

The effects of diet and caloric restriction on adipose tissue fatty acid signatures of tufted puffin (*Fratercula cirrhata*) nestlings

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Received: 9 October 2008 / Revised: 13 March 2009 / Accepted: 19 March 2009
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Abstract Fatty acid (FA) signature analysis is a powerful tool to investigate foraging ecology and food web dynamics in marine ecosystems. However, use of FA signatures to qualitatively or quantitatively infer diets is potentially complicated by effects of nutritional state on lipid metabolism. Estimation of diets using the quantitative fatty acid signature analysis (QFASA) model requires the use of calibration coefficients to account for predator metabolism of individual FAs. We conducted a captive feeding experiment to determine the effects of a 50% reduction in food intake on growth rate and adipose tissue FA signatures of tufted puffin (*Fratercula cirrhata*) nestlings, a species that routinely experiences food restriction during growth. FA signatures of chicks fed low- and high-calorie diets both exhibited a change in composition in response to the dietary shift with the direction of change in the composition of individual FAs matching the direction of change in the dietary FAs. Despite a growth rate in the restricted nestlings

that was 38% of those in the well-fed group, rates of FA turnover were not different between high and low-calorie treatments, and turnover was close to, but not entirely complete, after 27 days on both high-calorie and restricted diets. FA signatures of tufted puffin nestlings were significantly affected by caloric restriction, but these effects were much less pronounced than those of dietary turnover, and calibration coefficients of puffins fed low and high-calorie diets were highly correlated. Our results demonstrate that changes in physiological state can affect FA metabolism, but future research is required to better understand whether the size of these effects is sufficient to substantially alter diet estimation using the QFASA model.

Introduction

Analysis of fatty acids (FAs) is increasingly being used to study foraging ecology and food web dynamics in marine ecosystems (Dalsgaard et al. 2003; Budge et al. 2006; Iverson 2009). FAs are most commonly used in a qualitative manner to assess spatial and/or temporal differences in diet (e.g., Iverson et al. 1997; Williams et al. 2008a) or to confirm the importance of certain prey types in the diets of marine predators (e.g., Raclot et al. 1998; Käkälä et al. 2006; Budge et al. 2007). More recently, FAs have been used to quantitatively estimate predator diets using a statistical model to determine the combination of prey FA signatures that comes closest to matching the predator FA signature after accounting for the effects of predator lipid metabolism (i.e., quantitative FA signature analysis, QFASA; Iverson et al. 2004). The QFASA method uses calibration coefficients to account for predator lipid metabolism by weighting individual FAs according to their tissue deposition relative to diet (Iverson et al. 2004, 2007).

Communicated by G. Heldmaier.

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Calibration coefficients for each FA can be determined empirically by feeding predators diets of known FA composition until complete turnover of storage FA is thought to have occurred. Nevertheless, interpretation of FA data in studies of animal ecology remains potentially complicated by factors that may affect rates of biosynthesis, deposition and metabolism of specific FAs within the predator.

Nutritional state, for example, has the potential to affect biochemical pathways and physiological processes, thus altering predator FA signatures. Numerous free-living animals routinely experience changes in nutritional state associated with different life-history stages, including hyperphagia prior to migration, loss of body mass associated with reproduction and periodic fasting associated with breeding, molt or migration (Moreno 1989; Bairlein 2002). In addition to shifts in nutritional status associated with predictable life-history events, marine predators, including seabirds, may experience unpredictable changes in nutritional state associated with changes in prey availability (Anderson and Piatt 1999). However, studies examining whether these types of changes in physiological state alter the deposition of dietary FAs or the FA composition of endogenous lipid stores in predators are lacking.

The tufted puffin (*Fratercula cirrhata*, hereafter: puffin) is a pelagic diving seabird found in the North Pacific. As a higher predator, puffins can potentially serve as useful indicators of marine ecosystems. For instance, growth rates of puffin nestlings have been found to exhibit high rates of spatial and temporal variability (e.g., Williams et al. 2008b) and puffin reproduction in some locales is correlated with climate variability (Gjerdrum et al. 2003). FAs have been shown to provide important insights into seabird foraging (Iverson et al. 2007). Thus, analysis of FA signatures in puffins may furnish useful information regarding mechanistic links between environmental variability and reproductive parameters of puffins. For example, FA signatures could be used in a qualitative manner to determine whether diets of adults and/or nestlings are different in years characterized by low reproductive output. Estimating the diets of puffin nestlings using the QFASA method might offer insight into the importance of certain forage fishes in nestling diets or changes in the energy density of diets (e.g., Beck et al. 2007). Determination of diet quality would potentially be useful to evaluate bottom-up hypotheses, which postulate that recent declines in apex predator populations are due to a shift in the quality of prey available (Rosen and Trites 2000; Wanless et al. 2005). Thus, although diet has well-established effects on adipose tissue FA composition in higher monogastric predators, a better understanding of the consequences of energy intake on lipid metabolism is needed to evaluate the degree to which changes in physiological state affect FA signatures in free-living populations.

In this paper, we report the results of a captive feeding experiment designed to examine the effect of dietary FAs and energy intake on FAs in growing puffin nestlings. We fed puffin nestlings either a high-calorie diet or a low-calorie diet (50% of high-calorie treatment) and measured changes in adipose tissue FA signatures. The low-calorie diet was designed to mimic levels of food deprivation that puffin chicks routinely experience in the wild (Piatt and Kitaysky 2002). Because food restriction coincided with a shift in diet, we repeatedly sampled adipose tissue for a 27-day period to determine whether observed effects were due to differences in rates of FA turnover or were a consequence of altered FA metabolism. We then evaluated the degree to which FA turnover was complete at the end of the experiment by estimating calibration coefficients for the nestlings that were fed high- and low-calorie diets and compared these coefficients with those obtained for common murrelets (*Uria aalge*) in a previous captive study (Iverson et al. 2007).

