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Selective mobilization of fatty acids from adipose tissue in migratory birds

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SUMMARY

During times of high energy demand, stored fatty acids are mobilized from adipocytes. This mobilization has previously been shown to be a non-random process, with more hydrophilic fatty acids being mobilized most readily. The objectives of this study were to characterize the relative mobilization of fatty acids from adipocytes in two migratory bird species and to investigate possible changes in selective fatty acid mobilization associated with the migratory period. Captive ruffs (*Philomachus pugnax*) and white-crowned sparrows (*Zonotrichia leucophrys*) were studied. The sparrows were divided into two treatments: 'winter' (photoperiod 8 h:16 h L:D) and 'migrant' (in which migratory condition was induced with a photoperiodic manipulation of 8 h:16 h L:D, followed by 16 h:8 h L:D). Adipose tissue was removed from ruffs and sparrows and incubated for 90 min after stimulation with epinephrine. The proportions of individual fatty acid species released into the incubation medium were compared with their proportions in the adipocytes to determine relative mobilizations. We found that patterns of relative mobilization in ruffs and sparrows are similar to those of mammals, with shorter chain lengths and more double bonds leading to higher relative mobilization. Seasonal condition in sparrows did not alter this pattern. This pattern of relative mobilization from adipocytes seems to be a general rule amongst birds and mammals and should be considered before inferring functionality about selective retention or mobilization of certain fatty acids. The composition of adipose stores in birds may affect migratory performance; however, our results indicate that patterns of relative mobilization at the adipocytes do not vary with season in migratory birds.

Key words: migration, relative mobilization, unsaturated fatty acids, linoleic acid, hormone-sensitive lipase, *Zonotrichia*, *Philomachus*.

INTRODUCTION

During times of fasting or high energy demand, fatty acids are mobilized from adipocyte triacylglycerol (TRIG) stores and exported to the circulation. Inside the adipocytes, this process involves cleavage of TRIG by hormone-sensitive lipase (HSL) into constituent fatty acids and glycerol moieties. In recent studies, it has become clear that the proportions of the specific fatty acid species mobilized differ somewhat from their proportions in the TRIG from which they were released (Herzberg and Skinner, 1997; Hollenberg and Angel, 1963; Raclot and Groscolas, 1993; Soppela and Nieminen, 2002). This fatty acid selectivity has been demonstrated in both *in vivo* (Herzberg and Skinner, 1997; Nieminen et al., 2006; Soppela and Nieminen, 2002) and *in vitro* (Raclot and Groscolas, 1993; Raclot et al., 1995) studies and has led to adaptive explanations regarding the functions of the specific fatty acids that are selectively retained or mobilized (Falkenstein et al., 2001; Florant et al., 1990; Nieminen et al., 2006; Soppela and Nieminen, 2002). However, in a recent review, Raclot concluded that differential mobilization of fatty acids occurs primarily due to the molecular structure of each fatty acid species; fatty acids that are shorter, more unsaturated and have their double bonds closer to the methyl end are more polar and are preferentially mobilized (Raclot, 2003). This may occur due to a combination of enzyme specificity, access of HSL to polar lipids at the lipid/aqueous interface, and other lipid transport processes within the adipocyte (Raclot, 2003; Raclot et al., 2001).

During migratory flights, birds engage in endurance (often >8 h) exercise, achieving maximal rates of oxygen consumption that are more than double those achieved by similarly sized mammals, all while fasting (Butler and Woakes, 1990). Stored adipose TRIG serves as the primary fuel source during these flights (McWilliams et al., 2004; Odum et al., 1964; Ramenofsky, 1990), and birds undergo seasonal changes in physiology that allow them to utilize fat at high rates (Guglielmo et al., 2002a; Jenni-Eiermann and Jenni, 1992; Pelsers et al., 1999). The mobilization of lipids from adipocytes is an important step in this process (Johnston, 1973). The selective mobilization of particular fatty acids may also be important given that the fatty acid composition of adipose stores can affect migratory performance (Johnston, 1973; Pierce et al., 2005). Nonetheless, the phenomenon of selective fatty acid mobilization at the adipose tissue has not been studied in birds except in fasting penguins (Groscolas, 1990; Johnson and West, 1973). Moreover, to our knowledge, selective fatty acid mobilization has not been investigated *in vitro* in any animal during its different life history stages and periods of energy demand. The goals of this study, therefore, were to characterize the selective mobilization of fatty acids *in vitro* for two migratory bird species and to investigate any changes in selective fatty acid mobilization associated with the migratory period in one of the species. Specifically, we were interested in whether migratory state would result in an increase in mobilization of 18:2n6 (Soppela and Nieminen, 2002) or a selective retention of 18:2n6, perhaps to conserve this fatty acid for use during breeding (Florant et al., 1990; Mostafa et al., 1994).

