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Fatty acid composition of pectoralis muscle membrane, intramuscular fat stores and adipose tissue of migrant and wintering white-throated sparrows (*Zonotrichia albicollis*)

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SUMMARY

The fatty acid composition of muscle membrane phospholipids and fat stores may affect migration performance in birds. The purpose of this study was to investigate seasonal changes in the fatty acid composition of (1) pectoralis muscle phospholipids, (2) intramuscular triglyceride stores and (3) adipose tissue triglycerides in free-living white-throated sparrows (*Zonotrichia albicollis*). During migratory seasons there was an increase in the n-6:n-3 ratio of muscle membrane phospholipid fatty acids without a change in the proportion of unsaturated fatty acids. This change was driven mainly by an increase in the proportion of 18:2n-6 and a decrease in the proportion of 22:6n-3. An increase in the proportion of 18:2n-6 was also observed in the intramuscular and adipose tissue triglyceride stores during the migratory seasons. These increases in 18:2n-6 were offset by a decrease in 16:0; resulting in an elevated proportion of unsaturated fatty acids and elevated double bond index in both fat stores of migrants. The elevated levels of 18:2n-6 in migrant fat stores indicates a high dietary component of this fatty acid, as white-throated sparrows feed mainly on tree seeds and some insects during migration and may not have access to a diet high in n-3 fatty acids. We suspect that elevated dietary levels of 18:2n-6 also caused the observed increases in the proportion of this fatty acid in muscle phospholipids. Overall, we conclude that seasonal changes in adipose and muscle fatty acid composition are likely attributable to diet more than other factors such as migratory exercise or mitochondrial density.

Key words: natural doping, migration, polyunsaturated fatty acids, n-6, n-3, phospholipids, *Zonotrichia*.

INTRODUCTION

During a migratory flight, birds must maintain a high metabolic rate while fasting, sometimes for several days (McWilliams et al., 2004). Fat is the primary fuel for migratory flight (Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004), and therefore it is critical that birds store a large quantity of fat prior to migration. Previous studies have shown that the level of fat stored can sometimes reach 50% of body mass or more (Piersma, 1990; Ramenofsky, 1990; Lindström and Piersma, 1993). There is also evidence that the fatty acid (FA) composition of dietary lipids could affect migratory performance (Blem, 1976; Egeler and Williams, 2000; Guglielmo et al., 2002; Maillet and Weber, 2006; Maillet and Weber, 2007; Price et al., 2008; Price and Guglielmo, 2009).

Most studies of variation in the FA composition of adipose tissue in migratory birds have focused on the preferential mobilization of different types of FA (Johnston, 1973; Conway et al., 1994; Egeler and Williams, 2000; Price et al., 2008). For a given carbon chain length, higher degrees of unsaturation result in preferential mobilization (Groscolas, 1990; Raclot and Groscolas, 1993; Price et al., 2008; Raclot, 2003). Thus, preferential deposition of unsaturated FA might be important for meeting substrate demand during high-intensity exercise such as migratory flight. Supporting this hypothesis, several studies have shown that migrants have a higher proportion of unsaturated FA than non-migrants, and further, that migrants increase the relative unsaturation of fat stores prior to migration (Johnston, 1973; Conway et al., 1994; Egeler and Williams, 2000). Nonetheless, this observation has been inconsistent, and other researchers have found no difference between migrants and non-migrants with regard to adipose tissue FA composition (Hicks, 1967; Blem, 1976; McWilliams et al., 2004). We sought to extend this previous work by examining the seasonal changes in adipose and muscle FA composition in white-throated sparrows.

Our interest in the performance consequences of the phospholipid (PL) composition of muscles arises from work associating muscle FA with exercise performance in a number of species. Ayre and Hulbert (Ayre and Hulbert, 1997) observed an association between endurance and high levels of n-6 polyunsaturated fatty acids (PUFA) in the skeletal muscle membranes of rats. In their interspecific study, Ruf and colleagues (Ruf et al., 2006) found a high correlation between membrane n-6 content and maximal running speed in mammals. In contrast, studies in humans have reported decreases in the relative amount of n-6 PUFA and the n-6:n-3 ratio during exercise or endurance training, suggesting that n-3 PUFA in muscle membranes may enhance exercise ability (Andersson et al., 1998; Helge et al., 1998). Likewise, Infante and colleagues (Infante et al., 2001) found high levels of n-3 PUFA in the highly aerobic muscles of hummingbirds and rattlesnakes.

