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METABOLIC ROUTING OF DIETARY NUTRIENTS IN BIRDS: EFFECTS OF DIETARY LIPID CONCENTRATION ON $\delta^{13}C$ OF DEPOT FAT AND ITS ECOLOGICAL IMPLICATIONS

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Abstract.—During migration, many songbirds eat primarily fruit while depositing fat. Given that some fruits contain mostly carbohydrate and others contain mostly lipid, the ability of birds to fatten may depend on the macronutrient composition of the fruit. Stable isotopes of carbon may be useful in determining the source of nutrients used for synthesizing fat, because the enzyme that regulates the transfer of carbon skeletons from carbohydrate into fat synthesis has a higher affinity for ^{12}C than for ^{13}C, whereas dietary lipids can be directly incorporated into animal fat. Thus, fat stores of animals that are synthesized directly from dietary lipid should have isotopic signatures similar to dietary lipid, whereas biosynthesis of fats from dietary carbohydrates should produce changes in isotopic signatures. We tested these predictions by manipulating the concentrations and isotopic signatures of macronutrients in diets fed to Yellow-rumped Warblers (*Dendroica coronata*). The $\delta^{13}C$ of depot fat in birds fed high-lipid diets was similar to that of dietary lipid, whereas $\delta^{13}C$ of depot fat in birds fed low-lipid diets indicated that a combination of dietary lipid and carbohydrate were used to synthesize depot fat. Models that incorporated 8‰ discrimination between dietary carbohydrate and depot fat consistently estimated the proportion of dietary lipid and carbohydrate routed into depot fat. Stable-isotope analysis of macronutrients in the diet of wild birds combined with estimates of the effects of diet composition on the isotopic signature of depot fat in birds offer a method to identify the relative importance of nutritional resources used by songbirds to deposit fat. *Received 18 May 2005, accepted 6 August 2006.*

Key words: depot fat, migration, songbirds, stable isotopes, stopover.

Rutas Metabólicas de Nutrientes Dietarios en Aves: Efectos de la Concentración de Lípidos Dietarios sobre $\delta^{13}C$ en los Depósitos de Grasa y sus Implicancias Ecológicas

Resumen.—Durante la migración, muchas aves canoras se alimentan primariamente de frutas mientras acumulan grasas. Dado que algunas frutas contienen mayormente carbohidratos y que otras contienen principalmente lípidos, la habilidad de las aves para engordar podría depender de la composición de macronutrientes de las frutas. Los isótopos estables de carbono podrían ser útiles a la hora de determinar la fuente de los nutrientes usada para sintetizar las grasas, debido a que la enzima que regula la transferencia de esqueletos de carbono desde carbohidratos a síntesis de grasa tiene una afinidad mayor por el ^{12}C que por el ^{13}C, mientras que los lípidos de la dieta pueden ser incorporados directamente a la grasa animal. De este modo, las reservas de grasa de los animales que son sintetizadas directamente a partir de los lípidos dietarios deberían presentar características isotópicas similares a la de los lípidos dietarios, mientras que la biosíntesis de grasas a partir de carbohidratos

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dietarios debería producir cambios en las características isotópicas. Evaluamos estas predicciones manipulando las concentraciones y las características isotópicas de los macronutrientes en las dietas provistas a *Dendroica coronata*. El $\delta^{13}C$ en los depósitos de grasa de las aves alimentadas con dietas de alto contenido lipídico fue similar al de los lípidos dietarios, mientras que el $\delta^{13}C$ en los depósitos de grasa de las aves alimentadas con dietas de bajo contenido lipídico indicó el uso de una combinación de lípidos y carbohidratos dietarios para sintetizar los depósitos de grasa. Los modelos que incorporaron una discriminación del 8‰ entre los carbohidratos dietarios y los depósitos de grasa estimaron de forma consistente la proporción de lípidos dietarios y de carbohidratos destinados a los depósitos de grasa. Los análisis de isótopos estables de los macronutrientes en la dieta de aves silvestres, combinados con los estimados de los efectos de la composición de la dieta sobre la característica isotópica de los depósitos de grasa en las aves, ofrecen un método para identificar la importancia relativa de los recursos nutricionales usados por las aves canoras para almacenar grasa.

At stopover sites along the east coast of the United States, many songbirds eat mostly fruit while depositing fat in preparation for the flight to the next stopover site (Parrish 1997, 2000). Fruit species differ in nutritional composition, some being high in carbohydrates, whereas others are high in lipid and lower in carbohydrates (Izhaki and Safriel 1989, Biebach 1996, Witmer 1998). Stable-isotope analysis of the macronutrients within the diet of wild birds may offer a method to determine the dietary resource(s) used by songbirds to deposit furcular fat (Alexander et al. 1996, Pearson et al. 2003).