MATERIALS AND METHODS

Animals

Six ruffs (*Philomachus pugnax* Linnaeus) were obtained from a captive colony maintained at Simon Fraser University by Dr David Lank and housed communally at the University of Montana, MT, USA. They were maintained on a diet of trout pellets (Purina Mills, St Louis, MO, USA) supplemented occasionally with hard boiled eggs and supplied with water *ad libitum*. This diet contained a broad diversity of fatty acids that allowed us to examine how patterns of relative mobilization vary with chain length and unsaturation. Ruffs were maintained on a 12 h:12 h L:D light cycle (light 06.00–18.00 h) for the duration of the experiment, with a nightlight provided (<1 lux). The ruffs were weighed weekly, and sex (three males/three females) was determined by size dimorphism and plumage.

Twelve white-crowned sparrows (*Zonotrichia leucophrys gambelii* Nuttall) were captured during fall migration in Missoula, MT, USA in September 2004. Another five sparrows were captured in fall 2003 at Sunnyside, WA, USA and used for other diet experiments until April 2004 (Cerasale and Guglielmo, 2006), after which they were kept in an outdoor aviary in Missoula until our experiment. All sparrows were thus exposed to natural light conditions for an extended period before experiments and had completed feather molt and autumn migration before entering the short-day photoperiod conditioning. Sparrows were individually housed indoors in 38 cm×43 cm×41 cm cages at 22°C for the duration of the study. Sparrows were weighed in the morning before feeding every 2–3 days throughout the experiment. Ten sparrows were aged as 'hatch year' and the rest as 'after hatch year' according to Pyle (Pyle, 1997). Sex was determined after the experiment by inspection of the gonads. Sparrows were fed a diet of black oil sunflower seeds, and water was provided *ad libitum*, with supplemental grit and vitamins offered weekly. This limited diet ensured that sparrows on both treatments would have similar adipose stores so that relative mobilization rates would be more easily comparable. A nightlight (<1 lux) was provided to encourage nightly migratory behavior. Experimental protocols were approved by the University of Montana Institutional Animal Care and Use Committee and appropriate permits for collection, export/import and possession were obtained from the US Fish and Wildlife Service, Canadian Wildlife Service, and Montana State Department of Fish Wildlife and Parks.

Photoperiod manipulation

All sparrows were initially kept on a short day light cycle (8 h:16 h L:D) for 58 days to break photorefractoriness and simulate winter. Sparrows were then randomly assigned to treatment groups of long days (migrant=MIG; N=10; 16 h:8 h L:D) and short days (winter=WIN; N=7; 8 h:16 h L:D). All sparrows were then maintained for another 22–26 days before experiments were initiated. This manipulation of the light cycle in MIG sparrows induced zugunruhe, a captive analog of the migratory condition in which birds undergo nightly hopping and wing fluttering (Breuner et al., 1999; King and Farner, 1963). As evidence of this, nightly hopping activity, as measured by counters attached to the perches, was significantly elevated in the MIG sparrows compared to WIN sparrows (P<0.05).