Previous studies of migrating birds have found that n-6:n-3 of PUFA in pectoralis muscle membrane phospholipids can vary seasonally and during stopover refuelling. The ratio of n-6:n-3 in western sandpiper (*Calidris mauri*, Cabanis) pectoralis muscle decreased during migration (Guglielmo et al., 2002). These authors hypothesized that a high proportion of n-6 PUFA in muscle membrane PL enhances exercise performance, and migration causes a depletion of n-6 PUFA. In contrast, Maillet and Weber (Maillet and Weber, 2006) found that during migratory stopover,

semipalmated sandpipers (*Calidris pusilla*, Linnaeus) with access to high n-3 marine benthic prey (*Corophium volutator*, Pallas) incorporated high proportions of dietary n-3 PUFA into muscle membrane PL. Maillet and Weber (Maillet and Weber, 2007) also found that the proportion of n-3 PUFA was positively correlated with activities of oxidative enzymes, suggesting that high proportions of n-3 PUFA in muscle membrane PL (i.e. low n-6:n-3) enhance exercise performance in these birds. They proposed the natural doping hypothesis, whereby birds might enhance exercise performance by selecting diets high in n-3 FA that induce the expression of key genes in the oxidative metabolism of FA through peroxisome proliferator-activated receptor pathways or *via* incorporation into muscle PL (Maillet and Weber, 2007; Weber, 2009). In addition, a recent study by Nagahuedi and colleagues (Nagahuedi et al., 2009) showed that dietary supplementation with n-3 PUFA increased the representation of these FA in muscle membrane PL and greatly increased oxidative enzyme activities of bobwhite quail (*Colinus virginianus*, Linnaeus). Price and Guglielmo (Price and Guglielmo, 2009) found that white-throated sparrows (*Zonotrichia albicollis*, Gmellin) eating diets with high n-6:n-3 achieved greater peak metabolic rates during exercise than sparrows with low dietary n-6:n-3. They also found no effect of diet (high n-6 *versus* high n-3) on muscle oxidative enzyme activities or the expression of muscle FA transporters. Through independent manipulation of muscle and adipose FA composition they concluded that the performance enhancement was more likely related to adipose neutral lipid (NL) composition than muscle PL. However, the importance of muscle PL FA composition to migration performance remains unclear.

The goal of this study was to investigate seasonal changes in the FA composition of pectoralis muscle PL, intramuscular triglyceride and adipose tissue triglyceride in free-living white-throated sparrows. The white-throated sparrow is a long distance short-bout migrant that winters mainly in the southeastern United States and breeds throughout the boreal forests of North America (Falls and Kopachena, 1994). It feeds on terrestrial seeds and some insects, and may not have access to diets high in n-3 PUFA. It is thus a good model to examine seasonal changes in FA composition and the generality of the natural doping hypothesis in birds. In particular, we tested the hypothesis that seasonal changes in muscle PL composition are caused by migratory endurance exercise (Guglielmo et al., 2002). Accordingly we predicted that changes in muscle PL would mirror those in western sandpipers (Guglielmo et al., 2002), with decreased n-6 and increased n-3 FA during the migratory period compared with winter. On the other hand, if seasonal changes are a result of diet, then changes in PL FA should be similar to changes to triacylglycerol FA (likely increasing n-6 during migration).

MATERIALS AND METHODS

Animal collection

Migrating white-throated sparrows were caught by mist netting and ground trapping in April and May 2006 (spring, N=17, body mass 29.8±0.55 g) and in October 2006 (autumn, N=30, body mass 25.16±0.33 g) in Southwestern Ontario, Canada, approximately 10 km northwest of Long Point Provincial Park. Wintering white-throated sparrows were caught in February 2006 and January 2007 by the same methods in the Stoneville Wildlife Management Area, Delta Experimental Forest in Mississippi, USA (N=19, body mass 28.48±0.54 g). All birds were anaesthetized with isoflurane and killed by cervical dislocation. Samples of pectoralis muscle and adipose were immediately removed and weighed. They were then snap frozen in liquid nitrogen and stored in a –80°C freezer until analysed. Procedures were approved by the University of Western Ontario Animal Use Sub-committee (protocol no. 2005-060-08). A scientific collection permit was granted by the Canadian Wildlife Service (permit no. CA 0168), and the United States Fish and Wildlife Scientific Collection Permit (MB758364-1) to Dr Frank Moore, from the University of Southern Mississippi.

FA analysis

Total lipids were extracted from both pectoralis muscle (50–100 mg) and adipose (3–8 mg) with 15 ml chloroform:methanol (1:1 v/v) (Folch et al., 1957) containing butylated hydroxytoluene (BHT) (25 mg l^{-1}). The samples were first homogenized using a polytron homogenizer (3×10 s) and then centrifuged for 15 min at 2056 ***g***. Samples were then filtered (Whatman no. 1) and washed with 10 ml chloroform:methanol (2:1 v/v). Aqueous solutes were separated after the addition of 6 ml 0.25% KCl and 10 min incubation in a water bath at 70°C. The aqueous layer was suctioned off and the lipid layer was transferred into a pear flask for evaporation of the organic phase (Rotovapor, Buchi, Switzerland). Samples were either stored in 1 ml chloroform:methanol (1:1 v/v with BHT) at –20°C overnight or immediately resuspended in 100 µl chloroform for loading onto Supelclean solid phase extraction tubes (Supelco, Sigma-Aldrich Canada, Oakville, ON, Canada; LC-NH2, 100 mg). PL and NL (mainly triglycerides) were examined in muscle tissue. Only NL were examined in adipose samples. Columns were washed with hexane, samples were loaded and then the columns were centrifuged for 1 min at 1370 ***g*** (columns were centrifuged after each elution). NL were eluted with 1.8 ml chloroform:isopropanol (2:1 v/v). Non-esterified FA were eluted with 1.6 ml isopropyl ether:acetic acid (98:2 v/v), then discarded. PL were eluted with 3 ml of methanol. Heptadecanoic acid (17:0; 50 µl, 3 mg per 10 ml hexane) was added to each sample as an internal standard. Samples were then dried under N_2 and either stored in 1 ml chloroform:methanol (1:1 v/v with BHT) at –20°C overnight or immediately transesterified.

