Dietary Macronutrients Affect Lipid Metabolites and Body Composition of a Migratory Passerine, the White-Throated Sparrow (*Zonotrichia albicollis*)

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ABSTRACT

Plasma lipid metabolites can provide information about fat metabolism and storage in migrating birds, yet little is known about the influence of diet and nutrition or how they relate to intraindividual differences in body composition of songbirds. We investigated how dietary macronutrient composition affects plasma lipid concentrations and subsequent changes in fat accumulation in white-throated sparrows (*Zonotrichia albicollis*). Birds fed a low-protein diet with more glucose had higher plasma triglyceride levels and higher average fat mass compared with levels and mass in birds fed diets with less glucose, and birds fed diets with less glucose and more fat had the highest plasma B-hydroxybutyrate concentrations, regardless of protein content. Birds fed the low-protein, high-fat diet also had the highest plasma nonesterified fatty acids. Diet-related changes in plasma triglyceride and B-hydroxybutyrate were more strongly related to fat mass than to lean mass of birds. Nevertheless, diet-related changes in lipid metabolites were more strongly influenced by the intake of certain macronutrients than by body fatness. Thus, plasma lipid metabolites may reliably indicate fat mass and fattening rates of birds, although our results suggest that diet composition must be considered given that certain macronutrients, namely, dietary glucose, may enhance fat deposition in songbirds.

Introduction

Songbird migration is characterized by frequent periods of rapid fat deposition in order to replenish energy stores used to fuel migratory flight. Maximizing fat deposition rates can increase the speed and success of annual migrations (Alerstam and Lindström 1990); thus, indices of fattening are frequently used to assess habitat quality for migratory birds. Plasma lipid metabolites provide information about the opposing processes of fat storage and catabolism. High plasma concentrations of triglyceride indicate transport and storage of dietary or de novo synthesized fats, whereas high plasma B-hydroxybutyrate concentrations indicate increased fat degradation for energy, particularly during periods of fasting or restricted food intake (Klasing 1998). Nonesterified fatty acids are also a product of lipolysis, although some dietary fatty acids may be absorbed directly and circulate in the bloodstream (Sklan et al. 1984). Triglyceride and B-hydroxybutyrate are related to body mass changes over the previous several hours in small songbirds (Jenni-Eiermann and Jenni 1994; Jenni and Schwilch 2001; Cerasale and Guglielmo 2006). Consequently, these lipid metabolites have been used to evaluate refueling rates in many species of free-living songbirds and shorebirds during annual migrations (Schaub and Jenni 2001; Guglielmo et al. 2005; Acevedo Seaman et al. 2006; Williams et al. 2007).

The capacity of these lipid metabolites to directly indicate fat deposition is rarely validated. Instead, lipid metabolite concentrations in plasma of songbirds are usually related to overall body mass gain of birds or to coarse measures of body fat mass, such as visual subcutaneous fat (Totzke and Bairlein 1998; Gannes 2001). Total body electrical conductivity (TOBEC) has been used to determine body composition and whole-body fat mass in a variety of bird species (Castro et al. 1990; Scott et al. 1991; Skagen et al. 1993; Whitman 2002) and can provide information about changes in body fat over time. However, no previous study has directly related changes in lipid metabolites with measured whole-body lean or fat mass in small migratory songbirds.

One factor that may influence fattening rates and plasma metabolite levels in feeding songbirds is the nutritional quality of food resources. Dietary protein modifies the rate of in vitro lipogenesis and fat accumulation in poultry through regulation of insulin-like growth factor 1 (Rosebrough et al. 1996, 2004) and the activity of lipogenic enzymes such as hepatic malic enzyme (Adams and Davis 2001; Rosebrough et al. 2002). Therefore, chickens fed diets with low protein-calorie ratios have increased hepatic lipogenesis and body fat levels (Donaldson 1985; Rosebrough and Steele 1985). The proportion of protein, carbohydrate, and fat in poultry diets has also been related to plasma concentrations of some lipid metabolites, namely, triglyceride and free fatty acids (Malheiros et al. 2003; Swennen et al. 2005). Fattening rates of captive garden warblers

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Table 1: Composition of four semisynthetic experimental diets fed to white-throated sparrows for 41–44 d

	Low-Protein Diets				High-Protein Diets			
	High Carbohydrate		High Fat		Low Carbohydrate		Low Fat	
Ingredients	Wet (%)	Dry (%)	Wet (%)	Dry (%)	Wet (%)	Dry (%)	Wet (%)	Dry (%)
Casein[a]	2.8	10.0	2.8	10.0	14.9	60.0	14.9	60.0
D-glucose[b]	18.0	64.2	4.1	14.7	1.2	5.0	5.0	20.3
Oil[c]	2.3	8.1	8.5	30.3	3.0	12.0	1.3	5.2
Vitamin mix[d]	.4	1.5	.4	1.5	.4	1.5	.4	1.5
Salt mix[e]	1.4	5.2	1.4	5.2	1.3	5.2	1.3	5.2
Cellulose[f]	1.8	6.4	9.5	33.8	2.8	11.3	.7	2.9
Agar[g]	1.3	4.6	1.3	4.6	1.2	5.0	1.2	5.0
Water	72.0		72.0		75.0		75.0	

Note. All diets had identical energy density (16.3 kJ/g).

[a] Casein (high N); USB, Cleveland, OH.

[b] Fisher Scientific, Pittsburgh, PA.

[c] Corn oil.

[d] AIN-76 vitamin and minerals mix; MP Biomedicals, Irvine, CA.

[e] Salt mix—Briggs; MP Biomedicals.

[f] Celufil, non-nutritive cellulose filler; USB.

[g] Agar, bacteriological grade; USB.

(*Sylvia borin*), estimated by body mass gain, was hindered in birds fed high-protein diets (Bairlein 1998), though specific substrate use and metabolite differences were not the focus of that work.