Adipocyte incubation and fatty acid analysis

The protocol for determining relative mobilization from adipocytes generally followed that described by Raclot and Groscolas (Raclot and Groscolas, 1993). Birds were euthanized between 10.00 h and 14.30 h (2–6.5 h after lights on) by pentabarbitol overdose. Approximately 400 mg adipose tissue was removed immediately from the claviculo-corocoid depot and placed in Krebs Ringer buffer (made with 15 mmol l^{-1} $NaHCO_3$, 3.32 mmol l^{-1} $CaCl_2$ and 4% fatty-acid-free bovine serum albumen; Sigma, St Louis MO, USA) (KRBA/BSA). Tissue was minced with scissors and washed with KRBA/BSA three times. Washed tissue was then transferred to a polypropylene flask containing 4 ml fresh KRBA/BSA warmed to 37°C. We added epinephrine (Sigma) to a final concentration of 100 μmol l^{-1}, and the flask was flushed with 95%/5% O_2/CO_2 and capped. The flask was incubated for 90 min at 37°C in a shaking water bath. The contents were then filtered under vacuum with a glass microfiber filter (cat # 28297-978; VWR, West Chester, PA, USA). A sample of this filtrate was taken for glycerol analysis (described below). We added heptadecanoic acid (17:0; 200 μl 1.107 mmol l^{-1} hexane) as an internal standard. Three 1 ml aliquots of the incubation medium were each added to vials containing 15 ml chloroform:methanol (1:1 v/v) and shaken vigorously. For analysis of adipose lipids, 5–10 mg of subcutaneous adipose tissue was removed from the claviculo-corocoid depot, added to 15 ml chloroform:methanol (1:1 v/v) and homogenized at high speed for 3×10 s with a 1 cm generator (Polytron). Total lipid extracts from incubation medium and from adipocytes were then centrifuged for 15 min at 2056 ***g*** and filtered (Whatman no. 1), adding 10 ml of chloroform:methanol (2:1 v/v) to rinse. We added 6 ml 0.25% KCl to partition and remove aqueous solutes. The aqueous layer was vacuum pipetted off and the organic solvent was evaporated (Rotovapor, Buchi, Flawil, Switzerland). Lipids were resuspended in 100 μl chloroform for loading onto Supelclean solid-phase extraction tubes (LC-NH2; 100 mg; Supelco, Bellefonte, PA, USA). Neutral lipids (NL, primarily TRIG) were eluted with 1.8 ml chloroform:isopropanol (2:1 v/v). Non-esterified fatty acids (NEFA) were then eluted with 1.6 ml isopropyl ether:acetic acid (49:1 v/v). Columns were washed between samples with hexane and methanol. We added the internal standard (17:0; 200 μl 1.107 mmol l^{-1} hexane) to the adipocyte NL fraction and then evaporated each lipid fraction to dryness under N_2. NEFA were methylated at room temperature for 30 min following addition of 100 μl methanol, 1 ml dimethoxypropane and 40 μl concentrated HCl. The sample was dried under N_2 and resuspended in 40 μl isooctane before injection in the gas chromatograph (GC) column. The NL fraction was transesterified with 2 ml acetyl chloride in methanol (1 mol l^{-1}), heated for 2 h at 90°C. The solvent was evaporated under N_2, and fatty acid esters were resuspended in 60 μl isooctane for injection onto the GC column.

Fatty acids were separated on a Hewlett Packard HP 6890 (Hewlett Packard, Palo Alto, CA, USA) with a J&W scientific high-resolution gas chromatography column (DB-225 ms; Agilent Technologies, Palo Alto, CA, USA) and flame ionization detector. The carrier gas was N_2. The temperature program was 2 min at 120°C, then increase at 5°C per min for 16 min, hold at 200°C for 5 min, increase at 5°C per min for 4 min, then hold at 220°C for 13 min. Fatty acids with fewer than 16 carbons were excluded from statistical analysis because precautions to minimize volatization were not taken. Only fatty acids constituting more than 0.25% of the total neutral lipids in adipose tissue were considered in the analysis. The identities of fatty acids were determined by comparison of retention times to those of standard mixtures of fatty acid methyl esters (Supelco 37 component mix, Supelco PUFA No. 3; Sigma).

Glycerol concentration was measured in the medium after adipose incubation as a measure of overall lipolysis rate. Glycerol was assayed on a microplate spectrophotometer (BioTek Powerwave X340; Winooski, VT, USA) in 400 μl flat-bottomed microplates with an endpoint assay (Sigma; Trinder reagent A, 5 μl medium, 300 μl reagent). Glycerol is reported as (concentration in medium)/(mass of adipose incubated).

Statistical analysis

For each bird, relative mobilization for each fatty acid was calculated as (mass % in NEFA from the incubation)/(mass % in NL from adipose). Only fatty acids that were detected in both NL and NEFA were included in the analysis (infinite and zero relative mobilizations were excluded). The 18:1n9 and 18:2n6 chromatograph peaks were inseparable in one WIN sparrow's adipose NL. These fatty acids were removed from analysis for this animal.

In comparing the relative mobilization rates of different fatty acid species, normal statistical approaches are inappropriate due to the non-independence of data. For our analyses, we used a permutation approach aided by scripts written in S-Plus (Insightful Corp., Seattle, WA, USA). To test for a general effect of chain length, we measured a least-squares regression 'observed slope' of relative mobilization *vs* chain length on the relative mobilizations of all fatty acids for all individuals. We then performed a random permutation of the relative mobilizations within an individual and within a degree of unsaturation (i.e. the relative mobilizations for 16:0 and 18:0 were permuted for each animal, the relative mobilizations for 16:1, 18:1 and 20:1 were permuted for each animal, etc.). A new slope was determined for all the permuted data. This process was repeated 1000 times to obtain a distribution of slopes. We considered chain length to have a significant effect if the observed slope was as or more extreme than the most extreme 5% (two-sided) of the slopes in the permuted distribution. A similar process was conducted for analyzing the effect of degree of unsaturation (# of double bonds), permuting within individual and within a given chain length. While we recognize that the effects of these factors may not be linear and that these slopes may not have biological meaning, these tests give a general indication of the effects of chain length and unsaturation on relative mobilization.

