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Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eyed vireo

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Summary

Most migrating birds accumulate lipid stores as their primary source of energy for fueling long distance flights. Lipid stores of birds during migration are composed of mostly unsaturated fatty acids; whether such a fatty acid composition enhances exercise performance of birds is unknown. We tested this hypothesis by measuring metabolic rate at rest and during intense exercise in two groups of red-eyed vireos, a long-distance migratory passerine, fed either a diet containing 82% unsaturated fat (82%U), or one containing 58% unsaturated fat (58%U). Vireos fed the 82%U diet had fat stores containing (77%) unsaturated fatty acids, whereas vireos fed the 58% U diet had fat stores containing less (66%) unsaturated fatty acids. Blood metabolites measured prior to and immediately following exercise confirmed that vireos were metabolizing endogenous fat during intense exercise. Mass-specific resting metabolic rate (RMR) was similar for vireos fed the 58%U diet (2.75±0.32 ml O_2 g^{-1} h^{-1}) and for vireos fed the 82%U diet (2.30±0.30 ml O_2 g^{-1} h^{-1}). However, mass-specific peak metabolic rate (MR_{peak}) was 25% higher in vireos fed the 58%U diet (28.55±1.47 ml O_2 g^{-1} h^{-1}) than in vireos fed the 82%U diet (21.50±1.76 ml O_2 g^{-1} h^{-1}). Such whole-animal energetic effects of fatty acid composition of birds suggest that the energetic cost of migration in birds may be affected by the fatty acid composition of the diet.

Key words: red-eyed vireo, *Vireo olivaceous*, lipid, unsaturated fatty acid, migration, fatty acid composition, metabolic rate.

Introduction

Athletes train and eat to increase their capacity to perform on demand. Birds during migration are like well-trained mammalian athletes in that they prepare for migration by increasing the size of certain muscles (e.g. flight muscles), and they accumulate energy and nutrient stores (Piersma, 1990; Piersma and Jukema, 1990; Lindstrom and Piersma, 1993; Butler and Bishop, 2000; Bauchinger and Biebach, 2001). Mammalian athletes fuel high-intensity endurance exercise primarily by glycogen stored within muscle cells, with fatty acid oxidation contributing <20% to energy demand (Roberts et al., 1996; Weber et al., 1996a,b). In contrast to mammals, birds fuel high-intensity endurance exercise such as migratory flights using primarily fatty acid oxidation (McWilliams et al., 2004). Here we examine for the first time the effect of fatty acid composition of depot fat on peak metabolic rates during exercise in a migratory bird.

Reliance on fatty acid oxidation to fuel high-intensity endurance exercise in birds is remarkable because, unlike hydrophilic fuel substrates such as glucose, transport of fatty acids requires the action of soluble protein carriers at every step of fatty acid transport. Accordingly, the capacity of these carriers must be significantly upregulated during intense exercise to ensure adequate fatty acid transport, but few studies have focused on this type of physiological modulation (Guglielmo et al., 2002a; McWilliams et al., 2004). There are many possible ways that migratory birds could augment fatty acid utilization capacity during exercise, including mechanisms associated with adipose sources, circulatory pathways, myocyte uptake and intracellular disposal (esterification and oxidation; Roberts et al., 1996; Weber et al., 1996a,b; Hochachka and Somero, 2002). We focus here on how modulation of fatty acid composition of fat stores affects exercise performance in birds.

Although few studies document fatty acid composition of fat stores in birds during migration, evidence to date shows that the majority of lipids in migrating birds comprise 16- or 18-carbon fatty acids, and unsaturated fatty acids (mostly 16:1, 18:1, 18:2) usually predominate over saturated fatty acids (mostly 16:0 and 18:0; Blem, 1976; Conway et al., 1994; Egeler and Williams, 2000). Whether a certain fatty acid

composition enhances exercise performance of birds is unknown, although studies with exercising mammals provide a basis for developing some hypotheses. Mammalian adipocytes release fatty acids preferentially based on chain length and degree of unsaturation: fatty acids with the same number of carbon atoms but with more double bonds are preferentially released, and those with the same number of double bonds but with shorter chain lengths are preferred (Raclot and Groscolas, 1995; Raclot, 2003). In rats and humans, high levels of essential n-6 polyunsaturated fatty acids (e.g. 18:2n6) in muscle membrane phospholipids have been associated with improved endurance capacity, and n-6 fatty acids appear to be depleted from membranes by repetitive exercise (Ayre and Hulbert, 1996, 1997; Andersson et al., 1998). Such preferential mobilization and oxidation of unsaturated fatty acids has not been demonstrated in birds. If similar mechanisms exist in birds, then we predict that birds with more unsaturated fatty acids, in general, or more n-6 polyunsaturated fatty acids, in particular, would have enhanced exercise performance.

We tested this hypothesis by measuring metabolic rate at rest and during intense exercise in two groups of red-eyed vireos *Vireo olivaceous* fed one of two semi-synthetic diets for 4 months. Red-eyed vireos are abundant, medium-sized (13–25 g) neotropical migrants that spend the summer in northern United States and southern Canada and spend the winter in Central and South America (Cimprich et al., 2000). They store relatively large fat reserves during migration and thus are an excellent species in which to study composition of fat reserves and its energetic consequences.

Materials and methods

Capture and maintenance of birds

Hatch-year red-eyed vireos *Vireo olivaceous olivaceous* (N=10) were captured using mist-nets between 1 and 12 October 2001 on Block Island, Rhode Island (41°10′N, 71°34′W) (USFWS permit MB-003201, R.I. DEM permit 2001-75). In the laboratory, birds were housed individually in stainless-steel cages (59 cm×45 cm×36 cm), at constant temperature (23°C), and on a light cycle that simulated the natural light cycle at time of capture (11 h:13 h light:dark cycle, lights on at 07:00 h). All birds were provided with water *ad libitum* and one of two semi-synthetic diets, along with eight waxworms *Galleria mellonella* per day for 4 months. Nutrient content of the semi-synthetic diets simulated a high-lipid fruit diet (41% carbohydrate: 13% protein: 30% fat; Stiles and White, 1982; Johnson et al., 1985; Stiles, 1993). The more saturated fat diet (58%U) contained cottonseed and palm oil whereas the more unsaturated fat diet (82%U) contained olive oil (Table 1), so that the two diets differed in their fatty acid composition (Table 2; N=5 fed 58%U diet, N=5 fed 82%U diet). Use of such semi-synthetic diets makes the composition of the diets less ambiguous than diets compounded from raw foodstuffs (Murphy and King, 1982). Since waxworms comprised 4% of the total daily dry intake (13% of total daily lipid intake) of vireos fed the 58%U diet and 3% of the total daily dry intake (11% of total daily lipid intake) of vireos fed the 82%U diet, we report the fatty