Studies designed to examine dietary effects on intermediate metabolism and mechanisms of fattening in migratory birds are rare and often focus on the relationship between dietary fat intake and body mass gain. These studies have produced conflicting results in that dietary fat can influence plasma triglyceride and B-hydroxybutyrate in migratory shorebirds (Seaman et al. 2005) but not in certain other songbird species, such as yellow-rumped warblers (*Dendroica coronata*; Cerasale and Guglielmo 2006) and white-throated sparrows (*Zonotrichia albicollis*; Smith et al. 2007). Furthermore, diets that differ in dietary carbohydrate and protein but not fat content can influence plasma concentrations of these lipid metabolites in feeding white-throated sparrows (Smith et al. 2007). The inconsistency among studies may be attributed to differences in diet composition or energy density among diets. Most important would be studies that manipulate diet composition so that the interactive effects of dietary protein, carbohydrate, and fat on fattening rates of songbirds can be determined while controlling for potential confounding variables such as energy density. Such studies are particularly relevant given that many species of songbirds during their fall migration in the eastern United States and western Europe consume a wide variety of insects and fruits that differ in protein, fat, and sugar content (Herrera 1984; White 1989; Parrish 1997, 2000).

The objective of this study was to determine how macronutrient composition of isoenergetic diets influences plasma lipid metabolite concentrations and body composition in a temperate migrant, the white-throated sparrow. Specifically, we designed our study to examine the interacting effects of dietary protein, fat, and nonfiber carbohydrate on plasma triglyceride, B-hydroxybutyrate, nonesterified fatty acids, and lean and fat mass of sparrows. We tested the hypotheses that (1) high dietary protein-calorie ratios inhibit fattening of songbirds, (2) high-carbohydrate diets increase fat deposition, (3) high-fat diets increase dietary fat utilization, and (4) plasma triglyceride is positively related and B-hydroxybutyrate and NEFA are negatively related to fat mass of sparrows.

Material and Methods

Diets

White-throated sparrows were captured using mist nets on Block Island, Rhode Island (41°12′N, 71°35′W) and in Kingston, Rhode Island (41°5′N, 71°5′W) between October 30 and November 27, 2004. Birds were housed indoors at the University of Rhode Island in individual stainless steel cages (59 cm × 45 cm × 36 cm) and maintained on a 11L : 13D photoperiod (lights on at 0800 hours) and at constant temperature (22°C). Birds were weighed each morning between 0800 and 0830 hours.

Birds were initially provided ad lib. mealworms (*Galleria mellonella*) and water. During the first 2 wk in captivity, birds were gradually acclimated to a powdered semisynthetic diet identical in macronutrient composition to a grainlike diet previously fed to white-throated sparrows (Pierce and McWilliams 2004) but without addition of amino acid mix and choline chloride. Birds were fed ad lib. this acclimation diet and water for 66–70 d before experiments. Birds were then randomly assigned to one of four semisynthetic experimental diets that were isoenergetic (16.3 kJ/g dry calculated assuming energy density of nonfiber carbohydrate [17.6 kJ/g], protein [17.8 kJ/g], and fat [39.3 kJ/g]; Schmidt-Nielson 1997) and that were either high (60% dry mass) or low (10% dry mass) in protein. The two high- and low-protein diets were formulated to contain

Table 2: Mean ± SE body mass, food intake, and mass change of sparrows on blood sampling days when fed the acclimation diet for 66–70 d and then one of the experimental diets for 41–44 d

	Morning Mass[a] (g)	Afternoon Mass[b] (g)	Mass Change[c] (g)	Food Intake[d] (g)
Acclimation diet group:[e]				
Low protein, high carbohydrate	25.9 ± 1.1	27.2 ± 1.1	1.3 ± .2	1.7 ± .1
Low protein, high fat	25.6 ± .6	26.6 ± .7	1.0 ± .3	1.4 ± .3
High protein, low carbohydrate	26.1 ± 1.0	27.4 ± .9	1.3 ± .3	1.4 ± .2
High protein, low fat	25.9 ± 1.0	26.8 ± 1.1	1.0 ± .1	1.5 ± .1
Experimental diet group:				
Low protein, high carbohydrate	27.9 ± 1.1 A	29.5 ± .9 A	1.6 ± .2[f]	1.4 ± .2 A
Low protein, high fat	22.5 ± .8 B	24.0 ± .9 B	1.6 ± .2[f]	2.4 ± .3 B
High protein, low carbohydrate	22.8 ± .6 B	25.1 ± .7 B	2.3 ± .3[f]	1.8 ± .2 AB
High protein, low fat	24.5 ± 1.4 AB	26.7 ± 1.3 AB	2.1 ± .2[f]	1.5 ± .3 AB

Note. Different letters indicate significant group differences.

[a] Body mass at 0800–0830 hours.

[b] Body mass when bled at 1300–1335 hours.

[c] Mass change between 0800–0830 and 1300–1335 hours.

[d] Dry matter intake between 0800–0830 and 1300–1335 hours.

[e] Birds were assigned to these experimental diets after being fed the acclimation diet for 66–70 d.

[f] Significant overall ANOVA but nonsignificant follow-up pairwise comparisons.

the most carbohydrate or fat within the limits set by the requirement that all diets had certain dietary protein (60% or 10%), at least 5% dry mass of each macronutrient, and the same energy density (Table 1). This allowed us to examine the effects of dietary protein content while considering the interacting effects of dietary carbohydrate and fat. Birds were fed these semisynthetic experimental diets and water ad lib. for the next 41–44 d. Previous studies have shown that small songbirds can be maintained for many months on similar semisynthetic diets (McWilliams et al. 2002; Pierce and McWilliams 2004, 2005).

Blood Sampling, TOBEC, and Metabolite Analyses

We measured food intake, body composition, and blood metabolites at two times during the study: on the last day of feeding on the acclimation diet and again on the last day of feeding on a given experimental diet. Since all birds were fed identical diets during the acclimation period, we considered initial samples taken after 2 mo on the acclimation diet as baseline values for metabolites and body composition.

We measured body composition of all birds between 0800 and 0845 hours (<5 min/bird) and before food was offered on the final day of ad lib. feeding on acclimation and experimental diets. TOBEC was measured using an EM-SCAN SA-3000 small animal electrical conductivity analyzer (model 3044 detection chamber). Lean mass was then calculated using the following predetermined equation calibrated for white-throated sparrows: lean mass = −3.890 + (0.011 × TOBEC) + (0.563 × tarsus) + (0.475 × body mass), maximum SE ± 0.71 g (Whitman 2002). Fat mass was estimated by subtracting calculated lean mass from body mass measured immediately before TOBEC measurement. Predicted fat mass of one sparrow in our study was negative (−0.4 g). This bird had a fat class score of 0, so for subsequent analyses we instead assumed this bird had 0.0 g fat.

