeding in birds (Boswell and Dunn, 2015). Recently, a study of migrating garden warblers (*Sylvia borin*) showed that plasma concentrations of acylated ghrelin were positively correlated with body fat, and that peripheral injections of unacylated ghrelin inhibited feeding and stimulated nocturnal migratory restlessness (Goymann et al., 2017). Thus, ghrelin signaling may play a role in regulating fat storage and migratory behavior. Corticosteroids, acting through the low-affinity corticosteroid receptor stimulate feeding in mammals by increasing NPY and AGRP (Cornelius et al., 2013). In birds, the elevation of baseline corticosterone (CORT) appears to play a permissive role during migration seasons by stimulating feeding and mass gain, possibly through effects related to insulin (Boswell and Dunn, 2015; Holberton et al., 2007; Landys-Cianelli et al., 2004). During stopover refueling the effects of CORT are complex as plasma CORT levels are positively correlated with fat load and negatively correlated with the rate of feeding and mass gain in migrant wheatears (Eikenaar et al., 2013). High levels of CORT are associated with peak fatness at stopover and may signal departure readiness and, thus, the role of CORT in stopover fattening may be independent of or dependent on interactions with other hormones, such as ghrelin (Eikenaar et al., 2014, 2013). Cholecystokinin (CCK) is inhibitory to feeding, and recent studies in birds suggest that variation in central CCK signaling can alter the growth rate (Boswell and Dunn, 2015). Other factors that could be important for influencing long-term control of appetite and energy storage in migrant birds may be related to thyroid hormones, which can stimulate AGRP, or to mechanisms in the arcuate nucleus for sensing energy metabolites, such as lipids in plasma (Boswell and Dunn, 2015; Cornelius et al., 2013). Plasma triacylglycerol levels are very often correlated with individual body mass in migratory birds (Guglielmo et al., 2005) and could play such a signaling role. Like adiponectin expression in mammals, adiponectin is expressed by adipose and skeletal muscle of birds, and is present in plasma in high concentrations that increase with fatness (Ramachandran et al., 2013). Adiponectin receptors are present in bird skeletal muscle, liver, diencephalon, adipose and the anterior pituitary, and adiponectin appears to be involved in signaling information about fat stores peripherally to affect liver and muscle metabolism, and centrally through the pituitary to influence growth and reproduction (Ramachandran et al., 2013). Stuber et al. (2013) reported that the circadian pattern of plasma adiponectin changed during migration in white-throated sparrows, and that it was positively correlated with fat stores and nocturnal migratory restlessness. Plasma visfatin also increased during the migratory season, suggesting this adipokine may also be involved in migratory physiology (Stuber et al., 2013).

Lipid biosynthesis and storage is regulated locally in the liver and adipocytes by peroxisome proliferator-activated receptors (PPARs) and their co-factors (Wang, 2010). In gray catbirds, PPAR α transcript abundance increased threefold during spring migration in the liver compared with tropical wintering, and remained at intermediate levels through summer and autumn migration (Corder et al., 2016). However, neither PPAR λ nor any of the PPAR target genes measured in the liver changed significantly with migration. PPAR λ expression did not vary seasonally in adipose, and only perilipin3 (PLIP3) and plasma membrane fatty acid binding protein (FABPpm) showed any seasonal change, tending to be lowest during spring migration (Corder et al., 2016). Much more remains to be learned about how feeding, fat storage and lean mass dynamics are regulated in migratory birds.

How bats get fat during migration or potentially alter their fatty acid composition is almost completely unknown (McGuire and Guglielmo, 2009). Whether bats always get fatter during migration (particularly males – see above) is unclear, and whether they become hyperphagic in captivity or nature has not been demonstrated. Bats may favor higher PUFA insects to facilitate torpor (Schalk and Brigham, 1995), but whether they do this during migration or if liver *de novo* synthesis and desaturation capacities increase is not known. Like birds, the small intestine of bats also has a high capacity for passive nutrient absorption, which may be a general adaptation to minimize weight in flying vertebrates (Price et al., 2015). However, McGuire et al. (2013a) reported that the alimentary tract and liver of hoary bats were smaller during migration, suggesting that hyperphagia does not occur. Temperate bats are consummate heterotherms, and are well known for their extensive use of daily and seasonal torpor. Migrating hoary and silver-haired bats use torpor facultatively during stopovers (Cryan and Wolf, 2003; Jonasson, 2017; McGuire et al., 2014); in silver-haired bats, the depth and duration of torpor are adjusted depending on ambient temperature, such that fat loss and energy expenditure during daytime roosting at a stopover is independent of temperature (Baloun, 2017; McGuire et al., 2014). Female silver-haired bats appear to use less torpor and have greater roosting energy expenditure than males during spring stopovers, when they are pregnant, which may explain their greater fat loads in spring (Baloun, 2017; Jonasson, 2017). The low and predictable energy cost of stopping may reduce the need for refueling in migrating bats, contributing to short stopovers (McGuire et al., 2012, 2014). Furthermore, bats may feed before or during nocturnal migratory flight, thus using both endogenous and ingested fuels for flight (Voigt et al., 2012). Seasonal fat storage for migration could potentially be regulated by the leptin signaling system, which has been shown to be important during fattening of bats prior to hibernation (Kronfeld-Schor et al., 2000). Much basic research with captive and wild bats on the fundamental aspects of lipid storage and metabolism during migration remains to be undertaken.

Meeting the challenge of fat-fueled flight

Migratory birds are fat-burning machines. For decades, it was widely accepted that fat fueled the migratory flight of birds, without clear recognition of how impossible it should be based on what was known about mammalian exercise. Many studies of muscle enzymes of migrant birds emphasized that fatty acid oxidation capacity increased and how important this was, yet the key limitation is not oxidation but transport (Guglielmo, 2010). The paradox of fatty acids stored in adipose fat (triacylglycerol) as fuel for flying migrants such as birds and bats, is that the property that makes them ideal for storing energy (hydrophobicity), also makes

them difficult to metabolize at the very high rates needed for flight. Unlike soluble fuel substrates such as glucose and amino acids, fatty acids have aqueous solubility in the nanomolar range, meaning that their transport must be mediated by protein carriers from their source to the site of oxidation in muscle cells during exercise.

Migratory birds do not fit the crossover exercise model of running mammals (Brooks, 1998), which predicts that fatty acids can provide ~70% of the energy for low-intensity exercise (e.g. 40% $\dot{V}_{O_2, \max}$) but only ~10–20% of the energy needed for highly intense aerobic exercise (e.g. 85% $\dot{V}_{O_2, \max}$; McClelland, 2004). Most of the energy used by a mammal running near $\dot{V}_{O_2, \max}$ is derived from carbohydrate oxidation, mostly intramuscular glycogen (McClelland, 2004). Furthermore, circulatory fatty acids only seem to contribute approximately half of the total fatty acids oxidized during running in mammals, with the remainder originating from intramuscular triacylglycerol (Weber et al., 1996). Thus, if a flying bird exercises at nearly 80% $\dot{V}_{O_2, \max}$, then the mammal crossover model would predict that only 5–10% of its energy would come from the oxidation of fatty acids delivered from adipose tissue to muscles through the circulation; however, the value is closer to 90% (Guglielmo, 2010). Flight muscles of migratory birds have very high activities of mitochondrial enzymes involved in fatty acid oxidation, such as hydroxyacyl-CoA-dehydrogenase (HOAD) and carnitine palmitoyltransferase (CPT), that are greater in more migratory species (Lundgren and Kiessling, 1985), increase during migration seasons (Banerjee and Chaturvedi, 2016; Guglielmo et al., 2002a; Lundgren and Kiessling, 1985, 1986; Marsh, 1981; McFarlan et al., 2009; Zajac et al., 2011), and may increase at stopovers as birds refuel and prepare to depart (Driedzic et al., 1993; Maillet and Weber, 2007). However, increasing the mitochondrial fatty acid oxidation potential alone does not solve the problem with using adipose-derived fatty acids as fuel because the limitation in mammals appears to be due to constraints on transport through the circulation and uptake and transport of fatty acids by myocytes (Guglielmo, 2010; Vock et al., 1996). Hypotheses regarding the mechanisms that allow birds to use their fat stores for migration have focused on two aspects of fatty acid transport: (1) enhanced circulatory delivery and (2) enhanced myocyte uptake and intramyocyte transport capacity.