Table 1. *Composition of semi-synthetic fruit diet fed to red-eyed vireos for 4 months*

	Fruit diet	
Ingredients	% wet mass	% dry mass
Glucose	10.25	41.0
Casein[a]	2.50	10.0
Amino acid mix[b]	0.70	2.8
Vitamin and minerals mix[c]	0.25	1.0
Salt mix[d]	1.25	5.0
Cellulose[e]	1.30	5.2
Plant oil[f]	7.50	30.0
Agar[g]	1.25	5.0
Water	75.00	–

[a]Casein (high N): US Biochemical Corp., Cleveland, OH, USA.
[b]Amino acid mix: Murphy and King (1982), all amino acids supplied by Fisher Scientific, Pittsburgh, PA, USA.
[c]AIN-76 Vitamin and minerals mix, ICN Biomedicals, Inc., Irvine, CA, USA.
[d]Salt mix: Briggs-N Salt mixture, ICN Biomedicals, Inc.
[e]Celufil-hydrolyzed: US Biochemical Corp.
[f]Olive oil in 82%U diet and equal amounts of cottonseed and palm oil in 58%U diet (for sources, see Table 2).
[g]Agar bacteriological grade: US Biochemical Corp.

Table 2. *Fatty acid composition (%) of two experimental diets and waxworms* Galleria mellonella *that were eaten by red-eyed vireos* Vireo olivaceous

	Fatty acid composition[a]		
Fatty acids[b]	58%U diet[c]	82%U diet[d]	Waxworms[e]
10:0	3.8	3.6	–
14:0	1.3	–	0.4
16:0	33.2	10.9	28.4
16:1n7	<1	<1	1.3
18:0	3.6	3.3	1.9
18:1n9	28.6	72.2	48.0
18:1n7	<1	1.9	0.1
18:2n6	29.5	8.1	10.7
18:3n6	–	–	1.5
20:1n9	–	–	6.1

Both diets were similar in nutrient composition (41% carbohydrate, 13% protein, 30% fat; see Table 1).
[a]Fatty acid composition was directly measured using gas chromatography.
[b]Other fatty acids found in <1% of the lipid portion of the diet(s) were 8:0, 12:0, 18:3n3, 20:4n6 and 22:6n3.
[c]Lipid in 58%U diet was equal amounts of cottonseed oil (ICN Biomedicals, Inc., Irvine, CA, USA) and palm oil (Tuscan Sun brand from Sid Wainer, Inc., New Bedford, MA, USA).
[d]Lipid in 82%U diet was Rienzi-brand olive oil.
[e]Waxworm caterpillars (Grubco, Hamilton, OH, USA).

acid composition of the composite diet (i.e. semisynthetic diet plus waxworms; Table 3). Each day we measured body mass (±0.1 g) and food intake (±0.1 g wet mass) of each bird. All bird husbandry conformed to guidelines in Gaunt and Oring (1997) and was approved by URI IACUC (AN01-04-029).

Influence of diet on fatty acid composition of vireo body fat

To determine how dietary fatty acids influenced fatty acid composition of vireos, all vireos fed each diet were killed immediately following exercise and stored at –20°C for later analysis. Carcasses were thawed and whole liver and both pectoral muscles were removed, rinsed in distilled water, blotted dry and weighed (±0.1 mg). Intestines were removed, perfused with distilled water, blotted dry and weighed (±0.1 mg). We also collected and weighed ca. 1 g (±0.1 mg) fat from the furcular region of each bird. Each tissue sample was placed into a glass scintillation vial and stored at –20°C for later analysis.

All organic solvents used were of HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). All bird tissues and diet samples were freeze-dried, weighed (±0.1 mg), and cut into fine particles using surgical scissors. Lipids were extracted from ca. 100 mg of sample using a modified version of Folch et al. (1956) as described in Jackson and Place (1990). Briefly, samples were homogenized with 3.0 ml dichloromethane:methanol (2:1 CH_2Cl_2:methanol), centrifuged for 15 min at 537 ***g*** and the supernatant transferred to a large test tube. This procedure was repeated with 1:1 CH_2Cl_2:methanol and 2:1 CH_2Cl_2:methanol. Lipid extract was first washed with 0.88% potassium chloride water solution, and then with dichloromethane:methanol:water (3/48/47). Samples were dried under nitrogen, weighed (±0.1 mg), resuspended in 500 µl of 1:1 dichloromethane:methanol and capped under nitrogen.

Quantification of fatty acid methyl esters was achieved by hydrolyzing ca. 500 µg of extracted lipid with methanolic HCl, adding 25 µg of internal standard mixture of equal amounts of nonadecanoic acid (C19:0) and heinecosanoic acid (C21:0) (Nu-chek PreP Inc., Elysian, MN, USA) to each sample, and extracting the methyl esters into dichloromethane. An aliquot sample of the dichloromethane extract was subjected to gas chromatography directly on a Hewlett-Packard (Paolo Alto, CA, USA) model 6890 instrument equipped with a flame ionization detector at 300°C and a J&W DBWAX fused silica capillary column (30 m×0.25 mm i.d. with 0.25 mm film thickness; J. & W. Scientific Inc., Folsom, CA, USA). Helium was used as the carrier gas with a constant flow rate of 1.5 ml min^{-1}. Oven temperature was programmed from an initial temperature of 50°C for 0.5 min to 195°C for 15 min after ramping at 40°C min^{-1}, to 220°C for 7 min after ramping at 2°C min^{-1}, with a total run time of 38.13 min. Peaks were identified by comparison with retention times of quantitative standards from Nu-Check Prep, Inc. (stds 3B, GLC 17AA′) and expressed as percentages of fatty acid methyl esters. Fatty acids that comprised on average less than 1% of the fatty acids in all tissue and diet samples are reported but were excluded from statistical analysis.