To compare mobilization rates of two different fatty acids, we performed a standard permutation test. To evaluate the effects of sex, age and photoperiod on the relative mobilization of a particular fatty acid and glycerol we used Mann–Whitney tests. Results are presented as means ± s.e.m.

RESULTS

Ruff adipocyte relative mobilization

There was no difference in relative mobilization between males and females for any of the fatty acids ($P>0.1$) so data were pooled. The pattern of relative mobilization observed in ruffs generally followed the order of mobilization of fatty acids that was found previously in mammals and penguins (Fig. 1). The relative mobilization of fatty acids in ruffs increased with degree of unsaturation (Fig. 2) ($P<0.001$) and decreased with carbon chain length, particularly for lengths 18 and greater (Fig. 2) ($P<0.001$). The only pair of positional isomers that we measured was 18:3n3 and 18:3n6. There was no significant difference between the relative mobilizations of these two fatty acids ($P>0.9$).

Sparrow adipocyte relative mobilization

Due to the limited fatty acid composition of the sparrow diet, only four fatty acids were found in substantial amounts in adipocytes.

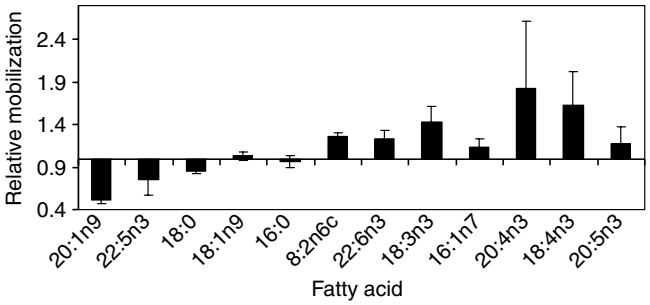

Fig. 1. Relative mobilization of fatty acids from ruff adipocytes. Fatty acids are ordered from least to most mobilized according to Raclot's studies in rats (Raclot, 2003).

These four fatty acids (16:0, 18:0, 18:1n9 and 18:2n6) constituted over 97% of the total present in adipose TRIG, and only two others (20:0 and 20:1n9) constituted more than 0.25% of the total. This limited variety of fatty acids precluded analysis of the effects of chain length and degree of unsaturation on relative mobilization in sparrows. However, the pattern of relative mobilization of fatty acids was similar to the pattern previously reported for mammals and penguins (Fig. 3) and was similar to the pattern seen in ruffs (present study). Sex did not affect the relative mobilization of any of the fatty acids measured ($P>0.1$), but age did have a significant effect on mobilization of 18:0 ($P=0.033$), with relative mobilization of this fatty acid being higher for hatch year birds. Relative mobilization was not affected by migratory state for the six most common fatty acids ($P>0.133$).

Treatment did not have a significant effect on glycerol concentration ($P=0.070$) although there was a trend towards higher glycerol in migrant birds (Fig. 4). Neither age ($P=0.536$) nor sex ($P=0.601$) had significant effects on glycerol concentration. The ratio of moles fatty acids released to moles glycerol was 2.28:1.

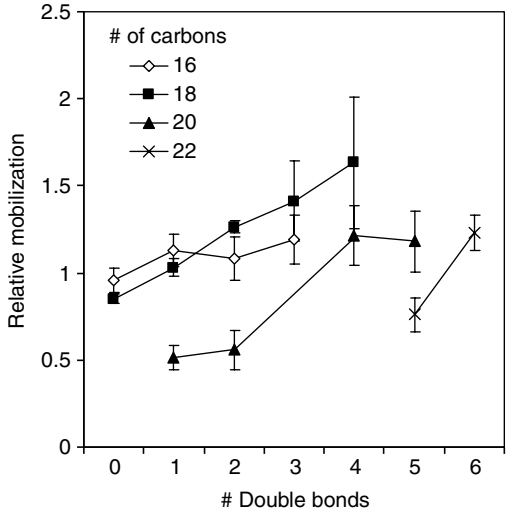

Fig. 2. Relative mobilization of fatty acids from ruff adipocytes in relation to the number of double bonds. The value for 18:3 is the mean of 18:3n3 and 18:3n6. The number of double bonds and chain length both had significant effects on relative mobilization ($P<0.001$). See text for statistical details.