Before transesterification, samples that were stored in chloroform:methanol (1:1 v/v with BHT) were first dried under N_2. Then 2 ml acetyl chloride (1 mol l^{-1}, dissolved in methanol) was added and samples were incubated for 2 h at 90°C. Samples were dried under N_2; 1 ml methanol was added then samples were dried again under N_2 to remove any residual HCl or water. Methylated samples were subsequently dissolved in dichloromethane for injection into the gas chromatograph.

FA methyl esters were separated on an Agilent Technologies 6890N gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a J&W Scientific High Resolution Gas Chromatography Column (DB-23, Agilent Technologies) and a flame ionization detector. The carrier gas was He. The temperature program was 2 min 80°C, then increase at 5°C min^{-1} for 20 min, hold at 180°C for 3 min, increase at 1.5°C min^{-1} for 13.3 min, hold at 200°C for 0 min, increase at 10°C min^{-1} for 4 min, then hold for 3 min at 240°C. FA were identified by comparison of relative retention time with known standards (Supelco 37 component FAME mix, and Supelco PUFA No.3, from Menhaden oil).

Statistical analysis

FA that comprised less than 0.5% of the total FA content were excluded from analysis. Data are expressed as mean mass per cent of total pectoralis muscle PL, intramuscular triglyceride and adipose tissue triglyceride ±s.e.m. We calculated double bond index (DBI) as the number of double bonds per 100 FA molecules, i.e. $DBI=\Sigma[(\text{Molar \% } FA_i)(\text{number of double bonds per } FA_i)]$. For adipose triglyceride the ratio of 18:1/18:2 and (16:1+18:1)/18:2 were

calculated. Additionally, the n-6:n-3 ratio for PL FA was calculated. For analysis of FA percentages the data were arcsine square root transformed. Seasonal changes of individual FA, monounsaturated FA (MUFA), PUFA and saturated FA (SAT; for PL and both triglyceride stores) were analysed using a one-way analysis of variance. In addition, changes during the migratory seasons were examined by combining the spring and autumn seasons (migrants) and comparing them with wintering birds with an independent sample *t*-test. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Muscle phospholipids

The FA composition of muscle membrane phospholipids changed in relation to season (Fig. 1). Significant changes between at least two seasons were observed in 16:0, 18:0, 18:1n-7, 18:2n-6, 20:4n-6, 20:5n-3 and 22:6n-3 (P<0.05, Fig. 1). All of the above FA were also significantly different when compared between migrants and wintering birds with the exception of 20:5n-3 (Fig. 1). Although there were no significant changes in MUFA, PUFA or SAT (Fig. 2A) in relation to season, there was a shift in the type of PUFA observed, as well as a decrease in DBI (Fig. 3) in migrating birds. The main change was an increase in 18:2n-6 and a decrease in 22:6n-3 during migration (Fig. 1). This caused the n-6:n-3 ratio during migration to increase significantly compared with that of wintering birds (Fig. 4).

Intramuscular triglycerides

The composition of triglyceride in muscle was dominated by 16–18 carbon FA. Significant differences between at least two seasons were observed in 16:0, 16:1n-7 and 18:2n-6 (P<0.05, Fig. 5). These FA also showed significant changes in their proportions when comparing migrant and wintering birds. There were no significant changes with season in the MUFA and SAT composition; however, there was an observed trend of decreased SAT during the migratory seasons and a corresponding significant increase in PUFA and DBI in migrating white-throated sparrows (Fig. 2B and Fig. 3).

Adipose triglycerides

There were seasonal changes in the FA composition of adipose triglycerides (Fig. 6). Similar to intramuscular triglycerides, the major components of the adipose FA were 16–18 carbon FA. There were significant changes between at least two seasons in 16:0, 16:1n-7, 18:0, 18:1n-7, 18:2n-6, 18:3n-3, 20:1n-9 and 20:4n-6 (P<0.05, Fig. 6). All of the above FA were also significant when compared between migrants and wintering birds with the exception of 18:3n-3 (Fig. 6). There was a significant decrease in SAT during the migratory seasons and a corresponding increase in PUFA and DBI (P<0.05, Fig. 2C and Fig. 3). The ratio of 18:1 to 18:2 was significantly higher in wintering birds (1.51±0.13) compared with that in migrants (1.00±0.062, P<0.001), with no difference between spring and autumn migrants (P=0.580). Similarly, the ratio of (16:1+18:1)/18:2 was significantly higher in wintering birds (1.72±0.12) compared with that in migrants (1.06±0.065, P<0.001), with no difference between spring and autumn migrants (P=0.536).