Table 3. *Fatty acid composition of two experimental diets and selected tissues from red-eyed vireos fed one of two diets*

		Tissues from vireos fed 58%U diet					Tissues from vireos fed 82%U diet				
Fatty acid	58%U diet with worms[a]	Fat	Pectoral muscle	Intestines	Liver	82%U diet with worms[a]	Fat	Pectoral muscle	Intestines	Liver	Tissue comparisons[b]
Four predominant fatty acids in diets and tissues											
16:0	32.57±0.00	30.49±0.53	26.14±2.19	29.26±1.42	32.72±0.89	12.79± 0.00	19.10±0.23	17.51±0.75	17.16±0.52	23.56±1.05	L>F P I
18:0	3.40±0.00	2.86±0.14	10.03±0.80	6.19±0.67	12.95±1.36	3.17±0.00	2.41±0.13	7.19±0.64	4.77±0.45	12.08±0.90	L>P>I>F
18:1 n9	31.12±0.00	40.04±0.73	28.88±2.61	36.15±0.90	29.06±1.61	69.52± 0.00	62.56±0.74	51.34±3.36	61.20±1.27	40.41±0.88	F I>P>L
18:2 n6	27.06±0.00	21.60±1.59	19.98±5.04	22.55±1.51	20.43±1.36	8.41± 0.00	10.32±0.09	10.48±2.64	10.99±0.21	11.90±0.62	L F P I
Other fatty acids detected in diets and tissues[c]											
10:0	3.27±0.00	0.71±0.46	1.33±0.81	0.81±0.67	1.07±0.66	3.16±0.00	–	–	0.83±0.52	0.77±0.48	
18:1 n7	1.22±0.00	–	0.55±0.33	–	–	1.76±0.00	–	1.82±0.07	0.46±0.28	0.74±0.31	
20:1 n9	0.79±0.00	4.29±0.64	1.03±0.47	2.99±0.62	0.92±0.41	0.67±0.00	4.06±0.76	1.45±0.38	2.63±1.04	0.48±0.48	
20:4 n6	–	–	3.81±1.18	1.47±0.69	2.01±0.88	–	–	3.59±0.37	1.58±0.68	5.83±0.60	
22:5	–	–	5.08±0.66	–-	0.84±0.60	–	–	1.58±0.51	–	2.03±0.25	
22:6 n3	–	–	3.19±0.74	–	–	–	–	3.81±0.52	–	2.20±0.15	

Values are % ± S.E.M. (tissues were obtained from *N*=5 vireos fed each diet).

Tissues from vireos fed the 58%U diet were significantly different than tissues from vireos fed the 82%U diet (see text for details). The only significant Diet×Tissue interaction was for 18:1n9 ($F_{(3,32)}$=7.03, P=0.001).

[a]Diet composition includes semi-synthetic diet plus 8 waxworms (Table 1); *N*=3; S.E. for diet composition are <0.001.

[b]Results of Tukey's HSD *post hoc* comparison of the proportion of a specific fatty acid in fat (F), pectoral muscle (P), intestine (I), and liver (L) of red-eyed vireos. Letters with a common underline are not significantly different.

[c]Statistical analyses were not performed on these six remaining fatty acids since many were undetected in several tissues from vireos fed either diet.

Analysis of blood metabolites

We measured concentrations of blood metabolites in resting and post-exercise birds to demonstrate that birds used fats to fuel their exercise in the enclosed running wheel. We sampled ca. 200 µl of blood from the brachial vein of vireos 1 week prior to and then immediately following their exercise trial. Blood was sampled between 12:00 and 15:00 h and after 90–120 min without food. Whole blood was centrifuged at 2817 ***g*** for 10 min. Plasma was stored in cryogenic vials at –80°C until further analysis.

Metabolites were measured using commercial kits modified for small volumes. Triacylglycerols (TAG) and free glycerol (GLYC) were measured by endpoint assay (Sigma, St Louis, MO, USA; Trinder reagent A, 300 µl to 5 µl plasma). Uric acid, phospholipids (PL), and non-esterified fatty acids (NEFA) were measured by endpoint assay (Wako Diagnostics, Richmond, VA, USA; 3 µl plasma, 120 µl reagent A, 240 µl reagent B). β-hydroxybutyrate was measured by kinetic assay (Roche Biopharma, Indianapolis, IN, USA). All samples (2.5–5 µl) were analyzed in duplicate on a microplate spectrophotometer (Biotek PowerwaveX 340, Winooksi, VT, USA). If coefficients of variation (CV) were greater than 10% for duplicate samples, additional samples were analyzed until CV<10%.

Resting and active metabolic rates of vireos

Oxygen consumption was measured using open-circuit respirometry. The same flow system was used for measuring both resting metabolic rates (RMR) and peak metabolic rates (MR_{peak}). We measured both RMR and MR_{peak} so that we could calculate the metabolic scope (MR_{peak}/RMR) of birds, which provides an indication of the intensity of exercise. Flow rates of dry, CO_2-free air (800 ml min^{-1} for RMR and 5000 ml min^{-1} for MR_{peak}) were regulated by thermal mass flowmeters (RMR; Brooks, Veenendaal, The Netherlands; MR_{peak}; Sable, Las Vegas, NV, USA). Approximately 100 ml min^{-1} of the excurrent air from the chambers was diverted, dried (using Drierite), passed through a CO_2 analyzer (Ametek CD-3A, Pittsburgh, PA, USA), redried, scrubbed of CO_2 (Ascarite), redried and passed through an oxygen analyzer (Sable FC-1). Outputs from these instruments were sampled by a MacIntosh computer equipped with an analog-to-digital converter and Warthog© software (Mark A. Chappell and the Regents of the University of California).

