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SEASONAL CHANGES IN COMPOSITION OF LIPID STORES IN MIGRATORY BIRDS: CAUSES AND CONSEQUENCES

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Abstract. It is well established that birds use fat stores to primarily fuel migration; however, few studies have focused on the causes and consequences of observed seasonal changes in fatty acid composition of fat stores in birds. We propose and test two hypotheses that address the causes of these seasonal changes in composition of fat stores: (1) diet composition determines fatty acid composition of fat stores, and (2) birds selectively metabolize and store certain fatty acids during migration in lieu of changing their diet. When we offered Red-eyed Vireos (*Vireo olivaceous*) choices between diets that differed only in fatty acid composition, vireos preferred diets with more triolein over diets with more tristearin and tripalmitin, and these preferences were similar between seasons. We also collected fat samples six times throughout the year from captive Red-eyed Vireos fed one of two diets differing in fatty acid composition, and found that fatty acid composition of stored fat differed by diet and changed over time, although these changes were not season-specific or consistent with the selective-metabolism hypothesis. Thus, fatty acid composition of stored fat was primarily a product of diet composition; selective metabolism possibly played a minor, but important, role. Given recent evidence that fatty acid composition of birds affects their energy expenditure during intense exercise, the implication is that birds at stopover sites can influence the fatty acid composition of their body fat by selective feeding, and this can significantly affect the energetic cost of migration.

Key words: *diet selection, fat metabolism, fatty acid composition, migratory birds.*

Cambios Estacionales en la Composición de las Reservas Lipídicas en Aves Migratorias: Causas y Consecuencias

Resumen. Se sabe que las aves utilizan reservas de grasa principalmente como fuente de energía durante la migración; sin embargo, pocos estudios han analizado las causas y consecuencias de los cambios estacionales en la composición de ácidos grasos de las reservas de grasa de las aves. Aquí proponemos y probamos dos hipótesis que se relacionan con las causas de los cambios estacionales en la composición de las reservas de grasa: (1) la composición de la dieta determina la composición de ácidos grasos de las reservas de grasa, y (2) las aves metabolizan y almacenan de manera selectiva ciertos ácidos grasos durante la migración, en lugar de cambiar sus dietas. Ofrecimos a individuos de la especie *Vireo olivaceous* para que elijan entre dietas que sólo diferían en la composición de ácidos grasos, los cuáles prefirieron dietas con más trioleina que dietas con más tristearina y tripalmitina, y estas preferencias fueron similares entre estaciones. Colectamos además muestras de grasa, en seis oportunidades a lo largo del año, de individuos en cautiverio alimentados con una de las dos dietas disponibles, las que difirieron en la composición de ácidos grasos. Encontramos que la composición de ácidos grasos de las reservas de grasa difirió en relación a las dietas y varió a lo largo del tiempo, aunque estos cambios no fueron estacionalmente específicos ni coherentes con la hipótesis de metabolismo selectivo. Por lo tanto, la composición de ácidos grasos de las reservas de grasa fue principalmente el producto de la composición de la dieta; el metabolismo selectivo probablemente jugó un papel menor, aunque importante. Existe evidencia reciente que sugiere que la composición de ácidos grasos en las aves afecta el gasto energético que tienen durante períodos de ejercicios intensos, lo que implica que las aves pueden influir sobre la composición de los ácidos grasos en sus reservas de grasa al alimentarse de manera selectiva en los sitios de parada migratoria, lo que puede afectar significativamente el costo energético de la migración.

INTRODUCTION

Many recent studies have demonstrated that birds, in preparation for migration, increase the size of certain muscles, and accumulate energy and nutrient stores (Piersma 1990, Piersma and Jukema 1990, Lindström and Piersma 1993, Butler and Bishop 2000, Bauchinger and Biebach 2001). Empirical evidence confirms the primary importance of adipose fat as a metabolic fuel in flying birds, with a variable contribution of protein. For example, body composition analyses show that migrants deposit fat loads as high