After TOBEC measurement, birds were returned to their cages and allowed to feed undisturbed until we sampled approximately 50 μL of blood at 1300–1335 hours. Blood was sampled by puncturing the brachial vein with a 27-gauge needle and then collecting blood in heparinized capillary tubes, and the time of day that blood was sampled was recorded (hereafter "bleed time"). Birds were then weighed and mass change between 0800 and 0830 hours and blood sampling (1300–1335 hours) was determined (hereafter "mass change"). Food intake (dry mass offered − dry mass remaining ± 0.1g) was also measured during this same ca. 5 h. We then calculated macronutrient intake (g eaten of protein, carbohydrate, and fat) for each bird given dry matter intake and diet composition. All procedures were approved under University of Rhode Island Institutional Animal Care and Use Committee protocol AN05-04-020.

Plasma was separated from red blood cells by centrifugation at 8,000 rpm for 10 min and then stored at −80°C until analysis. Plasma metabolites were assayed on a Bio-Tek Powerwave X340 microplate spectrophotometer. Triglyceride was measured by sequential endpoint assay (Sigma, St. Louis, MO; 5 μL plasma, 240 μL reagent A, 60 μL reagent B), where free glycerol was measured and subtracted from overall total triglyceride values to obtain triglyceride concentrations. Nonesterified fatty acids (NEFA) were also measured by endpoint assay (WAKO Diagnostics, Richmond, VA; 3 μL plasma, 120 μL reagent A, 240 μL reagent B). B-hydroxybutyrate (BUTY) was measured by kinetic assay (R-Biopharm, Marshall, MI; 5 μL sample, 150 μL working solution, and 3 μL B-hydroxybutyrate dehydrogenase read kinetically for 30 min) similar to the method of Guglielmo et al. (2005). Samples were diluted with 0.9% NaCl before all assays, except for NEFA analysis, and we repeated measurements until the coefficient of variation was <10% between replicates.

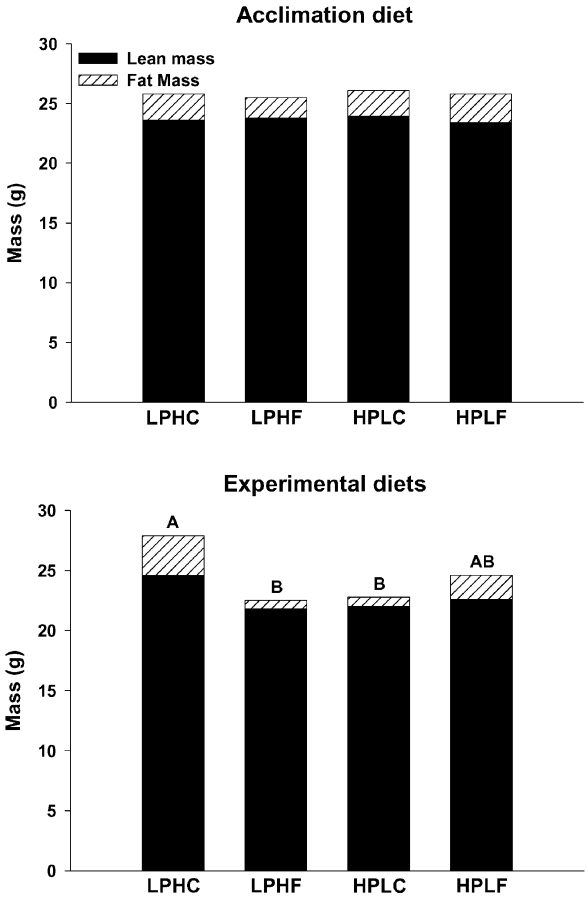

Figure 1. Average body composition (lean or fat mass) of sparrows in the low-protein, high-carbohydrate (LPHC); low-protein, high-fat (LPHF); high-protein, low-carbohydrate (HPLC); and high-protein, low-fat (HPLF) diet groups ($N = 5$/diet group) when fed the acclimation diet for 66–70 d or one of the four experimental diets for 41–44 d. Different letters above bars indicate significant group differences in fat mass.

Statistical Analyses

We used a linear mixed model (PROC MIXED with repeated-measures analysis and compound symmetric covariance structure; SAS Institute, Cary, NC) to compare morning body mass of all birds on the day of sampling with morning body mass 5 d before sampling. This allowed us to establish that birds were in a period of stable body mass when blood and body composition were sampled. We used one-way ANOVA with post hoc Tukey's HSD tests to analyze differences in morning body mass, lean mass, fat mass, body mass at blood sampling, mass change, bleed time, and dry intake among diet groups when birds were sampled while fed acclimation or experimental diets. We calculated the diet-related change in metabolite concentrations between baseline measurements (after all birds were fed the acclimation diet for ca. 2 mo) and the experimental diet measurements (after 41–44 d on a given diet). This allowed us to determine changes from baseline levels when all birds were fed identical diets and presumably were in similar physiological states and to control for intraindividual variation in lipid metabolites. We calculated Pearson's correlation coefficients to determine whether metabolite concentrations were correlated with body mass at blood sampling, mass change, or bleed time. Variables with significant correlations were then used as covariates in ANCOVA to compare blood metabolite concentrations among the four diet groups. Results of statistical analyses were the same with or without covariates in the model for triglyceride and BUTY; therefore, we report only the results of ANOVA for comparisons among diet groups for these two metabolites. However, we used ANCOVA with body mass at blood sampling to compare NEFA concentrations among diet groups.

We used an information-theoretic approach to evaluate the relative importance of four predictor variables (fat mass, glucose intake, fat intake, protein intake) for explaining variation in diet-related changes in metabolite concentrations after birds were fed on of the four experimental diets for 41–44 d. We used a candidate model set that included models with each variable alone and all possible combinations of predictor variables. We calculated the second-order Akaike's Information Criterion (AIC_c; Burnham and Anderson 2002) and ranked models where the model with the lowest AIC_c value had the most support given the data. We then calculated differences in AIC_c ($\Delta_i = AIC_{ci} - AIC_{c\,min}$), where models with $\Delta_i \leq 2$ are considered to have substantial support. We then calculated model weights (w_i), which provide an estimate of the likelihood of a model given the data (Burnham and Anderson 2002). We used AIC_c model weights to calculate the relative importance of each predictor variable ($w + (j)$) by summing w_i of all models that contain a given variable. Thus, variables that are included in highly ranked models receive high relative importance values. We then used simple linear regression to determine the direction of relationships between diet-related changes in metabolite concentrations and body composition or macronutrient intake.

