Comparative Biochemistry and Physiology, Part A 154 (2009) 165–172

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effect of dietary fatty acid composition on fatty acid profiles of polar and neutral lipid tissue fractions in zebra finches, *Taeniopygia guttata*

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ARTICLE INFO

Article history:
Received 20 April 2009
Received in revised form 4 June 2009
Accepted 4 June 2009
Available online 11 June 2009

Keywords:
Fatty acid methyl ester
Gas chromatography
Lipid metabolism
Omega-3 PUFA
Passerine birds
Phospholipids
Triacylglycerols
Unsaturation index

ABSTRACT

The growing awareness that the fatty acid (FA) composition of the diets of birds, and ultimately their tissues, influence physiological performance variables, such as aerobic capacity, thermosensitivity, digestive efficiency, *etc.*, underscores the need to understand how differences in dietary fatty acid composition actually translate into differences in the fatty acid composition of specific tissues. We quantified the fatty acid profiles of polar and neutral lipid fractions of several tissues in zebra finches (*Taeniopygia guttata*) and compared these profiles among birds fed either a control diet of only hulled millet, or one of two experimental diets of hulled millet supplemented with either 8% (by mass) sunflower seed oil (ω6-enriched diet) or linseed oil (ω3-enriched diet). We found that different lipid fractions vary widely in their diversity and complexity of FA composition, with neutral lipids being much less structurally diverse than those of polar lipids, for example, and that the fatty acid compositions of different organs exhibited different propensities to be altered by the diet, with brain and cardiac tissues having lower levels of flexibility than skeletal muscle and liver. We also present evidence suggesting that adipose tissue may be used to sequester essential FAs when they occur in the diet at levels that exceed immediate requirements. We conclude that the fatty acid composition of adipose tissue may not be a particularly useful indicator of the dietary FA composition of birds, and suggest that future studies investigating the relationships between the FA profiles of bird tissues and bird diets and/or physiological performance variables examine multiple tissues and distinguish between neutral and polar lipid fractions.

1. Introduction

Given their comparatively high mass-specific metabolic rates (Blem, 1990; Nagy et al., 1999; McNab, 2002), and the weight limitations associated with flapping flight (Tucker, 1971; Dawson et al., 1983; Ekman and Hake, 1990; Videler, 1995; Harrison and Roberts, 2000; Biebach and Bauchinger, 2003), small birds are particularly reliant on lipids, especially β-oxidation of fatty acids (FAs), for fuel. Previous studies have shown that the FA compositions of avian tissues are variable and dependent on diet and/or are influenced by season (Morton and Liebman, 1974; Conway et al., 1994; Diaz, 1996; Guglielmo et al., 2002; Maillet and Weber, 2007). While changes in diet may be confounded by seasonal events, they are generally thought to be driven by fruiting cycles of plants, breeding cycles of insects, and reproductive cycles of the birds themselves, as well as changes in foraging habitat in the case of migratory species. Seasonal changes in tissue FA composition are usually thought to result from the need to optimize the fluidity of fat depots in order to facilitate lipid mobilization and maintain viscosity of cell membranes under varying thermal conditions (Zar, 1977a,b; Yom-Tov and Tietz, 1978; Conway et al., 1994; Guglielmo et al., 2002; Newman et al., 2002; Egeler et al., 2003; Maillet and Weber, 2007).

The few reported comparative studies of lipid composition among birds provide compelling evidence that FA composition differs among species (Lovern, 1938; Walker, 1964; Moss and Lough, 1968; Caldwell, 1973). Attempts to compile the results of these studies to elucidate generalizable patterns of FA composition among birds have generally been unable to arrive at robust conclusions (see reviews by Blem, 1976 and McWilliams et al., 2004). Likely explanations for this are that most studies of the FA composition in birds, examined a single tissue, usually adipose or muscle, or the whole body (West and Meng, 1968; Johnston, 1970; Caldwell, 1973; Morton and Liebman, 1974; Yom-Tov and Tietz, 1978; Austin, 1993; Conway et al., 1994; Xu et al., 1994; Newman et al., 2002; Egeler et al., 2003; Nagahuedi et al., 2009). In other studies, no distinction was made between polar and neutral lipid fractions when non-adipose tissues were examined (Blem, 1976; Zar, 1977a,b; Crespo and Esteve-Garcia, 2001; Thil et al., 2003; Horbanczuk et al., 2004; Pierce et al., 2004, 2005). Our inability to identify clear comparative patterns about the FA composition of lipids

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doi:10.1016/j.cbpa.2009.06.002

is probably also related to natural differences in diets among the species examined.

Given that diets containing particular FA profiles have been shown to influence physiological performance variables such as growth rate, aerobic capacity, absorption efficiency, and enzyme activity in birds, a growing number of studies have suggested that birds might select foods containing particular FA compositions (Bairlein and Simons, 1995; Diaz, 1996; Crespo and Esteve-Garcia, 2001; Bairlein, 2002; McWilliams et al., 2002, 2004; Pierce et al., 2004, 2005; Maillet and Weber, 2007; Nagahuedi et al., 2009). Presently we understand less about how the FA composition of birds' diets affects the composition of FA pools in specific tissues than about the overall physiological effects of the diets.

Neutral acyl lipids, mainly triacylglycerols, are the major components of energy rich fat depots, whereas polar lipids, such as phospholipids, are major components of cell and organelle membranes (Masoro, 1968; Clarenburg, 1992; Christie, 2003; Murray et al., 2003; Berg et al., 2007; Karasov and Martinez del Rio, 2007). If one is guided by nomenclature, one might assume that FAs found in adipose 'depot' tissue are simply 'deposited' following absorption from the diet. From time to time, it has been suggested that the FA profile of depot fat might be a useful bioindicator of the dietary habits of wild birds (e.g., Lovern, 1938; Moss and Lough, 1968; Austin, 1993; Guglielmo et al. 2002). Given that birds preferentially route ingested and absorbed FAs to the liver as portomicrons (Noyan et al., 1964; Krogdahl, 1985), it has been suggested that birds are capable of substantial structural modification of exogenous FAs prior to deposition in tissues (Lovern, 1938; West and Meng, 1968; Zar, 1977b; Bairlein and Simons, 1995; Surai et al., 2000; Bairlein, 2002; Egeler et al., 2003; McWilliams et al., 2004). There is, however, scant empirical evidence for this possibility among non-domesticated birds.