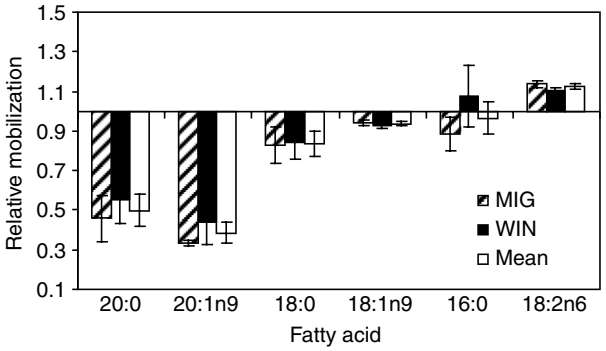

Fig. 3. Relative mobilization of fatty acids from white-crowned sparrow adipocytes. Fatty acids are ordered from least to most mobilized according to Raclot's studies in rats (Raclot, 2003). There are no significant differences in relative mobilization of any fatty acid according to migratory state or exercise (P>0.133). MIG=migrant, WIN=winter. Values are means ± s.e.m.

DISCUSSION

Fatty acid relative mobilization rates have not previously been measured *in vitro* in birds. Our results show that in these two bird species, adipose fatty acid relative mobilization rates are largely affected by structural factors such as chain length and the number of double bonds. This finding agrees with previous *in vitro* studies in mammals (Raclot, 2003), as well as *in vivo* measurements from fasting penguins (Groscolas, 1990). Although we have not studied subcellular processes such as hydrolysis of TRIG by HSL, this agreement generally suggests that the biochemical processes that result in selective mobilization of fatty acids are similar between mammals and birds. These mechanisms may include specificity of HSL for certain TRIG-bound fatty acids as well as access of HSL to more polar fatty acids at the lipid droplet interface (Hazel and Sidell, 2004; Raclot, 2003).

We used a photoperiodic manipulation to investigate the effects of migratory state on relative mobilization rates in sparrows. Our results indicate that the patterns of mobilization rates of individual fatty acids relative to the overall mobilization rate do not vary with migratory state. While we did not find a significant effect of migratory state on overall lipolysis rates, the trend we observed can fuel further hypotheses. Specifically, it appears that migratory state may alter adipose tissue such that it responds more strongly to stimulation by epinephrine. Similar results were found in *Junco hyemalis* (Savard et al., 1991). This could occur by an increase in receptor density (Breuner et al., 2003; Landys et al., 2004a). Additional findings include the observation that 18:2n6 was not selectively retained in the birds, contrary to the predictions of Mostafa et al. based on work in a congeneric species (Mostafa et al., 1994). Interestingly, young sparrows mobilized 18:0 at higher relative rates than their older counterparts, although the importance of this finding is unknown.

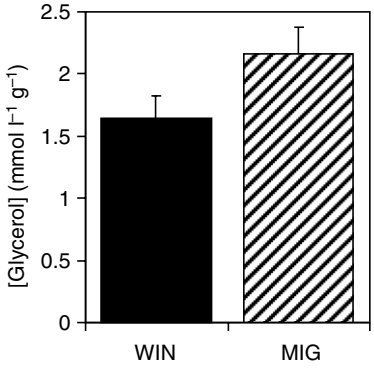

Fig. 4. Glycerol concentration of incubation medium at the end of incubation. There were no significant differences between treatment groups (P=0.070). MIG=migrant, WIN=winter.

Traditional use of photoperiod-manipulated captive birds as models for migration in endocrinology and orientation studies have relied on zugunruhe as evidence for migratory condition (Able and Cherry, 1986; Landys et al., 2004b). Data on bird zugunruhe indicate that the MIG sparrows did respond to the photoperiodic treatment by increasing nightly activity. This treatment may not, however, perfectly mimic the biochemical changes that occur in adipocytes during true migration. Using a captive protocol, however, provides the opportunity to study birds with very similar adipose stores by controlling diet. Additionally, it is possible that no change in relative mobilization was observed with migration due to the limited number of fatty acids available in abundance for this experiment, although this limited fatty acid composition may be typical for wild passerines (Blem, 1976; Conway et al., 1994). Further experiments could evaluate relative mobilization rates in free-living migrants across seasons, particularly in shorebirds that eat a varied diet and whose adipose depots would be expected to have a variable fatty acid composition.