DISCUSSION

Seasonal changes in muscle phospholipids

We found a pattern of increased n-6 and decreased n-3 FA in muscle phospholipids in migrating birds, relative to wintering birds. This pattern contrasts with that observed in the only other study of seasonal changes in muscle phospholipids in migratory birds, which found that n-3 FA increased at the expense of n-6 FA during migration in western sandpipers (Guglielmo et al., 2002). There are multiple hypotheses for both the causes and consequences of different phospholipid FA compositions in muscles (Ayre and Hulbert, 1996; Helge et al., 1998; Helge et al., 2001; Infante et al., 2001; Guglielmo et al., 2002; Maillet and Weber, 2007; Price and Guglielmo, 2009) (Price et al., in press). Guglielmo and colleagues (Guglielmo et al., 2002) speculated that the seasonal changes they observed were related to the high intensity endurance exercise associated with migration. They proposed that increased levels of n-6, specifically 20:4n-6, are beneficial for high levels of endurance activity and that the decreased levels of 20:4n-6 in muscle membrane PL are a physiological cost of migration (Guglielmo et al., 2002).

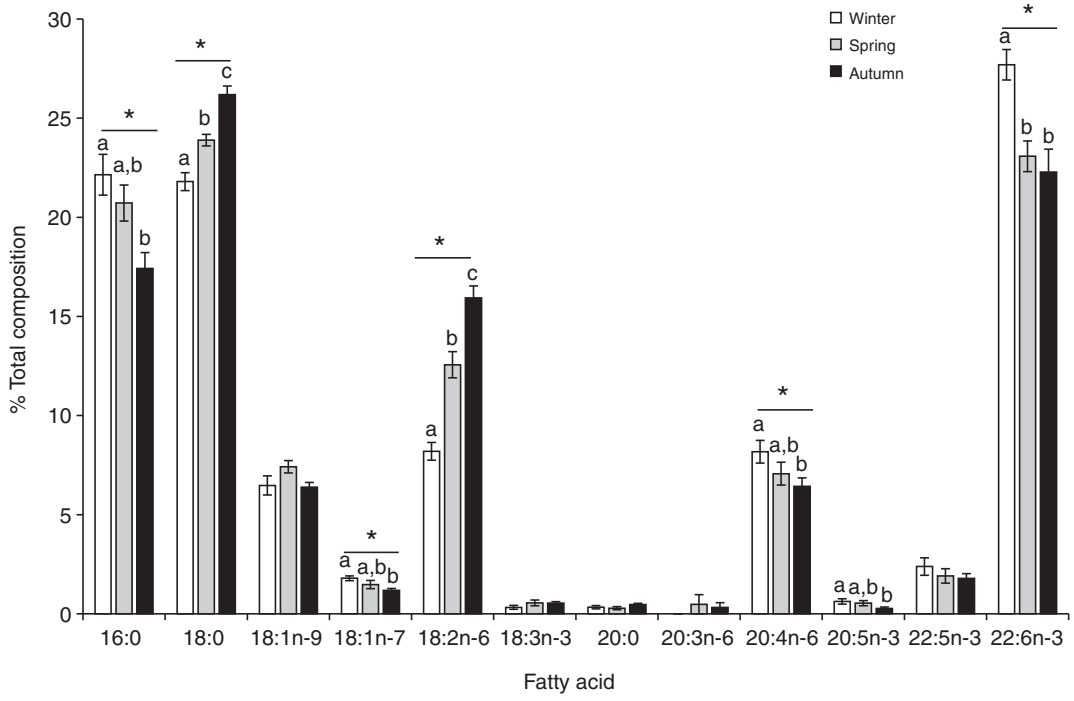

Fig. 1. Fatty acid (FA) composition of pectoralis muscle membrane phospholipids (PL). Adult white-throated sparrows were sampled from a wintering area in Mississippi (winter) and migratory stopover site in Southwestern Ontario (spring, autumn). Data are mean mass per cent of total PL ±s.e.m. For each FA, shared letters denote no significant difference between seasons (P<0.05). Bars with an asterisk represent significant changes between migrant (autumn and spring combined) and wintering birds (P<0.05). N_{winter}=19, N_{autumn}=30 and N_{spring}=17.