The isotopic value of fats synthesized from carbohydrates should be more depleted in ^{13}C than the isotopic value of fats stored directly from dietary fat because of biochemical pathway preferences for ^{12}C over ^{13}C (DeNiro and Epstein 1977). Because of negative feedback inhibition of enzymes used in fat synthesis, bacteria and animals on high-lipid diets downregulate the synthesis of dietary glucose into fat stores (Monson and Hayes 1980, Pierce et al. 2004). For organisms on a low-lipid diet, carbon skeletons from glucose enter the citric-acid cycle and are transformed into intermediates that are used in fat synthesis. Pyruvate dehydrogenase, the enzyme that regulates the transfer of carbon skeletons from glycolysis into the citric-acid cycle, has a higher affinity for ^{12}C than for ^{13}C, thereby leading to biosynthesized fats with relatively depleted isotopic signatures compared with the dietary carbohydrate substrate (Abelson and Hoering 1961; DeNiro and Epstein 1977; Monson and Hayes 1980, 1982). By contrast, fat stores that are synthesized from dietary lipids should have isotopic signatures similar to those of dietary lipids (Gannes et al. 1998). At this time, the fractionating steps in lipid synthesis have been quantified only in bacteria and yeast; however, lipid synthesis is similar in all organisms (DeNiro and Epstein 1977). Thus, one should be able to determine the dietary source of carbon used to rebuild depot fat in songbirds if the isotopic values of the macronutrients of the diet are different.

We investigated the metabolic routing of dietary macronutrients into depot fat of a migratory bird, the Yellow-rumped Warbler (*Dendroica coronata*; hereafter "warbler") We tested the hypothesis that the availability of dietary lipids affects the substrate used for lipid synthesis. Our first prediction was that warblers fed a high-lipid diet will preferentially route carbon from dietary lipid into depot fat. Our second prediction was that warblers fed a low-lipid diet will preferentially route carbon from both dietary lipid and dietary carbohydrate into depot fat.

Methods

We captured 48 warblers during fall migration in 2002, on Block Island, Rhode Island (41°12′N, 71°35′W). Birds were transferred to the animal-care facilities on the University of Rhode Island's Kingston campus and housed in stainless-steel cages (51 × 35.5 × 20.5 cm) in a room with a constant light schedule: 12 h light and 12 h dark (12L:12D) and temperature (21°C). All birds were initially fed a 30% lipid diet (diet 3; lipid, carbohydrate, and protein from C3 plant sources; Tables 1 and 2). Throughout the experiment, birds had *ad*

TABLE 1. Values of $\delta^{13}C$ (‰) and percentage of C (%C) (mean ± SD) of the ingredients in the acclimation diet fed to Yellow-rumped Warblers for 2.5 months and in the five other experimental diets fed to warblers during the 15-day feeding trial (*n* = number of different ingredient sources used during the experiment; all birds were supplemented with five waxworms per day).

Source	Macronutrient	$\delta^{13}C$ (‰)	%C	*n*
C3	Beet sugar	–24.5 ± 0.6	39.3 ± 0.2	3
	Casein	–26.0 ± 0.7	46.5 ± 0.2	4
	Olive oil	–28.9 ± 0.3	73.3 ± 1.8	3
C4	Corn sugar	–9.8 ± 0.1	37.8 ± 0.9	4
	Corn oil	–15.3 ± 0.6	70.5 ± 1.1	3
Waxworm	Protein	–18.6 ± 0.1	46.6 ± 0.6	3
	Lipid	–22.7 ± 0.1	73.7 ± 1.1	3

TABLE 2. Composition (percentages of dry mass), macronutrient source (C3 or C4), and whole diet $\delta^{13}C$ and percentage of C (%C) (means ± SD) of the experimental diets fed to Yellow-rumped Warblers during the 2.5-month acclimation period (diet 3) and during the 15-day feeding trial (*n* = number of batches sampled during the experiment; Carb = carbohydrate).

	Percentage of dry mass			Macronutrient source			Whole diet		
Diet	Lipid	Carb	Protein	Lipid	Carb	Protein	$\delta^{13}C$ (‰)	%C	*n*
1	30	28	31	C4	C3	C3	-19.9 ± 0.1^{a}	52.0 ± 0.8^{a}	2
2	5	53	31	C4	C3	C3	-22.9 ± 0.1^{b}	42.3 ± 0.3^{b}	2
3	30	28	31	C3	C3	C3	-25.7 ± 0.1^{c}	52.9 ± 0.6^{a}	4
4	5	53	31	C3	C3	C3	-24.3 ± 0.0^{d}	45.2 ± 0.1^{c}	2
5	30	28	31	C3	C4	C3	-22.8 ± 0.1^{b}	53.1 ± 0.5^{a}	2
6	5	53	31	C3	C4	C3	-17.5 ± 0.4^{e}	41.9 ± 0.1^{b}	2