Materials and methods

Experimental procedures

We conducted an experimental feeding trial using puffin nestlings reared in their burrows on Cliff Island in Chiniak Bay, Kodiak, AK (57°40'N, 152°20'W) in 2004. Tufted puffins are monogamous with both parents caring for a single chick raised in a burrow excavated in the soil. We located 13 puffin nestlings during the early stages of chick-rearing and excavated vertical access holes, which were patched with flat rocks to permit later access to the nesting chamber. When chicks were estimated to be approximately 10-days old, based on wing chord measurements, we blocked burrow entrances to prevent adults from provisioning their young and began feeding them one meal per day. We fed nestlings either 120 g/day (control group; 650 kJ/day, $n = 6$) or 60 g/day (restricted group; 325 kJ/day, $n = 7$) of Pacific herring (*Clupea pallasii*) plus a multivitamin supplement. We measured body mass using a Pesola spring scale (± 2 g) and biopsied subcutaneous adipose tissue according to the methods of Iverson et al. (2007) on days 0, 9, 18 and 27 of the experimental trial by extracting approximately 100–300 mg of adipose tissue from a <1 cm-long incision located at a site just anterior to the uropygial gland and 1 cm lateral to the spine. We alternated sampling locations between the right and left side of the spine so that each location was sampled only twice. Samples of adipose tissue were immediately placed in chloroform containing 0.01% BHT (butylated hydroxytoluene) as an antioxidant and stored on ice until frozen at -30°C . Blood samples were also taken for analysis of nitrogen and carbon stable

isotopes and these results are reported elsewhere (Williams et al. 2007). After the final biopsy, we fed all chicks ad libitum until they reached the age and size of wild fledglings, at which point we removed obstructions from the burrow entrance and allowed them to fledge on their own initiative or released them to the water.

Diets of free-living puffin nestlings

FA signatures measured in 10-day-old chicks were likely mostly reflective of diets fed by parents since hatch, although it is possible that there was a small residual influence of pre-hatch FA signatures at this stage. We did not determine diets of the 13 study chicks prior to the experiment on Cliff Island, because adult puffins are particularly susceptible to disturbance during incubation and early chick-rearing. However, we collected samples of chick meals from this colony by placing wire screens at burrow entrances (Hatch and Sanger 1992) for 5 weeks following initiation of the feeding trial. Burrow screening prevents adults from entering the burrow, permitting the collection of whole fish dropped at burrow entrances. Because we obtained only 26 meal samples from Cliff Island during this period, we also report on diets from burrows screened at Chiniak Island ($n = 158$ meals), located 22 km east of Cliff Island, and likely similar to those of Cliff Island; puffins are thought to forage within 100 km of their nesting colony (Piatt and Kitaysky 2002). We calculated the percentage composition by weight within each sample and reported the mean percent diet composition for each island.

Laboratory analyses

We extracted lipids from adipose tissue samples and from prey samples according to Folch et al. (1957), as modified by Iverson et al. (2001). We homogenized whole prey items and used a 1.5 g sub-sample for analysis. We used a subset of the prey items collected at both islands and supplemented this with 9 juvenile Pacific cod (*Gadus macrocephalus*) and 13 juvenile salmonids (*Onchorynchus* spp.) that were collected in beach seines within Chiniak Bay. FA methyl esters (FAMES) were prepared from ≤ 100 mg of the lipid extracts using 3.0 ml Hilditch Reagent (0.5 N H_2SO_4 in methanol) in 1.5 ml methylene chloride with BHT, capped under nitrogen and heated to 100°C for 1 h (Budge et al. 2006). We successfully obtained FAMES for all samples with the exception of one biopsy from a 10-day-old chick from the control group; the biopsy for this chick was too small to yield sufficient sample. Thin-layer chromatography revealed that FAMES generated from some prey samples contained fatty alcohols due to the presence of wax esters. We transformed these alcohols into FAs using modified Jones' reagent

(CrO_3 in H_2SO_4), as described by Budge and Iverson (2003), and repeated the transesterification using Hilditch reagent. Following transesterification, FAMES were extracted into hexane, concentrated using nitrogen gas, and then brought up to a final volume of 50 mg FAME/ml hexane. Identification and quantification of FAMES were performed in duplicate using temperature programmed gas-liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m \times 0.25 mm column coated with (50% cyanopropyl), methylpolysiloxane (DB-23), and linked to a computerized integration system (Tubochrom 4 software, PE Nelson, San Jose, CA, USA). Each chromatogram was manually assessed for correct identification of peaks and reintegrated where necessary.

Calibration coefficients

An integral part of quantitative diet estimation using QFASA modeling is the development of calibration coefficients to account for the effects of lipid metabolism within the predator. We used the assumption that turnover of adipose tissue FAs in puffin nestlings was complete by the end of the 27-day herring feeding trial (see "Results") and calculated calibration coefficients for the restricted and control calorie groups. The calibration coefficient of a particular FA is the ratio of the percentage composition of that FA in the adipose tissue of the puffin to the average percentage composition of that FA in herring (see details in Iverson et al. 2004).