Fatty acids are transported in plasma as non-esterified fatty acids (NEFA) bound to albumin, or as triacylglycerol, phospholipids and NEFA in lipoproteins (Ramenofsky, 1990). Adipocytes release NEFA and glycerol following hydrolysis of triacylglycerol by hormone-sensitive lipase and adipose triglyceride lipase. At rest and during intense shivering, ruff sandpipers (*Philomachus pugnax*) were found to have the highest adipose lipolytic rates measured in vertebrates (two- to threefold greater than mammals), and 50% of the fatty acids were re-esterified and retained within adipocytes (Vaillancourt and Weber, 2007). Basal adipose lipolysis tended to increase during spring and autumn migration compared with summer, but was lower than during wintering in gray catbirds (Corder et al., 2016). In mammals, lipolysis is not thought to limit fatty acid oxidation during exercise (Weber et al., 1996), and the very high lipolysis rates of birds suggest that this may also be true for birds. Changes in plasma albumin concentration are unlikely to be important for enhancing fatty acid transport because excess albumin would be detrimental to blood viscosity and fluid homeostasis (Jenni-Eiermann and Jenni, 1992). Migrating songbirds captured in mid-flight were found to have elevated plasma triacylglycerol and very low-density lipoproteins (VLDL) compared with fasted or fed birds (Jenni-Eiermann and Jenni, 1991, 1992), leading to the hypothesis that some fatty acids mobilized

from adipocytes during flight may be cleared from the plasma by the liver, re-esterified to triacylglycerol (and possibly phospholipids), and packaged into VLDL for re-release to the circulation (Jenni-Eiermann and Jenni, 1992). Once reaching the muscle capillaries, triacylglycerol would be hydrolyzed by lipoprotein lipase, releasing NEFA in high local concentrations for uptake by myocytes (Ramenofsky, 1990). This could increase overall fatty acid flux by keeping the availability of albumin binding sites high and creating an alternative pathway for the delivery of fatty acids to muscle by lipoproteins (Jenni-Eiermann and Jenni, 1992). Further support for the lipoprotein hypothesis was found in bar-tailed godwits (*Limosa lapponica taymyrensis*) that had greater plasma triacylglycerol concentrations immediately after arrival from a long migratory flight than after five hours of inactivity (Landys et al., 2005). The potential for lipoproteins to transport large amounts of lipids to muscles with a lesser osmotic challenge seems attractive and would make lipid transport of birds during exercise more similar to that of flying insects and some fish than mammals (Weber, 2009). However, although plasma NEFA generally increases during flight in birds (Bordel and Haase, 1993; Gerson and Guglielmo, 2013; Jenni-Eiermann et al., 2002; Pierce et al., 2005; Schwilch et al., 1996), the lipoprotein pathway of fatty acid delivery has not been empirically replicated in birds flying under controlled conditions. Plasma triacylglycerol and phospholipids remained the same or decreased during flight wheel exercise lasting 30–45 min in red-eyed vireos (Pierce et al., 2005) and yellow-rumped warblers (Guglielmo et al., 2017). Triacylglycerol tended to decrease and phospholipids decreased significantly in American robins (*Turdus migratorius*) flying for 18–55 min in a wind tunnel (Gerson and Guglielmo, 2013). Plasma triacylglycerol concentration either does not change or decreases in flying homing pigeons (*Columba livia*; Bordel and Haase, 1993; Schwilch et al., 1996). In red knots (*Calidris canutus*), plasma triacylglycerol decreased during the first hour and then remained stable for up to ten hours of wind tunnel flight, but was lower than time-matched fasting controls (Jenni-Eiermann et al., 2002). Similarly, in a small passerine, the yellow-rumped warbler, plasma triglyceride remained stable or declined slightly during wind tunnel flights of 40–360 min, and did not differ from time-matched fasting controls (Guglielmo et al., 2017). However, plasma phospholipids increased with flight and fasting duration (Guglielmo et al., 2017), and the triacylglycerol to phospholipid ratio tended to decrease ($P=0.06$; C.G.G., unpublished data) in warblers, suggesting a shift in lipoprotein composition that could reflect greater offloading of triacylglycerol and lipoprotein flux (Bussière-Côté et al., 2016). Even though these studies do not confirm the lipoprotein hypothesis, they consistently show that plasma concentrations of fatty acids in circulating triacylglycerol and phospholipids are maintained several-fold greater than in NEFA during endurance flights. This large pool of fatty acids could be important for fuel supply during flight. Additional field measurements of wild birds captured in mid-flight are needed to replicate earlier studies. Although isotopic tracing of lipoproteins and NEFA to measure flux during endurance flights will not be trivial (Magnoni et al., 2008; McCue et al., 2010; Vaillancourt and Weber, 2007), this could be performed to conclusively test the lipoprotein hypothesis.

The low capacity for fatty acid uptake by mammalian myocytes is thought to be the greatest factor limiting the use of circulatory fatty acids as fuel for running (Vock et al., 1996). Although mammals adapted or trained for high endurance running ability have an enhanced absolute fatty acid oxidation capacity, they rely more on intramyocyte triacylglycerol than circulatory fatty acids for energy

(McClelland, 2004), a pattern quite unlike that of migratory birds. A simple hypothesis that follows is that the flight muscles of migratory birds should have a very high capacity for fatty acid uptake, and that this may change between migration and non-migration seasons as the requirement for exogenous fatty acid oxidation changes. Approximately a third of the improved delivery of fatty acids to bird muscles could be related to their small fiber size and high capillary surface density; however, most of the improved delivery of fatty acids is likely to be due to changes in protein-mediated transport (Guglielmo et al., 2002a). The bulk of fatty acid transport across the sarcolemma is mediated by proteins, including fatty acid translocase (FAT/CD36) and plasma membrane fatty acid binding protein (FABPpm), and high intracellular concentrations of the heart-type fatty acid binding protein (H-FABP) promote rapid uptake, movement and disposal of fatty acids (Luiken et al., 1999; Sweazea and Braun, 2006). Pectoralis muscles of migrating western sandpipers have an approximately 10-fold greater concentration of H-FABP (up to 11% of cytosolic protein) than that of mouse soleus muscle, and H-FABP has been shown to increase by 70% during migratory seasons compared with winter (Guglielmo et al., 2002a). H-FABP also increases during migration at the transcript level by up to 30-fold and at the protein level by ~50% in the flight muscles of barnacle geese (Pelsers et al., 1999), wild and captive photostimulated white-throated sparrows (McFarlan et al., 2009; Zajac et al., 2011) and captive photostimulated yellow-rumped warblers (Dick, 2017). FAT/CD36 has been shown to increase at the transcript level by two to four times during migratory phases in wild and captive photostimulated white-throated sparrows, but protein changes could not be measured owing to a lack of antibody specificity (McFarlan et al., 2009; Zajac et al., 2011). In the same birds, FABPpm increased by fourfold and twofold at the transcript and protein levels, respectively, in wild migrants (McFarlan et al., 2009), but did not change at the transcript level in captive photostimulated sparrows (Zajac et al., 2011). H-FABP, FAT/CD36 and FABPpm protein concentrations of yellow warblers (*Setophaga petechia*) and warbling vireos (*Vireo gilvus*) mostly increased during spring and autumn migrations relative to the summer levels, although changes in transcript abundances were less consistent (Zhang et al., 2015). Price et al. (2010) did not find any changes in fatty acid transporters or oxidative enzymes in long-day-treated white-crowned sparrows, but attributed these results to a lack of response to photostimulation. Interestingly, fatty acid transporters and oxidative enzymes did not change in cardiac muscle during migration in captive photostimulated white-throated sparrows (Zajac et al., 2011). Oxidative enzyme activities in the heart were also constant in wild yellow warblers and warbling vireos; however, fatty acid transporters tended to decrease during migration in yellow warblers and increase during migration in warbling vireos (Zhang et al., 2015). These findings emphasize that the regulation of flight muscle fuel metabolism to favor exogenous fatty acids during migration may be decoupled from the heart. Overall, the myocyte uptake and transport hypothesis is well supported by data from both non-passerine and passerine birds.

The fuel metabolism of migratory bats presents a fascinating problem because if, like birds, they are able to fuel endurance flight almost completely with adipose fatty acids, then they are predicted to have much greater lipid transport and oxidation capacities than other mammals (McGuire and Guglielmo, 2009). Enzymes involved in fatty acid oxidation generally show high levels of activity in bat flight muscles (Suarez et al., 2009; Yacoe et al., 1982), and HOAD and CPT activities have been reported to increase by 53% and 32%, respectively, during migration in hoary bats

(McGuire et al., 2013b). The potential for fatty acid delivery via lipoproteins during migratory flight has not been studied in bats. All that is known is that little brown bats (*Myotis lucifugus*) show highly variable plasma triacylglycerol concentrations during aerial feeding, from barely detectable to >3 mmol l⁻¹, suggesting that lipoproteins can be present at high concentrations and available as fuel during flight (McGuire et al., 2009a,b). With respect to myocyte fatty acid transport capacity, the messenger ribonucleic acid (mRNA) transcript abundance of FAT/CD36 and FABPpm has not been found to change between summer residency and migration in hoary bats, and H-FABP mRNA increases in migratory females but not in males (McGuire et al., 2013b). Muscle concentrations of H-FABP, FAT/CD36 and FABPpm proteins have not been measured in bats, so it is not possible to compare the concentrations of these proteins in bats to those of birds or to test the hypothesis that they are more abundant than in other mammals. H-FABP expression has been shown to increase during hibernation in little brown bats, indicating that H-FABP expression responds to changing demands for fatty acid transport in other contexts (Eddy and Storey, 2004). Although respirometry studies show that fasted bats can fly while oxidizing fatty acids (Welch et al., 2008), the available evidence does not conclusively demonstrate that migratory bats can use solely exogenous fatty acids to fuel migratory flight or that they make seasonal adjustments to muscle fatty acid transporters. As aerial foragers, it is possible that they always maintain a high muscle fatty acid uptake capacity to use ingested lipids as fuel. Additional studies of migratory bat species are required to determine their metabolic and biochemical strategies.