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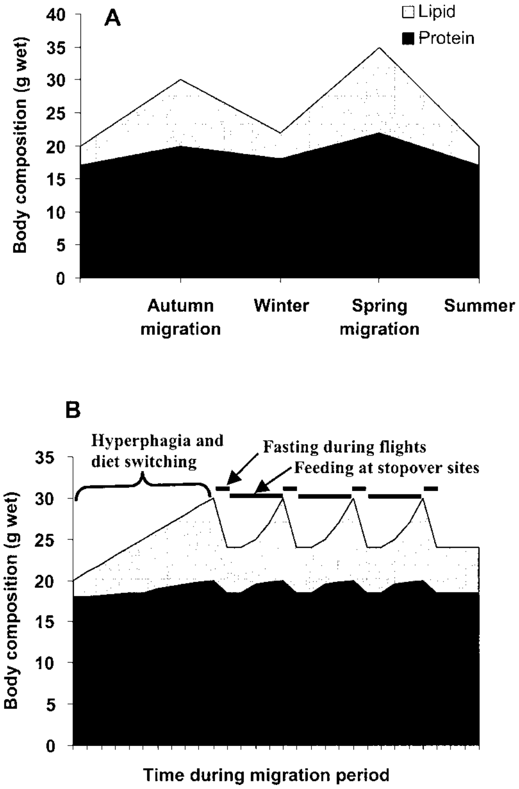

FIGURE 1. Change in body composition associated with migratory and nonmigratory periods of the annual cycle in a typical temperate-zone migratory songbird. (A) Body mass increases from approximately 20 g to 35 g between nonmigratory and migratory periods, and this increase in body mass is composed of primarily lipid and some protein stores. (B) In preparation for migration, birds accumulate fat stores primarily by increasing food intake and by carefully selecting their diets. Then during a given migration period, a typical migratory bird traveling between wintering and breeding areas must take several flights that are interspersed with layovers at stopover sites where the bird refuels in preparation for the next flight. Thus, birds during migration alternate between periods of high feeding rate at migratory stopover sites and periods without feeding as they travel between stopover sites.

as 50% of total body mass, that adipose fat can be severely depleted by migratory flight, and that body protein is used from muscles and digestive organs (Piersma and Jukema 1990, Ramenofsky 1990, Lindström and Piersma 1993, Battley et al. 2000, 2001, Bauchinger and Biebach 2001, McWilliams and Karasov 2001, McWilliams et al. 2004; Fig. 1). Despite the obvious importance of stopover sites along the migration route for recovery of stored energy and nutrients, the ecology and physiology of birds at stopover sites is poorly understood (Lindström 1995, Moore and Aborn 2000). In this paper, we review what is known about the fatty acid composition of fat stores in birds during migratory and nonmigratory periods, we propose and test hypotheses that address possible causes of the observed seasonal changes in composition of fat stores, and then discuss the implications of these results for our understanding of stopover ecology of birds and the energetic costs of their migrations.

BODY COMPOSITION DURING THE ANNUAL CYCLE: FAT IS THE PRIMARY FUEL FOR MIGRATION

Migratory birds accumulate fat stores primarily by increasing food intake and by selecting diets based in part, on nutrient content (Blem 1990, Stiles 1993, Bairlein and Gwinner 1994, Biebach 1996, McWilliams et al. 2004). Fattening rates in Garden Warblers (*Sylvia borin*), a long-distance migratory songbird, were affected by the relative amounts of dietary fat, carbohydrate, and protein (Bairlein 1990). Such studies of how diet composition affects rate of fattening and body-fat composition are particularly relevant because many temperate-zone breeding songbirds switch from feeding on insects (high protein-to-calorie ratio) to fruits (low protein-to-calorie ratio) during their migration (Bairlein 1990, 1991, Bairlein and Gwinner 1994, Parrish 1997, Parrish 2000, Levey and Martinez del Rio 2001). This dietary switch from insects to fruits during migration has been proposed as an energy-conservation strategy, in that fruits that are abundant are less energetically expensive to obtain compared to insects (Berthold 1976, Thompson and Willson 1979, Bairlein and Gwinner 1994, Parrish 2000). Fruits may be nutritionally adequate for replenishing fat stores, although several studies suggest that birds that eat only fruit restore fat more slowly than birds that eat other diets (Bairlein and Gwinner 1994, Parrish 2000, Pierce and McWilliams 2004). In a recent review, Levey and Martinez del Rio (2001) suggested that the frontier in research on nutritional ecology of fruit-eating birds should involve studies that focus on understanding micronutrients and secondary metabolites in fruits in relation to bird preferences and performance. Here, we present evidence that fatty acid composition of fat stores in migratory birds changes seasonally, is affected by diet composition, and has consequences for the energetic cost of intense exercise such as flight.

FATTY ACID COMPOSITION OF MIGRATING BIRDS

Given the primary importance of lipid stores for fueling migration, there has been some interest in the fatty acid composition of these lipid stores. In general, fat stores in a variety of birds captured during migration are composed of many fatty acids, although certain fatty acids usually predominate (McWilliams et al. 2004). Specifically, three fatty acids (16-carbon saturated (16:0), 18-carbon unsaturated with one [18:1] or two [18:2] double bonds) comprised 50%–90% of the body fat in birds captured during fall or spring migration. What is not clear from such work is whether the fat stores of these migratory birds always have this fatty acid composition or whether their fatty acid composition changes seasonally.