Statistical analyses

We performed all statistical analyses using SAS 9.1 (SAS Institute, Cary, NC, USA) and present data as means \pm SE. Because the number of identified FAs (69) greatly exceeded the number of samples, we restricted our analyses to 12 abundant FAs in adipose tissue with overall means $> 1.0\%$ of total FAs and known to reflect dietary intake (Iverson et al. 2004). The 12 FAs that met this criterion accounted for 88% by mass of the total FAs in puffin adipose tissue. Percentages of the 12 FAs were renormalized over 100% and then transformed into log ratios according to the following: $x_{\text{trans}} = \log(x_i/c_r)$ where x_i is the percentage of a given FA, x_{trans} is the transformed FA and c_r is 18:0, a reference FA (Budge et al. 2006). We selected 18:0 a priori, because this fatty acid occurs in high abundance in marine organisms and therefore will be little affected by measurement error. Because the log of zero cannot be taken, values of zero were changed to 0.005, a value considered to be below the minimum detection level of 0.01%. Transformation of raw percentages into log ratios was done to break the constraint that each observation must

sum to a constant (Aitchison 1986). We performed principal component analysis (PCA) on the 11 transformed FAs and extracted the first and second principal components (PC1 and PC2). We used repeated-measures mixed models to determine the effects of age, treatment and the interaction between age and treatment on PC1 and PC2. Use of repeated-measures mixed procedures allowed us to model the covariance structure of each data set and permitted inclusion of the individual for which we lacked FA data at age 10 days (Littell et al. 1998). Calibration coefficients of murre and puffins fed low- and high-calorie diets were examined using Pearson product-moment correlation coefficients.

We performed univariate and multivariate analyses on the prey data using the same 12 FAs previously selected for puffins. These FAs accounted for 85% by mass of the total FAs across all prey species. We performed a principal component analysis on the 11 transformed FA and extracted the first and second principal components. In order to visualize the relationship between prey items and puffin chicks, we computed PC scores of puffins based on the factor loadings from the PCA on prey data and plotted puffin PC scores on the same figure as fish PC scores. For fishes, we tested for differences between PC1 and PC2 using Kruskal–Wallis tests (ANOVA on ranks) followed by post-hoc Tukey HSD tests with alpha adjusted using a Bonferroni correction (e.g., $\alpha = 0.05/2 = 0.025$). We also conducted univariate analyses on the 12 selected FAs; we used Kruskal–Wallis tests followed by Tukey HSD tests with alpha adjusted using a Bonferroni correction ($\alpha = 0.05/12 = 0.0042$). Non-parametric Kruskal–Wallis tests were used because raw and log-ratio transformed data failed to meet the homogeneous variance assumption required for parametric methods. The Bonferroni correction controls the family-wise error rate, but is extremely conservative and increases the likelihood of making a type II error.

Results

Diets of free-living puffin nestlings

We collected 28 meal samples comprising 45 fish on Cliff Island and 158 meal samples comprising 718 fish on Chiniak Island. It was likely that most meal samples collected from Cliff Island were only partial meals; 18 of 28 meal samples comprised a single fish. At both islands, diets were dominated by Pacific sand lance (*Ammodytes hexapterus*) and, to a lesser extent, capelin (*Mallotus villosus*). The estimated diet composition based on burrow screening for nestlings at both islands is shown in Table 1.

Table 1 Mean diet composition (% by mass) of tufted puffin nestlings on Cliff and Chiniak Islands, as estimated using the burrow screening method in August 2004

Prey species	Diet composition (% by mass)	
	Cliff Island (<i>n</i> = 26 meals)	Chiniak Island (<i>n</i> = 158 meals)
Pacific sand lance <i>Ammodytes hexapterus</i>	76	65
Capelin <i>Mallotus villosus</i>	12	21
Pacific sandfish <i>Trichodon trichodon</i>	8	5
Pacific cod <i>Gadus macrocephalus</i>	0	4
Salmonid <i>Onchorynchus</i> spp.	4	3
Others	1	2

Growth rate and FA signatures in control-fed puffin nestlings

Food restriction severely decreased the rate of mass gain: mass gain of nestlings in high- and low-calorie groups was 17.1 g/day and 6.6 g/day, respectively (Fig. 1a). Mass was significantly affected by age ($F_{3,33} = 601.09$, $P < 0.0001$), treatment ($F_{1,11} = 212.77$, $P < 0.0001$), and the interaction between age and treatment ($F_{3,33} = 109.90$, $P < 0.0001$). We extracted two significant principal components from the PCA on transformed FAs; PC1 and PC2 accounted for 61 and 21% of the total variation in the 12 selected FAs, respectively. After the switch from fishes fed by parents to a herring diet, PC1 increased rapidly in both the restricted and control groups, but did not quite reach an asymptotic level in either group by the end of the experiment (Fig. 1b). PC1 was significantly affected by age ($F_{3,11} = 263.59$, $P < 0.0001$) and was positively affected by restriction ($F_{1,11} = 9.63$, $P = 0.010$). The interaction between age and treatment approached significance ($F_{3,11} = 3.20$, $P = 0.066$). PC2 only increased in the control group. PC2 was significantly affected by age ($F_{3,11} = 4.70$, $P = 0.024$) and was negatively affected by food restriction ($F_{1,11} = 15.86$, $P = 0.002$, Fig. 1c), but the interaction between age and treatment was not significant ($F_{3,11} = 1.45$, $p = 0.28$). Absolute levels of the 12 most abundant FAs changed in a similar manner in both treatment groups over the course of the feeding experiment, although some differences were evident between those on control versus restricted-calorie diets (Fig. 2). In both treatment groups, changes in the FAs were most pronounced early after the switch to the pure herring diet and appeared to have almost reached asymptotic levels by 28–37 days (Fig. 2).