Selective mobilization and seasonal changes in energetic demand

Several investigators have studied changes in adipose tissue fatty acid composition under conditions of fasting, nutritional stress and/or hibernation (Falkenstein et al., 2001; Florant et al., 1990; Groscolas, 1990; Nieminen et al., 2006; Soppela and Nieminen, 2002). When selective mobilization of fatty acids has been observed, it has often been interpreted as adaptive (for example to maintain metabolism and functions of essential PUFA or to maintain fluidity of fat reserves) (Falkenstein et al., 2001; Nieminen et al., 2006; Soppela and Nieminen, 2002). Given the apparent ubiquity and consistency in the pattern of selective mobilization of fatty acids (Groscolas, 1990; Groscolas and Herzberg, 1997; Hazel and Sidell, 2004; Raclot, 2003; Raclot et al., 1995) (present study), we recommend that such interpretations should be made in light of 'background' selective mobilization. For example, Soppela and Nieminen (Soppela and Nieminen, 2002) infer functional importance from the observation that 18:2n6 was highly mobilized from malnourished reindeer, but high mobilization should not be unexpected because, in general, 18:2n6 is preferentially mobilized relative to the other fatty acids that make up reindeer TRIG. On the other hand, the selective retention of 18:2n6 in hibernating marmots (Florant et al., 1990) and hibernating echidnas (Falkenstein et al., 2001) is notable as it demonstrates an exception to the general pattern. Valuable information about lipid metabolism might be gained from these and other species by comparing *in vitro* selective mobilization from adipocytes excised at different stages of the animals' life histories.

Importance of selective mobilization to migratory birds

Because birds use extramuscular lipids as their primary fuel during migratory flights (McWilliams et al., 2004), the composition of stored fats has long been of interest to physiologists. A particular focus has been on the amount of unsaturated fats stored (Conway et al., 1994; Egeler and Williams, 2000; Johnston, 1973; Yom-Tov

and Tietz, 1978). Although unsaturated fats are less energy dense than saturates, this energetic difference is relatively small (Bower and Helms, 1968), and whole-organism oxidation rates of unsaturates are higher than those of saturated fats in fed rats (Leyton et al., 1987). Thus, it might be advantageous for birds to store unsaturated fats because they are more easily transported from adipose stores to the muscle during energetically demanding flights. Johnston observed that migratory species tend to have more unsaturated fatty acids in adipose stores than non-migratory birds (Johnston, 1973), although Blem found no trend in saturation for migratory and non-migratory birds (Blem, 1976; Blem, 1980). Additionally, some researchers have noted a change in fatty acid composition leading up to migration, although this observation has not been consistent (Egeler and Williams, 2000; Hicks, 1967; McGreal and Farner, 1956) and does not seem to be the direct result of selective metabolism (Pierce and McWilliams, 2005). Many authors have noted that adipose fatty acid composition tends to reflect dietary composition, and changes in bird diets leading up to migration can alter adipose stores (Bower and Helms, 1968; Conway et al., 1994; Egeler and Williams, 2000; Morton and Liebman, 1974; Pierce and McWilliams, 2005; West and Meng, 1968). Experiments with sandpipers have indicated that diet is not solely responsible for changes to increased adipose unsaturation leading up to migration but that endogenous modification, preferential deposition and *de novo* synthesis can increase adipose unsaturation prior to and during migration (Egeler and Williams, 2000; Egeler et al., 2000; Egeler et al., 2003).

During a migratory flight, the utilization rate for fatty acids will depend on four processes: the rates of (1) mobilization from adipocyte, (2) transport through the blood, (3) uptake at the muscles and (4) intracellular oxidation (Egeler and Williams, 2000). Despite the importance of fat utilization to avian flight, none of these processes has previously been studied in migratory birds with respect to fatty acid composition. Leyton et al. have often been cited as reporting that oxidation rates of unsaturated fatty acids are high (Leyton et al., 1987). However, their study reported rates of whole-organism oxidation from fed rats and therefore their results could be due to preferential deposition of saturated dietary fatty acids rather than selective transport, uptake and oxidation of unsaturates. As such, their results may not be easily comparable to the process of fat utilization in fasting, migrating birds. Our results indicate that mobilization of fatty acids from adipocytes is generally more rapid for more highly unsaturated fatty acids (as well as for shorter fatty acids). This could have adaptive significance for the preferential storage of unsaturated fatty acids, but only if adipose mobilization is limiting [which does not appear to be the case (McWilliams et al., 2004)] or may be limiting under certain conditions [e.g. increased energetic demands due to adverse weather (Conway et al., 1994)]. Other processes may limit the supply of fats to fuel migratory flight, although it seems likely that unsaturated fatty acids would also be transported and utilized more quickly in these processes, due to their greater solubility in water. Additionally, the preferential mobilization of unsaturated fatty acids compared to saturates may not indicate substantially increased rates of overall lipolysis. Our results indicate a trend towards increased lipolysis in migratory-stage birds without a change in the pattern of selective mobilization of fatty acids.