Each day one vireo from each of the two diet groups was randomly selected and the resting metabolic rates (RMR) of both birds were measured. Starting at 20:00 h, 60 min prior to lights off, vireos were weighed (±0.1 g) and placed into individual stainless-steel metabolic chambers (volume 1.9 l). These chambers were then placed in a temperature-controlled cabinet at 32±1.0°C, which is within the thermoneutral zone of a songbird species similar in size to vireos (Root et al., 1991). Each vireo fasted and rested in the chamber for 3–4 h, then oxygen consumption was measured during the remaining 6–7 h of the overnight period. Excurrent air from the two chambers was routed to the carbon dioxide and oxygen analyzers through a computer-controlled airstream selector (Sable Systems Respiratory Multiplexer). Excurrent streams from each chamber were sampled for 30 min, followed by a 5 min reference sample, before switching to sample the alternate chamber for 30 min. This sequential sampling continued throughout the measurement period. Thus, the RMR values reported are for postabsorptive individuals, resting in the inactive phase of their daily cycle. The RMR of an individual was taken as the minimum 10 min mean of O_2 consumption during the overnight test period. After RMR determination, vireos were placed back into their regular cages at 06:45 h and given *ad libitum* food and water for 4 h. Since digesta retention time of lipids in red-eyed vireos fed similar diets is ca. 68 min (Pierce et al., 2004), we removed food 1 h before energetic trials to promote emptying of the gut and the use of endogenous energy reserves by vireos during exercise.

Oxygen consumption of vireos during exercise was measured using an enclosed running wheel modified for flying birds. The wheel was constructed of acrylic plastic, carpet-lined, manually driven, and contained four ping-pong balls to discourage birds from walking (Chappell et al., 1996, 1999). Birds were weighed (±0.1 g), placed in the wheel (which was covered with a cloth at this time in order to reduce stress in the bird), and allowed to acclimate for 10 min within the wheel. The cloth was then removed and the wheel was spun slowly for 10–15 s to initiate exercise. The wheel was then kept in constant motion so that vireos were forced to hop and hover for ca. 30–40 min. This type of exercise provides a significant aerobic challenge to the birds and allowed us to determine a peak metabolic rate during short-term intense exercise, as done in other studies of peak metabolic rates in running and flying birds (Chappell et al., 1996, 1999). The MR_{peak} of an individual was the maximum mean of 'instantaneous' O_2 consumption achieved during this exercise over a 1 min period, calculated using the equation of Bartholomew et al. (1981).

Statistical analysis

Fatty acid composition (%) of the diets and tissues were arcsine transformed and Hotelling's T multivariate analysis of variance (MANOVA) was used to compare the proportion of each fatty acid in body tissues of birds fed each diet. Analysis of variance (ANOVA) was used to compare the proportion of unsaturated fat in the diets and tissues of vireos fed those diets. Repeated-measures analysis of variance (RM-ANOVA) was used to compare blood metabolite levels of vireos before and after exercise. ANOVA was used to compare metabolic rates of vireos fed each diet and Student *t*-test was used to compare metabolic scope of vireos fed each diet. All ANOVA tests were performed using the general linear model in SPSS 11.0 (SPSS, Inc.) and Tukey's HSD (honestly significant difference) was used for all *post hoc* comparisons. Results are reported as means ± standard error (S.E.M.).

Results

Food intake and body mass of vireos

At capture, body mass of birds assigned to the 58%U diet (17.8±1.6 g) was similar to that of birds assigned to the 82%U diet (17.8±0.7 g; $F_{(1,8)}$ <0.001, P=1.0). During the 4 month feeding period, vireos fed the 58%U diet gained on average 0.54±1.48 g while vireos fed the 82%U diet gained on average 4.26±1.49 g; however, the average change in body mass throughout the 4 month feeding period was not significantly different between the two diet groups ($F_{(1,8)}$=4.515, P=0.066). After 4 months, birds fed the 82%U diet (22.1±1.1 g) were significantly heavier than birds fed the 58%U diet (18.4±1.1g; $F_{(1,8)}$=5.5, P=0.047). Despite these diet differences in body mass, daily intake of vireos fed the 82%U diet (20.7±1.5 g) was similar to that of vireos fed the 58%U diet (17.1±1.6 g; $F_{(1,8)}$=2.57, P=0.15).

Influence of diet on fatty acid composition of vireo body fat

Vireos fed the 82%U diet had significantly more unsaturated fat and less 18:2n6 in their furcular fat than birds fed the 58%U diet (Diet: $F_{(1,32)}$=182.6, P<0.001; Fig. 1). Fatty acids 16:0, 18:0, 18:1n9 and 18:2n6 comprised >94% of dietary fatty acids and were also the primary fatty acids (>85%) in tissues of birds fed either diet (Table 3). However, tissues from vireos fed the 58%U diet had significantly more 16:0 ($F_{(1,32)}$=169.9, P<0.001), 18:0 ($F_{(1,32)=}$7.04, P=0.01) and 18:2n6 ($F_{(1,32)}$=42.6, P<0.001) and significantly less 18:1n9 ($F_{(1,32)}$=266.2, P<0.001) than vireos fed the 82%U diet (Table 3). For vireos fed either diet, the proportions of 16:0 ($F_{(3,32)}$=11.8, P<0.001), 18:0 ($F_{(3,32)}$=65.1, P<0.001) and 18:1n9 ($F_{(3,32)}$=38.0, P<0.001) were significantly different among tissues (Table 3). In general, liver contained more 16:0 and 18:0 than other tissues, and furcular fat and intestine contained more 18:1n9 than pectoral muscle and liver (Table 3). Fatty acids 10:0, 18:1n7, 22:5 and 22:6n3 comprised <6% of either diet, and none of these fatty acids comprised >5% of the lipids in the furcular fat, intestine and liver of birds fed these diets (Table 3). However, these four fatty acids combined comprised ca. 10% and 7% of the fatty acids in the pectoral muscle of birds fed the 58%U and the 82%U diets, respectively (Table 3). Of the remaining less-common fatty acids in the tissues of vireos, furcular fat contained the largest proportion of 20:1n9, and pectoral muscle and liver contained the largest proportions of 20:4n6 (Table 3).

Blood metabolites

Diet did not influence plasma metabolite concentrations prior to or immediately following exercise (P>0.21 for each metabolite; the Kolmogorov–Smirnov test revealed 9 of 12 metabolites were normally distributed and transformation of data did not effect level of significance). TAG and phospholipid levels decreased by 42% and 11%, respectively, after exercise ($F_{(1,8)}$=53.64, P<0.001; $F_{(1,8)}$=6.47, P=0.035, respectively) and NEFA and β-hydroxybutyrate increased by 68% and 67%, respectively, after exercise ($F_{(1,8)}$=134.78, P<0.001; $F_{(1,8)}$=23.17, P=0.001, respectively; Fig. 2). In addition, glycerol levels decreased by 82% and uric acid increased by 30% after exercise ($F_{(1,8)}$=12.15, P=0.008; $F_{(1,8)}$=12.12, P=0.008, respectively; Fig. 2).