In their laboratory study of redpolls (*Acanthis flammea*) West and Meng (1968) reported that differences in dietary FA composition did not alter the FA profile of adipose tissues, however, in contrast, it was reported that dietary FA composition in European starlings (*Sturnus vulgaris*) and chickens influenced that of their adipose tissues (Yom-Tov and Tietz, 1978; Crespo and Esteve-Garcia, 2001). Because of the paucity of studies on the FA composition of several tissues at once, the extent to which birds other than poultry can postabsorptively modify exogenous FAs prior to allocation to extra-hepatic tissues is not well known (McWilliams et al., 2004).

Given the potential links between physiological performance and fatty acid composition, we hypothesized that dietary FA composition influences the FA composition of tissues of zebra finches (*Taeniopygia guttata*). To explore this hypothesis, we characterized the FA composition of polar and neutral lipid fractions of various organs and tested the following predictions concerning the relationships between the FA profiles of the diet and that of specific tissue fractions: 1) polar lipid fractions of a given tissue will comprise a more diverse FA profile and contain higher levels of long-chain polyunsaturated FAs than neutral lipid fractions; 2) FA profiles of adipose tissue will more closely reflect that of the diet compared than other tissues; and 3) the FA profiles of different tissues vary in the degree that they can be influenced by diet.

2. Materials and methods

Twenty-four adult zebra finches (*T. guttata*) were purchased from a local dealer (Hamakor Hedera, Israel) in the spring of 2008. The finches were maintained in outdoor aviaries (4 m × 3 m × 2 m; $L \times W \times H$) at the Jacob Blaustein Institutes for Desert Research, Midreshet Ben-Gurion, Israel. During the summer of 2008, birds were acclimated to a diet of whole millet seeds, water, and cuttlefish bone, ad libitum. Fresh lettuce was offered to the birds once a week.

Immediately before the feeding experiments described below, birds were weighed to ±0.1 g and individually identified with color-coded, numbered leg bands. The birds were sexed and randomly assigned to one of three diets, each containing a different FA composition (Table 1). The control diet consisted of hulled pearl millet composed of 10.3% moisture, 9.6% crude protein, 3.1% lipids, and 73.4% carbohydrates (Akeredolu et al., 2005). The two experimental diets consisted of hulled millet supplemented with 8% (by wet mass) sunflower seed oil (ω6-enriched diet) or linseed oil (ω3-enriched diet). Water supplemented with multivitamins, and cuttlebone were available ad libitum. Orts were replaced daily with fresh food. Birds were then transferred to six adjacent aviaries (1.5 m × 1.5 m × 2.2 m). To prevent mating, male and female birds were housed separately, but still visible to each other. After 60 days of feeding on their respective diets, eight birds from each group (males and females approx. 1:1 ratio) were weighed, and killed by decapitation. Pectoral muscle, liver, heart, brain, and claviculocoracoid adipose tissue were harvested, placed into individually labeled glass vials, and frozen. Tissue samples were later freeze-dried, and then homogenized using mortar and pestle.

Total lipids were extracted from bird tissues over ice in a 2:1:0.8 ratio of methanol:chloroform:water, following Bligh and Dyer (1959). Lipids were recovered in the chloroform phase, transferred to a separate glass vial, and dried under a stream of N_2. Samples were stored at −20 °C for up to 2 weeks (Christie, 2003) before analysis. Polar and neutral lipid fractions were separated by passing samples through a 500 mg silica column (Bond Elut™; Varian USA) with chloroform for neutral lipids, followed by methanol for polar lipids (Khozin-Goldberg et al., 2005). A C-17 FA standard (Sigma-Aldrich) was added to the evaporated lipid fractions followed by transmethylation for 1 h in anhydrous methanol (Sigma-Aldrich), containing 2% H_2SO_4 (v/v) at 85 °C. The reaction was terminated by addition of water, and fatty acid methyl esters (FAMEs) were extracted in hexane and transferred to 300 μL autosampler vials. FAMEs were separated in a gas chromatograph (GC Ultra, Thermo, Italy) with a capillary column (ZBwax, Phenomonex, USA), programmed temperature vaporization (PTV) injector, and flame ionization detector, using helium as a carrier gas. The temperature program was first a 1 min hold at 130 °C followed by a linear increase of 2 °C min^{-1} to 240 °C, and final hold of 10 min at the high temperature. FAMEs ranging in length from 14 to 24 carbons were identified by comparing chromatograms with retention times of FAMEs from a fish oil standard (Avanti Polar Lipids) resolved in parallel with the unknown samples. All FA analyses were done within 2 months of tissue preparation (Christie, 2003).

The absolute fatty acid compositions of the diets were calculated based on the amount of C-17 tracer added to each sample. Fatty acid proportions are reported as percentages of total FA by mass. Major FAs were considered those that accounted for an average of ≥1% of the total in respective tissue-lipid fractions (Smith et al. 2003; McCue

Table 1
Fatty acid profiles of the three experimental diets (μg FA per mg diet) fed to zebra finches.

	Control diet		ω3 diet		ω6 diet	
	μg/mg	(% total FAs)	μg/mg	(% total FAs)	μg/mg	(% total FAs)
16:0	4.2	11.8	6	8.1	7.9	10.2
16:1	0.1	0.2	0.1	0.1	0.2	0.3
18:0	0.7	2	2.3	3.3	2.3	3
18:1[†]	7.6	21.5	14.1	19.4	23.8	31.2
18:2ω6	22.2	62.2	24.2	33.5	41.8	53.6
18:3ω3	0.4	1	25.6	34.7	0.5	0.6
20:0	0.2	0.5	0.2	0.4	0.3	0.4
20:1ω9	0.2	0.5	0.2	0.3	0.2	0.3
22:0	0.1	0.4	0.2	0.2	0.4	0.5
Total	35.6	100	72.8	100	77.3	100

The control diet consisted of hulled millet, and the ω3- and the ω6-enriched diets consisted of hulled millet supplemented with 8% (by mass) of linseed and sunflower oils, respectively.
[†]Refers to the sum of 18:1ω9 and 18:1ω7 isomers.