New insights of the -omics age

The risk in formulating hypotheses based on prior knowledge of physiology and biochemistry is that cherry picking obvious or well-known pathways or regulatory systems may be biased, leading one to overlook novel and crucial information. With respect to how migratory birds achieve exceptionally high rates of fatty acid oxidation in muscle, attention has been focused on particular enzymes (CS, HOAD and CPT) and transporters (H-FABP, FAT/CD36 and FABPpm) that seem likely to be important regulatory steps or indicators of overall flux capacity. Transcriptomic and proteomic analyses provide unbiased assessments of changes in gene transcription and protein levels. Proteomic comparison of pectoralis muscles between non-migratory (short day) and migratory (long day) phenotypes of the black-headed bunting (*Emberiza melanocephala*) revealed significant upregulation of H-FABP, myoglobin and creatine kinase, and upregulation of H-FABP and myoglobin transcripts were also evident (Srivastava et al., 2014). Such changes did not occur in the Indian weaverbird (*Ploceus philippinus*), a full-year resident species (Srivastava et al., 2014). A proteomic analysis of the red-headed bunting revealed 24 proteins that were upregulated in the pre-migratory phase compared with non-migrants, and two proteins that were only expressed in pre-migrants (Banerjee and Chaturvedi, 2016). The upregulated proteins served a wide variety of functions, including fatty acid transport and oxidation and roles in the citric acid cycle, electron transport chain, glycogen metabolism, glycolysis, amination–deamination, heat shock and calcium regulation (Banerjee and Chaturvedi, 2016). An analysis of lipid-related genes of pectoralis muscle in gray catbirds throughout the annual cycle showed that transcript abundance of transcription factors PPAR α , PPAR δ , estrogen-related nuclear receptors (ERR β , ERR λ), PGC-1 α and PGC-1 β tended to be greater during the late-summer pre-migratory period and autumn migration than during spring migration and

breeding, whereas the opposite was generally apparent in some of the downstream targets of these transcription factors, such as hormone sensitive lipase (HSL), adipose triglyceride lipase (ATGL), FABPpm, medium-chain acyl CoA dehydrogenase (MCAD) and perilipins (PLIN2, PLIN3) but not FAT/CD36 or LPL (DeMoranville, 2015). High levels of expression of the transcription factors and variable expression of the targets were apparent during winter in the tropics (DeMoranville, 2015). Pectoralis muscle transcriptomes were studied in migratory and resident subspecies of the dark-eyed junco after spring photoperiod exposure, thus contrasting the migratory and reproductive states (Fudickar et al., 2016). In total, 366 genes were differentially expressed between subspecies, and gene ontology (GO) terms identified notable increases in genes related to lipid transport, fatty acid catabolism, ketone metabolism, peroxisomes and mitochondria in the migrant juncos. Dick (2017) found 455 genes to be differentially expressed between pectoralis muscles of autumn migratory and winter-condition captive yellow-rumped warblers. Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway analysis revealed that only four pathways were significantly upregulated, three of which were related to fatty acid metabolism and one to ribosomes. These included genes such as PGC-1 α , PGC-1 β , PPARs, FABP, acyl-CoA-dehydrogenase and a peroxisome-specific fatty acid transporter ABCD-1 (Dick, 2017). A surprising 45 pathways were downregulated in the migratory state, such as those related to muscle growth, inflammation and immune function, circadian rhythm and hormone signaling, suggesting that migratory preparation may require trade-offs in other muscle functions. These new studies generally verify that augmented fatty acid transport and oxidation pathways are very prominent features of avian migration, and also highlight aspects, such as mitochondrial biogenesis, microsomal fatty acid oxidation, protein synthesis capacity, inflammation and oxidative stress that should be explored in more detail in future studies. Transcriptomic and proteomic changes associated with migration in bats have not been examined and should be explored.

Potential benefits of unsaturated fatty acids for migrants

An intriguing question is whether unsaturated fatty acids in fat stores and muscle membranes improve the exercise and migratory performance of birds and other animals. If this is true for n-3 and n-6 PUFA, then selection for diets high in PUFA could be important for successful migration. Similar arguments have been made for diet shifts in mammals preparing to hibernate (Ruf and Arnold, 2008). The evidence regarding the effects of unsaturation in general, and PUFA in particular, on migratory birds has been reviewed previously (Guglielmo, 2010; Pierce and McWilliams, 2014; Price, 2010; Weber, 2009), so I will only cover this briefly here and discuss new findings.

One way that unsaturated fatty acids could improve endurance flight is by being better fuels (fuel hypothesis, *sensu* Price, 2010). For a given carbon chain length, the addition of double bonds generally increases the relative rate of fatty acid mobilization from adipocytes and the rate of oxidation by muscles (Fig. 4A). For example, in ruff sandpipers and white-crowned sparrows, 16:0 and 18:1n-9 are released from adipocytes in proportion to their abundance in adipocyte triacylglycerol, whereas fatty acids such as 20:0 and 20:1 are retained and PUFA are preferentially mobilized (Price et al., 2008). Similarly, the rate of CPT catalysis in flight muscles of five species of migratory and non-migratory birds decreased dramatically among saturated fatty acids as substrate chain length increased (16:0>18:0, 20:0 undetectable), but was

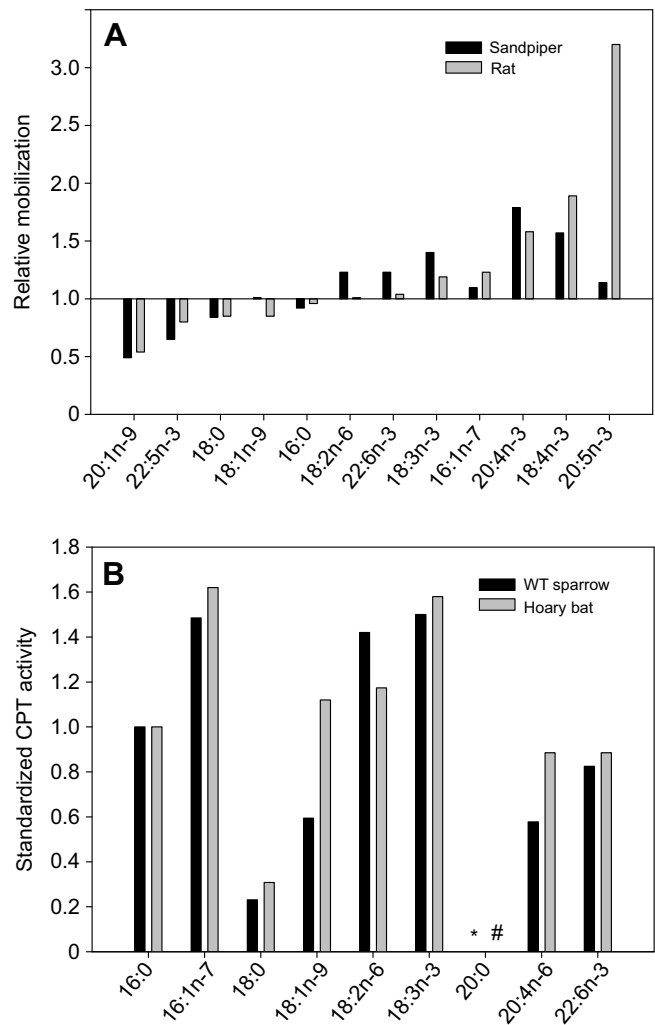


Fig. 4. Shorter and more unsaturated fatty acids may be better fuels for exercise. Fatty acid relative mobilization from (A) adipocytes and (B) catalysis by carnitine palmitoyltransferase (CPT) depends on the carbon chain length and the degree of unsaturation. Data in A were quantitatively estimated from fig. 1 of Price et al. (2008) and from fig. 3 of Raclot (2003). A value of 1 indicates that a fatty acid was released from adipocytes in direct proportion to its abundance in adipocyte triacylglycerol, whereas values greater and less than 1 indicate preferential mobilization and retention, respectively. Data in B were quantitatively estimated for white-throated (WT) sparrows from fig. 1 of Price et al. (2011) and for hoary bats from fig. 1 of Price et al. (2014). CPT activity was standardized in both studies to 16:0. * indicates a CPT activity of 20:0 was measured but not detectable in the white-throated sparrow. # indicates a CPT activity of 20:0 was not measured in the hoary bat. There is remarkable concordance between birds and mammals for both processes.

rescued by the addition of double bonds (Price et al., 2011). CPT activities measured with 18:2n-6 and 18:3n-3 surpassed the activity measured with 16:0, and 20:4n-6 and 22:6n-3 were used at nearly the same rate as 16:0 (Fig. 4B). The rate of oxidation of fatty acids by wing muscles of white-throated sparrows increased in the order 18:0<18:1n-9<18:2n-6. Mammals also preferentially mobilize and oxidize unsaturated fatty acids (Leyton et al., 1987; Raclot, 2003; Fig. 4A), and the flight muscle CPT of hoary bats reacted faster with unsaturated fatty acids in a similar pattern to that measured in birds (Price et al., 2014; Fig. 4B). Thus, the ease of mobility could make unsaturated fatty acids better fuel for endurance flights of birds and bats, and this could explain why their fat stores generally have high levels of unsaturation.