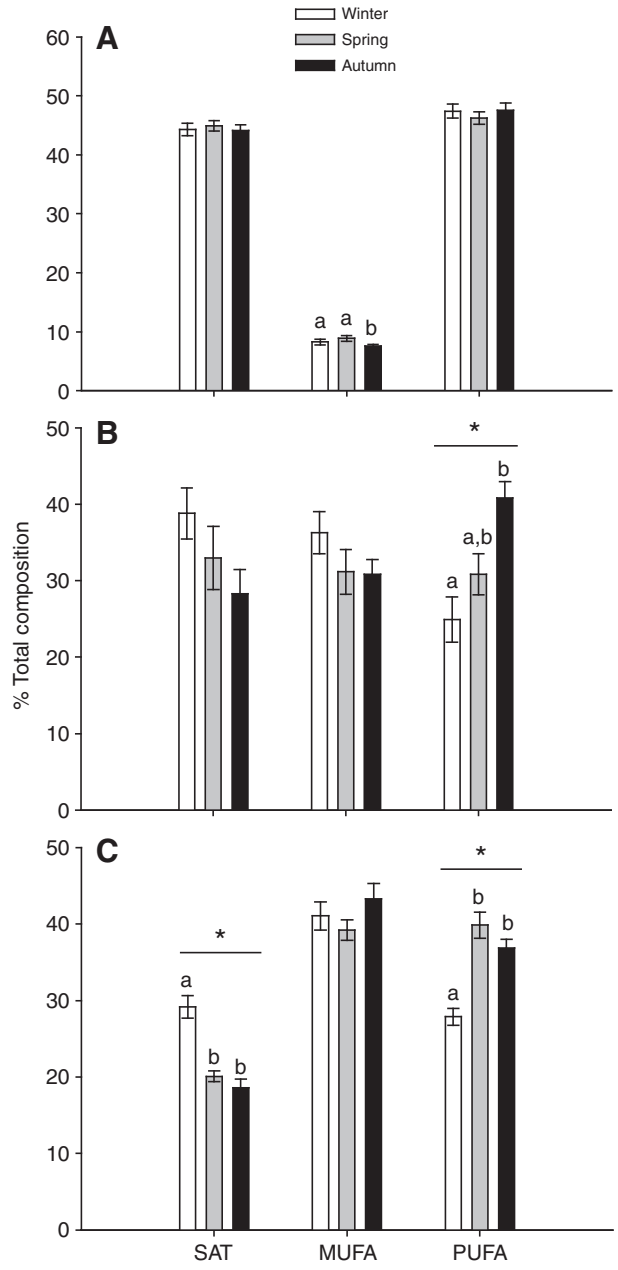

Fig. 2. FA class composition (saturated, SAT; monounsaturated, MUFA; and polyunsaturated, PUFA) of (A) pectoralis muscle membrane PL, (B) intramuscular neutral lipids (NL) and (C) adipose NL. Data are mean mass per cent ±s.e.m. For each FA, shared letters denote no significant difference between seasons and bars with asterisks denote significant differences between migrant (autumn and spring combined) and wintering birds (P<0.05). (A) N_{winter}=19, N_{autumn}=30 and N_{spring} =17; (B) N_{winter}=17, N_{autumn}=27 and N_{spring}=17; (C) N_{winter}=19, N_{autumn}=30 and N_{spring}=17.

However, given the largely contrasting results observed in the current study, we suggest that seasonal changes in avian muscle PL FA composition instead arise primarily from dietary sources. It may be that the western sandpipers studied by Guglielmo and colleagues (Guglielmo et al., 2002) ate a diet more enriched in n-3 PUFA during migration as they fed on benthic invertebrates of colder, northern latitudes. This would be similar to the patterns of n-3 incorporation in refuelling semipalmated sandpipers observed by Maillet and Weber (Maillet and Weber, 2006).

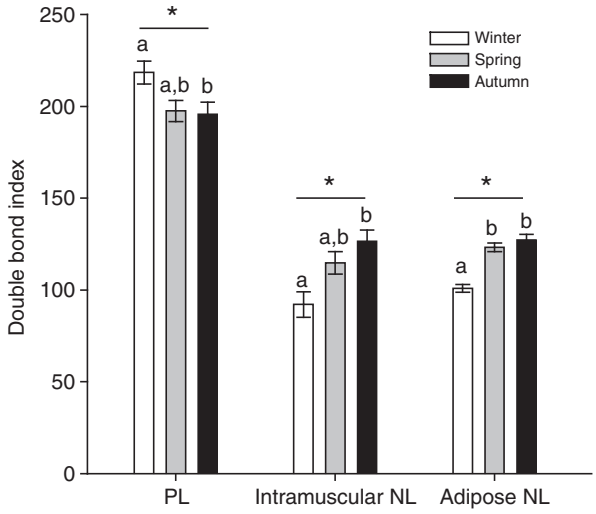

Fig. 3. Double bond index (DBI) for pectoralis muscle membrane PL, intramuscular NL and adipose NL. Data are means ± s.e.m. Within a tissue, similar letters denote no significant difference between seasons and bars with asterisks denote significant differences between migrant (autumn and spring combined) and wintering birds (P<0.05). PL: N_{winter}=19, N_{autumn}=30 and N_{spring}=17; intramuscular NL: N_{winter}=17, N_{autumn}=27 and N_{spring}=17; adipose NL: N_{winter}=19, N_{autumn}=30 and N_{spring}=17.