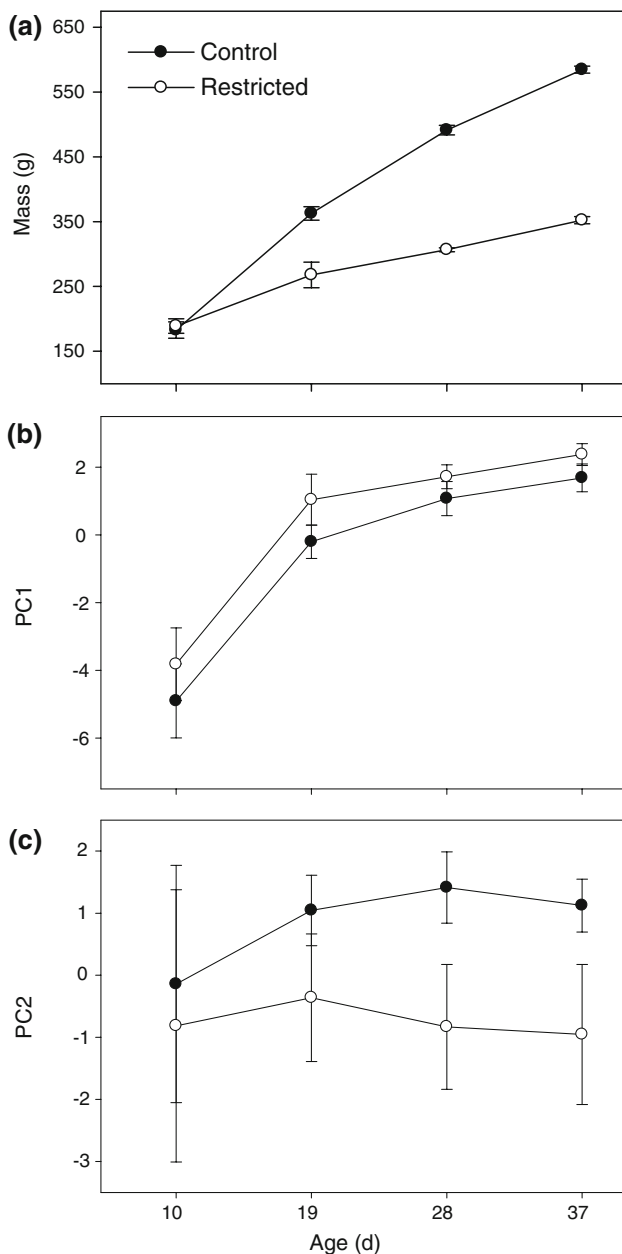


Fig. 1 Changes in the values of: **a** mass, **b** PC1, and **c** PC2 (mean \pm SE) of tufted puffins with age for high-calorie (650 kJ per day, $n = 6$, filled circles) and low-calorie diet (325 kJ per day, $n = 7$, open circles). PC1 and PC2 are the first and second principal components output from a PCA on transformed fatty acids (FAs). PC1 and PC2 account for 61 and 21% of the total variation in select FAs, respectively. Nestlings were fed by their parents until 10 days of age and then switched to a diet consisting exclusively of herring for the duration of the experiment

Prey FA signatures

Univariate analyses revealed that prey FA signatures differed significantly between species ($P < 0.0042$ for all 12 FAs except 18:1n-9, Fig. 3). Differences in the levels of

FAs that were most abundant in sandlance and capelin, the two primary prey fed to chicks prior to the start of the experiment (Table 1), compared to the herring, fed from 10–37 days, were reflected similarly in the increases or decreases observed in these FAs in the puffin chicks following the diet switch (Figs. 2, 3). Absolute levels of these FAs were also similar between prey species eaten and the puffins. The first and second principal components from a PCA of prey species using the 11 transformed FAs accounted for 60 and 16% of the total variation, respectively (Fig. 4). Following the switch to a herring-only diet, PC scores of puffin chicks moved towards the PC scores of herring, although differences between puffins fed high- and low-calorie diets and between puffins and herring were evident (Fig. 4). PC1 differed among species ($F_{5,104} = 27.61$, $P < 0.0001$); post-hoc Tukey tests indicated no significant difference in PC1 among herring, sandlance and capelin, but all three species differed ($P < 0.025$) from sandfish, cod and salmon, which formed a second group of fishes that were not significantly different from one another. PC2 also differed among species ($F_{5,104} = 4.54$, $P < 0.001$); post-hoc Tukey tests indicated the mean PC2 value of cod was significantly different ($P < 0.025$) from that of herring and salmonids.

FA turnover and calibration coefficients

Estimated calibration coefficients calculated for puffins fed a low-calorie constant diet of herring from 10–37 days were significantly correlated with those of chicks fed a high-calorie diet ($r = 0.93$, $P < 0.0001$; Fig. 5). Puffin calibration coefficients for the 12 most abundant FAs were also correlated between low- and high-calorie groups ($r = 0.90$, $P < 0.0001$). The full set of puffin calibration coefficients were correlated with those previously obtained for murres (low calorie: $r = 0.73$ and $P < 0.0001$; high calorie: $r = 0.57$ and $P < 0.0001$). The largest difference between calibration coefficients of murres and puffins was associated with 20 and 22 carbon monounsaturated FAs, which were higher in murres. If calibration coefficients for these particular FAs were discarded, puffin calibration coefficients were more highly correlated with murre calibration coefficients (low calorie: $r = 0.80$ and $P < 0.0001$; high calorie: $r = 0.80$ and $P < 0.0001$).