PUFA could improve exercise performance by influencing membrane function (the phospholipid hypothesis *sensu* Price, 2010), by activating PPAR pathways or by altering eicosanoid profiles (Pierce and McWilliams, 2014; Price, 2010; Weber, 2009). Membrane fatty acid composition responds to variation in dietary PUFA, and high PUFA membranes are more fluid, more permeable, and may allow enzymes and transporters to function at higher rates than low PUFA membranes, potentially increasing the metabolic rate or efficiency (Price, 2010). Fatty acids are natural ligands for PPAR (Wang, 2010). PUFA are generally stronger PPAR ligands than MUFA or saturated fatty acids (Desvergne and Wahli, 1999), and high PUFA availability could increase the expression of genes that increase the fatty acid oxidation capacity of muscles. PUFA are precursors for eicosanoid synthesis with n-6 PUFA-derived eicosanoids generally being more pro-inflammatory than those derived from n-3 PUFA (Price, 2010). The ratio of n-3 to n-6 PUFA could therefore influence inflammation and repair processes during and after flight.

One of the most energizing ideas relating to lipids and migratory birds has been the natural doping hypothesis (Maillet and Weber, 2007; Weber, 2009). Natural doping refers to the possibility that birds that eat high n-3 PUFA diets during fueling can enhance their endurance flight ability by increasing membrane functionality and activating PPAR to increase the expression of proteins involved in fatty acid transport and oxidation. The hypothesis was originally based on studies of semipalmated sandpipers refueling at the Bay of Fundy, Canada, which became highly enriched in long-chain n-3 PUFA (20:5n-3 and 22:6n-3) while feeding on *Corophium volutator* (see earlier discussion), and showed positive correlations between tissue n-3 PUFA and pectoralis activities of CS and HOAD but not CPT (Maillet and Weber, 2007). Wintering and migratory western sandpipers showed strong positive correlations between plasma n-3 PUFA and pectoralis HOAD and CPT activities, but not CS activity or H-FABP protein content (Guglielmo, 2010). Further support for natural doping came from a dosing study of non-migratory bobwhite quail (*Colinus virginianus*), which responded to daily supplementation with oils high in 20:5n-3 (59%) and 22:6n-3 (70%) by increasing pectoralis activities of CS, CPT, HOAD and COX (Nagahuedi et al., 2009). However, it is not certain that the correlations measured in sandpipers represent cause and effect, or that the variation in muscle enzyme activities meaningfully affect flight performance. Various studies suggest that either n-3 or n-6 PUFA improve exercise (reviewed in Price, 2010; Guglielmo, 2010). Definitive testing of the natural doping hypothesis requires experimental studies where exercise performance outcomes are measured.

Only a few experiments have been carried out to test the natural doping hypothesis. The study of Pierce et al. (2005) preceded the advent of the natural doping hypothesis but contains relevant data. Red-eyed vireos fed a lower total unsaturation diet that was enriched in PUFA (24 versus 14%) had greater aerobic scope while exercising in a flight wheel respirometer, suggesting that PUFA in general could be beneficial (Guglielmo, 2010; Pierce et al., 2005). White-throated sparrows were treated with feeding/fasting protocols with high n-3 and high n-6 PUFA diets, which produced either birds with fat stores and muscle membranes enriched in n-6 or n-3 PUFA or birds with fat stores with a similar n-3/n-6 composition but different muscle membrane PUFA (Price and Guglielmo, 2009). When the sparrows were exercised in a flight wheel, birds with high n-6 PUFA had an increased level of exercise performance, which was not evident when the fat stores were similar but muscles differed in PUFA composition. These findings indicated that n-6 were better

than n-3 PUFA for exercise in birds and supported the fuel hypothesis over the phospholipid hypothesis. Recent experiments tested large numbers of yellow-rumped warblers ($N=15$ per group) that were photostimulated into a migratory state, fed carefully controlled MUFA, high n-3 (22:6n-3) or high n-6 (20:4n-6) diets, and flown for up to six hours in a bird wind tunnel (Dick, 2017). Diet changed the n-3 to n-6 ratio of adipose triacylglycerol in the expected way, but the fat contained few 20 or 22 carbon fatty acids (<1%), showing that in a terrestrial songbird, even when diet is enriched in long-chain PUFA, fat stores contain predominately 16 and 18 carbon fatty acids. The fatty acid composition of pectoralis phospholipids changed greatly in response to the diet, notably in the amounts of 20:4n-6 (MUFA=10%, n-3=0.7%, n-6=19%) and 22:6n-3 (MUFA=8%, n-3=25%, n-6=7%). In contrast to the hypothesis of natural doping, in relation to the MUFA diet, pectoralis CPT activity was reduced by both PUFA diets, CS activity was significantly increased by the n-6 diet and reduced by the n-3 diet, HOAD was reduced by the n-3 diet, LDH was increased by the n-3 diet, and diet had no effect on H-FABP expression. PPAR δ (PPAR β) expression in the pectoralis was also decreased by the n-3 diet, whereas PPAR α and PPAR γ expression were unaffected. Diet did not affect metabolic and flight performance measures: BMR, peak metabolic rate in a flight wheel, aerobic scope in a flight wheel, voluntary flight duration, flight energy cost, or the relative contribution of protein to energy during flight.

Overall, the experimental evidence does not support natural doping in songbirds, and two studies suggest that high n-3 PUFA diets may be detrimental to some performance traits (Dick, 2017; Price and Guglielmo, 2009). Studies of captive songbirds kept on constant diets also show that photoperiod changes alone are sufficient to induce changes in metabolic enzymes and fatty acid transporters in flight muscle (Dick, 2017; Zajac et al., 2011), so that when songbirds are in the migratory state they may not respond strongly or positively to dietary PUFA (i.e. they cannot go any higher). The strong response of bobwhite quail muscles to n-3 PUFA may be because their muscles are normally dominated by anaerobic fibers, which may be induced to a more oxidative phenotype by PPAR and associated signaling pathways. Migrating shorebirds have some of the highest muscle activities of oxidative enzymes and concentrations of fatty acid transporters measured in any birds (Guglielmo et al., 2002a; Maillet and Weber, 2007). Whether they can or need to go higher by doping with high n-3 PUFA diets, or whether high n-3 PUFA diets are what take them to this highest level of performance is not completely resolved. Perhaps marine-associated birds have reduced elongase and desaturase activity compared with terrestrial birds, making them more dependent on the dietary input of 20 and 22 carbon PUFA, as is the case for marine compared with freshwater fish (Glencross, 2009). Further comparative studies and experiments with shorebirds and perhaps bats, which have not been considered in this context, will be required to answer these questions.

Conclusions

Migratory birds and bats are high-performance exercise machines, but rather than burning what exercise physiologists consider an easy fuel (glucose), they use the equivalent of diesel oil; hard to transport and metabolize fatty acids with a bit of their precious protein mixed in as a spark. These animals show us that fatty acids are indeed fantastic high-energy fuels that can be used to power the highest aerobic metabolic rates, given the right adaptations to the energy supply system.

Much has previously been written about fat, particularly in birds, and so my objective in this Review was to cover key patterns and processes, to update information with recent studies, and to try to draw out new generalities and ideas. Another goal was to thoroughly discuss experimental studies that have tested important ideas about lipid metabolism in migrants, such as lipoprotein transport, unsaturated fatty acids as fuel and natural doping. Physiologists are empiricists and they must seek to experimentally verify patterns that have been described in nature and then delve deeper into mechanisms and regulation. If migratory species are to be managed to provide particular foods for fueling that are believed to improve fitness, then experimental studies must be conducted to determine the most relevant information and to provide a solid justification for managing migratory species in this way. There is much yet to be discovered about fat metabolism in migratory birds and bats, from the most basic descriptive information to complex metabolic and regulatory networks.

Acknowledgements

I thank Raul Suarez and Hans Hoppeler for their invitation to participate in the 2017 JEB Symposium on the Biology of Fat. I am grateful to the symposium participants for their informative and thought-provoking presentations, and for many stimulating conversations that followed. I thank my present and former graduate students, Morag F. Dick, Edwin R. Price, Alexander R. Gerson, Liam P. McGuire, Kristin Jonasson and Dylan Baloun for their excellent research and valuable insights, and for allowing me to include their published and unpublished findings.

Competing interests

The author declares no competing or financial interests.

Funding

Funding for the symposium was provided by JEB and my research is supported by the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation and the Ontario Research Fund.