Only six studies have documented how fatty acid composition of free-living migratory birds changes seasonally and especially during migration (Table 1). The same three fatty acids (16:0, 18:1 and 18:2) that were most common in a variety of birds captured during migration (McWilliams et al. 2004) also predominated in fat stores from the same species captured during migratory and nonmigratory periods (Table 1). However, in some cases other fatty acids (e.g., 14:0, 16:1 and 18:0) were just as common. For example, fat stores of Red-eyed Vireos captured during fall migration were primarily composed of 16:1 and 18:1, and White-crowned Sparrows (*Zonotrichia leucophrys*) captured during spring migration were primarily composed of 14:0 and 18:1. Regardless of when during the annual cycle they were captured, Western Sandpipers (*Caldiris mauri*) had remarkably less 18:2 in their fat stores compared to other species that have been investigated.

Using the data in Table 1, we can evaluate several alternative hypotheses that have been proposed to explain how fatty acid composition of fat stores in birds changes between migratory and nonmigratory periods. Given that 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), one hypothesis is that the proportion of unsaturated fatty acids in fat stores will increase during migration. Results from only three of the six species studied (Red-eyed Vireos, Wood Thrush [*Hylocichla mustelina*], and Western Sandpiper) support this hypothesis. Blem (1980) suggested that the ratio of 18:1 to 18:2 was higher in migratory birds. Results from only three of the six species studied (Wood Thrush, Slate-colored Junco [*Junco hyemalis*], and White-crowned Sparrow) support this alternative hypothesis. However, the ratio of long-chain monounsaturated fatty acids (16:1, 18:1) to an essential long-chain polyunsaturated fatty acid (18:2) increased during migratory periods for four of the six species. Thus, the two principle patterns that emerge from these few studies is that (a) there are seasonal changes in fatty acid composition associated with migration, and (b) the change is primarily more long-chain monounsaturated fatty acids (16:1, 18:1) relative to the amount of the essential fatty acid, 18:2. Below, we discuss the possible causes of these seasonal changes in fatty acid composition of fat stores in birds and its ecological consequences.

DETERMINANTS OF FATTY ACID COMPOSITION IN BIRDS DURING MIGRATION

Fatty acid composition of diet can influence fatty acid composition of fat stores in birds and other animals (Bower and Helms 1968, West and Meng 1968a, 1968b, Landau 1970, Morton and Liebman 1974, Thomas and George 1975, Yom-Tov and Tietz 1978, Heitmeyer and Fredrickson 1990, Pierce et al. 2004, 2005), although some conversion of dietary fatty acids occurs and this selective metabolism may cause differences between fatty acid composition of diet and body fat (Blem 1990, Klasing 1998, Pierce et al. 2004). Given that free-living migratory birds exhibit seasonal changes in the fatty acid composition of their body fat (Table 1), we tested two hypotheses that address the causes of seasonal changes in fatty acid composition: (1) behavioral preferences for diets with specific fatty acids, and (2) selective metabolism and storage of specific fatty acids.

Changes in diet selection may at least in part be driven by seasonal changes in preference for diets with specific fatty acids. Diet preferences are most easily investigated by allowing an animal equal access to certain food types and measuring relative use (Johnson 1980). Three previous studies have directly tested whether mi-

TABLE 1. Fatty acid composition (%) of subcutaneous fat or whole carcass for birds captured during the breeding season, during fall or spring migration, and during winter. Fatty acid nomenclature is number of carbon atoms to number of double bonds. Several alternative hypotheses have been proposed to explain how fatty acid composition of fat stores in birds changes between nonmigratory and migratory periods: an increase in the proportion of unsaturated fatty acids, an increase in the ratio of 18:1 to 18:2, or an increase in the ratio of long-chain monounsaturated (16:1, 18:1) to an essential long-chain polyunsaturated fatty acid (18:2).

Species	Season	14:0	16:0	16:1	18:0	18:1
Red-eyed Vireo (*Vireo olivaceous*)	Breeding	–	29.8	2.1	6.9	39.7
	Fall migration	–	17.5	27.9	2.5	32.8
Wood Thrush (*Hylocichla mustelina*)	Breeding	–	24.0	4.7	13.8	34.4
	Fall migration	–	15.2	2.4	7.6	61.6
Slate-colored Junco (*Junco hyemalis*)	Winter	0.7	14.3	3.1	6.2	27.5
	Spring migration	0.6	19.2	3.1	7.3	33.7
White-crowned Sparrow (*Zonotrichia leucophrys*)	Winter	1.5	22.3	2.8	11.6	31.1
	Spring migration	31.8	16.6	4.2	5.8	24.4
Western Sandpiper (*Calidris mauri*)	Winter	6.0	35.0	8.0	17.0	18.0
	Spring migration	4.0	34.0	13.0	10.0	27.0
Canada Geese (*Branta canadensis*)	Breeding	0.2	24.3	2.5	5.1	54.5
	Spring migration	0.3	24.7	4.1	4.9	45.7