All data were evaluated for normality and homogeneity of variance before statistical analyses. In the few cases where these assumptions were not satisfied (BUTY and NEFA concentrations, fat mass of birds fed the acclimation diet, and protein and fat intake of birds fed the experimental diets), we conducted statistical analyses on both transformed and untransformed data. In all cases except one, transformation satisfied the assumptions but did not change the results of statistical tests, so we report the results from the analyses using the untransformed values. However, fat intake of birds fed the experimental diets was square root transformed to improve normality and variance of residuals for regression analyses. Significance level was set at $P \leq 0.05$ for all analyses.

Results

Food Intake and Body Mass

We determined that birds were in a period of stable body mass immediately before sampling because there were no differences between morning body mass on the fifth day before sampling and body mass on the morning of sampling for birds fed the

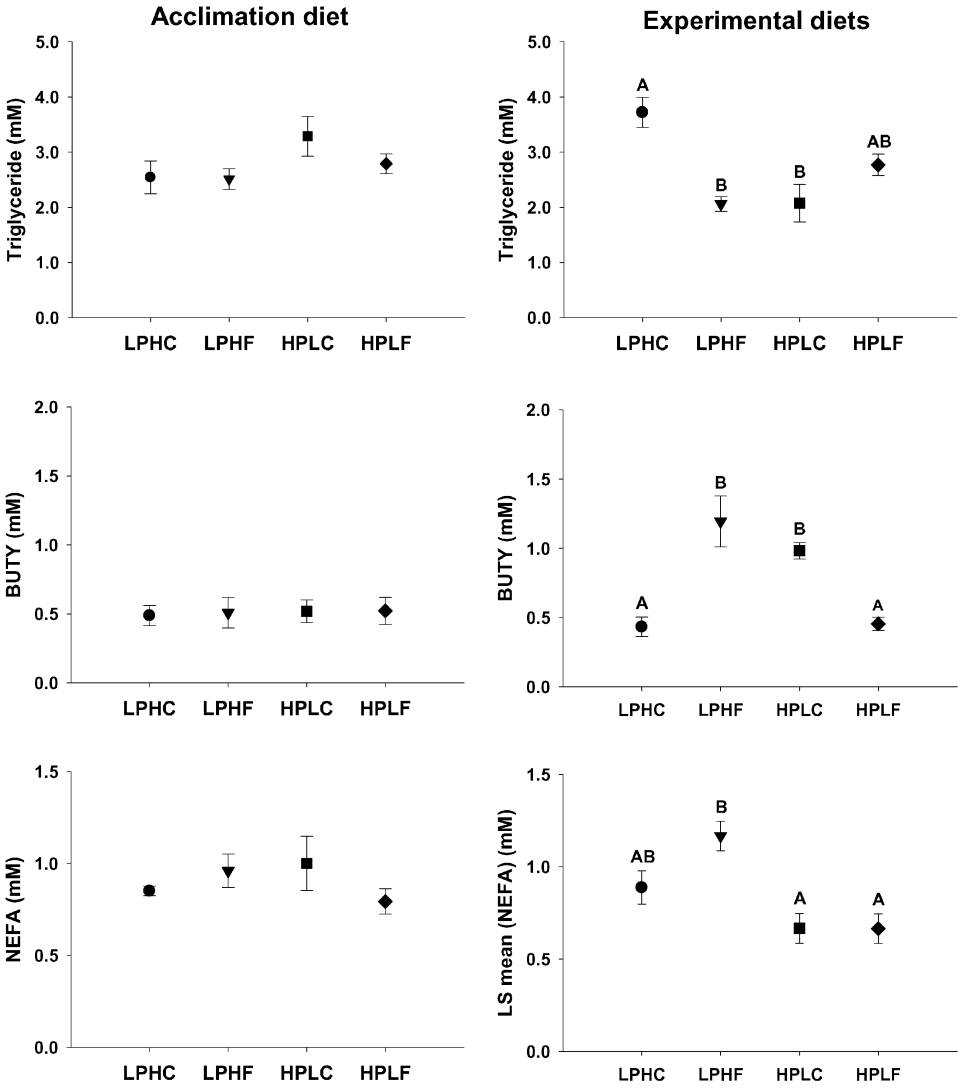

Figure 2. Mean ± SE plasma concentrations of triglyceride, B-hydroxybutyrate (BUTY), and nonesterified fatty acids (NEFA) of sparrows in the low-protein, high-carbohydrate (LPHC); low-protein, high-fat (LPHF); high-protein, low-carbohydrate (HPLC); and high-protein, low-fat (HPLF) diet groups ($N = 5$/diet group) when fed the acclimation diet for 66–70 d or experimental diets for 41–44 d. Least squares means ± SE are provided for NEFA concentrations of birds when fed the experimental diets to correct for body mass at blood sampling. Different letters above points indicate significant group differences.

acclimation diet ($F_{1,16} = 0.6$, $P = 0.47$) or experimental diets ($F_{1,16} = 0.2$, $P = 0.70$). When fed the acclimation diet, all birds had similar morning body mass ($F_{3,16} = 0.1$, $P = 0.98$), body mass when bled ($F_{3,16} = 0.1$, $P = 0.94$), mass change ($F_{3,16} = 0.6$, $P = 0.63$), and food intake ($F_{3,16} = 0.6$, $P = 0.65$; Table 2). After >40 d on their respective experimental diets, birds fed the low-protein, high-carbohydrate diet were significantly heavier in the morning ($F_{3,16} = 5.5$, $P < 0.01$) and at blood sampling ($F_{3,16} = 5.9$, $P < 0.01$) than birds fed the low-protein, high-fat and high-protein, low-carbohydrate diets ($F_{3,16} = 3.3$, $P = 0.05$; Table 2). Birds fed the low-protein, high-fat diet also ate more than birds fed the low-protein, high-carbohydrate diet ($F_{3,14} = 3.5$, $P = 0.05$; Table 2). Although there was a significant effect of diet on mass change of birds when fed the experimental diets ($F_{3,16} = 3.3$, $P = 0.05$; Table 2), follow-up Tukey's HSD tests did not reveal significant pairwise differences among the four diet groups.