References

- Ackman, R. G., Nash, D. M. and McLachlan, J. (1979). Lipids and fatty acids of *Corophium volutator* from the Minas Basin. *Proc. N.S. Inst. Sci.* **29**, 501-516.
- Afik, D., Caviedes-Vidal, E., Martinez del Rio, C. and Karasov, W. L. (1995). Dietary modulation of intestinal hydrolytic enzymes in yellow-rumped warblers. *Amer. J. Physiol. Reg. Comp. Integr. Physiol.* **268**, R413-R420.
- Afik, D., Darken, B. W. and Karasov, W. H. (1997a). Is diet shifting facilitated by modulation of intestinal nutrient uptake? Test of an adaptational hypothesis in yellow-rumped warblers. *Physiol. Zool.* **70**, 213-221.
- Afik, D., McWilliams, S. R. and Karasov, W. H. (1997b). A test for passive absorption of glucose in yellow-rumped warblers and its ecological implications. *Physiol. Zool.* **70**, 370-377.
- Alerstam, T. and Lindström, Å. (1990). Optimal bird migration: the relative importance of time, energy and safety. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 331-351. New York: Springer-Verlag.
- Bairlein, F. (1992). Recent prospects on trans-Saharan migration of songbirds. *Ibis* **134**, 41-46.
- Bairlein, F. (2002). How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. *Naturwissenschaften* **89**, 1-10.
- Bairlein, F., Norris, D. R., Nagel, R., Bulte, M., Voigt, C. C., Fox, J. W., Hussell, D. J. T. and Schmaljohann, H. (2012). Cross hemisphere migration of a 25g songbird. *Biol. Lett.* **8**, 505-507.
- Baker, R. R. (1978). *The Evolutionary Ecology of Animal Migration*. New York: Holmes and Meier.
- Baloun, D. E. (2017). Energetics of migratory bats during stopover: a test of the torpor-assisted migration hypothesis. *M.Sc. Thesis*, University of Western Ontario, London, ON.
- Banerjee, S. and Chaturvedi, C. M. (2016). Migratory preparation associated alterations in pectoralis muscle biochemistry and proteome in Palearctic-Indian emberizid migratory finch, red-headed bunting, *Emberiza bruniceps*. *Comp. Biochem. Physiol. D* **17**, 9-25.
- Battley, P. F., Piersma, T., Dietz, M. W., Tang, S., Dekinga, A. and Hulsman, K. (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proc. R. Soc. B* **267**, 191-195.
- Bauchinger, U. and McWilliams, S. R. (2009). Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. *Physiol. Biochem. Zool.* **82**, 787-797.
- Bauchinger, U., Wohlmann, A. and Biebach, H. (2005). Flexible remodeling of organ size during spring migration of the garden warbler (*Sylvia borin*). *Zoology* **108**, 97-106.
- Berthold, P. (1993). *Bird Migration, a General Survey*. New York: Oxford University Press.
- Biebach, H. (1996). Energetics of winter and migratory fattening. In *Avian Energetics and Nutritional Ecology* (ed. and C. , Carey), pp. 280-323. New York: Chapman and Hall.
- Blem, C. R. (1976). Patterns of lipid storage and utilization in birds. *Amer. Zool.* **16**, 671-684.
- Blem, C. R. (1980). The energetics of migration. In *Animal Migration, Orientation, and Navigation* (ed. S. A. Gauthreaux), pp. 175-224. New York: Academic Press.
- Blem, C. R. (1990). Avian energy storage. In *Current Ornithology*, Vol. 7 (ed. D. M. Power), pp. 59-113. New York: Plenum Press.
- Bordel, R. and Haase, E. (1993). Effects of flight on blood parameters in homing pigeons. *J. Comp. Physiol. B* **163**, 219-224.
- Boswell, T. (2005). Regulation of energy balance in birds by the neuroendocrine hypothalamus. *J. Poult. Sci.* **42**, 161-181.
- Boswell, T. and Dunn, I. C. (2015). Regulation of the avian central melanocortin system and the role of leptin. *Gen. Comp. Endocr.* **221**, 278-283.
- Brooks, G. A. (1998). Mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol. B* **120**, 89-107.
- Burns, J. G. and Ydenberg, R. C. (2002). The effects of wing loading and gender on the escape flights of least sandpipers (*Calidris minutilla*) and western sandpipers (*Calidris mauri*). *Behav. Ecol. Sociobiol.* **52**, 128-136.
- Bussi re-C t , S., Omlina, T., Pinheiro, E. C. and Weber, J.-M. (2016). Gemfibrozil disrupts the metabolism of circulating lipids in bobwhite quails. *Comp. Biochem. Physiol. C* **179**, 137-143.
- Butler, P. J. and Woakes, A. J. (1990). The physiology of bird flight. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 300-318. New York: Springer-Verlag.
- Butler, P. J. and Woakes, A. J. (2001). Seasonal hypothermia in a large migrating bird: saving energy for fat deposition? *J. Exp. Biol.* **204**, 1361-1367.
- Calvert, A. M., Bishop, C. A., Elliot, R. D., Krebs, E. A., Kydd, T. M., Machtans, C. S. and Robertson, G. J. (2013). A synthesis of human-related avian mortality in Canada. *Avian Conserv. Ecol.* **8**, 11.
- Carpenter, F. L., Hixon, M. A., Beuchat, C. A., Russell, R. W. and Paton, D. C. (1993). Biphasic mass gain in migrant hummingbirds: body composition changes, torpor, and ecological significance. *Ecology* **74**, 1173-1182.
- Caviedes-Vidal, E., McWhorter, T. J., Lavin, S. R., Chediack, J. G., Tracy, C. R. and Karasov, W. H. (2007). The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. *Proc. Nat. Acad. Sci. USA* **104**, 19132-19137.
- Cerasale, D. J., Zajac, D. M. and Guglielmo, C. G. (2011). Behavioural and physiological effects of photoperiod-induced migratory state and leptin on a migratory bird, *Zonotrichia albicollis*: I. Anorectic effects of leptin administration. *Gen. Comp. Endocrinol.* **174**, 276-286.
- Christians, J. K. (1999). Controlling for body mass effects: is part-whole correlation important? *Physiol. Biochem. Zool.* **72**, 250-253.
- Corder, K. R., DeMoranville, K. J., Russell, D. E., Huss, J. M. and Schaeffer, P. J. (2016). Annual life-stage regulation of lipid metabolism and storage and association with PPARs in a migrant species: the gray catbird (*Dumetella carolinensis*). *J. Exp. Biol.* **219**, 3391-3398.
- Cornelius, J. M., Boswell, T., Jenni-Eiermann, S., Breuner, C. W. and Ramenofsky, M. (2013). Contributions of endocrinology to the migration life history of birds. *Gen. Comp. Endocrinol.* **190**, 47-60.
- Cryan, P. M. (2003). Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *J. Mammal.* **84**, 579-593.
- Cryan, P. M. and Wolf, B. O. (2003). Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. *J. Exp. Biol.* **206**, 3381-3390.
- DeLuca, W. V., Woodworth, B. K., Rimmer, C. C., Marra, P. P., Taylor, P. D., McFarland, K. P., Mackenzie, S. A. and Norris, D. R. (2015). Transoceanic migration by a 12 g songbird. *Biol. Lett.* **11**, 20141045.
- DeMoranville, K. J. (2015). Characterization and molecular regulation of metabolic and muscle flexibility in a neotropical migrant, *Dumetella carolinensis* (gray catbird). *MAS Thesis*, Miami University, Oxford, OH.
- Desvergne, B. and Wahli, W. (1999). Peroxisome proliferator activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20**, 649-688.
- Dick, M. F. (2017). The long haul: migratory flight preparation and performance in songbirds. *PhD Thesis*, University of Western Ontario, London, ON.
- Dingle, H. (1996). *Migration. The Biology of Life on the Move*. New York: Oxford University Press.
- Drent, R. and Piersma, T. (1990). An exploration of the energetics of leap-frog migration in arctic breeding waders. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 399-412. New York: Springer Verlag.
- 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 semipalmated sandpipers (*Calidris pusilla*). *Can. J. Zool.* **71**, 1602-1608.