[a] Numbers correspond to the following references: 1) This study, 2) Conway et al. 1994, 3) Bower and Helms 1968, 4) Morton and Liebman 1974, 5) Egeler and Williams 2000, and 6) Thomas and George 1975.

gratory songbirds prefer diets with specific fatty acids (Bairlein 1991, McWilliams et al. 2002, Pierce et al. 2004). These studies showed that warblers and vireos preferred diets containing long-chain unsaturated fatty acids over diets containing long-chain saturated fatty acids. However, no previous study has examined whether such diet preferences for specific fatty acids change seasonally with fatty acid composition of fat stores in birds. Given that diet selection is important in determining fatty acid composition of birds, and that a few studies have shown birds prefer 18:1, we predicted that bird preferences for diets with 18:1 would increase during migratory periods of the annual cycle.

An alternative explanation for seasonal changes in fatty acid composition of fat stores is that birds selectively metabolize specific fatty acids. We know little about selective metabolism of fatty acids in wild birds (McWilliams et al. 2004). No previous study has examined whether selective metabolism for specific fatty acids changes seasonally with fatty acid composition of fat stores in birds. Given the evidence that fat stores in birds during migration contained more long-chain monounsaturated fatty acids (16:1, 18:1) than the essential fatty acid, 18:2 (Table 1), the selective-metabolism hypothesis predicts that vireos maintained on the same diet would metabolically increase production of monounsatured fatty acids or selectively use polyunsaturated fatty acids during migratory compared to nonmigratory periods.

METHODS

CAPTURE AND MAINTENANCE OF BIRDS

Red-eyed Vireos ($n = 40$) were captured using mist nets during October 2000 on Block Island, Rhode Island (41°10′N, 71°34′W) and immediately banded and weighed (±0.1 g). In the laboratory, birds were housed individually in stainless-steel cages (59 × 45 × 36 cm), and housed in a room kept at constant temperature (23°C) and initially on a light cycle that simulated the natural light cycle at time of capture (11 hr light: 13 hr dark cycle [11L:13D], with lights on at 07:00). Light cycles were then adjusted to those that the vireos would have experienced throughout their natural cycle (i.e., increased to 12L:12:D during autumn to simulate winter conditions, and then increased to 14L:10D during the summer). Each day, we measured body mass (±0.1 g) and food intake (±0.1 g wet) of each bird.

All birds were initially provided *ad libitum* water and semi-synthetic diet (composed of 82% unsaturated fatty acids [82%U]) along with eight waxworms (*Galleria mellonella*). On 2 April 2001, 18 birds were randomly selected from the captive population and switched to a different semi-synthetic diet (58%U diet). Both semi-synthetic diets were 41% carbohydrate, 13% pro-

TABLE 1. Extended.

18:2	Percent unsaturated	18:1 18:2	16:1 & 18:1 18:2	Tissue	Source[a]
14.4	56.2	2.8	2.9	Subcutaneous fat	1
16.1	76.8	2.0	3.8	Subcutaneous fat	1
12.0	51.1	2.9	3.3	Whole animal	2
7.6	71.6	8.1	8.4	Whole animal	2
41.8	72.4	0.7	0.7	Whole animal	3
31.1	67.9	1.1	1.2	Whole animal	3
29.3	63.2	1.1	1.2	Whole animal	4
10.6	39.2	2.3	2.7	Whole animal	4
1.0	27.0	18.0	26.0	Subcutaneous fat	5
2.0	42.0	13.5	20.0	Subcutaneous fat	5
12.8	69.8	4.3	4.5	Subcutaneous fat	6
19.1	68.9	2.4	2.6	Subcutaneous fat	6

tein, and 30% fat which is similar in macronutrient content to high-lipid fruits (Stiles and White 1982, Johnson et al. 1985, Stiles 1993). The only difference between the two diets, besides the level of unsaturated fat composition, was that the dietary fat was cottonseed and palm oil for the less unsaturated fat diet (58%U) whereas it was olive oil for the more unsaturated fat diet (82%U). Pierce et al. (2005) present diet recipes and fatty acid composition of both diets.