Note: Diets differed in their $\delta^{13}C$ ($F = 843.13$, df = 5 and 8, $P < 0.001$) and %C ($F = 272.43$, df = 5 and 8, $P < 0.001$). Within a column, values that do not share the same superscript are significantly different from one another ($P < 0.05$).

libitum access to food and water, and each day they were provided five waxworms (*Galleria mellonella*; ~0.46 g dry weight; waxworms were 48% lipid [$\delta^{13}C = -20.79$‰]), to ensure that birds maintained body mass (Frazer and McWilliams 2002). All birds were weighed every day and provided new food at 0800 hours.

After birds were fed this acclimation diet for 2.5 months, we manipulated the photoperiod starting on 14 January 2003 to stimulate hyperphagia and fattening in these birds (McWilliams and Karasov 1998). We first changed the daily light schedule from 12L:12D to 10.5L:13.5D by decreasing light 0.5 h each week over three consecutive weeks. We then changed the daily light schedule to 16L:8D by increasing light 0.5 h approximately every 4 days over 36 consecutive days. We began the fasting part of the experiment when most of the birds had gained ≥5% mass while on the 16L:8D schedule (percentage of increase during the seven days before fasting was, on average, 8.3 ± 9.0%; range: –1.8% to 32.0%).

Once the birds had responded to the photoperiod manipulations, each bird was fasted and then refed one of the six experimental diets that differed in macronutrient signatures (Table 2). Using this experimental design, we can determine how dietary macronutrients are metabolically routed to depot fat. We measured $\delta^{13}C$ of depot fat in birds before fasting using samples of furcular fat from six birds killed at 1700 hours on day 0 (9 days after the light was increased to 16 h). Starting at 1200 hours on day 1, we fasted all birds for 21–44 h until they lost 15–20% of their starting mass or until

they reached a minimum body mass of 8.5 g. All birds had *ad libitum* access to water throughout the fast. After the fast, six birds were killed, and six birds were returned to the same diet (diet 3) they had been fed before the fast. The remaining birds (n = 30) were switched to one of five other experimental diets (Table 2). Assignment of birds to treatment groups was stratified by body mass and order of exit from the fast. Three of the experimental diets had 30% lipid and 28% carbohydrate, whereas the other three experimental diets had 5% lipid and 53% carbohydrate. All diets had 31% protein (Table 2). All batches of the 30% lipid diets contained 168 g beet sugar or corn sugar, 180 g olive oil or corn oil, 186 g casein, and 1,800 g water. All batches of the 5% lipid diets contained 318 g beet sugar or corn sugar, 30 g olive oil or corn oil, 186 g casein, and 1,800 g water. All diets also contained essential vitamins (0.25 g per 100 g wet food; AIN-76 vitamin mix, ICN Biomedicals, Irvine, California), salts (1.25 g per 100 g wet food; Briggs-N Salt Mix, ICN Biomedicals), and water (75 g per 100 g wet food). All diets were agar-based (1.25 g per 100 g wet food; Afik et al. 1997, Podlesak et al. 2005). We calculated daily food intake (g dry) during the eight days after the fast for birds fed each of the six experimental diets. We estimated metabolic routing of dietary macronutrients to depot fat by measuring the carbon isotope signature of furcular fat from the birds killed 15 days after the fast. We were unable to obtain furcular fat samples from three birds killed at the end of the fast.

Isotopic analysis.—All samples were freeze-dried before isotopic analysis. All samples were analyzed at the Atlantic Ecology Division of the Environmental Protection Agency using a Carlo-Erba NA 1500 Series II Elemental Analyzer attached to a continuous-flow isotope-ratio Micromass Optima Spectrometer (CF-IRMS). Samples were converted to CO_2 gas in oxidation–reduction furnaces and separated by gas chromatography. After separation, samples were measured for $^{13}C/^{12}C$ ratios on the mass spectrometer. Stable carbon isotope ratios are reported in delta notation as parts per thousand (‰) deviations from Pee Dee belemnite (the international standard for C) for $\delta^{13}C$. Powdered dogfish muscle (DORM-1) reference material (National Research Council, Institute for Environmental Chemistry, Ottawa, Ontario) was used as a working standard. All samples were analyzed in duplicate. The same reference material analyzed over a period of several months was measured with ±0.3‰ precision. Percentage of C (%C) was calculated by deriving the masses of C in each sample by integrating the ion-beam concentrations and comparing the integrals to standards with known concentrations of C, and then dividing the mass of C in the sample by the sample weight and multiplying by 100.