- Eddy, S. F. and Storey, K. B.** (2004). Up-regulation of fatty acid-binding proteins during hibernation in the little brown bat, *Myotis lucifugus*. *Biochim. Biophys. Acta* **1676**, 63-70.
- 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.
- Egeler, O., Williams, T. D. and Guglielmo, C. G.** (2000). Modulation of lipogenic enzymes, fatty acid synthase and D9-desaturase, in relation to migration in the western sandpiper (*Calidris mauri*). *J. Comp. Physiol. B* **170**, 169-174.
- Egeler, O., Seaman, D. A. and Williams, T. D.** (2003). Influence of diet on fatty acid composition of depot fat in western sandpipers (*Calidris mauri*). *Auk* **120**, 337-345.
- Eikenaar, C., Fritzsche, A. and Bairlein, F.** (2013). Corticosterone and migratory fueling in Northern wheatears facing different barrier crossings. *Gen. Comp. Endocr.* **186**, 181-186.
- Eikenaar, C., Bairlein, F., Stöwe, M. and Jenni-Eiermann, S.** (2014). Corticosterone, food intake and refueling in a long-distance migrant. *Horm. Behav.* **65**, 480-487.
- Evans, P. R., Davidson, N. C., Uttley, J. D. and Evans, R. D.** (1992). Premigratory hypertrophy of flight muscles: an ultrastructural study. *Ornis Scand.* **23**, 238-243.
- Ewing, W. G., Studier, E. H. and O'Farrell, M. J.** (1970). Autumn fat deposition and gross body composition in three species of *Myotis*. *Comp. Biochem. Physiol.* **36**, 119-129.
- Fleming, T. H. and Eby, P.** (2003). Ecology of bat migration. In *Bat Ecology* (ed. T. H. Kunz and M. B. Fenton), pp. 157-208. Chicago: University of Chicago Press.
- Fraser, R., Heslop, V. R., Murray, F. E. M. and Day, W. A.** (1986). Ultrastructural studies of the portal transport of fat in chickens. *Brit. J. Exp. Path.* **67**, 783-791.
- Friedman-Einat, M., Cogburn, L. A., Yosefi, S., Hen, G., Shinder, D., Shirak, A. and Seroussi, E.** (2014). Discovery and characterization of the first genuine avian leptin gene in the rock dove (*Columba livia*). *Endocrinology* **155**, 3376-3384.
- Fudickar, A. M., Peterson, M. P., Greives, T. J., Atwell, J. W., Bridge, E. S. and Ketterson, E. D.** (2016). Differential gene expression in seasonal sympatry: mechanisms involved in diverging life histories. *Biol. Lett.* **12**, 20160069.
- Gerson, A. R. and Guglielmo, C. G.** (2011). Endurance flight at low ambient humidity increases protein catabolism in migratory birds. *Science* **333**, 1434-1436.
- Gerson, A. R. and Guglielmo, C. G.** (2013). Energetics and metabolite profiles during early flight in American robins (*Turdus migratorius*). *J. Comp. Physiol. B* **183**, 983-991.
- Gill, R. E., Tibbitts, T. L., Douglas, D. C., Handel, C. M., Mulcahy, D. M., Gottschalk, J. C., Warnock, N., McCaffery, B. J., Battley, P. F. and Piersma, T.** (2009). Extreme endurance flights by landbirds crossing the Pacific Ocean: ecological corridor rather than barrier? *Proc. R. Soc. B* **276**, 447-457.
- Glencross, B. D.** (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture* **1**, 71-124.
- Goymann, W., Lupi, S., Kaiyad, H., Cardinale, M. and Fusani, L.** (2017). Ghrelin affects stopover decisions and food intake in a long-distance migrant. *Proc. Nat. Acad. Sci. USA* **114**, 1946-1951.
- Guglielmo, C. G.** (2010). Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integ. Comp. Biol.* **50**, 336-345.
- 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., 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. *Amer. J. Physiol. Reg. Comp. Integr. 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.
- Guglielmo, C. G., Cerasale, D. J. and Eldermire, C.** (2005). A field validation of plasma metabolite profiling to assess refueling performance of migratory birds. *Physiol. Biochem. Zool.* **78**, 116-125.
- Guglielmo, C. G., McGuire, L. P., Gerson, A. R. and Seewagen, C. L.** (2011). Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *J. Ornithol.* **152**, 75-85.
- Guglielmo, C. G., Gerson, A. R., Price, E. R. and Hays, Q. R.** (2017). The effects of dietary macronutrients on flight ability, energetics, and fuel metabolism of yellow-rumped warblers *Setophaga coronata*. *J. Avian Biol.* **48**, 133-148.
- Gurr, M. I., Harwood, J. L., Frayn, K. N., Murphy, D. J. and Michell, R. H.** (2016). *Lipids: Biochemistry, Biotechnology and Health*. Oxford: Wiley Blackwell.
- Hedenström, A.** (1993). Migration by soaring or flapping flight in birds: the relative importance of energy cost and speed. *Phil. Trans. R. Soc. Lond. B* **342**, 353-361.
- Hedenström, A. and Alerstam, T.** (1992). Climbing performance of migrating birds as a basis for estimating limits for fuel-carrying capacity and muscle work. *J. Exp. Biol.* **164**, 19-38.
- Hiebert, S.** (1993). Seasonal changes in body mass and use of torpor in a migratory hummingbird. *Auk* **110**, 787-797.
- Holberton, R., Wilson, C. M., Hunter, M., Cash, W. and Sims, C.** (2007). The role of corticosterone in supporting migratory lipogenesis in the dark-eyed Junco, *Junco hyemalis*: a model for central and peripheral regulation. *Physiol. Biochem. Zool.* **80**, 125-137.
- Huang, G., Li, J., Wang, H., Lan, X. and Wang, Y.** (2014). Discovery of a novel functional leptin protein (LEP) in zebra finches: evidence for the existence of an authentic avian leptin gene predominantly expressed in the brain and pituitary. *Endocrinology* **155**, 3389-3396.
- Jenni, L. and Jenni-Eiermann, S.** (1998). Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521-528.
- Jenni, L., Jenni-Eiermann, S., Spina, F. and Schwabl, H.** (2000). Regulation of protein breakdown and adrenocortical response to stress in birds during migratory flight. *Amer. J. Physiol. Reg. Comp. Integr. Physiol.* **278**, R1182-R1189.
- 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., Kvist, A., Lindström, Å., Jenni, L. and Piersma, T.** (2002). Fuel use and metabolic response to endurance exercise: a windtunnel study of a long-distance migrant shorebird. *J. Exp. Biol.* **205**, 2453-2460.
- Jonasson, K. A.** (2017). Energy balance and movement ecology of migratory bats. PhD Thesis, University of Western Ontario, London, ON.
- Jonasson, K. A. and Guglielmo, C. G.** (2016). Sex differences in spring migration timing and body composition of *Lasionycteris noctivagans*. *J. Mammal.* **97**, 1535-1542.
- Kaiser, A.** (1993). A new multi-category classification of subcutaneous fat deposits of songbirds. *J. Field Ornithol.* **64**, 246-255.
- Karasov, W. H. and Pinshow, B.** (1998). Changes in lean mass and in organs of nutrient assimilation in a long-distance passerine migrant at a springtime stopover site. *Physiol. Zool.* **71**, 435-448.
- Kennedy, L. V.** (2012). Dynamics of Fat and Lean Mass in Refuelling Migrant Passerines Measured Using Quantitative Magnetic Resonance. MSc Thesis, The University of Western Ontario, London, ON.
- Kerlinger, P.** (1989). *Flight Strategies of Migrating Hawks*. Chicago: University Press.
- Klaassen, M.** (1996). Metabolic constraints on long-distance migration in birds. *J. Exp. Biol.* **199**, 57-64.
- Klaassen, R. H. G., Alerstam, T., Carlsson, P., Fox, J. W. and Lindström, Å.** (2011). Great flights by great snipes: long and fast non-stop migration over benign habitats. *Biol. Lett.* **7**, 833-835.
- Klaiman, J. M., Price, E. R. and Guglielmo, C. G.** (2009). Fatty acid composition of pectoralis muscle membrane, intramuscular fat stores and adipose tissue of migrant and wintering white-throated sparrows (*Zonotrichia albicollis*). *J. Exp. Biol.* **212**, 3865-3872.
- Korine, C., Daniel, S., van Tets, I. G., Yosef, R. and Pinshow, B.** (2004). Measuring fat mass in small birds by dual-energy x-ray absorptiometry. *Physiol. Biochem. Zool.* **77**, 522-529.
- Kronfeld-Schor, N., Richardson, C., Silva, B. A., Kunz, T. H. and Widmaier, E. P.** (2000). Dissociation of leptin secretion and adiposity during prehibernatory fattening in little brown bats. *Amer. J. Physiol. Reg. Comp. Integr. Physiol.* **279**, R1277-R1281.
- Kunz, T. H., Arnett, E. B., Erickson, W. P., Hoar, A. R., Johnson, G. D., Larkin, R. P., Strickland, M. D., Thresher, R. W. and Tuttle, M. D.** (2007). Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. *Front. Ecol. Environ.* **5**, 315-324.
- Kvist, A. and Lindström, Å.** (2000). Maximum daily energy intake: it takes time to lift the metabolic ceiling. *Physiol. Biochem. Zool.* **73**, 30-36.
- Kvist, A. and Lindström, Å.** (2003). Gluttony in migratory waders—unprecedented energy assimilation rates in vertebrates. *Oikos* **103**, 397-402.
- Landys, M. M., Piersma, T., Guglielmo, C. G., Jukema, J., Ramenofsky, M., Wingfield, J. C.** (2005). Metabolic profile of long-distance migratory flight and stopover in a shorebird. *Proc. R. Soc. B* **272**, 295-302.
- Landys-Cianelli, M. M., Ramenofsky, M., Guglielmo, C. G. and Wingfield, J. C.** (2004). The low-affinity glucocorticoid receptor regulates feeding and lipid breakdown in the migratory Gambel's white-crowned sparrow *Zonotrichia leucophrys gambelii*. *J. Exp. Biol.* **207**, 143-154.
- Langlois, L. A. and McWilliams, S. R.** (2010). Protein requirements of an omnivorous and a granivorous songbird decrease during migration. *Auk* **127**, 850-862.
- 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. and Alerstam, T.** (1992). Optimal fat loads in migrating birds: a test of the time-minimization hypothesis. *Am. Nat.* **140**, 477-491.
- Lindström, Å. and Piersma, T.** (1993). Mass changes in migrating birds: the evidence for fat and protein storage re-examined. *Ibis* **135**, 70-78.
- Lindström, Å., Kvist, A., Piersma, T., Dekinga, A. and Dietz, M. W.** (2000). Avian pectoral muscle size rapidly tracks body mass changes during flight, fasting and fuelling. *J. Exp. Biol.* **203**, 913-919.