DIET PREFERENCES FOR SPECIFIC FATTY ACIDS

To determine if diet preferences changed seasonally, we offered Red-eyed Vireos simultaneous choices between three semi-synthetic diets differing only in fatty acid composition at times of the year that coincided with distinct periods of the annual cycle of free-living vireos: spring migration (April 2001), breeding season (early June 2001), fall migration (mid-October 2001), and winter season (late January 2002). During each of the four three-way preference trials, we offered birds (n = 9 per acclimation diet) equal amounts of three different semi-synthetic diets for four consecutive days. Each three-way preference trial involved providing the birds each morning with 30 g (±0.1 g wet) of three different diets in adjacent Petri dishes. Some food remained in all dishes after one day thus ensuring *ad libitum* feeding conditions. We measured dry matter food intake (±0.1 g) each day during preference trials and report the percent of total intake for each diet on day 4 of each preference trial. After each 4-day preference trial, birds were provided their acclimation diet (82%U or 58%U) until the next preference trial.

The three diets used in preference trials were 41% carbohydrate, 13% protein, and 30% fat, but the fatty acid composition of the dietary fat in each diet was unique (>90% of 16:0, 18:0, or 18:1) because we used purified triglycerides instead of olive, cottonseed, or palm oil for dietary fat. Pierce et al. (2004) present diet recipes and fatty acid composition of these three diets. Purified forms of triglycerides were used in each diet because fatty acids in foods eaten by wild birds are mostly in the triglyceride form (Zurovchak 1997).

SELECTIVE METABOLISM OF SPECIFIC FATTY ACIDS

To determine if selective metabolism of specific fatty acids was different between birds in migratory and nonmigratory state, we randomly sampled a subset of captive vireos fed either the 58%U diet (n = 18) or 82%U diet (n = 22) during spring (April 2001, n = 6 per diet), breeding (June 2001, n = 2 per diet), fall (October 2001, n = 2 per diet), winter (January 2001, n = 2 and 6, respectively), pre-migration (mid-March 2002, n = 3 per diet), and a second spring (mid-April 2002, n = 3 per diet). Selected vireos were killed and about 1.0 g (±0.1 mg) of fat from the furcular region of each bird was collected and stored in a glass scintillation vial

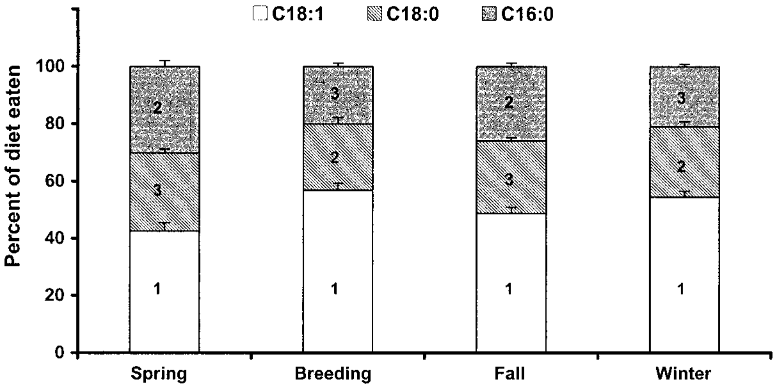

FIGURE 2. Percent (± SE) of total food intake of Red-eyed Vireos (n = 18 birds trial^{-1}) during each three-way preference trial given at four different times of the year. The three diets were 41% carbohydrate, 13% protein, and 30% fat, but the fatty acid composition of the dietary fat in each diet was unique (>90% 16-carbon saturated [16:0], or 18-carbon unsaturated with one [18:1] or two [18:2] double bonds) because we used purified triglycerides. Vireos ate significantly more of the 18:1 diet compared to the 18:0 and 16:0 diets in all seasons ($P < 0.02$). Numbers within the bars denote the rank order of preference based on compositional analysis.

at −20°C for later analysis. We also captured free-living vireos in May 2001 ($n = 5$) and October 2002 ($n = 4$) in Rhode Island and collected approximately 1.0 g of their furcular fat to examine the fatty acid composition of free-living vireos during breeding and fall migration. In the laboratory, furcular fat and diet samples were freeze-dried, weighed (±0.1 mg), and cut into fine particles using surgical scissors. Lipids were extracted from approximately 100 mg of sample using a modified version of Folch et al. (1956, described in Jackson and Place 1990). Quantification of fatty acid methyl esters was achieved by further chemical extraction and analysis with gas chromatography as described by Pierce et al. (2004, 2005).