2007, 2008). In most cases, it was impossible to resolve reliably 18:1ω9 and 18:1ω7, therefore proportions of these two FAs were combined. Results of the FA profiles of tissues from males and female finches were combined within respective treatment groups once it was determined that there were no detectable differences between them. Analyses of variance (ANOVA) with critical *P*-values Bonferroni corrected for multiple comparisons were used to compare lipid fractions among the three diet treatments, where $P_{critical} = 0.05/$ number of tests (Sokal and Rohlf, 1987). For ANOVAs yielding significant outcomes Fisher's PLSD *post hoc* tests were used to compare the proportions of FAs between pairs of dietary treatments. Ratios of ω3 and ω6 FAs were calculated for the various diets and tissue fractions, and compared among diets using ANOVA and *post hoc* tests as described above. Unsaturation indices (UI) were calculated as the sum of the percent mass of each unsaturated FA (N_i) multiplied by its respective degree of unsaturation (i) and by 100 (Jezierska et al., 1982; Geiser et al., 1992; Pan and Storlien, 1993; Stuart et al., 1998) following McCue (2008):

$$\mathrm{UI} = \left[\sum_{i=1}^{6} N_i \cdot i\right] 100 .$$

3. Results

The total lipid content (%±S.D.) of dry brain, liver, heart, and pectoral muscle averaged 52±3%, 34±3%, 37±6%, and 37±4% respectively, and did not differ significantly among diet treatments. Masses of claviculocoracoid adipose depots were not quantified, but by visual assessment (Gosler, 1996; Jenni et al., 2000; Costantini et al., 2007) appeared substantially greater in the birds fed the ω3- and ω6-enriched diets. Nevertheless according to ANOVA the body masses of birds from the treatment groups were not significantly different.

3.1. Fatty acids of neutral lipids

The FA profiles of the neutral lipids differed among the tissues examined. For example, the liver contained four major FAs that were 20 or more carbon atoms in length, whereas the pectoral muscle contained only one FA that was 20 or more carbon atoms in length. In contrast, adipose tissue contained no major fatty acids that were 20 or more carbon atoms in length (Table 2). All significant diet-induced differences in neutral lipids were found in FAs that had 18 or fewer carbons atoms.

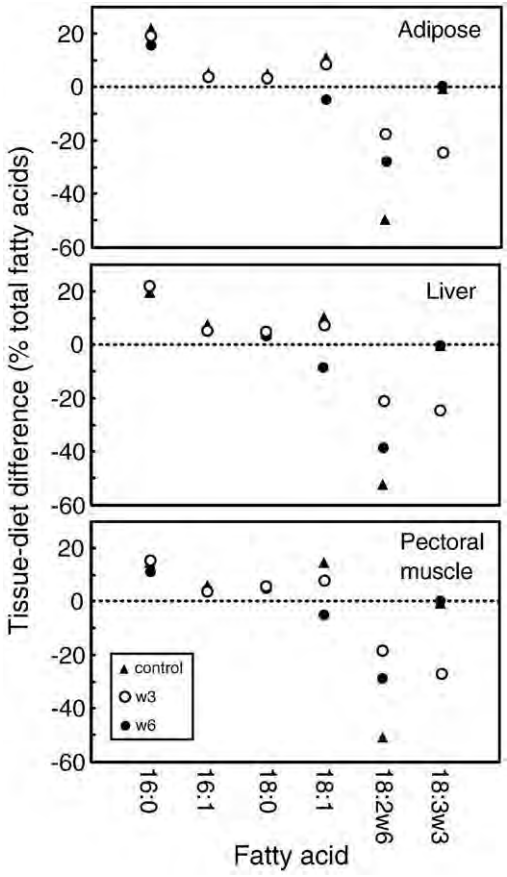

Fig. 1. Differences between the proportions of six dietary FAs compared to the proportion of the same FAs in neutral lipid fractions in zebra finches. Values indicate the proportion of each FA in the tissue of birds fed the experimental diets minus the proportion of measured in birds fed the control diet. Positive values indicate that the FA is found at a disproportionately higher level in the tissue than in the diets, whereas negative values indicate the opposite.

Some FAs, namely 16:0, 16:1, 18:0, and 18:1, were found in higher proportions in the birds' tissues than in their diets (Fig. 1), however,

Table 2
FA composition, as mean% of total FAs (SD) of neutral lipids extracted from organs of zebra finches fed one of three diets (see text and Table 1).