Previous work has found that dietary FA composition, and particularly the dietary PUFA composition, has a large influence on the FA composition of muscle membranes in rats, humans and birds (Ayre and Hulbert, 1997; Pan and Storlien, 1993; Pierce et al., 2005; Maillet and Weber, 2006; Price and Guglielmo, 2009). Furthermore, Turner and colleagues (Turner et al., 2004) investigated the effects of diet and exercise training on FA muscle membrane PL composition in rats and found that diet has a larger role than exercise training in the overall composition of skeletal muscle membranes. White-throated sparrows feed mainly on tree seeds (Falls and Kopachena, 1994), which have high proportions of n-6

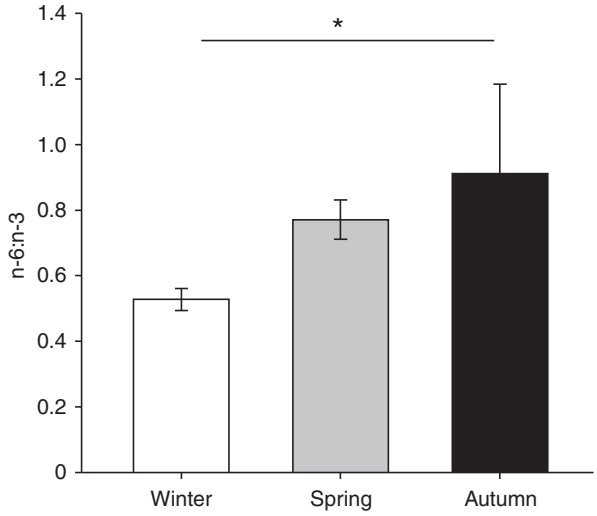

Fig. 4. The ratio of n-6:n-3 FA of pectoralis muscle membrane PL. Adult white-throated sparrows sampled from a wintering area in Mississippi (winter) and migratory stopover site in Southwestern Ontario (spring, autumn). Error bars represent s.e.m. The asterisk denotes a significant difference between migrants (autumn and spring combined) and wintering birds (P<0.05). N_{winter}=19, N_{autumn}=30 and N_{spring}=17.