STATISTICAL ANALYSES

We used compositional analysis (Aebischer et al. 1993) to assess vireo preferences for specific fatty acids because it accounts for nonindependence of data and overcomes the unit-sum constraint common to other statistical methods that are used to analyze compositional, proportion data (Neu et al. 1974). We used Greenhouse-Geisser repeated-measures analysis of variance (RMANOVA) to compare differences in diet preferences among diet groups and seasons. Fatty acid composition (%) of the tissues was arcsine transformed and Levine's test for homogeneity was applied to determine normality of the data. Hotelling's T multivariate analysis of variance (MANOVA) was used to compare fatty acid composition of furcular fat in captive birds fed each diet between the six time periods, and to compare the fatty acid composition of stored fat in free-living vireos between the two seasons. All ANOVA tests, were performed using the general linear model in SPSS 11.0 (Norusis 2002) and Tukey's HSD was used for all *post-hoc* comparisons. Results are reported as mean ± SE.

RESULTS

DIET PREFERENCES FOR SPECIFIC FATTY ACIDS

Vireos maintained on the 58%U diet or 82%U diet ate proportionately similar amounts of each diet (16:0 diet, $F_{1,14} = 0.3$, $P = 0.59$; 18:0 diet, $F_{1,14} = 0.8$, $P = 0.39$; 18:1 diet, $F_{1,14} = 1.2$, $P = 0.30$). Contrary to our prediction, vireos ate significantly more of the 18:1 diet compared to the 18:0 ($F_{2,31} = 4.5$, $P = 0.02$) and 16:0 ($F_{2,23} = 11.4$, $P < 0.01$) diets in all seasons (Fig. 2). Vireos ate similar proportions of 16:0 and 18:0 in each season ($F_{2,26} = 3.3$, $P = 0.06$) although their order of preference varied between migratory and nonmigratory periods (Fig. 2).

SELECTIVE METABOLISM OF SPECIFIC FATTY ACIDS

As expected, given the importance of diet in determining fat composition, vireos fed the 82%U diet had significantly more unsaturated fatty acids and less saturated fatty acids in their furcular fat than birds fed the 58%U diet (Diet: $F_{1,27}$ =

TABLE 2. Percent of the three most common fatty acids (mean ± SE) in furcular fat from Red-eyed Vireos fed a diet containing mostly unsaturated fat (82%U) or a diet containing proportionately less unsaturated fat (58%U) over a one-year period. Sample sizes (*n*) refer to the number of birds that were fed each diet.

		Season					
Fatty acid[a]	Diet[b] $n = 3$	Spring $n = 6$	Breeding $n = 2$	Fall $n = 2$	Winter $n = 6, 2$[c]	Pre-migration $n = 3$	Second Spring $n = 3$
82%U							
16:0	12.6 ± 0.1	24.9 ± 0.8	25.2 ± 1.1	23.5 ± 1.0	18.4 ± 0.5	19.1 ± 0.4	18.2 ± 0.9
18:1 n9	69.8 ± 0.3	54.4 ± 0.5	53.4 ± 0.6	56.0 ± 0.1	62.3 ± 0.7	62.2 ± 1.4	62.5 ± 0.4
18:2 n6	8.4 ± 0.1	11.0 ± 0.3	11.5 ± 0.1	10.6 ± 0.0	10.1 ± 0.2	10.2 ± 0.3	9.5 ± 0.2
58%U							
16:0	32.7 ± 0.2	28.3 ± 0.6	32.9 ± 0.2	30.0 ± 0.2	27.5 ± 0.8	28.1 ± 1.4	30.5 ± 0.5
18:1 n9	30.5 ± 0.1	47.3 ± 2.6	43.3 ± 0.8	43.1 ± 0.3	42.6 ± 1.6	40.8 ± 0.9	40.1 ± 0.3
18:2 n6	27.6 ± 0.2	11.2 ± 1.6	15.3 ± 0.5	18.1 ± 0.1	20.4 ± 0.1	21.7 ± 0.7	21.5 ± 0.6

[a] Unsaturated fatty acid nomenclature designates the carbon atom at which the double bond occurs with an ''n'' (e.g., the double bond for 18:1 n9 is at the ninth carbon position).
[b] Percent of the three most common fatty acids in the 82%U and 58%U diets.
[c] Furcular fat was collected from six birds fed the 82%U diet and two birds fed the 58%U diet.