Resting and active metabolic rates of vireos

We report metabolic rates on a whole-animal basis (per bird) and in mass-specific units (g^{-1}) because both sets of data demonstrate the effect of fatty acid composition of birds on metabolic rate during exercise, although variation in body mass between treatment groups influenced the level of statistical significance for estimates of whole-animal metabolic rate. Whole-animal RMR of vireos fed the 58%U diet (0.83±0.09 ml O_2 min^{-1}) was similar to that of vireos fed the 82%U diet (0.81±0.07 ml O_2 min^{-1}; $F_{(1,8)}$=0.04, P=0.8). Whole-animal MR_{peak} of vireos fed the 58%U diet (8.67±0.22 ml O_2 min^{-1}) was higher than whole-animal MR_{peak} of vireos fed the 82%U diet (7.68±0.56 ml O_2 min^{-1}). However, the variance in whole-animal MR_{peak} between individuals on the same diet was noticeably higher for birds fed the 82%U diet compared to birds fed the 58%U diet (Levene's test: $F_{(1,8)}$=4.7, P=0.06) so that the difference in MR_{peak} was not statistically significant ($F_{(1,8)}$=2.7, P=0.14).

We calculated mass-specific MR using the average body mass of a vireo before and after the RMR trial (i.e. 18.4±1.17 g for vireos fed the 58%U diet and 21.7±1.18 g for vireos fed the 82%U diet). Mass-specific RMR of vireos fed the 58%U diet (2.75±0.32 ml O_2 g^{-1} h^{-1}) was similar to that of vireos fed the 82%U diet (2.30±0.30 ml O_2 g^{-1} h^{-1}; $F_{(1,8)}$=1.09, P=0.33; Fig. 3). However, the mass-specific MR_{peak} of vireos fed the 58%U diet (28.55±1.47 ml O_2 g^{-1} h^{-1}) was significantly higher than the MR_{peak} of vireos fed the 82%U diet (21.50±1.76 ml O_2 g^{-1} h^{-1}) $F_{(1,8)}$=9.45, P=0.015; Fig. 3). Thus, metabolic rate of vireos during exercise was different between diet groups, indicating that fatty acid composition of birds

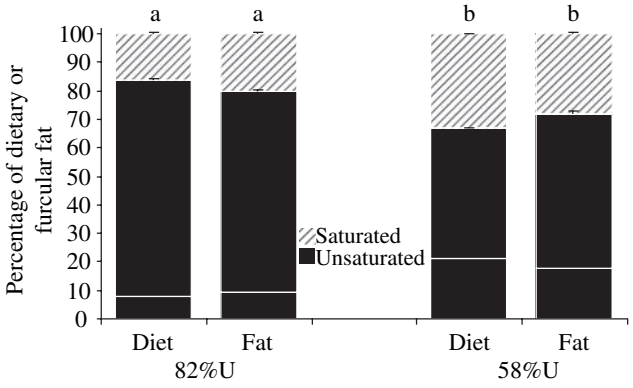

Fig. 1. Percentage of dietary fat and furcular fat in birds composed of saturated and unsaturated fat for red-eyed vireos fed the 58%U and 82%U diets. Values are means ± S.E.M. (diet N=3, fat N=5). Inset white line within each bar is the percentage of dietary fat and furcular fat in birds composed of 18:2n6 fatty acid (S.E.M. is less than bar width). Different letters above the bars denote significant differences (P<0.05) in percentage unsaturated fat and 18:2n6 between diet groups.

affected peak metabolic rate of vireos. This increase in mass-specific MR was 10.89±1.19 times the RMR for birds fed the 58%U diet and 9.95±1.41 times RMR for birds fed the 82%U diet and was not significantly different between the two diet groups (t=0.51, P=0.31).

Discussion

Below we discuss the effect of dietary fatty acids on the composition of body fat in birds, how changes in certain blood metabolites during exercise indicate fat metabolism is fueling the exercise, and how fatty acid composition of vertebrates influences their metabolic rate during exercise. We conclude with a discussion of the implications of these results for free-living birds during migration.

Influence of diet on fatty acid composition of vireo body fat

As expected, diet significantly influenced the fatty acid composition of body fat in vireos. The four predominant fatty acids in the diets (16:0, 18:0, 18:1n9 and 18:2n6) also predominated in the body fat of birds fed these diets. When vireos were fed a diet with mostly 18:1n9, their tissues (fat, breast, intestines, liver) also contained large proportions of this fatty acid. Likewise, when vireos were fed a diet with relatively equal proportions of 16:0, 18:1n9 and 18:2n6, their tissues also contained relatively similar proportions of these fatty acids. Lipids are the only dietary component that is deposited intact into tissues (Klasing, 1998). Consequently, the fatty acid composition of the diet can primarily determine the fatty acid composition of fat stores in birds (West and Meng, 1968; Thomas and George, 1975; West and Peyton, 1980), although some conversion of dietary fatty acids occurs and this selective metabolism can create some differences between fatty acid composition of diet and body fat, as is evident in our results and those of others (Blem, 1990; Klasing, 1998). For example, the livers of vireos fed each diet contained more 16:0 and 18:0, and less 18:1n9, than the other three tissues. This may be due, in part, to differences in oxidation rates of fatty acids in the liver (Leyton et al., 1987) as compared to other tissues. The majority of the long-chain unsaturated fatty acids in the breast, intestines and liver are likely to be found in the phospholipids of the cell membranes (Mead et al., 1986), although we cannot confirm this because we did not distinguish the different lipid classes (i.e. triacylglycerols, phospholipids) in our analysis.