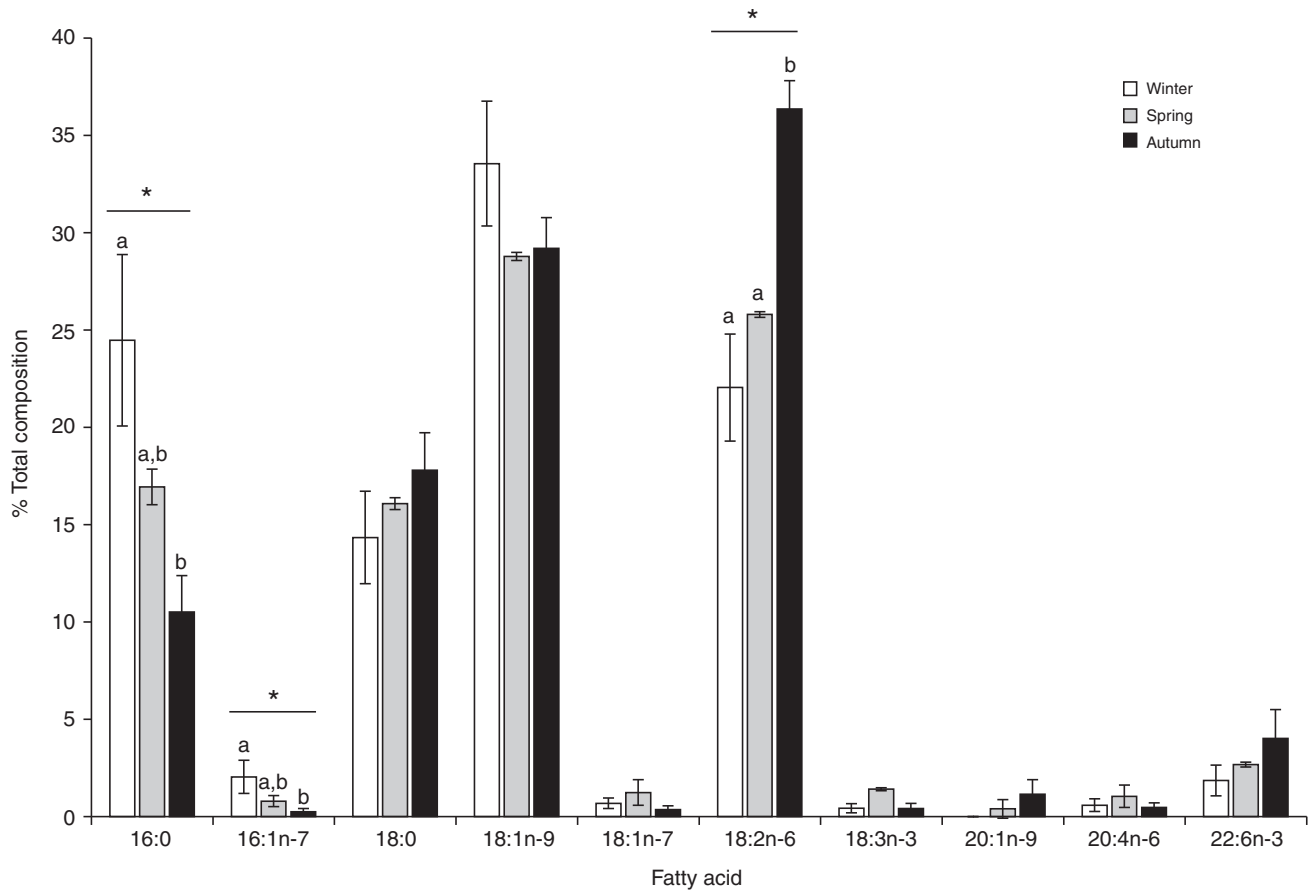

Fig. 5. FA composition of pectoralis intramuscular NL. Adult white-throated sparrows sampled from a wintering area in Mississippi (winter) and migratory stopover site in Southwestern Ontario (spring, autumn). Data are mean mass per cent of total NL ±s.e.m. For each FA, shared letters denote no significant difference between seasons and bars with asterisks denote significant differences between migrant (autumn and spring combined) and wintering birds (P<0.05). N_{winter}=17, N_{autumn}=27 and N_{spring}=17.

FA, specifically linoleic acid (18:2n-6; for example, this FA represents 25–45% of the triglyceride FA in various species of oak seeds and 56% in maple seeds) (Fietz et al., 2005). In addition, the composition of the migrants' fat stores in the current study was relatively enriched with n-6 FA compared with those of wintering sparrows, further indicating that during migration they feed increasingly on n-6 FA-rich foods (see below). On the other hand, the diet of semipalmated sandpipers refuelling at the Bay of Fundy is primarily composed of marine benthic invertebrates (*Corophium volutator*) which are typically high in n-3 FA (Napolitano and Ackman, 1990; Maillet and Weber, 2006; Maillet and Weber, 2007). Thus, it seems likely that the differences between sparrows and sandpipers in seasonal PL composition patterns are driven by dietary differences between the two species during migration.

Exercise is also known to affect the composition of muscle PL. Price and colleagues (Price et al., in press) found that exercise can increase the proportion of 22:6n-3 in muscle membranes in the congener *Z. leucophrys*, resulting in a decrease in n-6:n-3 ratio. Thus, the 'exercise' component of migration would likely have resulted in opposite changes from that observed in our study. Furthermore, the effect of exercise on muscle PL composition has been variable and inconsistent (Turner et al., 2004; Petridou et al., 2005). Helge and colleagues (Helge et al., 1999) found that the FA muscle membrane PL composition of trained rats was significantly different from that of sedentary rats fed the same diet (increased 18:2n-6, decreased 20:4n-6 and 22:6n-3). However, in another study, Helge and colleagues (Helge et al., 2001) observed an increase in 22:6n-3 and a decrease in the n-6:n-3 ratio in humans that were trained for 4 weeks. Andersson and colleagues (Andersson et al., 1998) reported decreases in 18:2n-6, 20:4n-6 and the n-6:n-3 ratio in response to a 10 week low intensity training regime in the leg muscle of humans. A possible explanation for the differences between the above studies may be the difference in training regime, diet, species and muscle fibre type under investigation (Ayre et al., 1998; Turner et al., 2004).

Another possible explanation for the variation between the above studies is the location of the phospholipids within the cells. None of the above studies have investigated subcellular membrane changes. It is known that during migration there are increases in mitochondrial density and activity (Evans et al., 1992; Guglielmo et al., 2001). Thus, differences between mitochondrial and sarcolemmal membranes coupled with changes in mitochondrial density may contribute to the overall change in whole muscle PL. Previous studies on mitochondrial membrane composition report high levels of n-6 PUFA that were associated with an increased oxidative capacity of mitochondria (Guderley et al., 2005). However, a recent study by Maillet and Weber (Maillet and Weber, 2007) found a positive correlation between increased citrate synthase activity (a measure of mitochondrial activity) and high levels of n-3 PUFA in muscle membrane PL in migrating shorebirds. Nagahuedi