210.1, $P < 0.01$; Table 2 and 3). Percent of unsaturated fatty acids in furcular fat of free-living vireos was significantly higher during fall migration than during the breeding season ($F_{1,5} = 50.7$, $P < 0.01$; Table 3). Percent of unsaturated fatty acids in furcular fat of vireos fed the 58%U diet was not significantly different among seasons ($F_{5,11} = 0.9$, $P = 0.47$) because as the percent of 18:1n9 decreased that of 18:2n6 increased (Table 2). However, percent of unsaturated fatty acids in furcular fat of vireos fed the 82%U diet was significantly higher during the winter, premigration, and second spring than during spring, breeding, and fall seasons ($F_{5,16} = 23.0$, $P < 0.01$) primarily because of an increase in the percent of 18:1n9 over time (Table 2). Despite these changes in fatty acid composition over the year, birds during migration periods (spring, fall, second spring) did not selectively increase the proportion of long-chain monounsaturated fatty acids or decrease the percent of 18:2 in their stored fat compared to nonmigratory periods as predicted by the selective-metabolism hypothesis.

DISCUSSION

WHAT CAUSES THE OBSERVED SEASONAL CHANGES IN FATTY ACID COMPOSITION?

Three previous studies investigating songbird diet preferences for specific fatty acids (Bairlein 1991, McWilliams et al. 2002, Pierce et al. 2004) during the nonmigratory period reported that Red-eyed Vireos, Garden Warblers, and Yellow-rumped Warblers (*Dendroica coronata*)

TABLE 3. Percent of most common fatty acids (mean ± SE) in furcular fat from free-living Red-eyed Vireos, captive vireos fed a diet containing mostly unsaturated fat (82%U), and captive vireos fed a diet containing less unsaturated fat (58%U) during breeding and fall seasons.

	Free-living birds		Captive birds fed 82%U		Captive birds fed 58%U	
Fatty acid[a]	Breeding $n = 3$	Fall $n = 4$	Breeding $n = 2$	Fall $n = 2$	Breeding $n = 2$	Fall $n = 2$
16:0	29.9 ± 1.6	17.5 ± 0.9	25.2 ± 1.1	18.2 ± 0.9	32.9 ± 0.2	30.5 ± 0.5
16:1	2.1 ± 0.3	28.0 ± 4.7	1.4 ± 0.2	<1.0	<1.0	<1.0
18:0	7.0 ± 0.3	2.5 ± 0.6	2.4 ± 0.2	2.4 ± 0.1	2.9 ± 0.1	3.0 ± 0.2
18:1n9	39.7 ± 2.8	24.9 ± 3.4	53.4 ± 0.6	62.5 ± 0.4	43.3 ± 0.8	40.1 ± 0.3
18:2n6	14.5 ± 1.6	16.1 ± 3.3	11.5 ± 0.1	9.5 ± 0.2	15.3 ± 0.5	21.5 ± 0.6
18:3	4.2 ± 1.1	<1.0	<1.0	<1.0	<1.0	<1.0

[a] Unsaturated fatty acid nomenclature designates the carbon atom at which the double bond occurs with an ''n'' (e.g., the double bond for 18:1 n9 is at the ninth carbon position.

consistently preferred diets containing long-chain unsaturated fatty acids over diets containing long-chain saturated fatty acids. When we offered Red-eyed Vireos simultaneous choices between three semi-synthetic diets differing only in fatty acid composition during migratory and nonmigratory periods, vireos consistently preferred the 18:1 diet throughout the year. If these results for vireos apply to other migratory birds, then diet preferences cannot fully account for the observed changes in fatty acid composition of migrating and nonmigrating birds.

Egeler et al. (2003) suggested that selective metabolism might be, in part, responsible for the seasonal changes in fatty acid composition of Western Sandpipers; however, we found no support for this hypothesis in our study of Red-eyed Vireos. Although selective metabolism of fatty acids produced changes in the fatty acid composition of Red-eyed Vireos' fat stores over the year, we did not observe the predicted change in fatty acid composition of vireos during migratory and nonmigratory periods. If these results for vireos apply to other migratory birds, then selective metabolism cannot explain the observed changes in fatty acid composition of fat stores in birds during migration.

Thus far we have demonstrated that neither diet preferences for certain fatty acids nor selective metabolism of specific fatty acids can adequately account for the observed seasonal changes in fatty acid composition of fat stores in birds during migratory and nonmigratory periods. Our recent experiments, where captive Red-eyed Vireos were fed diets that differed only in certain fatty acids, demonstrates the effect of diet composition on bird fatty acid composition: fat stores of birds fed either experimental diet were primarily composed of the same three fatty acids that comprised >90% of their dietary fatty acids. Fat stores of free-living Red-eyed Vireos captured during summer were also primarily composed of the same three fatty acids as our captive vireos, whereas free-living Red-eyed Vireos captured during fall migration had remarkably more 16:1 in their fat stores. Studies suggest that fatty acids in fat stores of birds are primarily determined by the fatty acid composition of birds' diet (Bower and Helms 1968, West and Meng 1968a, 1968b, Landau 1970, Morton and Liebman 1974, Thomas and George 1975, Yom-Tov and Tietz 1978, Heitmeyer and Fredrickson 1990, Pierce et al. 2004, 2005). If this is the case in Red-eyed Vireos, then their diet during fall migration must contain more 16:1 than that of vireos during summer.