Discussion

Puffin nestlings fed a calorically restricted diet had severely reduced growth rates (38% compared to those fed a high-calorie diet). Nevertheless, for 11 of the 12 selected FAs, the change in percent composition was in the same direction for puffins fed low- and high-calorie diets, following a

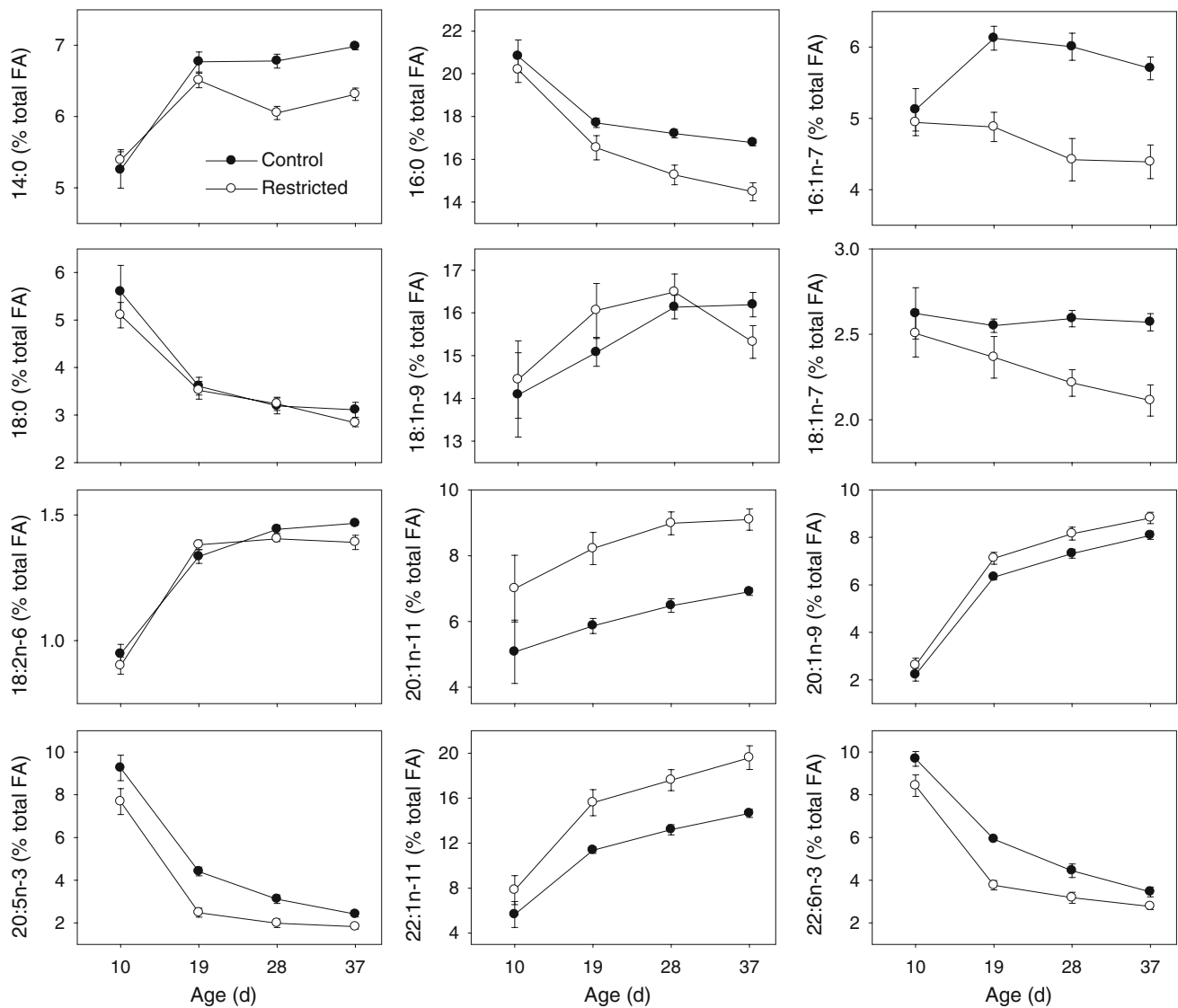


Fig. 2 Changes in the 12 most abundant fatty acids (mean mass percentage of total FAs \pm SE) in adipose tissue of tufted puffins with age for high-calorie (650 kJ per day, $n = 6$, filled circles) and low-

calorie diet (325 kJ per day, $n = 7$, open circles). Nestlings were fed by their parents until 10 days of age and then switched to a diet consisting exclusively of herring for the duration of the experiment

switch from a diet that was likely composed primarily of sandlance and capelin to a diet composed exclusively of herring. Turnover of FA signatures was close to, but not entirely, complete after 27 days on the controlled diet. However, based on the second principal component (PC2, Fig. 1), and some individual FA patterns (Fig. 2), we suggest that nutritional restriction affects adipose tissue FA signatures. Examination of changes in individual FAs supports our inferences based on PCA: turnover was not entirely complete at the end of the experiment, yet some FAs from food-restricted and control groups appeared to be approaching different asymptotic levels. Nevertheless, using the assumption that turnover was complete after 27 days on the controlled diet, calibration coefficients estimated for puffins fed high- and low-calorie diets were correlated (Fig. 5). FA

calibration coefficients calculated for puffins were also correlated with coefficients calculated for common murres fed a silverside (*Menidia menidia*) diet since hatch.