Although many researchers have speculated that the higher mobility of unsaturated fatty acids is beneficial to migratory performance, Pierce et al. found that very high concentrations of dietary and adipose 18:1 and total unsaturates resulted in poorer exercise performance in captive vireos (Pierce et al., 2005). Conversely, vireos with higher dietary 18:2n6 had improved performance. This finding has been replicated in rats (Ayre and Hulbert, 1997) and fish (McKenzie et al., 1998) and may be due to diet-influenced changes in phospholipid fatty acid composition (Ayre and Hulbert, 1997; Guglielmo et al., 2002b). Thus, the composition of adipose stores may not be the only mechanism by which dietary lipids can affect performance. The relative importance of dietary, adipose and phospholipid fatty acid composition to performance in migratory birds deserves further study.

LIST OF ABBREVIATIONS

GC	gas chromatograph
HSL	hormone-sensitive lipase
KRBA/BSA	Krebs-Ringer buffer with 4% bovine serum albumin
MIG	migratory treatment group
NEFA	non-esterified fatty acids
NL	neutral lipids
TRIG	triacylglycerol
WIN	winter treatment group

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REFERENCES

Able, K. P. and Cherry, J. D. (1986). Mechanisms of dusk orientation in white-throated sparrows (*Zonotrichia albicollis*): clock-shift experiments. *J. Comp. Physiol. A* **159**, 107-113.

Ayre, K. J. and Hulbert, A. J. (1997). Dietary fatty acid profile affects endurance in rats. *Lipids* **32**, 1265-1270.

Blem, C. R. (1976). Patterns of lipids storage and utilization in birds. *Am. 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.

Bower, E. B. and Helms, C. W. (1968). Seasonal variation in fatty acids of the slate-colored junco (*Junco hyemalis*). *Physiol. Zool.* **41**, 157-168.

Breuner, C. W., Wingfield, J. C. and Romero, L. M. (1999). Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *J. Exp. Zool.* **284**, 334-342.

Breuner, C. W., Orchinik, M., Hahn, T. P., Meddle, S. L., Moore, I. T., Owen-Ashley, N. T., Sperry, T. S. and Wingfield, J. C. (2003). Differential mechanisms for regulation of the stress response across latitudinal gradients. *Am. J. Physiol.* **285**, R594-R600.

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.

Cerasale, D. J. and Guglielmo, C. G. (2006). Dietary effects on prediction of body mass changes in birds by plasma metabolites. *Auk* **123**, 836-846.

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

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 Δ9-desaturase, in relation to migration in the western sandpiper (*Calidris mauri*). *J. Comp. Physiol. B* **170**, 169-174.

Egeler, O., Seaman, D. and Williams, T. D. (2003). Influence of diet on fatty-acid composition of depot fat in western sandpipers (*Calidris mauri*). *Auk* **120**, 337-345.

Falkenstein, F., Körtner, G., Watson, K. and Geiser, F. (2001). Dietary fats and body lipid composition in relation to hibernation in free-ranging echidnas. *J. Comp. Physiol. B* **171**, 189-194.

Florant, G. L., Nuttle, L. C., Mullinex, D. E. and Rintoul, D. A. (1990). Plasma and white adipose tissue lipid composition in marmots. *Am. J. Physiol.* **258**, R1123-R1131.

Groscolas, R. (1990). Metabolic adaptations to fasting in Emperor and King penguins. In *Penguin Biology* (ed. L. S. Davis and J. T. Darby), pp. 269-296. San Diego: Academic Press.

Groscolas, R. and Herzberg, G. R. (1997). Fasting-induced selective mobilization of brown adipose tissue fatty acids. *J. Lipid Res.* **38**, 228-238.