Body Composition and Plasma Metabolites

Birds fed the acclimation diet for ca. 2 mo had similar lean mass ($F_{3,16} = 0.1$, $P = 0.96$) and fat mass before being offered their experimental diets ($F_{3,16} = 0.6$, $P = 0.62$; Fig. 1). After being offered their experimental diets for 41–44 d, birds fed the low-protein, high-carbohydrate diet had more fat mass ($F_{3,16} = 10.1$, $P < 0.001$) but not lean mass ($F_{3,16} = 3.0$, $P = 0.06$) compared with the mass of birds fed the low-protein, high-fat or high-protein, low-carbohydrate diets (Fig. 1).

There were no differences in bleed time among the four diet groups when birds were fed the acclimation ($F_{3,16} = 0.4$, $P =$

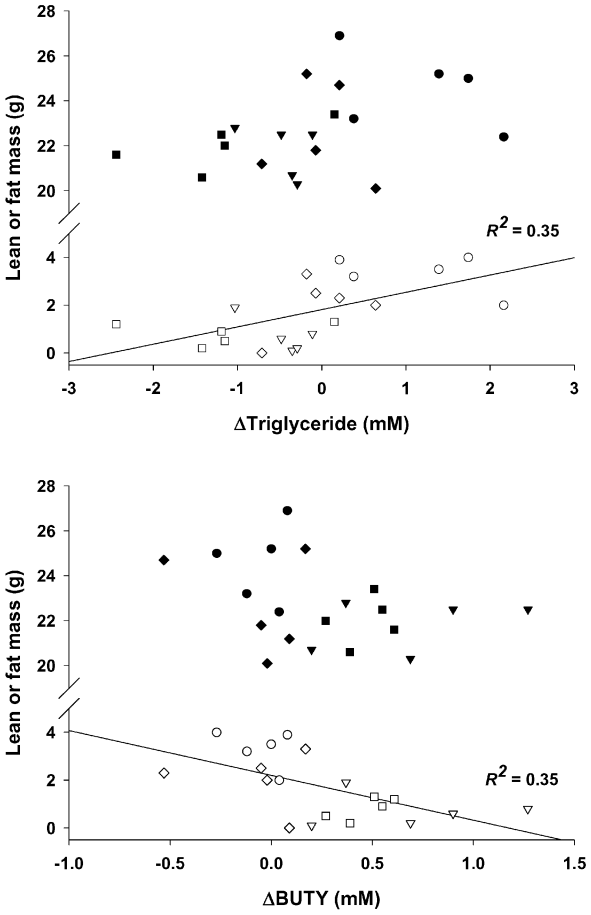

Figure 3. Relationship between change in triglyceride (Δtriglyceride) or change in B-hydroxybutyrate (ΔBUTY) and body composition of sparrows fed the low-protein, high-carbohydrate (*circles*); low-protein, high-fat (*inverted triangles*); high-protein, low-carbohydrate (*squares*); and high-protein, low-fat (*diamonds*) diets for 41–44 days ($N = 5$/diet group). Filled symbols correspond to lean mass, and open symbols correspond to fat mass. The R^2 values and regression lines are provided for significant relationships from simple linear regression.

0.75) or experimental ($F_{3,16} = 0.2$, $P = 0.88$) diets. Plasma concentrations of triglyceride ($F_{3,16} = 1.8$, $P = 0.19$; Fig. 2), BUTY ($F_{3,16} = 0.1$, $P = 0.94$; Fig. 2), and NEFA ($F_{3,14} = 0.9$, $P = 0.49$; Fig. 2) were similar among all birds fed the acclimation diet for ca. 2 mo. After being offered their experimental diets for 41–44 d, triglyceride concentrations were significantly higher in birds fed the low-protein, high-carbohydrate diet compared with concentrations in birds fed the low-protein, high-fat or high-protein, low-carbohydrate diets ($F_{3,16} = 10.0$, $P < 0.001$; Fig. 2). In addition, BUTY concentrations were significantly lower in birds fed the low-protein, high-carbohydrate and high-protein, low-fat diets compared with concentrations in birds fed the low-protein, high-fat and high-protein, low-carbohydrate diets ($F_{3,16} = 13.1$, $P < 0.01$; Fig. 2), and NEFA concentrations were higher in birds fed the low-protein, high-fat diet compared with concentrations in birds fed the two high-protein diets ($F_{3,13} = 15.9$, $P < 0.001$; Fig. 2).

Diet-related change in triglyceride after birds were fed the experimental diets for 41–44 d was not significantly related to lean mass of birds ($F_{1,18} = 3.5$, $P = 0.08$; Fig. 3) but was positively related to fat mass of these same birds ($F_{1,18} = 9.8$, $P < 0.01$, $R^2 = 0.35$; Fig. 3). Diet-related change in BUTY after birds were fed the experimental diets for 41–44 d was also not related to lean mass of birds ($F_{1,18} = 2.4$, $P = 0.14$; Fig. 3) but was negatively related to fat mass of these same birds ($F_{1,18} = 9.6$, $P < 0.01$, $R^2 = 0.35$; Fig. 3). Change in NEFA was not related to either lean mass ($F_{1,15} = 0.0$, $P = 0.88$) or fat mass ($F_{1,18} = 0.1$, $P = 0.73$). However, simple linear regression revealed that intake of certain macronutrients was also significantly related to changes in these metabolites. Glucose intake was positively related to change in triglyceride concentrations ($F_{1,16} = 18.3$, $P < 0.001$, $R^2 = 0.53$; Fig. 4*A*) and negatively related to change in BUTY ($F_{1,16} = 7.8$, $P = 0.01$, $R^2 = 0.33$; Fig. 4*C*). In contrast, fat intake was positively related to change in BUTY ($F_{1,16} = 7.8$, $P = 0.01$, $R^2 = 0.33$; Fig. 4*D*) and change in NEFA ($F_{1,14} = 5.2$, $P = 0.04$, $R^2 = 0.28$; Fig. 4*E*). Protein intake was negatively related to change in triglyceride ($F_{1,16} = 5.2$, $P = 0.04$, $R^2 = 0.25$; Fig. 4*B*) and change in NEFA ($F_{1,16} = 7.8$, $P = 0.01$, $R^2 = 0.33$; Fig. 4*F*).