	Adipose				Liver				Pectoral muscle			
Fatty acid	Control	ω3 diet	ω6 diet		Control	ω3 diet	ω6 diet		Control	ω3 diet	ω6 diet	
14:0	**2.7 (0.2)a**	**2.0 (0.2)b**	**2.0 (0.4)b**	*	**2.3 (0.3)**	**1.9 (0.9)**	**2.0 (1.4)**		**1.2 (0.1)**	**1.5 (0.7)**	**1.1 (0.2)**	
14:1	0.6 0.2)	0.8 (0.1)	0.5 (0.5)		0.2 (0.3)	0.2 (0.4)	0.7 (1.1)		**1.1 (0.2)**	**1.2 (1.0)**	**1.1 (0.8)**	
15:0	0.8 (0.2)	trace	0.9 (0.5)		**0.8 (0.6)**	**1.0 (0.7)**	**1.7 (1.3)**		**1.0 (0.3)**	**1.9 (1.7)**	**1.7 (2.2)**	
16:0	**33.6 (1.8)a**	**26.2 (3.7)b**	**25.9 (3.1)b**	*	**31.3 (2.3)**	**29.6 (5.2)**	**32.2 (4.3)**		**26.2 (1.0)**	**22.7 (3.1)**	**20.7 (2.6)**	
16:1	**5.2 (2.2)**	**4.0 (0.5)**	**4.0 (1.1)**		**7.8 (1.5)a**	**5.1 (1.5)b**	**5.4 (0.6)b**	*	**6.2 (1.3)**	**4.4 (1.4)**	**3.9 (1.0)**	
18:0	**6.9 (1.0)**	**6.1 (1.2)**	**6.3 (0.9)**		**6.0 (1.0)**	**6.5 (1.9)**	**7.8 (1.6)**		**7.8 (2.7)**	**8.3 (2.3)**	**8.7 (2.7)**	
18:1†	**32.6 (2.3)**	**29.0 (1.7)**	**29.7 (1.4)**		**31.7 (3.6)a**	**27.0 (3.3)b**	**23.6 (3.5)b**	*	**36.0 (2.7)a**	**27.3 (3.8)b**	**27.9 (2.5)b**	*
18:2ω6	**12.8 (1.7)a**	**15.6 (2.6)a**	**25.8 (4.7)b**	*	**9.8 (1.6)**	**12.4 (2.9)**	**14.8 (4.1)**		**11.6 (1.5)a**	**14.5 (2.1)a**	**24.5 (4.8)b**	*
18:3ω3	**0.4 (0.2)a**	**10.4 (1.1)b**	**0.4 (0.3)a**	*	**0.6 (1.0)a**	**9.8 (2.0)b**	**0.5 (0.7)a**	*	**0.3 (0.1)a**	**8.2 (2.8)b**	**0.4 (0.3)a**	*
20:4ω6	0.2 (0.2)	trace	trace		**2.4 (1.8)**	**0.7 (1.4)**	**2.3 (1.6)**		**2.4 (1.6)**	**1.4 (1.5)**	**2.3 (1.9)**	
22:1ω9	0.7 (0.1)	0.7 (0.5)	0.5 (0.5)		**2.1 (1.1)**	**1.3 (1.4)**	**2.4 (1.3)**		0.4 (0.2)	0.7 (0.5)	0.5 (0.4)	
22:5ω6	0.4 (0.2)	0.5 (0.4)	0.7 (0.7)		**1.1 (0.8)**	**0.9 (1.0)**	**1.4 (1.0)**		0.9 (0.3)	0.7 (0.9)	0.8 (0.4)	
24:1	0.6 (0.1)	0.7 (0.5)	0.6 (0.7)		**1.0 (0.9)**	**0.7 (0.9)**	**1.5 (1.2)**		0.6 (0.7)	0.4 (0.6)	0.4 (0.7)	
% ω3	0.4	10.4	0.4		0.6	9.8	0.5		0.3	8.3	0.4	
% ω6	13.4	16.1	26.5		13.3	14.0	18.5		14.9	16.6	27.6	

Values in bold-face are those FA accounting for ≥1% of the total FAs. Asterisks denote significance between diets and within tissues according to ANOVAs. Different letters refer to significant differences between diets according to Fisher's PLSD ($P<0.05$). The following FA were detected but comprised <1% of the total FAs: 18:3ω6, 18:4ω3, 20:0, 20:1ω9, 20:2ω6, 20:3ω6, 20:4ω3, 22:0, 20:5ω3, 22:5ω3, and 22:6ω3. 'Trace' refers to values ≤0.1%.
†Refers to the sum of 18:1ω9 and 18:1ω7 isomers.

the proportions of linoleic acid (18:2ω6) were lower. The proportion of linolenic acid (18:3ω3) was variable and dependent on diet; only birds fed the ω3-enriched diets had lower proportions than that found in the diet (Fig. 1).

The FA profiles of birds fed the two experimental diets generally contained lower proportions of FAs composed of 14–16 carbons and oleic acid (18:1) than birds fed the control diet (Fig. 2). The tissues of birds fed ω3- and ω6-enriched diets had consistently higher proportions of linolenic and linoleic acid, respectively, than those fed control diets (Fig. 2).

According to a two-way ANOVA with tissue type and lipid fraction as factors the UIs of FAs from neutral tissue lipids were significantly different from those of polar lipids ($P<0.001$) however differences in UI due to tissue type were not significant. Birds fed the ω3-enriched diet had higher UI than birds fed the control diet (Fisher's PLSD; $P=0.0100$; Fig. 3A). Ratios of total ω3:ω6 FAs were 0.01, 0.02, and 1.03 for control, ω6-, and ω3-enriched diets respectively. The ratios of ω3:ω6 FAs found in tissue lipids were influenced by diet ($P=0.0003$; Fig. 3B), and *post hoc* analysis also indicated that the ω3:ω6 ratio of neutral FAs was significantly higher in birds fed the ω3-enriched diet ($P<0.001$).

3.2. Fatty acids of polar lipids

The FA composition of polar lipids from liver, brain, heart, and pectoral muscle had greater diversity of chain length and number of double bonds than neutral lipids. Moreover, the proportions of several

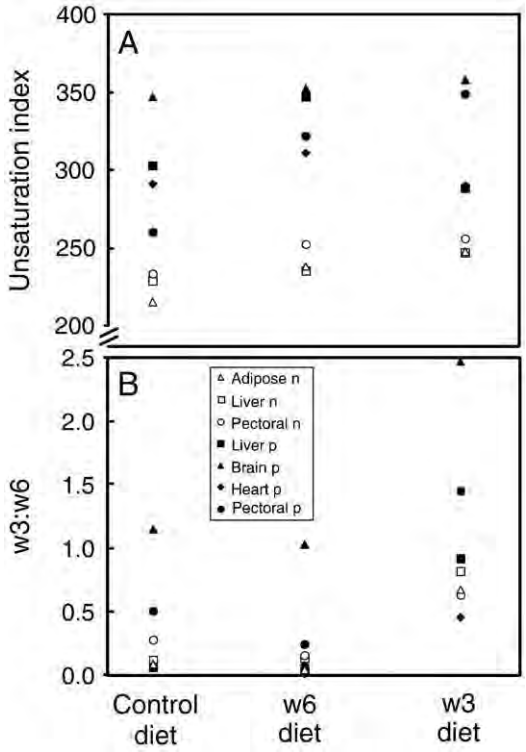

Fig. 3. The difference in (A) unsaturation indices and (B) ω3:ω6 ratios of polar (p) and neutral (n) lipid fractions in zebra finches fed one of three diets. Unsaturation indices for control, ω6- and ω3-enriched diet were 149, 140, and 191, respectively. Ratios of ω3:ω6 for control, ω6-, and ω3-enriched diets were 0.1, 0.2, and 1.0.