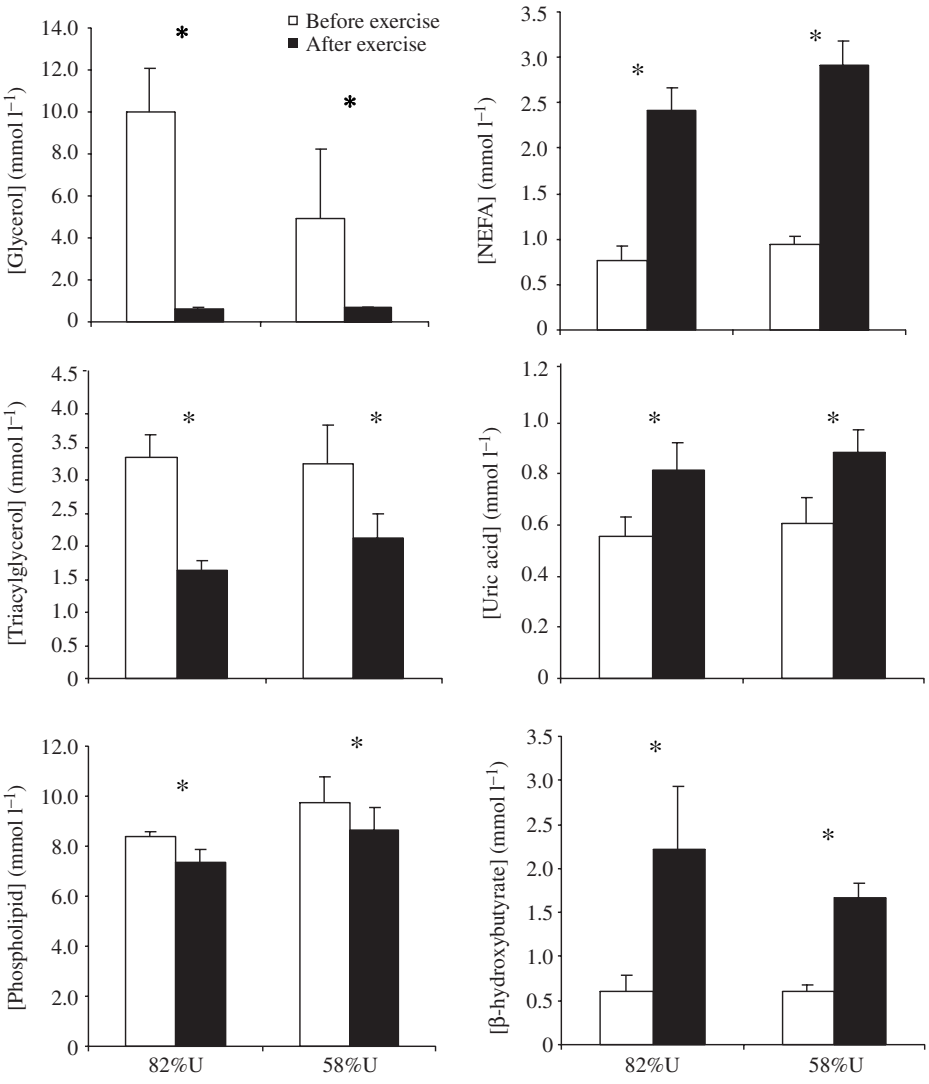

Fig. 2. Plasma metabolite concentrations in red-eyed vireos prior to and immediately following exercise. Values are means ± S.E.M. (N=5). Asterisks above the bars denote significant differences in plasma metabolite concentrations (P<0.05) just prior to and immediately following exercise. Diet did not influence plasma metabolite concentrations prior to or immediately following exercise (P>0.21 for each metabolite) and so is not shown.

Fuel use during exercise

Few studies have documented how concentrations of plasma metabolites change in a migratory songbird following intense exercise (Jenni-Eiermann and Jenni, 1991, 1992, 1996, 2001). Plasma metabolite changes in a migratory songbird are most commonly studied by comparisons of free-living birds captured at night during their annual migration (post-exercise) and free-living birds captured and maintained overnight (at rest). The increased levels of NEFA and β-hydroxybutyrate, and the decreased levels of PL, in the plasma of vireos after exercise demonstrate that vireos were metabolizing lipids while exercising in the running wheel. NEFA levels of vireos before

(ca. 0.85 mmol l^{-1}) and after exercise (ca. 2.5 mmol l^{-1}) were similar to those found for garden warblers *Sylvia borin* at rest (1.0 mmol l^{-1}) and during migratory flight at night (2.0 mmol l^{-1}; Jenni-Eiermann and Jenni, 1992). The levels of β-hydroxybutyrate (ca. 2.0 mmol l^{-1}) and uric acid (0.7 mmol l^{-1}) in vireos after exercise were also similar to those found in migrating garden warblers (2.2 and 0.6 mmol l^{-1}, respectively; Jenni-Eiermann and Jenni, 1996). Plasma GLYC levels in vireos after exercise (ca. 0.7 mmol l^{-1}) were similar to those found in garden warblers during migratory flight at night (0.9 mmol l^{-1}; Jenni-Eiermann and Jenni, 1992, 2001). However, vireos had significantly higher GLYC plasma levels prior to exercise (ca. 5–10 mmol l^{-1}) than did garden warblers at rest (0.5 mmol l^{-1}; Jenni-Eiermann and Jenni, 1992). High GLYC levels in vireos prior to exercise might be the result of substantial recent catabolism of exogenous fats in the high-lipid diet, since vireos were not in a fasted state when blood samples were taken.

Our results are relevant when considering an interesting supplementary mode of circulatory delivery of exogenous fatty acids to muscles that was proposed by Jenni-Eiermann and Jenni (1992) based on their studies of migratory passerines. They found that plasma TAG and very-low-density-lipoproteins (VLDL) were elevated in migrants captured in mid-flight, compared to birds fasted for 60 min or overnight. They suggested that the high lipid uptake and processing capacity of the liver allows it to act as an alternative sink for exogenous fatty acids originating from adipose tissue, thus freeing plasma albumin to transport more fatty acids per unit time. Fatty acids taken up by the liver would be re-esterified and released back to the plasma in VLDL. This pathway could provide large amounts of fatty acids to muscle without the osmotic effects of increased plasma albumin. However, elevated plasma TAG levels during flight have not been further confirmed in controlled field or laboratory studies with other species. Plasma TAG concentration declined in flying pigeons (Bordel and Haase, 1993; Schwilch et al., 1996), and declined initially and stabilized in red knots *Calidris canutus* flying for up to 10 h in a wind tunnel (Jenni-Eiermann et al., 2002). We found that plasma TAG levels also declined during flight wheel exercise in the red-eyed vireo. These results show that plasma TAG concentration is not necessarily elevated in birds during exercise, and so do not support the supplementary mode of fatty acid transport proposed by Jenni-Eiermann and Jenni (1992).