The explanatory model containing only glucose intake was ranked the highest for triglyceride concentrations, although a model containing both glucose and fat intake also had substantial support (Table 3). Relative importance values confirmed that glucose intake had a dominant effect on triglyceride concentrations (Table 4). There was considerable model selection uncertainly for plasma BUTY; the top-ranked model included fat mass, glucose intake, and protein intake, but three other models had considerable support with $\Delta_i < 2$ (Table 3), and glucose intake and protein intake had higher relative importance than fat intake and fat mass (Table 4). Models containing fat intake or protein intake alone, and including both fat and protein intakes had substantial support for explaining variation in plasma NEFA (Table 3). Relative importance values revealed that fat intake explained the most variation in plasma NEFA concentrations, followed by protein intake, whereas glucose intake and fat mass had relatively low importance (Table 4).

Discussion

We altered the nutritional content of diets while holding the energy density constant to ascertain the effects of the three primary dietary macronutrients on both plasma indicators of fattening and body composition of sparrows. Plasma lipid metabolites are integrative measures that are influenced by many variables, including changes in the physiological state of the animal (fasting, fattening), body condition, diet, and exercise. We show that certain changes in dietary macronutrient composition can affect fat metabolism and body fat mass in a temperate migrant, the white-throated sparrow.

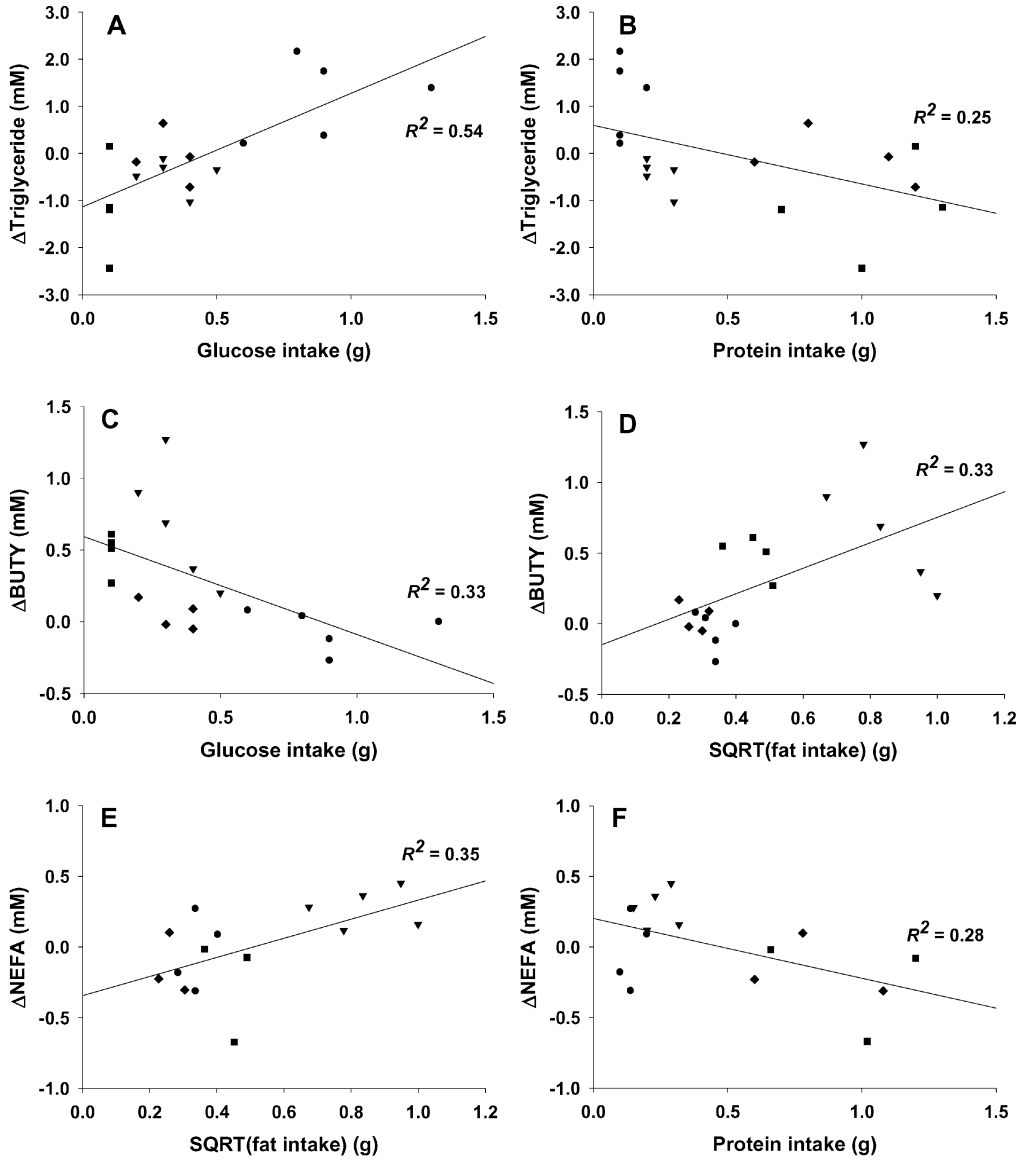

Figure 4. Relationships between change in triglyceride (Δtriglyceride; *A*, *B*), change in B-hydroxybutyrate (ΔBUTY; *C*, *D*), or change in nonesterified fatty acids (ΔNEFA; *E*, *F*) and macronutrient intake during the morning on the day of blood sampling for sparrows fed low-protein, high-carbohydrate (*circles*); low-protein, high-fat (*inverted triangles*); high-protein, low-carbohydrate (*squares*); and high-protein, low-fat (*diamonds*) diets for 41–44 d. Macronutrient intakes with nonsignificant results from simple linear regressions for each metabolite are not shown.