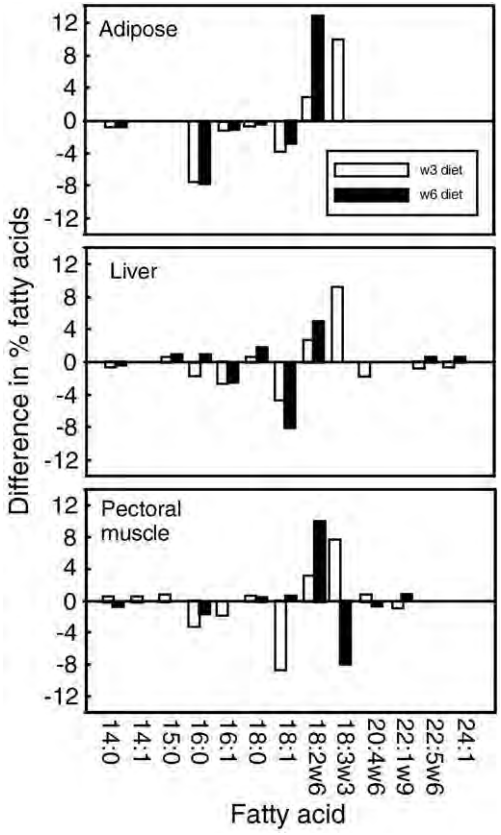

Fig. 2. The effects of two experimental diets on the relative FA composition of neutral lipids in adipose, liver, and pectoral muscle of zebra finches. Values are the mean proportion of FAs in tissues of birds fed ω6-enriched diet (solid bars) and ω3-enriched diet (open bars) subtracted from those measured in birds fed the control diets.

FAs containing 20 or more carbon atoms were significantly influenced by diet (Table 3). Despite having the greatest diversity of major lipid components, the FAs of the brain showed fewer diet-induced changes than other tissues. For example, brain tissue had fewer significant, diet-induced changes among individual FAs (Table 3; Fig. 4) that accounted for at least 1% of the total FAs. This 'conservative' pattern was also observed in heart muscle lipids, albeit to a lesser degree. Of the four polar fractions examined, the FA composition of pectoral muscle changed the most in response to diet (Fig. 4).

The effect of diet on the FA profiles of the polar lipids in the four tissues was generally similar to that observed for neutral lipids from the same tissues. For example, the saturated FAs, 16:0 and 18:0, accounted for larger proportions in the tissues than in the respective diets, whereas the proportions of linoleic acid in tissues were consistently lower than those of the diets (Fig. 5). As in the neutral lipid fractions, the proportion of linolenic acid in tissue lipids was lower than that found in the ω3-enriched diet.

The UI of polar lipid fractions was consistently higher than that of neutral lipid fractions. On average, the FA profile of the brain had the highest UIs, mainly due to the abundance of long-chain polyunsaturated fatty acids (PUFAs), and appeared to be the least influenced by diet. Ratios of ω3:ω6 FAs were highest in birds fed ω3-enriched diet, and the heart lipids had the lowest measured ω3:ω6 ratio in birds fed all three diets (Fig. 5).

4. Discussion

We found that in the zebra finches neutral lipid fractions generally contained higher proportions of palmitic (16:0) and oleic acid (18:1), and contained lower proportions of PUFAs than the polar lipid fractions. This supports our first prediction that neutral and polar lipid

Table 3
FA composition, as mean% of total FAs (SD) of polar lipids extracted from organs of zebra finches fed one of three diets (see text and Table 1).