Resting metabolic rate and active metabolic rate in red-eyed vireos

Average peak metabolic rate of flying birds is 16 times resting metabolic rate (Hinds et al., 1993). Average peak metabolic rates in our vireos were ca. 10 times higher than resting metabolic rates and similar to those of adult house sparrows *Passer domesticus* (10.8±2.11 ml O_2 g l^{-1} min^{-1}) exercised in a comparable enclosed running wheel (Chappell et al., 1999). In addition, mass-specific RMR of vireos fed either diet (2.3–2.75 ml O_2 g l^{-1} min^{-1}), was similar to those found for house sparrows (2.5–2.8 ml O_2 g l^{-1} min^{-1}; Chappell et al., 1999). However, whole-animal RMR of vireos fed the 58%U diet (0.83 ml O_2 min^{-1}) and 82%U diet (0.81 ml O_2 min^{-1}) were ca. 20% lower than those predicted by an allometric equation for passerine birds (1.02–1.15 ml O_2 min^{-1} depending on diet treatment; Lasiewski and Dawson, 1967). Mass-specific MR_{peak} of our vireos fed the 58%U diet (28.5±1.5 ml O_2 g^{-1} h^{-1}) was higher than those of adult (26.9 ml O_2 g^{-1} min^{-1}) and juvenile house sparrows (22.9 ml O_2 g^{-1} min^{-1}; Chappell et al., 1999), whereas mass-specific MR_{peak} of our vireos fed the 82%U diet (21.5±1.8 ml O_2 g^{-1} h^{-1}) were lower than those of adult and juvenile house sparrows (Chappell et al., 1999).

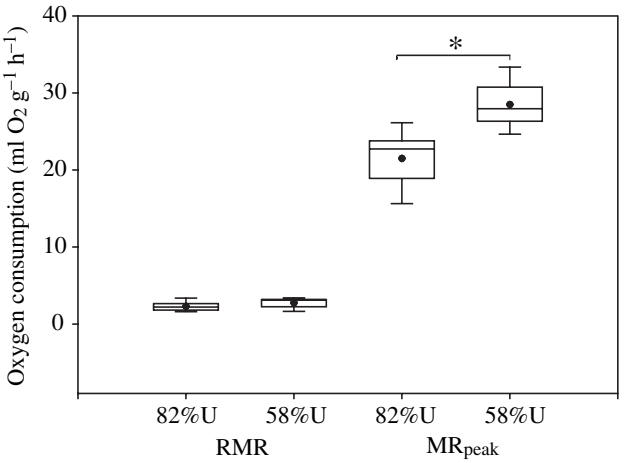

Fig. 3. Mass-specific resting metabolic rate (RMR) and mass-specific peak metabolic rate (MR_{peak}) for two groups of red-eyed vireos fed diets with different fatty acid composition (see Table 2). The asterisk denotes a significant difference (P<0.05, N=5) between diet groups. Boxes show the 25th, 50th and 75th percentile, whiskers show the 10th and 90th percentile, and means are closed circles.

Influence of fatty acid composition on energetic performance

Fatty acid composition of fat reserves affects exercise performance in rats and fish in part because specific unsaturated fatty acids are preferentially used during metabolism over saturated fatty acids (Leyton et al., 1987; Raclot and Groscolas, 1995; McKenzie et al., 1997, 1998). Ours is the first study to examine the influence of fatty acid composition of depot fat on the energetic performance in birds. Our results contradict the simple hypothesis that migratory birds with more unsaturated fatty acids comprising their fat depot have enhanced aerobic performance during intense exercise compared to birds with less unsaturated fatty acids. Determining which specific fatty acids were responsible for the differences in peak metabolic rate between birds fed each diet was not the goal of this study, although our results can be used to suggest and cautiously evaluate a few possible hypotheses. Below we discuss two alternative hypotheses that seem most likely, given the observed differences in fatty acid composition of our birds and the results from studies of other vertebrates.

Our results suggest that migratory birds with more

essential n-6 polyunsaturated fatty acids (i.e. 18:2n6) comprising their fat depot have improved exercise performance. The hypothesis that the amount of essential n-6 polyunsaturated fatty acids such as 18:2n6 improves exercise performance in birds is also supported by a recent field study of Western sandpipers *Calidris mauri*. Muscle phospholipids in sandpipers were more monounsaturated during migration and n-6 fatty acids decreased between premigration and migration periods, suggesting that n-6 fatty acids may be depleted by migratory flight (Guglielmo et al., 2002b).

Alternatively, animal performance may be enhanced when fat stores comprise some intermediate amount of unsaturated fatty acids. For example, McKenzie et al. (1998) found that sturgeon fed a diet containing menhaden oil (rich in polyunsaturated fatty acids) had reduced maximum swimming speed compared to fish fed a diet containing canola oil (rich in monounsaturated fatty acids). In addition, it is known that the polyunsaturated fatty acids in mammalian cells undergo lipid peroxidation (producing toxic lipid peroxides) more readily than saturated and monounsaturated fatty acids (Mead et al., 1986). Frank et al. (1998) found that golden-mantled ground squirrels *Spermophilus lateralis*, when given choices of diets with various proportions of unsaturated and saturated fatty acids, restricted their intake of polyunsaturated fatty acids to the minimal level required for proper hibernation. Similar processes may be occurring in migratory songbirds, although little is known about lipid peroxidation in avian tissues, and no study has tested whether birds combine diets with certain ratios of unsaturated and saturated fatty acids to achieve a specific fatty acid composition in their body fat prior to a certain energetically demanding event (e.g. migration, egg-laying, cold-tolerance).

Fatty acid storage and use: implications for the ecology of migratory songbirds

We have demonstrated that the fatty acid composition of the diet largely determines the composition of a migratory bird and this, in turn, affects the bird's energetic performance during intense exercise. These results suggest that birds during migration could benefit from selecting foods with certain fatty acids. Unfortunately, we know little about the fatty acid composition of foods eaten by free-living birds and the extent to which diet selection in birds is influenced by composition of dietary fats. Migratory birds prefer diets with more long-chain monounsaturated fatty acids (e.g. 18:1; Bairlein, 1991; McWilliams et al., 2002; Pierce et al., 2004). What remains to be demonstrated is how these diet preferences interact with food availability and composition to determine the fatty acid composition of birds during migration. We find particularly intriguing the untested hypothesis that there is some 'optimum' fatty acid composition of migratory birds that enhances performance of birds during migratory flight and which the birds attempt to achieve by carefully choosing their diet, thus influencing the pace of their migration.