In summary, evidence to date suggests that fatty acid composition of stored fat in birds is primarily a product of diet composition with selective metabolism of specific fatty acids playing a possibly important but minor role. Thus, birds can influence the fatty acid composition of their body fat by selectively feeding on foods with certain fatty acids.

CONSEQUENCES OF FATTY ACID COMPOSITION FOR MIGRATORY BIRDS

If diet selection during migration is primarily responsible for the seasonal changes in fatty acid composition of wild birds, then what benefit might birds gain by selectively feeding on foods with certain fatty acids? We have recently shown that differences in fat composition affect exercise performance of a songbird (Pierce et al. 2005). By feeding Red-eyed Vireos for four months either the 82%U diet or the 58%U diet, we produced one group of vireos that had fat stores comprised of mostly (77%) unsaturated fatty acids, and a second group of vireos that had fat stores comprised of less (66%) unsaturated fatty acids. We then measured metabolic rate at rest and during intense exercise in these two groups and found that although mass-specific resting metabolic rate was similar, the mass-specific peak metabolic rate of vireos fed the 58%U diet was 25% higher than vireos fed the 82%U diet (Pierce et al. 2005). Thus, fatty acid composition of birds can affect energy expenditure during intense exercise, implying that diet selection of birds at stopover sites can affect their energy expenditure during migratory flights.

FUTURE DIRECTIONS

More study is needed in three key areas of avian stopover ecology as it relates to the use of fatty acids to fuel migration. First, relatively little is known about the fatty acid composition of natural avian diets. Only one study has examined the fatty acid composition of natural avian diets in relation to that of fat stores in birds (Zurovchak 1997). Zurovchak (1997) found that 18:1 was the dominant unsaturated fatty acid and 16:0 was the dominant saturated fatty acid in five fruit species eaten by Wood Thrushes and in the stored fat of Wood Thrushes captured during fall migration. Perhaps birds consistently discriminate between diets that differ only in their fatty acid composition because certain fatty acids are rare in natural diets yet are necessary to satisfy specific nutrient requirements. Clearly, if birds are selectively choosing their diets based on fatty acid composition, then we need to know more about the fatty acid composition of wild foods and how it influences diet selection of birds.

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

Understanding fatty acid metabolism in wild birds may also help us understand why birds often switch to eating primarily fruit during migration. A potential cost associated with metabolizing stored polyunsaturated fatty acids to fuel energy expenditure during exercise is production of lipid peroxides. Exercising vertebrates can defend against antioxidation by upregulating antioxidant enzymes and by increasing intake of dietary antioxidants. Fruits eaten by wild birds contain flavonoids and carotenoids that, in addition to providing color, can also serve an antioxidant function (Lawlor and Obrien 1995), as can other compounds in wild fruits (Klasing 1998, Halliwell and Gutteridge 1999, Bramley et al. 2000). Thus, we envision a cost-benefit tradeoff where the nutritional benefits of a high-polyunsaturated fat diet are balanced by an increased risk of autoxidation so that wild vertebrates may seek to optimize rather than maximize their intake of polyunsaturated fats and dietary antioxidants (Munro and Thomas 2004). Perhaps the combination of nutrient complementarity (i.e., low protein-to-calorie ratio), lipid composition (i.e., mostly long-chain unsaturated fatty acids), and high concentrations of dietary antioxidants makes fruit-eating particularly advantageous for birds during migration. Although studies of fishes (Hamre et al. 1994, Sant'Ana and Mancini-Filho 2000, Tocher et al. 2003) and mammals (Frank and Storey 1995a, 1995b, Harlow and Frank 2001) demonstrate that dietary polyunsaturated fats and antioxidants are important for the health of these heterothermic vertebrates, whether these results apply to birds is unclear.

Finally, in contrast to mammals (Roberts et al. 1996, Weber et al. 1996), birds that fuel high-intensity endurance exercise, such as migratory flights, use primarily fatty acid oxidation (McWilliams et al. 2004). Although we recently demonstrated that fatty acid composition of fat stores affects energy expenditure during exercise in a migratory songbird (Pierce et al. 2005), much remains to be discovered. Given that these results contradicted the simple hypothesis that migratory birds with more unsaturated fatty acids have enhanced aerobic performance during intense exercise compared to birds with less unsaturated fatty acids, future research should test alternative hypotheses about how fatty acid composition of birds affects their exercise performance.

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