Statistical analyses.—We used one-way analysis of variance (ANOVA) with Bonferroni *post hoc* tests (SPSS) to compare differences between body mass of birds before and after the fast and to compare differences in $\delta^{13}C$ of furcular fat between the different treatment groups. We also used one-way ANOVAs with Bonferroni *post hoc* tests to compare differences between dry mass of food consumed by birds fed one of the six experimental diets and to compare differences between $\delta^{13}C$ and %C of the six experimental diets. We used repeated-measures ANOVA (SYSTAT, San Jose, California) to determine whether change in body mass after the fast differed for birds fed one of the six diets. Values reported below are means ± SD, and $P \leq 0.05$ was deemed significant for all statistical tests.

Results

Before fasting and the diet switch, mass of birds on day 0 for all treatment groups was not significantly different and was, on average, 12.3 ± 1.4 g ($F = 0.18$, df = 7 and 40, $P = 0.99$). After fasting and before refeeding, minimum body mass was not significantly different between treatment groups and was, on average, 10.6 ± 1.2 g ($F = 0.23$, df = 6 and 35, $P = 0.97$). Birds in all diet groups quickly gained mass during the refeeding period, and mass gain was similar for birds switched to each of the six experimental diets (diet treatment: $F = 0.52$, df = 5 and 30, $P = 0.76$; time: $F = 196.00$, df = 1 and 30, $P < 0.001$; diet treatment*time: $F = 1.01$, df = 5 and 30, $P = 0.43$). Refed birds were significantly heavier on day 18 than on day 1 (paired t-test: $t = -7.73$, df = 35, $P < 0.001$), which indicates that fasting stimulated birds to fatten.

During the eight days after fasting, birds switched to the low-lipid diets consumed, on average, ~40% more food per day than birds switched to the high-lipid diets ($F = 18.14$, df = 5 and 275, $P < 0.001$). Birds fed the low-lipid diets

ate, on average, 3.3 ± 1.0 g of food (dry) per day, and birds fed the high-lipid diets ate, on average, 2.3 ± 0.8 g of food (dry) per day. In general, $\delta^{13}C$ of furcular fat from fasted birds and birds refed one of the six experimental diets were significantly different ($F = 394.04$, df = 7 and 37, $P < 0.001$; Fig. 1). The $\delta^{13}C$ of furcular fat from birds before the fast was more negative than $\delta^{13}C$ of furcular fat from birds at the end of the fast. The $\delta^{13}C$ of furcular fat from birds refed the 30% C3 lipid + 28% C3 carbohydrate diet (diet 3) and birds switched to the 5% C3 lipid + 53% C3 carbohydrate diet (diet 4) were similar to $\delta^{13}C$ of furcular fat from birds sampled before the fast. By contrast, birds switched to the 30% C4 lipid + 28% C3 carbohydrate diet (diet 1) had the most enriched $\delta^{13}C$, and birds switched to the low-lipid diets (diets 2 and 6) with lipid and carbohydrate from different carbon sources (C3 or C4) had $\delta^{13}C$ values of furcular fat that were intermediate between postfast birds and birds fed the 30% C4 lipid + 28% C3 carbohydrate diet (diet 1; Fig. 1).

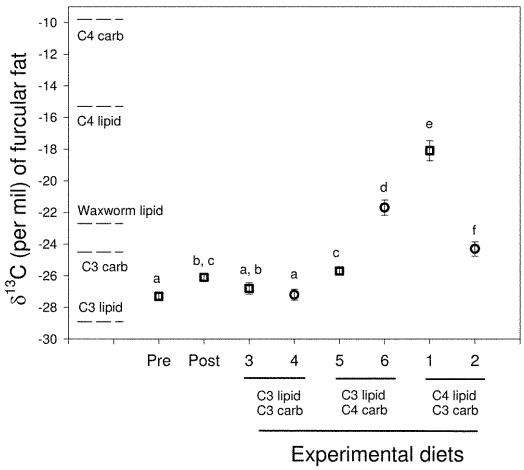

FIG. 1. The $\delta^{13}C$ (mean ± SD) of furcular fat sampled from Yellow-rumped Warblers before the 21- to 44-h fast (Pre), at the end of the fast (Post), and after eating one of the six experimental diets for 15 days. Diets are paired by macronutrient carbon source; diets 1, 3, and 5 contained 30% lipid (squares), and diets 2, 4, and 6 contained 5% lipid (circles). Values that share the same letter are not significantly different from one another ($P \geq 0.05$). Dotted lines represent $\delta^{13}C$ of the relevant macronutrient components of the experimental diets.