Diet Affects Body Composition

The relationship between diet composition and fattening is widely documented in a variety of poultry studies, especially the inhibitory effects of dietary protein on body fat accumulation (Donaldson 1985; Rosebrough and Steele 1985). In contrast to the proposed hypothesis that high dietary protein-calorie ratios inhibit fattening of birds, we found that high-protein diets did not consistently inhibit fat accumulation of sparrows when fed these diets over a period of ca. 1.5 mo. Although birds with the most body fat were fed a low-protein diet, this low-protein effect was only evident when dietary glucose was high and dietary fat was low. Our results agree with recent research emphasizing the role of dietary carbohydrates in fat metabolism of humans. Numerous clinical studies have demonstrated that adherence to a low-carbohydrate diet is accompanied by body fat loss and decreased basal plasma triglyceride concentrations (Westman et al. 2002, 2003; Yancy et al. 2004), which may in part be attributed to increased de novo lipogenesis associated with high-carbohydrate intake (Schwarz et al. 2003). We suggest that body fat accumulation was highest in sparrows fed the low-protein, high-carbohydrate diets in part because the availability of simple sugars (glucose) provided excess carbon substrates for de novo lipogenesis (Stevens 1996; Klasing 1998). Given that birds have a high capacity for de novo lipogenesis

Table 3: Model selection results for the five highest-ranked candidate models explaining variation in the change in metabolite concentrations after birds were fed experimental diets for 41–44 d

Parameters[a]	df	n	K[b]	RSS	$\log_e(L)$[c]	AIC_c	Δ_i	w_i
Triglyceride:								
Glucose	1	18	3	9.75	5.52	−3.32	.00	.445
Glucose + fat	2	18	4	8.94	6.30	−1.53	1.79	.182
FM + glucose	2	18	4	9.37	5.87	−.67	2.65	.118
Glucose + protein	2	18	4	9.64	5.62	−.16	3.16	.092
Glucose + fat + protein	3	18	5	8.23	7.04	.91	4.23	.054
B-hydroxybutyrate:								
FM + glucose + protein	3	18	5	.92	26.76	−38.53	.00	.261
Glucose + fat	2	18	4	1.19	24.42	−37.77	.76	.178
Glucose + protein	2	18	4	1.21	24.31	−37.55	.98	.160
Glucose + fat + protein	3	18	5	.98	26.16	−37.32	1.21	.143
FM	1	18	3	1.65	21.53	−35.35	3.18	.053
Nonesterified fatty acids:								
Fat	1	15	3	.83	21.66	−35.15	.00	.269
Fat + protein	2	15	4	.66	23.40	−34.80	.34	.227
Protein	1	15	3	.91	20.98	−33.78	1.37	.136
FM + protein	2	15	4	.76	22.35	−32.70	2.44	.079
Glucose + fat	2	15	4	.76	22.35	−32.70	2.45	.079

Note. Fat intake was square root transformed before analyses. n = number of plasma samples; RSS = residual sum of squares; AIC_c = second-order Akaike's Information Criterion; w_i = model weight.

[a] FM = fat mass; glucose = glucose intake during the morning before afternoon blood sampling; fat = fat intake during the morning before afternoon blood sampling; protein = protein intake during the morning before afternoon blood sampling.

[b] Calculated as the number of estimatable parameters plus the intercept and variance.

[c] Maximized log-likelihood function calculated as $[-0.5n \times \log_e(\mathrm{RSS}/n)]$.

when energy intake exceeds requirements (Klasing 1998), more simple sugars in the diet may strongly affect fat metabolism in hyperphagic migrants during periods of rapid refueling.

Diet Affects Plasma Lipid Metabolites

Macronutrient composition of the diet directly affected plasma lipid metabolites of sparrows in our study, consistent with previous research demonstrating significantly higher triglyceride and lower B-hydroxybutyrate levels in white-throated sparrows fed a high-carbohydrate grain diet compared with a low-carbohydrate insect diet (Smith et al. 2007). Our results also provide support for the hypothesis that high-fat diets increase dietary fat utilization and circulating concentrations of B-hydroxybutyrate and nonesterified fatty acids. The effect of dietary fat on triglyceride and B-hydroxybutyrate has also been demonstrated in western sandpipers (*Calidris mauri*; Seaman et al. 2005) but not in yellow-rumped warblers (Cerasale and Guglielmo 2006), and previous work with white-throated sparrows found no diet effect on nonesterified fatty acids (Smith et al. 2007). Thus, our study provides new evidence that dietary macronutrient content, independent of energy density, can affect lipid metabolites in small songbirds.

A major problem with earlier studies that used plasma lipid metabolites to indicate mass change in birds is that diet composition and energy intake were not controlled, so there may have been simultaneous changes in the diets of birds. Additionally, in most studies where macronutrient content has been manipulated, energy density was not held constant among diets, and food intake was usually not measured. We formulated isoenergetic diets with distinct differences in dietary protein, carbohydrate, and fat. This experimental design allowed us to demonstrate that macronutrient intake explained much of the variation in plasma lipid metabolites across diets. For example, plasma triglyceride and nonesterified fatty acids were positively related to glucose intake and fat intake, respectively, and both were negatively related to protein intake. Although we found some support for the hypothesis that high dietary fat intake increases dietary fat utilization, we also found that B-hydroxybutyrate was negatively related to glucose intake. These relationships with triglyceride and B-hydroxybutyrate support the hypothesis that simple carbohydrates can have a significant effect on plasma indices fat metabolism in songbirds.

Dietary protein affects fattening in poultry by inhibiting de novo lipogenesis and fat deposition when protein intake is high (Donaldson 1985; Rosebrough and Steele 1985; Rosebrough and McMurty 2000; Rosebrough et al. 2002, 2004). Low dietary protein intake results in generally higher plasma triglyceride and can result in higher plasma nonesterified fatty acids, though diet effects on nonesterified fatty acids have not been consistently demonstrated in these poultry studies (Rosebrough et al.

Table 4: Relative importance of predictor variables used in model selection analyses

Model Set	Fat Mass	Glucose Intake	Fat Intake	Protein Intake
ΔTriglyceride	.199	.940	.297	.212
ΔB-hydroxybutyrate	.465	.852	.444	.640
ΔNonesterifed fatty acids	.223	.174	.704	.526

Note. Relative importance values were calculated as the sum of second-order Akaike's Information Criterion weights of all models that contain a given variable. Fat intake was square root transformed before analyses.