	Liver				Brain				Heart				Pectoral muscle			
	Control	ω3 diet	ω6 diet		Control	ω3 diet	ω6 diet		Control	ω3 diet	ω6 diet		Control	ω3 diet	ω6 diet	
16:0	**18.9 (2.3)a**	**15.7 (1.7)b**	**14.5 (0.7)b**	*	**21.5 (4.4)**	**20.1 (1.6)**	**19.3 (1.0)**		**15.4 (2.1)a**	**14.5 (1.9)a**	**11.2 (2.0)b**	*	**23.2 (2.6)a**	**19.3 (2.3)b**	**15.0 (0.9)c**	*
16:1	**3.2 (0.9)a**	**1.9 (0.4)b**	**2.0 (0.4)b**	*	**1.4 (0.2)**	**1.2 (0.2)**	**1.0 (0.4)**		**1.9 (0.5)a**	**1.3 (0.3)b**	**0.8 (0.5)b**	*	**4.9 (1.5)a**	**0.7 (0.1)b**	**0.9 (0.4)b**	*
18:0	**18.2 (1.8)a**	**23.3 (1.9)b**	**23.5 (1.7)b**	*	**18.2 (0.9)**	**19.3 (1.4)**	**19.6 (1.9)**		**24.3 (2.5)**	**26.5 (1.9)**	**25.0 (2.4)**		**16.7 (2.3)**	**21.9 (2.3)**	**23.0 (2.0)**	
18:1†	**19.9 (2.0)a**	**12.5 (2.4)b**	**13.0 (2.9)b**	*	**15.1 (1.8)**	**14.6 (2.2)**	**14.4 (2.9)**		**13.5 (2.2)**	**10.5 (3.3)**	**9.2 (2.4)**		**20.5 (3.3)a**	**7.2 (0.9)b**	**9.5 (2.2)b**	*
18:2ω6	**14.6 (1.7)a**	**15.5 (1.3)a**	**20.3 (1.3)b**	*	**1.7 (0.4)**	**1.8 (0.6)**	**1.8 (0.7)**		**17.0 (0.8)a**	**18.8 (1.8)b**	**23.6 (0.7)c**	*	**11.7 (3.3)a**	**11.9 (1.7)a**	**19.1 (1.3)b**	*
18:3ω3	**0.2 (0.3)a**	**8.4 (1.3)b**	**0.3 (0.3)a**	*	0.3 (0.3)	1.1 (0.4)	0.3 (0.2)		**0.4 (0.3)a**	**7.5 (1.4)b**	**0.5 (0.2)a**	*	**0.7 (0.5)a**	**5.8 (0.7)b**	**0.4 (0.4)a**	*
20:3ω6	**1.4 (0.4)**	**0.6 (0.6)**	**1.3 (0.3)**		**1.3 (0.3)**	**1.0 (0.3)**	**1.2 (0.3)**		0.4 (0.2)	0.3 (0.1)	0.3 (0.2)		0.6 (0.4)	0.2 (0.2)	0.3 (0.2)	
20:4ω6	**16.8 (2.5)a**	**5.8 (0.4)b**	**19.0 (2.2)a**	*	**10.1 (1.2)a**	**5.6 (0.4)b**	**10.7 (0.8)a**	*	**19.4 (3.1)a**	**10.4 (3.4)b**	**21.4 (2.3)a**	*	**3.4 (0.6)a**	**6.6 (1.3)b**	**17.2 (1.5)c**	*
20:5ω3	**0.3 (0.3)a**	**10.8 (1.5)b**	**0.2 (0.4)a**	*	**0.6 (0.5)a**	**3.4 (0.5)b**	**0.9 (0.5)a**	*	**0.3 (0.2)a**	**4.0 (1.7)b**	**0.3 (0.3)a**	*	**0.6 (0.4)a**	**2.7 (0.6)b**	**0.1 (0.2)a**	*
22:5ω6	0.7 (0.4)	0.3 (0.5)	0.8 (0.2)		**4.3 (1.1)**	**2.5 (1.4)**	**5.4 (1.6)**		0.9 (0.3)	0.3 (0.4)	1.0 (0.1)		1.2 (0.6)	0.3 (0.2)	1.4 (0.3)	
22:5ω3	0.7 (1.3)	0.9 (2.4)	trace		**1.7 (1. 4)a**	**3.6 (0.5)b**	**1.3 (0.5)a**	*	0.3 (0.3)	0.2 (0.4)	0.2 (0.2)		**0.2 (0.2)a**	**2.4 (0.6)b**	**0.5 (0.1)a**	*
22:6ω3	0.3 (0.4)	0.8 (0.4)	trace		**16.9 (2.1)**	**19.3 (2.7)**	**16.9 (2.6)**		**1.4 (0.7)**	**1.5 (0.8)**	**2.0 (1.1)**		**6.8 (0.5)a**	**17.0 (3.7)b**	**8.1 (1.7)a**	*
24:1	0.5 (0.4)	0.5 (0.4)	0.8 (0.5)		**2.8 (1.8)**	**2.2 (1.5)**	**2.6 (1.4)**		0.5 (0.4)	0.6 (0.3)	0.7 (0.40)		0.6 (0.7)	1.2 (0.2)	0.8 (0.3)	
% ω3	1.1	20.9	0.5		5.4	27.4	19.4		2.4	13.2	3.0		8.3	27.9	9.1	
% ω6	32.8	22.2	41.4		17.4	10.9	19.1		37.7	29.8	46.3		16.9	19.0	38.0	

Values in bold-face are those FA accounting for ≥1% of the total FAs. Asterisks denote significance between diets and within tissues according to ANOVAs. Different letters refer to significant differences between diets according to Fisher's PLSD ($P<0.05$). The following FA were detected but comprised <1% of the total FAs: 14:0, 14:1, 15:0, 18:3ω6, 18:4ω3, 20:0, 20:1ω9, 20:2ω6, 20:4ω3, 22:0, and 20:1ω9. 'Trace' refers to values ≤0.1%.
†Refers to the sum of 18:1ω9 and 18:1ω7 isomers.

fractions have characteristic differences in FA composition. A similar pattern was found in the tissues of fishes (Castell et al., 1972). Most previous studies of FA in birds did not distinguish between polar and neutral FAs, and we are aware of only one other data set from birds that can be used to test this prediction (Surai et al., 2000), but the diets of the lesser black-backed gulls (*Larus focus*) examined by the researchers were not controlled. The dissimilarity between the FA composition of polar and neutral lipid fractions was independent of dietary lipid supplement, even after the finches were fed a diet containing unusually high levels of PUFAs, as in the ω3-enriched diet.

Our second prediction, that the FA profiles of neutral adipose tissue better reflect the FA composition of diets than the other tissue lipid fractions, was not supported by the data. Although all seven lipid fractions from the finches were in some ways similar in their proportions of FAs to their diets of origin, namely elevated proportions of palmitic and stearic (18:0) acids and reduced proportions of linoleic acid, none of the individual tissue fractions that we examined completely mirrored the FA profiles of the diets. Given that the FA profiles of animal tissues are influenced by differential uptake and turnover rates of individual FAs (Bloom et al., 1951; Greenberger et al.,