An important consideration when manipulating energy intake in growing animals is determining whether experimental procedures effectively mimic natural conditions. In our study, mass gain of nestlings during the linear growth phase (age 10–30 days; Gjerdrum 2001) in high- and low-calorie groups was 17.15 and 6.55 g/day, respectively. Piatt and Kitaysky (2002) review 49 colony years of nestling growth data and report an overall mean growth rate of 10.9 g/day \pm 4.7 SD (range: -0.6 to 19 g/day). Thus, growth rates of food-restricted birds in our experiments were less than 1 SD below the overall mean and well within the range reported for wild birds. Growth rates of

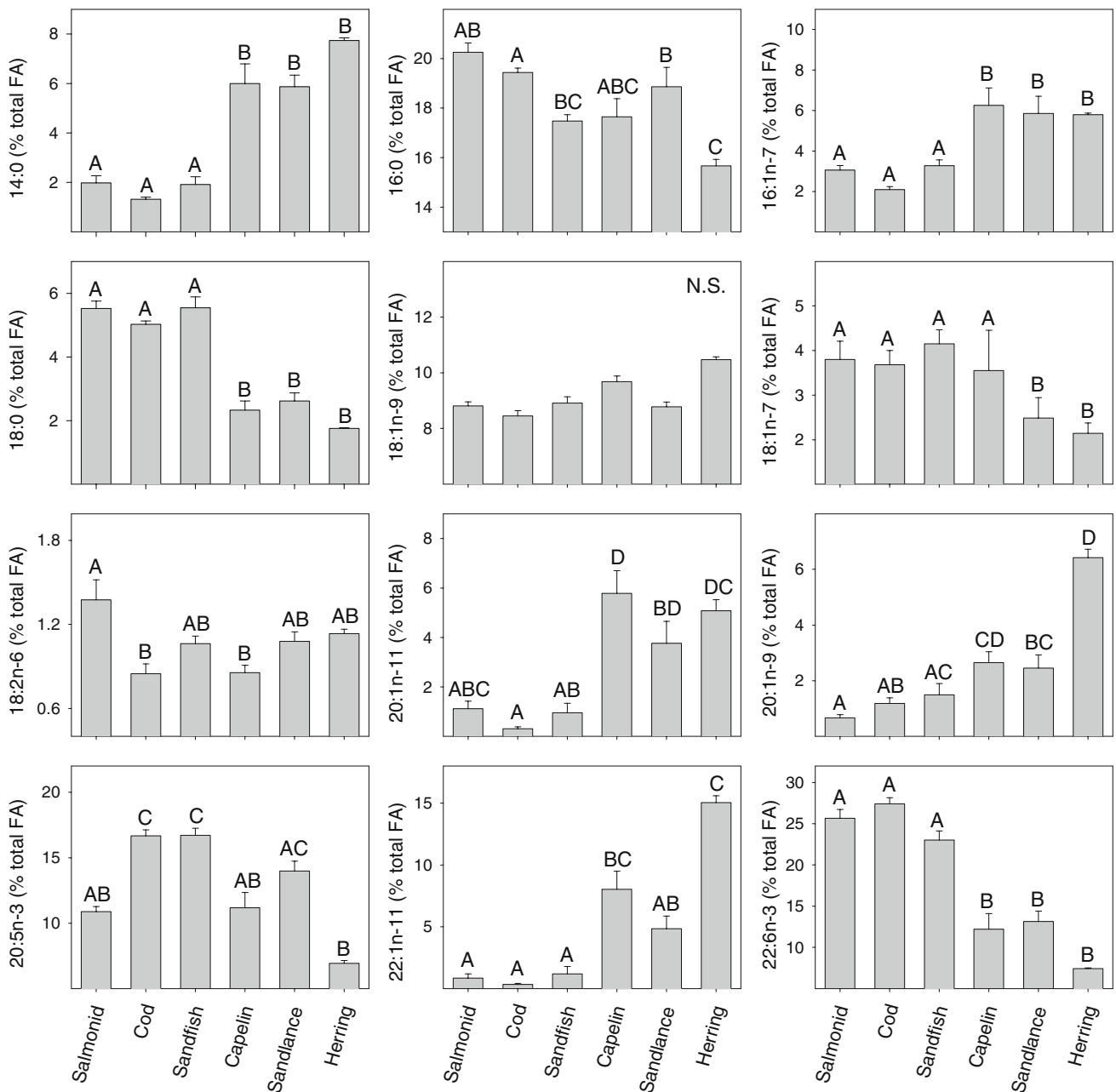


Fig. 3 Levels of the 12 most abundant FAs (mean mass percentage of total FAs \pm SE) in prey items fed to tufted puffin nestlings in 2004; FAs listed are the same as those used in puffins (Fig. 2). Prey species fed to nestlings by their parents from 0 to 10 days are presented in increasing order of importance (see Table 1), followed by herring that

was fed by hand to chicks from 10 to 37 days. In each panel, different letters indicate significant differences among species for that particular FA (Kruskal–Wallis tests followed by Tukey HSD tests with alpha adjusted using a Bonferroni correction. e.g., $\alpha = 0.05/12 = 0.0042$). Sample sizes for each group are shown in Fig. 4

the high-calorie group approached the maximum level observed in the wild.