List of abbreviations

58%U	saturated fat diet
82%U	unsaturated fat diet
GLYC	free glycerol
MR_{peak}	peak metabolic rate
NEFA	non-esterified fatty acid
PL	phospholipid
RMR	resting metabolic rate
TAG	triacylglycerol

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References

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

Ayre, K. J. and Hulbert, A. J. (1996). Dietary fatty acid profile influences the composition of skeletal muscle phospholipids in rats. *J. Nutr.* **126**, 653-662.

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

Bairlein, F. (1991). Nutritional adaptations to fat deposition in the long-distance migratory garden warbler *Sylvia borin*. *Intl. Ornith. Congr.* 2149-2158.

Bartholomew, G. A., Vleck, D. and Vleck, C. M. (1981). Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J. Exp. Biol.* **90**, 17-32.

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

Blem, C. R. (1976). Patterns of lipid storage and utilization in birds. *Am. Zool.* **16**, 671-684.

Blem, C. R. (1990). Avian energy storage. In *Current Ornithology*, vol. 7 (ed. 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.

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

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

Chappell, M. A., Zuk, M. and Johnsen, T. S. (1996). Repeatability of aerobic performance in red junglefowl: effects of ontogeny and nematode infection. *Funct. Ecol.* **10**, 578-585.

Cimprich, D. A., Moore, F. R. and Guilfoyle, M. P. (2000). Red-eyed vireo. In *The Birds of North America* No. 527 (ed. A. Pool and F. Gill), pp. 1-24. Academy of Natural Sciences, Philadelphia and The American Ornithologists Union, Washington, DC.

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.

Folch, J., Lees, M. and Sloane Stanley, G. H. (1956). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Frank, C. L., Dierenfeld, E. S. and Storey, K. B. (1998). The relationship between lipid peroxidation, hibernation, and food selection in mammals. *Am. Zool.* **38**, 341-349.

Gaunt, A. S. and Oring, L. W. (1997). *Guidelines to the Use of Wild Birds in Research*, pp. 56. Washington, DC: North American Ornithological Council.

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 migratory 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.

Hinds, D. S., Baudinette, R. V., Macmillen, R. E. and Halpern, E. A. (1993). Maximum metabolism and the aerobic factorial scope of endotherms. *J. Exp. Biol.* **182**, 41-56.

Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation – Mechanisms and Process in Physiological Evolution*. New York: Oxford University Press.

Jackson, S. and Place, A. R. (1990). Gastrointestinal transit and lipid assimilation efficiencies in three species of high latitude seabird. *J. Exp. Zool.* **255**, 141-154.

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

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

Jenni-Eiermann, S. and Jenni, L. (1996). Metabolic differences between the postbreeding, moulting and migratory periods in feeding and fasting passerine birds. *Funct. Ecol.* **10**, 62-72.

Jenni-Eiermann, S. and Jenni, L. (2001). Postexercise ketosis in night-migrating passerine birds. *Physiol. Biochem. Zool.* **74**, 90-101.

Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindström, Á., Piersma, T. and Visser, G. (2002). Fuel use and metabolic response to endurance exercise: a wind tunnel study of a long-distance migrant shorebird. *J. Exp. Biol.* **205**, 2453-2460.

Johnson, R. A., Willson, M. F., Thompson, J. N. and Bertin, R. I. (1985). Nutritional values of wild fruits and consumption by migrant frugivorous birds. *Ecology* **66**, 819-827.

Klasing, K. C. (1998). Nutritional strategies and adaptations. In *Comparative Avian Nutrition*, pp. 71-124. New York, New York: CAB International.

Lasiewski, R. C. and Dawson, W. R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13-23.

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.

Lindstrom, A. and Piersma, T. (1993). Mass changes in migrating birds: the evidence for fat and protein storage re-examined. *Ibis* **135**, 70-78.

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

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

McWilliams, S. R., Guglielmo, C., Pierce, B. J. and Klaassen, M. (2004). Flying, fasting, and feeding in birds during migration: a physiological ecology perspective. *J. Avian Biol.* **35**, 377-393.

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

Mead, J. F., Alfin-Slater, R. B., Howton, D. R. and Popjak, G. (1986). *Lipids: Chemistry, Biochemistry, and Nutrition*. New York: Plenum Press.

Murphy, M. E. and King, J. R. (1982). Semi-synthetic diets as a tool for nutritional ecology. *Auk* **99**, 165-167.

Pierce, B. J., McWilliams, S. R., O'Connor, T. P., Place, A. R. and Guglielmo, C. G. (2004). Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory red-eyed vireos (*Vireo olivaceous*). *Comp. Biochem. Physiol.* **138A**, 503-514.

Piersma, T. (1990). Pre-migratory 'fattening' usually involves more than the deposition of fat alone. *Ringing Migration* **11**, 113-115.

Piersma, T. and Jukema, J. (1990). Budgeting the flight of a long-distance migrant: changes in nutrient reserve levels of bar-tailed godwits at successive spring staging sites. *Ardea* **78**, 315-338.

Raclot, T. (2003). Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Prog. Lipid Res.* **42**, 257-288.

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

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

Root, T. L., O'Connor, T. P. and Dawson, W. R. (1991). Standard metabolic level and insulative characteristics of eastern house finches, *Carpodacus mexicanus*. *Physiol. Zool.* **64**, 1279-1295.

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

Stiles, E. W. (1993). The influence of pulp lipids on fruit preference by birds. *Vegetatio* **107/108**, 227-235.

Stiles, E. W. and White, D. W. (1982). Additional information on temperate bird-disseminated fruits: response to Herrera's comments. *Am. Nat.* **120**, 823-827.

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

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

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

West, G. C. and Meng, M. S. (1968). The effect of diet and captivity on the fatty acid composition of redpoll (*Acanthis flammea*) depot fats. *Comp. Biochem. Physiol.* **25**, 535-540.

West, G. C. and Peyton, L. J. (1980). Fatty acids of depot lipids in migrating lapland longspurs. *J. Field Ornith.* **51**, 138-143.