- 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.
- Maggini, I., Kennedy, L. V., Bursian, S. J., Dean, K. M., Gerson, A. R., Harr, K. E., Link, J. E., Pritsos, C., Pritsos, K. L. and Guglielmo, C. G.** (2017a). Toxicological and thermoregulatory effects of feather contamination with artificially weathered MC 252 oil in western sandpipers (*Calidris mauri*). *Ecotox. Environ. Safety*. **146**, 118-128.
- Maggini, I., Kennedy, L. V., Macmillan, A., Elliott, K. H., Dean, K. and Guglielmo, C. G.** (2017b). Light oiling of feathers increases flight energy expenditure in a migratory shorebird. *J. Exp. Biol.* **220**, 2372-2379.
- Magnoni, L., Vaillancourt, E. and Weber, J.-M.** (2008). High resting triacylglycerol turnover of rainbow trout exceeds the energy requirements of endurance swimming. *Amer. J. Physiol. Reg. Comp. Integrat. Physiol.* **295**, R309-R315.
- Maillet, D. and Weber, J.-M.** (2006). Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird, the semipalmated sandpiper. *J. Exp. Biol.* **209**, 2686-2695.
- Maillet, D. and Weber, J.-M.** (2007). Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. *J. Exp. Biol.* **210**, 413-420.
- 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.
- Marsh, R. L.** (1983). Adaptations of the gray catbird *Dumetella carolinensis* to long-distance migration: energy stores and substrate concentrations in plasma. *Auk* **100**, 170-179.
- Marsh, R. L.** (1984). Adaptations of the gray catbird *Dumetella carolinensis* to long-distance migration: flight muscle hypertrophy associated with elevated body mass. *Physiol. Zool.* **57**, 105-117.
- Marshall, T. J., Dick, M. F. and Guglielmo, C. G.** (2015). Seasonal dietary shifting in songbirds is not caused by changing nutritional targets. *Comp. Biochem. Physiol. A* **192**, 57-63.
- McClelland, G. B.** (2004). Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. *Comp. Biochem. Physiol. B* **139**, 443-460.
- McCue, M. D., Sivan, O., McWilliams, S. R. and Pinshow, B.** (2010). Tracking the oxidative kinetics of carbohydrates, amino acids and fatty acids in the house sparrow using exhaled ¹³CO₂. *J. Exp. Biol.* **213**, 782-789.
- McFarlan, J. T., Bonen, A. and Guglielmo, C. G.** (2009). Seasonal up-regulation of protein mediated fatty acid transporters in flight muscles of migratory white-throated sparrows (*Zonotrichia albicollis*). *J. Exp. Biol.* **212**, 2934-2940.
- McGuire, L. P. and Guglielmo, C. G.** (2009). What can birds tell us about the migration physiology of bats? *J. Mammal.* **90**, 1290-1297.
- McGuire, L. P. and Guglielmo, C. G.** (2010). Quantitative magnetic resonance: a rapid, non-invasive body composition analysis technique for live and salvaged bats. *J. Mammal.* **91**, 1375-1380.
- McGuire, L. P., Fenton, M. B., Faure, P. A. and Guglielmo, C. G.** (2009a). Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. *Physiol. Biochem. Zool.* **82**, 812-818.
- McGuire, L. P., Fenton, M. B. and Guglielmo, C. G.** (2009b). Effect of age on energy storage during pre-hibernation swarming in little brown bat (*Myotis lucifugus*). *Can. J. Zool.* **87**, 515-519.
- McGuire, L. P., Guglielmo, C. G., Mackenzie, S. A. and Taylor, P. D.** (2012). Migratory stopover in the long-distance migrant silver-haired bat, *Lasiurus noctivagans*. *J. Anim. Ecol.* **81**, 377-385.
- McGuire, L. P., Fenton, M. B. and Guglielmo, C. G.** (2013a). Phenotypic flexibility in migrating bats: seasonal variation in body composition, organ sizes and fatty acid profiles. *J. Exp. Biol.* **216**, 800-808.
- McGuire, L. P., Fenton, M. B. and Guglielmo, C. G.** (2013b). Seasonal upregulation of catabolic enzymes and fatty acid transporters in the flight muscle of migrating hoary bats, *Lasiurus cinereus*. *Comp. Biochem. Physiol. B* **165**, 138-143.
- McGuire, L. P., Jonasson, K. A. and Guglielmo, C. G.** (2014). Bats on a budget: torpor-assisted migration saves time and energy. *PLoS ONE* **9**, e115724.
- 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. A* **128**, 579-593.
- McWilliams, S. R. and Karasov, W. H.** (2014). Spare capacity and phenotypic flexibility in the digestive system of a migratory bird: defining the limits of animal design. *Proc. R. Soc. B* **281**, 20140308.
- McWilliams, S. R. and Whitman, M.** (2013). Non-destructive techniques to assess body composition of birds: a review and validation study. *J. Ornithol.* **154**, 597-618.
- McWilliams, S. R., Kearney, S. B. and Karasov, W. H.** (2002). Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. *J. Avian Biol.* **33**, 167-174.
- McWilliams, S. R., Guglielmo, C., Pierce, B. and Klaassen, M.** (2004). Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. *J. Avian Biol.* **35**, 377-393.
- Moore, F. R. 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. A* **48**, 329-335.
- Mujires, F. T., Johansson, C. L., Bowlin, M. S., Winter, Y. and Hedenström, A.** (2012). Comparing aerodynamic efficiency in birds and bats suggests better flight performance in birds. *PLoS ONE* **7**, e37335.
- Nagahuedi, S., Popescu, J. T., Trudeau, V. L. and Weber, J.-M.** (2009). Mimicking the natural doping of migrant sandpipers in sedentary quails: effects of dietary n-3 fatty acids on muscle membranes and PPAR expression. *J. Exp. Biol.* **212**, 1106-1114.
- Napolitano, G. E. and Ackman, R. G.** (1990). Anatomical distribution of lipids and their fatty acids in the semipalmated sandpiper *Calidris pusilla* L. from Shepody Bay, New Brunswick, Canada. *J. Exp. Mar. Biol. Ecol.* **144**, 113-124.
- Newton, I.** (2008). *The Migration Ecology of Birds*. London: Academic Press.
- Owen, M.** (1981). Abdominal profile: a condition index for wild geese in the field. *J. Wildl. Manage.* **45**, 227-230.
- Parrish, J. D.** (1997). Patterns of frugivory and energetic condition in Nearctic landbirds during autumn migration. *Condor* **99**, 681-697.
- Pelsters, 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. *Amer. J. Physiol. Reg. Comp. Integrat. Physiol.* **276**, R637-R643.
- Pennycuik, C. J.** (1998). Computer simulation of fat and muscle burn in long-distance bird migration. *J. Theor. Biol.* **191**, 47-61.
- Pierce, B. J. and McWilliams, S. R.** (2005). Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. *Condor* **107**, 269-279.
- Pierce, B. J. and McWilliams, S. R.** (2014). The fat of the matter: how dietary fatty acids can affect exercise performance. *Integ. Comp. Biol.* **54**, 903-912.
- Pierce, B. J., McWilliams, S. R., O'Connor, T., Place, A. R. and Guglielmo, C. G.** (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo. *J. Exp. Biol.* **208**, 1277-1285.
- Piersma, T. and Everaarts, J. M.** (1996). Build-up of red blood cells in refuelling bar-tailed godwits in relation to individual migratory quality. *Condor* **98**, 363-370.
- Piersma, T. and Gill, R. E. J.** (1998). Guts don't fly: small digestive organs in obese bar-tailed godwits. *Auk* **115**, 196-203.
- Piersma, T. and van Gils, J. A.** (2011). *The Flexible Phenotype*. New York: Oxford University Press.
- 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.
- Pond, C. M.** (1978). Morphological aspects and the ecological and mechanical consequences of fat deposition in wild vertebrates. *Annu. Rev. Ecol. Syst.* **9**, 519-570.
- Price, E. R.** (2010). Dietary lipid composition and avian migratory flight performance: development of a theoretical framework for avian fat storage. *Comp. Biochem. Physiol. A* **157**, 297-309.
- Price, E. R. and Guglielmo, C. G.** (2009). The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis*). *Amer. J. Physiol. Reg. Comp. Integrat. Physiol.* **297**, R775-R782.
- Price, E. R., Krokfors, A. and Guglielmo, C. G.** (2008). Selective mobilization of fatty acids from adipose tissue in migratory birds. *J. Exp. Biol.* **211**, 29-34.
- Price, E. R., McFarlan, J. T. and Guglielmo, C. G.** (2010). Preparing for migration? The effects of photoperiod and exercise on muscle oxidative enzymes, lipid transporters, and phospholipids in white-crowned sparrows. *Physiol. Biochem. Zool.* **83**, 252-262.
- Price, E. R., Staples, J. F., Milligan, C. L. and Guglielmo, C. G.** (2011). Carnitine palmitoyl transferase activity and whole muscle oxidation rates vary with fatty acid substrate in avian flight muscles. *J. Comp. Physiol. B* **181**, 565-573.
- Price, E. R., McGuire, L. P., Fenton, M. B. and Guglielmo, C. G.** (2014). Flight muscle carnitine palmitoyl transferase activity varies with substrate chain length and unsaturation in the hoary bat (*Lasiurus cinereus*). *Can. J. Zool.* **92**, 173-176.
- Price, E. R., Brun, A., Caviedes-Vidal, E. and Karasov, W. H.** (2015). Digestive adaptations of aerial lifestyles. *Physiology* **30**, 69-78.
- Raclot, T.** (2003). Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257-288.