DISCUSSION

Estimating the metabolic routing of dietary macronutrients into depot fat.—To estimate metabolic routing of dietary macronutrients into furcular fat in warblers, we compared the observed $\delta^{13}C$ of furcular fat from birds switched to new diets for 15 days with the predicted $\delta^{13}C$ of furcular fat from concentration-dependent mixing models (Phillips 2001, Phillips and Koch 2002, Martínez del Rio and Wolf 2005; Figs. 2 and 3). We used the mixing models to predict $\delta^{13}C$ of furcular fat given the carbon signatures of the dietary macronutrients and assuming 0–100% of the dietary lipid contributed to synthesis of furcular fat. These estimates of metabolic routing were then used to test predictions about how dietary composition influenced routing of dietary macronutrients into depot fat of birds.

Lipid stores are generally 4–8‰ more depleted than whole tissue (Hammer et al. 1998), and so our mixing models must include some estimate of discrimination. We created three models for each group of birds that were switched to one of the six experimental diets so that we could investigate how different discrimination factors between dietary carbohydrate and depot fat might influence our estimates of metabolic routing. We use the term "discrimination" to refer to the difference between macronutrient isotopic values and furcular fat rather than fractionation, because fractionation is only one process that can lead to differences between macronutrient isotopic values and furcular fat (Martínez del Rio and Wolf 2005). The first model assumed no discrimination between dietary carbohydrate and synthesized furcular fat. We included a 4‰ and 8‰ discrimination factor in the second and third models, respectively. We decreased the discrimination factors incrementally to zero as the amount of carbohydrate used to synthesize furcular fat decreased to 0% and the amount of dietary lipid used to synthesize fat increased to 100% because we expected little difference between dietary lipid and furcular fat (Gannes et al. 1998). Given that all birds were fed both semisynthetic diet and five waxworms each day, we estimated the relative contribution of dietary lipid from both semisynthetic diet and waxworms based on daily dry matter intake of each. We estimated that birds switched to the diets with 30% lipid received approximately

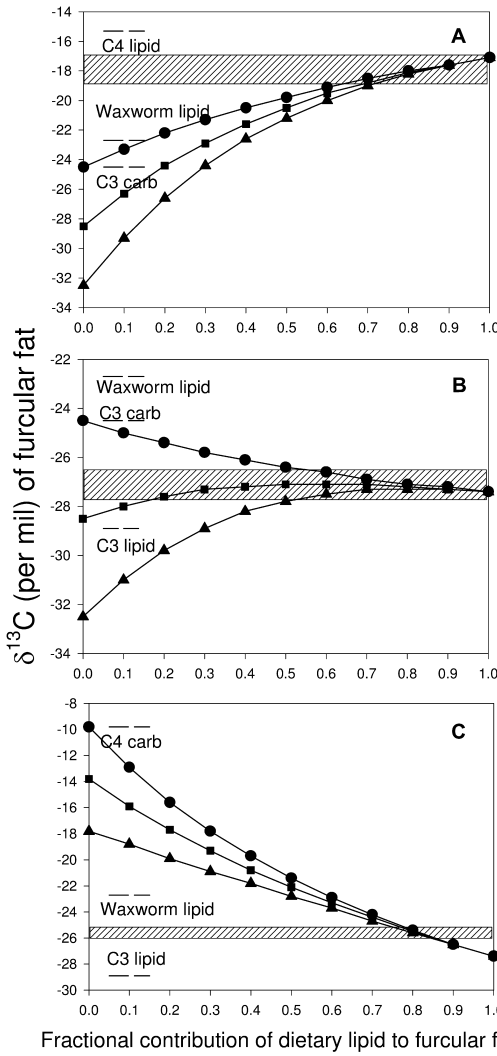

FIG. 2. Observed (shaded areas) and predicted (lines) $\delta^{13}C$ of furcular fat for Yellow-rumped Warblers fed (A) the 30% C4 lipid + 28% C3 carbohydrate diet, (B) the 30% C3 lipid + 28% C3 carbohydrate diet, and (C) the 30% C3 lipid + 28% C4 carbohydrate diet, as a function of the fractional contribution of dietary lipid to furcular fat. Predicted $\delta^{13}C$ for concentration-dependent mixing models (solid lines with circles, solid lines with squares, and solid lines with triangles) assumes 0‰, 4‰, and 8‰ discrimination between dietary carbohydrate and furcular fat, respectively. Shaded area in each panel is the range in $\delta^{13}C$ for furcular fat sampled from captive birds fed each diet; short, dashed horizontal lines are $\delta^{13}C$ of dietary macronutrients in each diet.