We used a repeated sampling design to determine whether the observed effects were due to differences in turnover rates or to changes in the characteristics of lipid metabolism. We did not find a significant interaction between age and treatment for either PC1 or PC2, suggesting that the turnover was similar among groups, although

our sample size was small. Turnover of FAs is a function of both metabolic turnover and accretion of additional fat stores during growth. We predicted that a greater accumulation of fat stores in chicks fed a high-calorie diet would have a greater dilution effect, resulting in more rapid turnover. Oyan and Anker-Nilssen (1996) determined that the amount of subcutaneous fat stored by Atlantic puffin (*Frat-ercula arctica*) chicks depends on the caloric intake; yet,

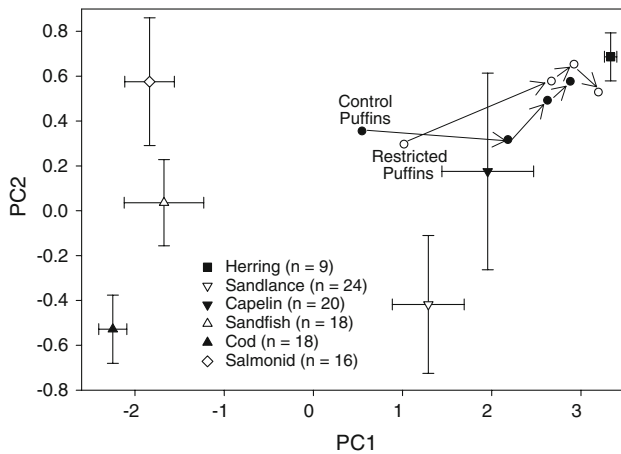


Fig. 4 Principal component analysis (PCA) of the FAs in prey items fed to chicks in 2004. PCA was performed using transformed FAs. Herring was fed by hand to chicks in the experiment (10–37 days), whereas other fishes were fed prior to 10 days to nestlings by their parents at Cliff and Chiniak Islands. PC scores of tufted puffins fed high-calorie ($n = 6$) and low-calorie ($n = 7$) diets were calculated using the loading factors from the PCA of prey species. *Arrows* indicate the direction of change in FA signatures of puffin chicks. Chicks were sampled at ages 10, 19, 28 and 37 days. The first (PC1) and second (PC2) principal components accounted for 60 and 16% of the total variation in fish FAs, respectively. Values are mean \pm SE except for puffins where SE is omitted for clarity

we found no evidence for more rapid turnover in the high-calorie group. The apparent lack of the effect of energy intake on FA turnover may indicate that food-restricted chicks mobilized more fat stores than well-fed chicks to

meet their energetic demands, resulting in a similar turnover rate between the two groups.

In addition to dietary FA composition, predator FA signatures depend on (1) metabolism of ingested FAs for energy, (2) potential modification of exogenous FAs through elongation and desaturation, (3) possible selective mobilization and/or deposition of stored FAs, and (4) de novo synthesis of FAs. Changes in nutritional state may alter FA signatures through any of these mechanisms. For example, in general, de novo FA synthesis occurs in animals on low-fat/high-carbohydrate diets and is inhibited by high-fat diets or during fasting. A decrease in de novo FA synthesis has also been demonstrated during fasting in neonatal chicks fed high-carbohydrate diets (e.g., Back et al. 1986). In our study, four of the six FAs (14:0, 16:0, 16:1n-7, and 18:1n-7) that are likely to be synthesized de novo within birds or mammals were less abundant (measured as percentage of total FA) in food-restricted puffins, which is consistent with rates of de novo synthesis being depressed with poor nutritional state in growing chicks. The two remaining FAs (of the 12 selected) that are likely to be synthesized de novo (18:0 and 18:1n-9) did not show a consistent difference between the high- and low-calorie groups. Thus, more research is required to determine the effects of nutritional state on de novo synthesis of FAs in rapidly growing animals fed diets rich in protein and lipids.

Effects of nutritional state on adipose tissue FAs might also occur through mechanisms other than de novo synthesis. Fasting in laboratory rats, for instance, alters adipose