- Ramachandran, R., Maddineni, S., Ocón-Grove, O., Hendricks, G., III, Vasilatos-Younken, R. and Hadley, J. A. (2013). Expression of adiponectin and its receptors in avian species. *Gen. Comp. Endocr.* **190**, 88-95.
- Ramenofsky, M. (1990). Fat storage and fat metabolism in relation to migration. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 214-231. New York: Springer-Verlag.
- 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. A* **122**, 385-397.
- Reynolds, P. S. and Lee, R. M. (1996). Phylogenetic analysis of avian energetics: passerines and non-passerines do not differ. *Am. Nat.* **147**, 735-759.
- Rousseau, K., Atcha, Z. and Loudon, A. S. I. (2003). Leptin and seasonal mammals. *J. Neuroendocrinol.* **15**, 409-415.
- Ruf, T. and Arnold, W. (2008). Effects of polyunsaturated fatty acids on hibernation and torpor: a review and hypothesis. *Amer. J. Physiol. Reg. Comp. Integrat. Physiol.* **294**, R1044-R1052.
- Salewski, V., Kery, M., Herremans, M., Liechti, F. and Jenni, L. (2009). Estimating fat and protein fuel from fat and muscle scores in passerines. *Ibis* **151**, 640-653.
- 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.
- Schalk, G. and Brigham, R. M. (1995). Prey selection by insectivorous bats: are essential fatty acids important? *Can. J. Zool.* **73**, 1855-1859.
- Schmaljohann, H., Liechti, F. and Bruderer, B. (2007). Songbird migration across the Sahara: the non-stop hypothesis rejected! *Proc. R. Soc. B* **274**, 735-739.
- 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.
- Schwilch, R., Grattarola, A., Spina, F. and Jenni, L. (2002). Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint. *J. Exp. Biol.* **205**, 687-695.
- Seewagen, C. L. and Guglielmo, C. G. (2011). Quantitative magnetic resonance analysis and a morphometric predictive model reveal lean body mass changes in migrating Nearctic-Neotropical passerines. *J. Comp. Physiol. B* **181**, 413-421.
- Shah, R. V., Patel, S. T. and Pilo, B. (1978). Glucose-6-phosphate dehydrogenase and 'malic' enzyme activities during adaptive hyperlipogenesis in migratory starling (*Stumus roseus*) and white wagtail (*Motacilla alba*). *Can. J. Zool.* **56**, 2083-2087.
- Sillet, T. S. and Holmes, R. T. (2002). Variation in survivorship of a migratory songbird throughout its annual cycle. *J. Anim. Ecol.* **71**, 296-308.
- Smith, S. B. and McWilliams, S. R. (2009). Dietary macronutrients affect lipid metabolites and body composition of a migratory passerine, the white-throated sparrow (*Zonotrichia albicollis*). *Physiol. Biochem. Zool.* **82**, 258-269.
- Srivastava, S., Rani, S. and Kumar, V. (2014). Photoperiodic induction of pre-migratory phenotype in a migratory songbird: identification of metabolic proteins in flight muscles. *J. Comp. Physiol. B* **184**, 741-751.
- Stein, R. W., Place, A. R., Lacourse, T., Guglielmo, C. G. and Williams, T. D. (2005). Digestive organ sizes and enzyme activities of refueling western sandpipers (*Calidris mauri*): contrasting effects of Season and age. *Physiol. Biochem. Zool.* **78**, 434-446.
- Stuber, E. F., Verpeut, J., Horvat-Gordon, M., Ramachandran, R. and Bartell, P. A. (2013). Differential regulation of adipokines may influence migratory behavior in the white-throated sparrow (*Zonotrichia albicollis*). *PLoS ONE* **8**, e59097.
- Suarez, R. K., Welch, K. C., Hanna, S. K. and Herrera, M. L. G. (2009). Flight muscle enzymes and metabolic flux rates during hovering flight of the nectar bat, *Glossophaga soricina*: further evidence of convergence with hummingbirds. *Comp. Biochem. Physiol. A* **153**, 136-140.
- Sweazea, K. L. and Braun, E. J. (2006). Oleic acid uptake by in vitro English sparrow skeletal muscle. *J. Exp. Zool.* **305A**, 268-276.
- Tracy, C. R., McWhorter, T. J., Wojciechowski, M., Pinshow, B. and Karasov, W. H. (2010). Carbohydrate absorption by blackcap warblers (*Sylvia atricapilla*) changes during migratory refuelling stopovers. *J. Exp. Biol.* **213**, 380-385.
- Vaillancourt, E. and Weber, J.-M. (2007). Lipid mobilization of long-distance migrant birds in vivo: the high lipolytic rate of ruff sandpipers is not stimulated during shivering. *J. Exp. Biol.* **210**, 1161-1169.
- Videler, J. J. (2005). *Avian Flight*. New York: Oxford University Press.
- 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.
- Voigt, C. C., Sörgel, K., Šuba, J., Keiš, O. and Pétersons, G. (2012). The insectivorous bat *Pipistrellus nathusii* uses a mixed-fuel strategy to power autumn migration. *Proc. R. Soc. B* **279**, 3772-3778.
- Wang, Y.-X. (2010). PPARs: diverse regulators in energy metabolism and metabolic diseases. *Cell Res.* **20**, 124-137.
- Weber, J.-M. (2009). The physiology of long-distance migration: extending the limits of endurance metabolism. *J. Exp. Biol.* **212**, 593-597.
- Weber, J.-M., Brichon, G., Zwingelstein, G., McClelland, G., Saucedo, C., Weibel, E. R. and Taylor, C. R. (1996). Design of the oxygen and substrate pathways. IV. Partitioning energy provision from fatty acids. *J. Exp. Biol.* **199**, 1667-1674.
- Welch, K. C., Herrera, M. L. G. and Suarez, R. K. (2008). Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. *J. Exp. Biol.* **211**, 310-316.
- Wiersma, P. and Piersma, T. (1995). Scoring abdominal profiles to characterize migratory cohorts of shorebirds: an example with red knots. *J. Field Ornithol.* **66**, 88-98.
- 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.
- Wingfield, J. C., Schwabl, H. and Mattocks, P. W. Jr. (1990). Endocrine mechanisms of migration. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 232-256. New York: Springer Verlag.
- Wirestam, R., Fagerlund, T., Rosén, M. and Hedenström, A. (2008). Magnetic resonance imaging for non-invasive analysis of fat storage in migratory birds. *Auk* **125**, 965-971.
- Witter, M. S. and Cuthill, I. C. (1993). The ecological costs of avian fat storage. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **340**, 73-92.
- Wojciechowski, M. S. and Pinshow, B. (2009). Heterothermy in small, migrating passerine birds during stopover: use of hypothermia at rest accelerates fuel accumulation. *J. Exp. Biol.* **212**, 3068-3075.
- Wojciechowski, M. S., Yosef, R. and Pinshow, B. (2014). Body composition of north and southbound migratory blackcaps is influenced by the lay-of-the-land ahead. *J. Avian Biol.* **45**, 264-272.
- Yacoe, M. E., Cummings, J. W., Myers, P. and Creighton, G. K. (1982). Muscle enzyme profile, diet, and flight in South American bats. *Amer. J. Physiol. Reg. Comp. Integrat. Physiol.* **242**, R189-R194.
- Zajac, D. M., Cerasale, D. J., Landman, S. and Guglielmo, C. G. (2011). Behavioural and physiological effects of photoperiod-induced migratory state and leptin on *Zonotrichia albicollis*: II. Effects on fatty acid metabolism. *Gen. Comp. Endocr.* **174**, 269-275.
- Zhang, Y., King, M. O., Harmon, E., Eyster, K. and Swanson, D. L. (2015). Migration-induced variation of fatty acid transporters and cellular metabolic intensity in passerine birds. *J. Comp. Physiol. B* **185**, 797-810.