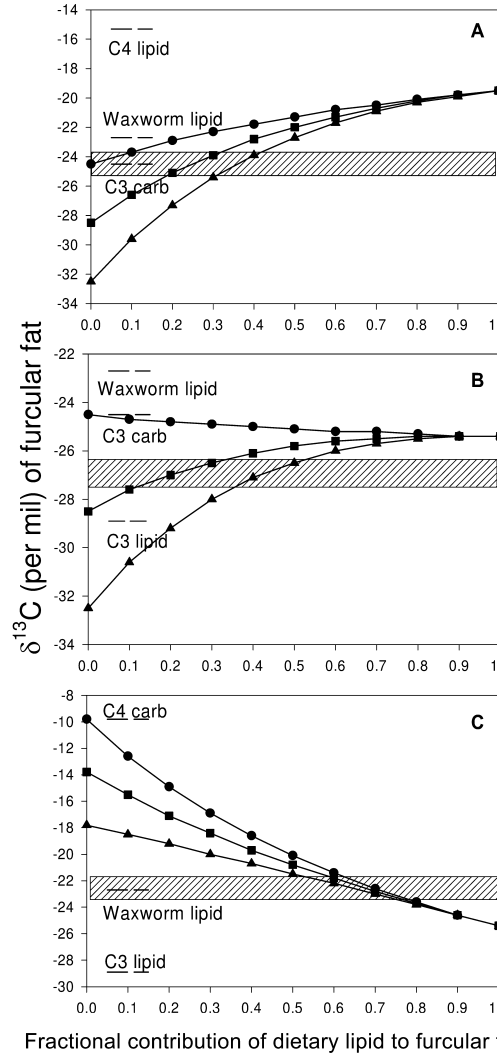

FIG. 3. Observed (shaded areas) and predicted (lines) $\delta^{13}C$ of furcular fat for Yellow-rumped Warblers fed (A) the 5% C4 lipid + 53% C3 carbohydrate diet, (B) the 5% C3 lipid + 53% C3 carbohydrate diet, and (C) the 5% C3 lipid + 53% C4 carbohydrate diet, as a function of the fractional contribution of dietary lipid to furcular fat. Predicted $\delta^{13}C$ for concentration-dependent mixing models (solid lines with circles, solid lines with squares, and solid lines with triangles) assumes 0‰, 4‰, and 8‰ discrimination between dietary carbohydrate and furcular fat, respectively. Shaded area in each panel is the range in $\delta^{13}C$ for furcular fat sampled from captive birds fed each diet; short, dashed horizontal lines are $\delta^{13}C$ of dietary macronutrients in each diet.

76% and 24% of daily lipid intake from the semisynthetic diet and waxworms, respectively, and that birds switched to the diets with 5% lipid received approximately 44% and 56% of daily lipid intake from the semisynthetic diet and waxworm, respectively (see Appendix for model equations and assumptions).

Testing prediction 1: Birds fed high-lipid diets primarily route dietary lipid to synthesize depot fat.—Our results support the prediction that warblers fed a high-lipid diet will route carbon primarily from dietary lipid into rebuilt depot fat. Observed $\delta^{13}C$ of furcular fat from warblers fed all three high-lipid diets was between the $\delta^{13}C$ of the dietary lipid source and the $\delta^{13}C$ of the lipid from the waxworm (Fig. 2). If warblers fed high-lipid diets had synthesized furcular fat primarily from dietary carbohydrate, the models predicted that $\delta^{13}C$ of furcular fat would have been 0–8‰ depleted compared with the signature of the dietary carbohydrate (Fig. 2). We observed that birds fed the 30% C3 lipid + 28% C3 carbohydrate diet had furcular fat signatures 2–3‰ depleted compared with dietary carbohydrate (Fig. 2B). However, warblers fed the 30% C4 lipid + 28% C3 carbohydrate diet had furcular fat signatures 5–6‰ enriched compared with dietary carbohydrate (Fig. 2A), whereas birds fed the 30% C3 lipid + 28% C4 carbohydrate had furcular fat signatures >15‰ depleted compared with dietary carbohydrate (Fig. 2C). Given that all three diets were similar in macronutrient concentration and differed only in isotopic signatures of the macronutrients, these large differences in the estimated routing of dietary carbohydrates by birds fed these different diets suggest that warblers fed high-lipid diets did not use primarily dietary carbohydrate to synthesize furcular fat but instead used primarily dietary lipid to synthesize depot fat. On the basis of the observed and predicted $\delta^{13}C$ of furcular fat for the warblers fed the high-lipid diets with the lipid and carbohydrate from different sources (Fig. 2A, C), we estimate that dietary lipid contributed ≥60% of the carbon used to synthesize furcular fat. Feeding birds diets composed of macronutrients with distinct isotopic signatures allowed us to quantify the amount of carbon routed from dietary carbohydrate and lipid into furcular fat, whereas the routing of dietary carbon was not as clear for the birds fed the diet composed of macronutrients with similar isotopic signatures (Fig. 2B).