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

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

Hazel, J. R. and Sidell, B. D. (2004). The substrate specificity of hormone-sensitive lipase from adipose tissue of the Antarctic fish *Trematomus newnesi*. *J. Exp. Biol.* **207**, 897-903.
Herzberg, G. R. and Skinner, C. (1997). Differential accumulation and release of long-chain *n*-3 fatty acids from liver, muscle, and adipose tissue triacylglycerols. *Can. J. Physiol. Pharmacol.* **75**, 945-951.
Hicks, D. L. (1967). Adipose tissue composition and cell size in fall migratory thrushes (Turdidae). *Condor* **69**, 387-399.
Hollenberg, C. H. and Angel, A. (1963). Relation of fatty acid structure to release and esterification of free fatty acids. *Am. J. Physiol.* **205**, 909-912.
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.
Johnson, S. R. and West, G. C. (1973). Fat content, fatty acid composition and estimates of energy metabolism of Adélie penguins (*Pygoscelis adeliae*) during the early breeding season fast. *Comp. Biochem. Physiol.* **45B**, 709-719.
Johnston, D. W. (1973). Cytological and chemical adaptations of fat deposition in migratory birds. *Condor* **75**, 108-113.
King, J. R. and Farner, D. S. (1963). The relationship of fat deposition to zugunruhe and migration. *Condor* **65**, 200-223.
Landys, M. M., Ramenofsky, M., Guglielmo, C. and Wingfield, J. C. (2004a). 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.
Landys, M. M., Wingfield, J. C. and Ramenofsky, M. (2004b). Plasma corticosterone increases during migratory restlessness in the captive white-crowned sparrow *Zonotrichia leucophrys gambelli*. *Horm. Behav.* **46**, 574-581.
Leyton, J., Drury, P. J. and Crawford, M. A. (1987). Differential oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *Br. J. Nutr.* **57**, 383-393.
McGreal, R. D. and Farner, D. S. (1956). Premigratory fat deposition in the Gambel White-crowned Sparrow: some morphological and chemical observations. *Northwest Sci.* **30**, 12-23.
McKenzie, D. J., Higgs, D. A., Dosanjh, B. S., Deacon, G. and Randall, D. J. (1998). Dietary fatty acid composition influences swimming performance in Atlantic salmon (*Salmo salar*) in seawater. *Fish Physiol. Biochem.* **19**, 111-122.
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.
Morton, M. L. and Liebman, H. A. (1974). Seasonal variations in fatty acids of a migratory bird with and without a controlled diet. *Comp. Biochem. Physiol.* **48A**, 329-335.
Mostafa, N., Bhat, B. G. and Coleman, R. A. (1994). Adipose monoacylglycerol:acyl-coenzyme A acyltransferase activity in the white-throated sparrow (*Zonotrichia albicollis*): characterization and function in a migratory bird. *Lipids* **29**, 785-791.
Nieminen, P., Rouvinen-Watt, K., Collins, D., Grant, J. and Mustonen, A.-M. (2006). Fatty acid profiles and relative mobilization during fasting in adipose tissue depots of the American marten (*Martes americana*). *Lipids* **41**, 231-240.
Odum, E. P., Rogers, D. T. and Hicks, D. L. (1964). Homeostasis of the nonfat components of migrating birds. *Science* **143**, 1037-1039.
Pelsers, M. M. A. L., Butler, P. J., Bishop, C. M. and Glatz, J. F. C. (1999). Fatty acid binding protein in heart and skeletal muscles of the migratory barnacle goose throughout development. *Am. J. Physiol.* **276**, R637-R643.
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., McWilliams, S. R., O'Connor, T. P., 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.
Pyle, P. (1997). *Identification Guide to North American Birds, Part 1*. Bolinas, CA: Slate Creek Press.
Raclot, T. (2003). Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257-288.
Raclot, T. and Groscolas, R. (1993). Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. *J. Lipid Res.* **34**, 1515-1526.
Raclot, T., Mioskowski, E., Bach, A. C. and Groscolas, R. (1995). Selectivity of fatty acid mobilization: a general metabolic feature of adipose tissue. *Am. J. Physiol.* **269**, R1060-R1067.
Raclot, T., Holm, C. and Langin, D. (2001). Fatty acid specificity of hormone-sensitive lipase: implication in the selective hydrolysis of triacylglycerols. *J. Lipid Res.* **42**, 2049-2057.
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.
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. Pharmacol.* **69**, 1443-1447.
Soppela, P. and Nieminen, M. (2002). Effect of moderate wintertime undernutrition on fatty acid composition of adipose tissues of reindeer (*Rangifer tarandus tarandus* L.). *Comp. Biochem. Physiol.* **132A**, 403-409.
West, G. C. and Meng, M. S. (1968). Seasonal changes in body weight and fat and the relation of fatty acid composition to diet in the willow ptarmigan. *Wilson Bull.* **80**, 426-441.
Yom-Tov, Y. and Tietz, A. (1978). The effect of diet, ambient temperature and day length on the fatty acid composition in the depot fat of the european starling (*Sturnus vulgaris*) and the rock partridge (*Alectoris chucar*). *Comp. Biochem. Physiol.* **60A**, 161-164.