1992; Malheiros et al. 2003; Collin et al. 2003, Swennen et al. 2005). However, fat metabolism in sparrows in this study was not simply a product of protein intake because both dietary glucose and fat intake were more strongly related to changes in triglyceride and nonesterified fatty acid concentrations, respectively, and plasma B-hydroxybutyrate was not directly related to protein intake. B-hydroxybutyrate is a ketone body formed through oxidation of fatty acids and is principally used as an energy source by the brain and kidney in birds during fasting and when carbohydrate intake is low (Stevens 1996). Therefore, feeding on carbohydrate-rich diets may result in decreased B-hydroxybutyrate formation because ample glucose is available for energy oxidation (Robinson and Williamson 1980; Stevens 1996), and B-hydroxybutyrate synthesis may be enhanced when ample dietary free fatty acids are available (Robinson and Williamson 1980; Ramenofsky 1990). Thus, it may be a combination of low dietary carbohydrate and free fatty acid availability from dietary fat that results in high circulating B-hydroxybutyrate levels in sparrows and other birds.

Relative Importance of Diet and Body Composition on Lipid Metabolites

We have shown that diet can affect indices of fat metabolism and deposition in songbirds; changes in macronutrient intake presumably caused changes in intermediary metabolism (lipid metabolites) and subsequent body fat storage. However, concentrations of some lipid metabolites can fluctuate with changes in body mass of passerines (Totzke and Bairlein 1998) and are directly related to body mass changes over the previous several hours in small songbirds (Jenni-Eiermann and Jenni 1994; Cerasale and Guglielmo 2006) and over several days in shorebirds (Williams et al. 1999). Because these metabolites are indicators of fat metabolism in birds (Ramenofsky 1990; Stevens 1996), it is implied that the positive relationship between mass change and plasma triglyceride is indicative of fat deposition, and negative relationship with B-hydroxybutyrate is indicative of fat catabolism. To our knowledge, ours is the first study that relates plasma triglyceride and B-hydroxybutyrate concentrations to measured lean and fat mass of songbirds. We found that both of these lipid metabolites were related to fat mass but not lean mass, demonstrating that these metabolites correspond to fat metabolism in sparrows. However, we did not examine how these metabolites relate to short-term changes in body composition as our birds were sampled while in a period of stable body mass and after a period of fat deposition or catabolism of fat stores had occurred. Thus, differences in lipid metabolites among birds at this point may also relate to fat mass where fatter birds have higher triglyceride and lower B-hydroxybutyrate than lean birds while maintaining current fat stores, similar to the observation in some migrating birds that body mass is positively related to refueling rates (Schaub and Jenni 2001).

The relative contribution of diet composition versus body fat mass to concentrations of circulating plasma lipid metabolites must be determined before these metabolites can be used to accurately predict short-term changes in body condition of birds. Our study provides evidence that the effects of macronutrient intake on certain lipid metabolites are stronger than the potential influence of current body fat mass. Patterns of metabolite concentrations did not consistently follow patterns of significant intraindividual differences in overall body mass and fat mass. In addition, even though both macronutrient intake and fat mass were independently related to changes in certain lipid metabolite concentrations (triglyceride and B-hydroxybutyrate), AIC_c analyses revealed that intake of certain macronutrients before blood sampling was more important than body fat mass for explaining the variation in these lipid metabolites. The exception was B-hydroxybutyrate, for which AIC_c analyses suggested important roles for both body fat mass and diet that differed from independent analyses of these variables. The contrasting results of these two analyses may be attributed to limitations of the statistical tests, which did not model specific interactions, or B-hydroxybutyrate may have been more sensitive to the combined effects of several variables. Further study is needed to clarify the interacting effects of body composition and diet composition on B-hydroxybutyrate concentrations in feeding songbirds. Despite this, the dominant effects of diet over body composition on plasma triglyceride and B-hydroxybutyrate are supported by previous research on birds with similar body mass conducted over shorter timescales (ca. 6 d; Smith et al. 2007) and on plasma triglyceride in free-living birds during migratory flight (Gannes 2001). Thus, it appears that diet habits of birds can have a significant influence on certain lipid metabolites in passerines regardless of their current fat mass.

Implications for Migrating Songbirds

Seasonal changes in diet preferences and food selection may provide nutritional advantages for optimization of fattening in migratory birds (Bairlein 1990, 2002; Bairlein and Simons 1995). Many species of migratory songbirds supplement their diet with a variety of fruits during fall migrations in the eastern United States (Baird 1980; Parrish 1997; Suthers et al. 2000),

and these fruits are often low in protein but high in fat or sugar (Johnson et al. 1985; White 1989). The potential benefits of including such fruits in the diet may include increased fat synthesis through de novo lipogenesis, particularly when birds consume high-sugar fruits that provide highly digestible dietary hexoses, primarily glucose and fructose. In migratory birds, the activity of certain lipogenic enzymes, such as liver malic enzyme, may increase during premigratory fattening (Ramenofsky 1990). This increased activity of lipogenic enzymes could allow birds to rapidly convert dietary simple sugars to fatty acids if energy intake exceeds energy demands, though this is a less energetically efficient means of fat storage than assimilating fats directly from the diet (Stevens 1996; Klasing 1998). Our results suggest that white-throated sparrows fed high-glucose diets increase synthesis and accumulation of fat regardless of dietary protein or fat content. High-fructose diets may be particularly beneficial because fructose can allow for even higher rates of hepatic lipogenesis than glucose in birds (Pearce 1983). Therefore, consumption of high-sugar fruits could facilitate increased fat synthesis and subsequent storage in migrating songbirds.

Plasma lipid metabolites have recently been applied as a tool for investigating habitat quality for migrating birds given their relationship to refueling rates at stopover sites (Schaub and Jenni 2001; Guglielmo et al. 2005; Acevedo Seaman et al. 2006; Williams et al. 2007). We have confirmed that these metabolites are indicative of fat mass rather than lean mass, but large differences in diet composition must be considered because certain dietary macronutrients can have a stronger effect on lipid metabolites than body fat mass in feeding birds. For example, our data suggest that diets with different protein content should have little effect on the utility of plasma lipid metabolites for qualitative comparisons of refueling among individual birds, provided fat and carbohydrate content is similar. However, if birds consume foods with a wide range of dietary carbohydrate content, such as sugary or high-fat fruits or high-protein insects with low sugar content, then diet composition must be considered since it affects plasma lipid metabolites as much or more than intraindividual differences fat mass.

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