Testing prediction 2: Birds fed low-lipid diets route both dietary lipid and carbohydrate into depot fat.—Our results support the prediction that warblers fed a low-lipid diet route carbon from both dietary lipid and dietary carbohydrate into rebuilt depot fat (Fig. 3). If warblers had synthesized furcular fat exclusively from dietary carbohydrate, we would expect discrimination between dietary carbohydrate and furcular fat to be similar for birds fed each low-lipid diet. Birds fed the 5% C4 lipid + 53% C3 carbohydrate diet had furcular fat signatures similar to dietary carbohydrate (Fig. 3A). By contrast, warblers fed the 5% C3 lipid + 53% C3 carbohydrate diet had furcular fat signatures 2–3‰ depleted compared with dietary carbohydrate (Fig. 3B), and birds fed the 5% C3 lipid + C4 carbohydrate had furcular fat signatures >12‰ depleted compared with dietary carbohydrate (Fig. 3C). Given that all three diets were similar in macronutrient concentration and differed only in isotopic signatures of the macronutrients, these large differences in the estimated routing of dietary carbohydrates by birds fed these different diets suggest that warblers fed low-lipid diets did not use primarily dietary carbohydrate to synthesize furcular fat.

Likewise, if warblers had only deposited dietary lipid as furcular fat, we would expect $\delta^{13}C$ of furcular fat to be between $\delta^{13}C$ of the dietary lipid source and $\delta^{13}C$ of the lipid from the waxworms (Fig. 3). We observed this in birds fed the 5% C3 lipid + 53% C3 carbohydrate diet (Fig. 3B), but $\delta^{13}C$ of furcular fat was more depleted (Fig. 3A) or more enriched (Fig. 3C) than dietary lipid for birds fed the other two low-lipid diets. Thus, these results suggest that warblers fed low-lipid diets did not exclusively use dietary lipid or carbohydrate to build fat stores, but instead used a combination of both to synthesize furcular fat.

Given that all three diets had the same macronutrient composition, we expected little difference in discrimination between dietary carbohydrate and depot fat between birds fed each of the three diets. The model that assumed no discrimination predicted that dietary lipid supplied 0–10%, 0%, and 60–80% of the carbon in furcular fat for warblers fed each diet, respectively (lines with solid circles in Fig. 3A, B, C). The model that assumed 4‰ discrimination predicted that dietary lipid supplied 20–30%, 10–35%, and 60–75% of the carbon in furcular

fat for warblers fed each diet, respectively (lines with solid squares in Fig. 3A, B, C). The model that assumed 8‰ fractionation predicted that dietary lipid supplied 30–40%, 35–55%, and 50–75% of the carbon in furcular fat for warblers fed each diet, respectively (lines with solid triangles in Fig. 3A, B, C). Thus, the model assuming 8‰ discrimination consistently estimated that warblers fed low-lipid diets preferentially route carbon about equally (~50%) from dietary lipid and dietary carbohydrate to synthesize their fat stores.

Conclusions and ecological implications.—The concentration-dependent mixing models coupled with measures of stable-isotope signatures of songbird body fat and food resources would allow researchers to answer a long-standing, important ecological question: what food resources are most important for songbirds at stopover sites as they store lipids for the next leg of their migration? Lipid stores in animals are generally assumed to be 4–8‰ more depleted than whole tissue, primarily because the enzyme that regulates the transfer of carbon skeletons from carbohydrate into lipid synthesis has a higher affinity for ^{12}C than for ^{13}C (DeNiro and Epstein 1977, Hammer et al. 1998). However, biosynthesis of fats from dietary carbohydrates is minimized when the diet contains sufficient fatty acids (Mead et al. 1986, Pierce et al. 2004), and lipid stores of animals that are synthesized directly from dietary lipid should have isotopic signatures similar to those of dietary lipid sources (Gannes et al. 1998). We found that $\delta^{13}C$ of depot fat in warblers fed high-lipid diets was similar to that of dietary lipid, whereas $\delta^{13}C$ of depot fat in birds fed low-lipid diets indicated that a combination of dietary lipid and dietary carbohydrate were used to synthesize their depot fat. Thus, our results clearly show that the carbon signature of body fat is predictable, given that one knows the carbon signatures of dietary macronutrients and the macronutrient composition of the diet. In general, if dietary lipids are abundant, carbon in dietary lipid metabolically routes to build body fat and we would expect limited discrimination between dietary lipid and body fat. By contrast, when dietary lipids are limited, dietary carbohydrates are increasingly used to build fat stores.