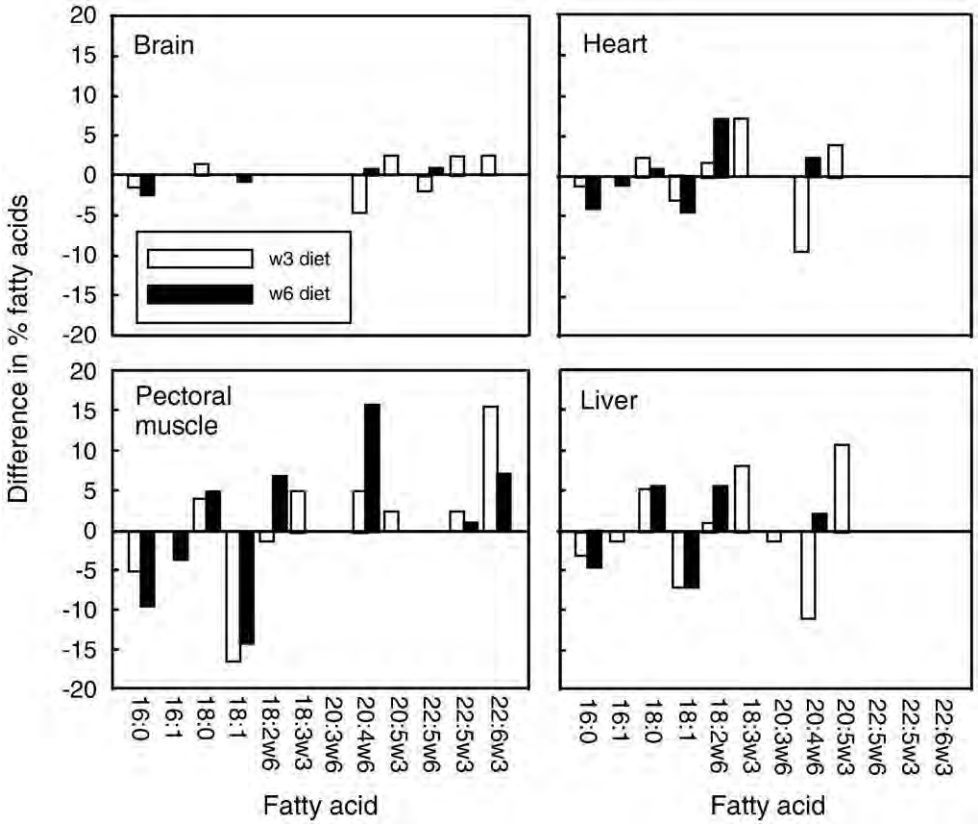

Fig. 4. The effects of two experimental diets on the relative FA content in polar lipids in brain, heart, pectoral muscle, and liver in zebra finches. Values are the mean proportion of FAs in tissues of birds fed ω6-enriched diet (solid bars) and ω3-enriched diet (open bars) subtracted from those measured in birds fed the control diets.

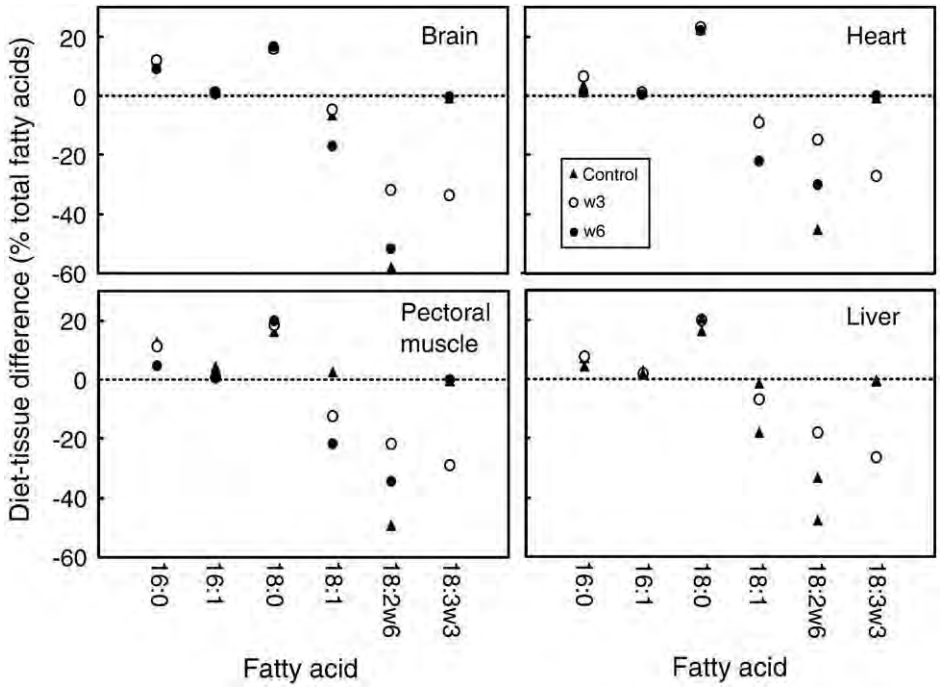

Fig. 5. The differences between the proportions of six dietary FAs compared to the proportion of the same FAs as they occur in polar tissue fractions in zebra finches. Positive values indicate that a given FA is found at disproportionately higher levels in tissue compared to diet, whereas negative values indicate the opposite.

1966; Zollitsch et al., 1997; Mu and Hoy, 2000; Sanz et al., 2000) and the total fat content of the diet (Greenberger et al., 1966; Agradi et al., 1995; Durazo-Beltran et al., 2004; Huang et al., 2005), the results of this study provide additional grounds that challenge the paradigm that lipid profiles of bird tissues can provide a reliable indication of dietary FA composition (Moss and Lough, 1968; Austin, 1993; Thil et al., 2003).

The data do support our third prediction that the FA profiles of different tissues are different in their propensity to be affected by dietary FA composition. All three neutral lipid fractions were highly flexible in their FA composition. However, among the four polar lipid fractions examined, there were fewer significant changes in brain and heart in birds fed the different diets. It was also found that the FA profile of polar brain lipids reflected fewer diet-induced differences in FA composition than other tissues in fish and lizards (Castell et al., 1972; Simandle et al., 2001). A similar observation about the 'refractory' nature of brain tissue FAs was made in penguin chicks (Thil et al., 2003) although the study did not distinguish between polar and neutral lipids.

The brain was unique in that, unlike the polar lipid fractions of other tissues, the proportion of linolenic acid did not increase in birds fed the ω3-enriched diet. However, given that linolenic acid is a precursor for longer chain polyunsaturated FAs that are required for proper brain function (Zar, 1977a; Conner et al., 1990; Guyton and Hall, 2000; Barcarolo et al., 2003), it is possible that the unchanging proportion of linolenic acid in the brain of the birds fed ω3-enriched diet resulted from conversion of linolenic acid to other ω3 PUFAs (e.g. EPA, 20:5ω3; DPA, 22:5ω3; and DHA, 22:5ω3; Table 3) (Conner et al., 1990; Jumpsen et al., 1997; Carrie et al., 2000). Eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) proportions were significantly higher in brain tissues in birds fed the ω3-enriched diet, but the proportions of docosahexaenoic acid (DHA) did not differ among treatments despite being 2.4% higher in the birds fed the ω3-enriched diet. Although the FA profile of brain was similar to that reported for *L. fucus* (Surai et al., 2000), emperor penguins (*Aptenodytes forsteri*) (Zar, 1977b), king penguin chicks (*A. patagonicus*) (Thil et al., 2003), and house sparrows (*Passer domesticus*) (Zar, 1977a), the FA compositions of other polar tissue fractions are apparently more variable among bird species.