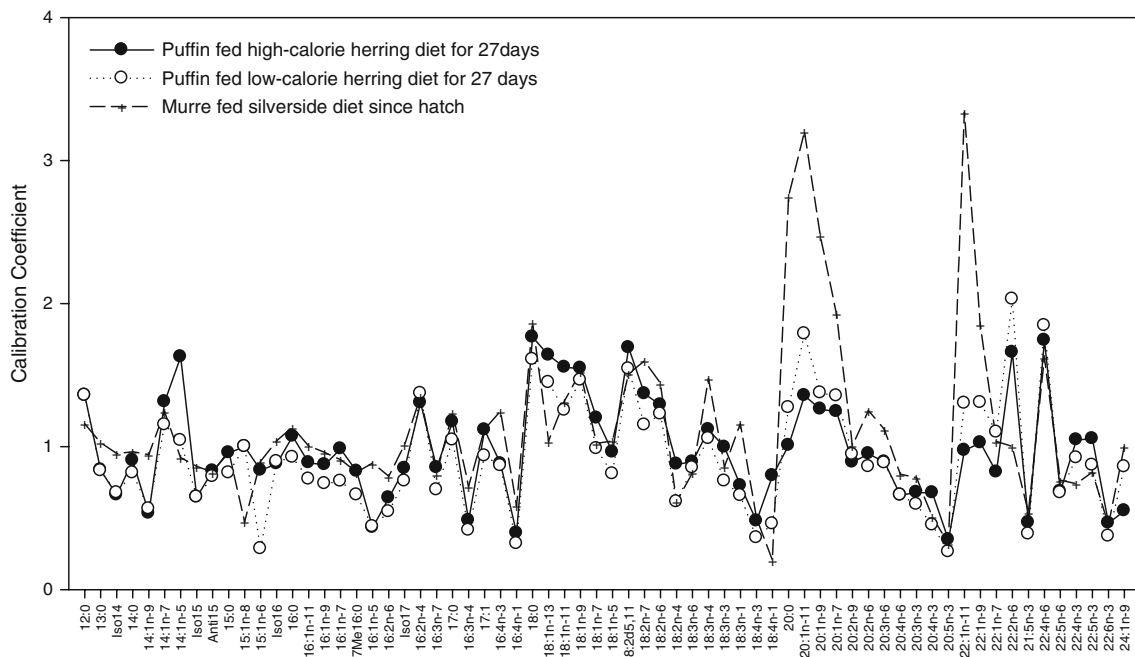


Fig. 5 Calibration coefficients for tufted puffins fed a high-calorie herring diet (650 kJ per day, *filled circles*) and a low-calorie herring diet (325 kJ per day, *open circles*). Calibration coefficients for common

murre fed a diet of silverside (247 kJ per day, *plus symbol*) are shown for comparison (from Iverson et al. 2007)

tissue FA signatures (Raclot and Groscolas 1995) apparently through selective re-uptake of FAs during lipolysis (Raclot and Oudart 2000). Although 20:5n-3 (one of the most readily mobilized FAs in rats) did not appear to be strongly affected by restriction in puffins, we did find higher levels of 22:1n-11 and 20:1n-11 in restricted chicks and these two FAs tend to be among the least mobilized FAs (reviewed in Raclot 2003). Effects of restriction on shorter chain 14–16 carbon saturated and mono-unsaturated FAs may have been the result of selective mobilization in restricted chicks, rather than increased biosynthesis in well-fed chicks.

Effects of nutritional restriction on the biochemical pathways responsible for modification of FAs via elongation and desaturation of FAs could also influence FA signatures. In birds, dietary triacylglycerides are packaged into portomicrons prior to secretion into the portal vein and subsequent uptake by the liver. Within the liver, dietary lipids are repackaged with FAs synthesized de novo as very low-density lipoproteins (VLDL), which are secreted into the blood (reviewed in Klasing 1998). Because dietary lipids are routed through the liver in birds, it is possible that FAs are modified, via elongation and desaturation, to a greater extent than in mammals. However, predicting the consequences of nutritional restriction on FA metabolism in piscivorous seabirds is difficult, because within birds these pathways have been studied extensively only in Galliformes (poultry). Given the large amounts of long-chain PUFAs available in their marine diet, it seems likely that the tendency of seabirds to synthesize and modify certain PUFAs may be greatly reduced (Iverson 2009).

Our results indicate that FA signatures of adipose tissue can be affected by changes in nutritional state in growing seabirds. However, the size of these effects was small relative to the change in dietary FAs. Thus, FA signatures of chicks fed low- and high-calorie diets both changed in response to the diet switch. When a particular FA had a higher concentration in herring compared to sandlance and capelin, then the concentration of that FA in puffin adipose tissue increased following the diet switch, and when the concentration was lower in herring, the concentration of that FA decreased in puffin tissue. PC scores of puffin FAs calculated from the loading factors of the fish PCA also moved towards herring PC scores during the experiment (Fig 4). Finally, calibration coefficients of chicks on low- and high-calorie diets were highly correlated. Future work is needed to determine how robust diet estimation, using the QFASA model, is to the effects of nutritional state on lipid metabolism observed in our study. For example, Iverson et al. (2004) found that diet estimation based on QFASA modeling produced similar results when slightly different sets of calibration coefficients were used. Ultimately, the error in diet estimation will depend on the

degree of inter-specific variability in potential prey species being modeled. The correlation between puffin and murre calibration coefficients (Fig. 5) suggest that metabolism of ingested lipids is comparable between these two species of alcids. However, murre calibration coefficients were exceptionally high for 20–22 carbon mono-unsaturated FAs (i.e., 20:1 and 22:1). This difference may be due to the nature of the FA signatures of silverside fed to murre (in silverside, absolute levels of these 20:1 and 22:1 FAs were extremely low: 0.1–0.8%), especially in comparison with those found in herring (up to 15%, Fig. 3), and thus estimations of calibration coefficients for these FAs in murre may be affected.

Our experimental approach did not allow us to determine the underlying mechanism(s) responsible for either the overall high similarity between high- and restricted-calorie nestlings in turnover and deposition of dietary FAs or the differences that remained in certain FAs. Without question, dietary FAs were the primary determinant of changes in adipose tissue FAs in both groups. However, effects associated with caloric restriction were evident. Future studies using such techniques as radiolabeled precursors (e.g., Budge et al. 2004) could help elucidate the processes responsible for these differences. It will also be important to assess influences of such effects on the eventual QFASA estimates of diets. Although FA signature analysis shows great potential for diet assessment in marine ecosystems, such controlled experiments will clearly help to identify refinements in the methods.

Acknowledgments Funding was provided by the North Pacific Research Board (NPRB #04-13) to CLB and CTW and by NOAA/NMFS (NA16FX1270) to CLB. Additional financial support was provided to CTW by AK EPSCoR, and to SJI by Natural Sciences and Engineering Research Council (NSERC), Canada. We thank T. Cooper, R. Orben, S. Runck and E. Whidden for assistance with field and laboratory work. We are grateful to S. Al-Shaghay and S. Lang for assistance with FA signature analysis. We thank the three anonymous reviewers for their helpful comments on an earlier version of this manuscript. All procedures were approved by the Institutional Animal Care and Use Committees at the University of Alaska Fairbanks (#05-43) and authorized under state and federal permits.

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