By manipulating the concentrations and isotopic signatures of the macronutrients in the bird's diets, we were able to estimate which dietary macronutrients were used by birds to synthesize their depot fat. The same models we used to estimate the relative contribution of dietary macronutrients to synthesis of depot fat can be used to predict the isotopic signature of depot fat in wild birds, given that one knows the concentration of lipids and carbohydrates in their diet. For example, viburnum (*Viburnum dentatum*), pokeweed (*Phytolacca americana*), and bayberry (*Myrica pensylvanica*) are three species of fruit that are commonly consumed by birds at stopover sites along the east coast (Parrish 1997). These three species of fruit vary markedly in their nutrient composition. Viburnum and bayberry are 40–50% lipid, 40–50% carbohydrate, and 3% protein, whereas pokeweed is 3% lipid, 80% carbohydrate, and 6% protein (S. R. McWilliams unpubl. data). On the basis of our models, we predict that $\delta^{13}C$ of depot fat in birds consuming viburnum and bayberry would be similar to $\delta^{13}C$ of the lipid in viburnum and bayberry. By contrast, because pokeweed contains only ~3% lipid, $\delta^{13}C$ of depot fat in birds consuming primarily pokeweed would be ~8‰ depleted compared with $\delta^{13}C$ of the carbohydrate in pokeweed. Such applications of these models require, however, that the carbon signatures of dietary macronutrients are relatively unique. In summary, concentration-dependent mixing models that incorporate the isotopic signatures of the macronutrients within the diet of wild birds are a viable method for identifying the nutritional resources birds use to rebuild necessary fat stores during migration.

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Appendix

Concentration-dependent Mixing Models

We used concentration-dependent mixing models to predict the $\delta^{13}C$ of furcular fat for songbirds based on the concentration of the macronutrients in the various diets. See Tables 1 and 2 for the composition, concentrations, and $\delta^{13}C$ for the diets, dietary macronutrients, and waxworm macronutrients.

Assumptions

(1) Birds used dietary lipid, waxworm lipid, and dietary carbohydrate as carbon sources for lipid synthesis. Birds may have incorporated carbon skeletons from dietary protein into furcular fat; however, to decrease complexity in the models and because all diets contained the same composition and concentration of dietary protein, we did not include dietary protein in our concentration-dependent mixing models.

(2) Birds assimilated dietary lipid and waxworm lipid proportionately to the level in the diet. Birds fed the 30% lipid diets received 76% of daily lipid intake from the semisynthetic diet and 24% of the daily lipid intake from the waxworms. Birds fed the 5% lipid diets received 44% of daily lipid intake from the semisynthetic diet and 56% of the daily lipid intake from the waxworms.

(3) We generated three models for each diet. Model 1 assumed no discrimination between dietary carbohydrate and synthesized furcular fat. Model 2 assumed 4‰ discrimination between dietary carbohydrate and synthesized furcular fat. Model 3 assumed 8‰ discrimination between dietary carbohydrate and synthesized furcular fat.

(4) Discrimination factors were decreased incrementally to zero as the amount of carbohydrate used to synthesize fat increased to 100%. We expected little fractionation between dietary lipid and furcular fat (our prediction 1).

Equation 1

Concentration-dependent mixing model for birds fed the 30% lipid diets:

$$\delta^{13}C_{\text{fat}} = \left(\frac{1}{p(0.76[C_1] + 0.24[C_2]) + (1-p)(C_3)}\right) \times \begin{pmatrix} p(0.76[C_1]\delta^{13}C_1 + 0.24[C_2]\delta^{13}C_2) + \\ (1-p)(C_3)(\delta^{13}C_3 + \Delta \end{pmatrix}$$

Equation 2

Concentration-dependent mixing model for birds fed the 5% lipid diets:

$$\delta^{13}C_{\text{fat}} = \left(\frac{1}{p(0.44[C_1] + 0.56[C_2]) + (1-p)(C_3)}\right) \times \begin{pmatrix} p(0.44[C_1]\delta^{13}C_1 + 0.56[C_2]\delta^{13}C_2) + \\ (1-p)(C_3)(\delta^{13}C_3 + \Delta) \end{pmatrix}$$

In these equations, p is the fraction of carbon assimilated from lipid sources and $(1 - p)$ is the fraction of carbon assimilated from dietary carbohydrate. C_1, C_2, and C_3 are the concentrations of carbon in dietary lipid, waxworm lipid, and dietary carbohydrate, respectively; $\delta^{13}C_1$, $\delta^{13}C_2$, and $\delta^{13}C_3$ are the isotopic composition of dietary lipid, waxworm lipid, and dietary carbohydrate, respectively; Δ is the discrimination between $\delta^{13}C$ of dietary carbohydrate and $\delta^{13}C$ of furcular fat.