We cannot offer a plausible explanation for the conservation of the FA profile of cardiac muscle, particularly in light of the diet-induced flexibility demonstrated by the polar FA fractions in flight muscle. However, based on his study of rats fed diets differing in FA composition, Hirai et al. (2001) suggested that the reason might be that the FA profile of the phospholipids in the heart is particularly critical to maintenance of proper cardiac function. Surai et al. (2000) found that the phospholipids of wild birds and chickens contained nearly equal proportions of linoleic and arachidonic acid (20:4ω6). Interestingly, this pattern was only observed in the zebra finches that were fed the ω6-enriched diet. Ratios of linoleic acid to arachidonic acid in polar pectoral FA fractions were approximately 10:1 and 2:1 for finches fed control and ω3-enriched diets, respectively.

4.1. Coping with excess fatty acids

Given that the control diet offered to the finches should satisfy their FA requirements for development, maintenance, and reproduction (Bradbury and Blakey, 1998; Harper et al., 1998; Rutstein et al., 2005), our results can be used to determine how birds allocate essential PUFAs (e.g. linoleic and linolenic) among lipid pools once they are available in excess in the diet. For example, Miller et al. (2007) found that salmon fed diets supplemented with 14% stearidonic acid (18:4ω3) stored excess stearidonic acid in their muscle tissues possibly for conversion to other PUFAs later. In the present study, zebra finches fed ω6- and ω3-enriched diets, may have preferentially sequestered large amounts of linoleic and linolenic acids, respectively, within polar and neutral fractions of liver and pectoral muscle. Although we did not measure them directly, the FA profiles of circulating free fatty acids might offer additional insight into the fates of excess fatty acids.

The proportions of 16- and 18-carbon FAs were generally reduced in neutral tissue lipids of birds fed the ω3-enriched diet. On the one hand, we observed increases of over 15-fold in the proportion of linolenic acid in pectoral, adipose, and liver tissues. Such increased levels of linolenic acid were not found in neutral lipids in tissues of *L. fucus* thought to feed on marine fish rich in ω3 FAs (Surai et al., 2000). On the other hand, similar proportions of linolenic acid were reported

in the adipose of capercaillie, *Tetrao urogallus* (Moss and Lough, 1968). Unfortunately, Moss and Lough (1968) did not include information about the FA profile of the birds' diet. But, since in winter capercaillie eat a nutrient-poor diet (Summers et al., 2004), we suggest that as a physiological strategy, preferentially sequestering essential fatty acids may be of adaptive benefit if the birds consume diets deficient in essential FAs during leaner seasons.

4.2. Energy considerations

In contrast to the typical mammalian strategy of initially fueling exercise with carbohydrates, followed by a mixture of carbohydrate, lipid, and protein (Guyton and Hall, 2000; Burnley et al., 2006), birds have an exceptional ability to rapidly mobilize and oxidize lipids even during short-term bouts of exercise (Dawson et al., 1983; Jenni and Jenni-Eiermann, 1998; Jenni-Eiermann et al., 2002; McWilliams et al., 2004). The physiological abilities that enable birds to effectively cope with lipid fluxes that exceed the capacity of most mammals probably involves a combination of adaptations associated with storage, circulatory transport, uptake, and oxidation capacities that have yet to be elucidated (Dawson et al., 1983; McWilliams et al., 2004; Pierce et al., 2005).

It is well known that FAs of neutral lipids serve as rapidly mobilizable stores that are able to meet energy demands of birds. However, given that the energy density of linoleic acid is lower than that of other FAs of equal chain length (e.g. oleic and stearic acid) (Johnston, 1970; Blem, 1990; Karasov and Martinez del Rio, 2007), and the fact that essential fatty acids like linoleate have one of the highest rates of mobilization from adipose tissues of ruffs (*Philomachus pugnax*) exposed to hormonally induced lipolysis in vitro (Price et al., 2008) this underscores the possibility that neutral lipids, like those found at high levels in adipose tissue, may also serve an adaptive function in storing essential FAs for modification and/or allocation to other tissues (e.g. brain and heart), as they are needed.

In summary, we found that the tissue FA profiles of zebra finches are influenced by the FA composition of their diets. However, different tissues and lipid fractions have distinct capacities to be altered by dietary FA composition, with brain and heart tissues showing fewer significant differences than pectoral muscle, liver, and adipose tissues. Zebra finches are apparently able to convert essential FAs from the diet, such as linoleic and linolenic acids, into longer and more unsaturated PUFAs, and we suggest that these birds may have the ability to preferentially store excess essential FAs over nonessential FAs in their adipose tissue for later use. The proportions of the two classes of PUFAs, namely ω3 and ω6, in tissues were generally indicative of the proportion of ω3 and ω6 FAs in the diets although the extent to which this occurred depended on the particular lipid fraction, supporting our contention that future studies examining the relationship between FA profiles of tissues and diet should distinguish between polar and neutral lipid fractions.

Acknowledgments

We thank Shosh Didi-Cohen for indispensable help with fatty acid methyl ester analyses, Ulf Bauchinger for helpful suggestions about the experimental design and Agus Munoz-Garcia and two anonymous reviewers for comments on the manuscript. We also thank Anton Khalilieh, Miri Ben-Hamo, Ortal Mizrahy, and Sarah Aamidor for their help in maintaining the birds prior to experiments. This project was funded by the US–Israel Binational Science Foundation Grant 2005119 to BP and SM. During the study MM was a Blaustein Postdoctoral Fellow. This study was done under the auspices of the animal care and ethics committee of Ben-Gurion University. This is paper #645 of the Mitrani Department of Desert Ecology.

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