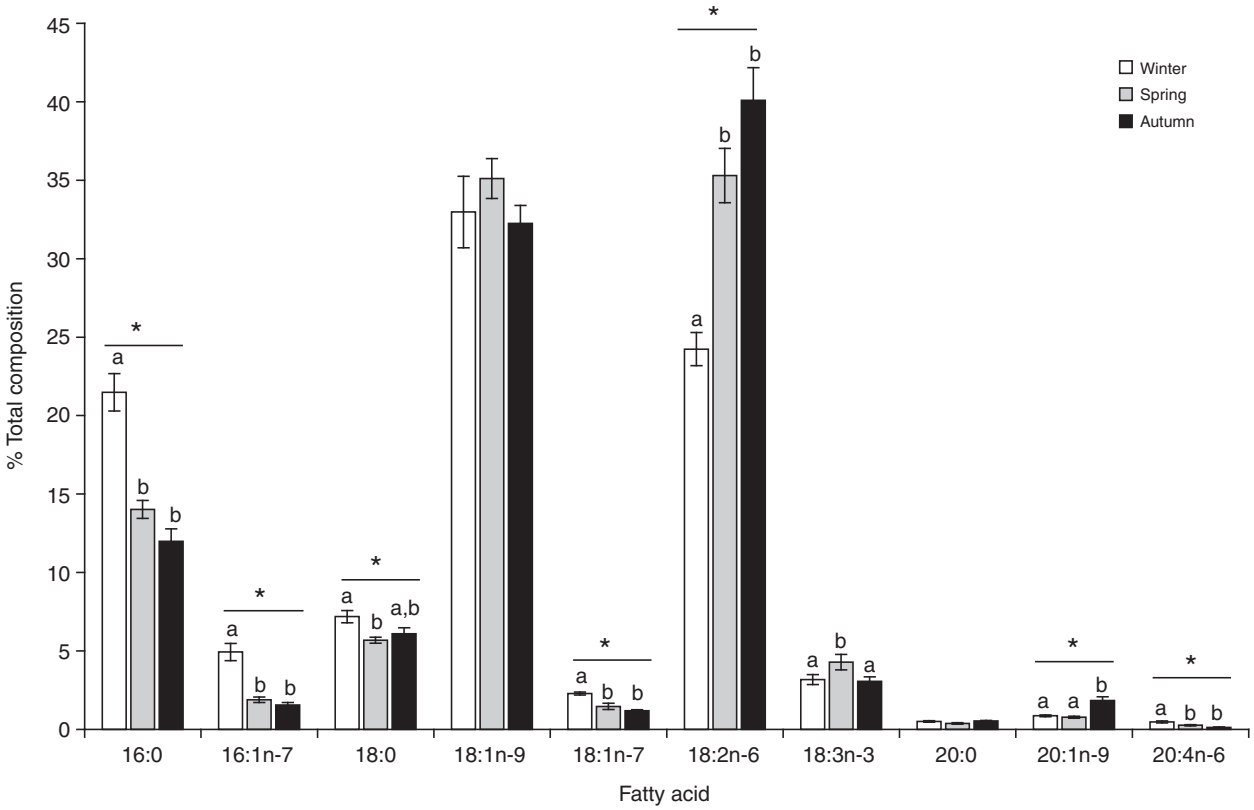

Fig. 6. FA composition of adipose NL. Adult white-throated sparrows sampled from a wintering area in Mississippi (winter) and migratory stopover site in Southwestern Ontario (spring, autumn). Data are mean mass per cent of total NL ±s.e.m. For each FA, shared letters denote no significant difference between seasons and bars with asterisks denote significant differences between migrant (autumn and spring combined) and wintering birds. N_{winter}=19, N_{autumn}=30 and N_{spring}=17.

and colleagues (Nagahuedi et al., 2009) recently found that both cell and subcellular muscle membranes in quail are affected by diet in similar ways. The role of n-6 and n-3 PUFA in mitochondrial membranes deserves to be investigated further.

Temperature is known to affect the FA composition of membranes. Sinensky (Sinensky, 1974) showed homeoviscous adaptation in *E. coli*, whereby membrane viscosity was maintained throughout a range of temperatures by changing the ratios of saturated and unsaturated FA. Membrane composition can also be affected by environmental temperature in homeotherms, particularly in animals that cool their body extremities during the winter (Valencak et al., 2003). However, this is not likely to be important in our study, as the tissues were sampled from the body core. Moreover, mean daily ambient temperatures were about 10°C during the spring and autumn seasons near Long Point and about 7°C during the wintering season near Stoneville (data obtained from Environment Canada and The US National Oceanographic and Atmospheric Administration).

Possible protective factor

One trend that emerged from the study of Guglielmo and colleagues (Guglielmo et al., 2002) and the current study is a decrease in 20:4n-6 during migration. We speculate that this specific FA change represents a possible protective mechanism in flight muscles of migratory birds in which a small degree of oxidative damage occurs to the pectoralis muscles (Guglielmo et al., 2001). Arachidonic acid (20:4n-6) can be converted into prostaglandins that are associated with inflammation (Simopoulos, 1999), which is itself associated with impairment of muscle recovery (Pizza et al., 2005). Thus, decreases in the level of 20:4n-6 in muscle phospholipids could decrease the pool of this substrate in the prostaglandin biosynthetic pathway, thereby reducing inflammation of damaged muscles and allowing for quicker muscle repair at the end of a migratory flight. This result deserves further investigation.

Seasonal changes in adipose and intramuscular fat stores

There has long been interest in the types of fat that birds store, especially in relation to migration (Blem, 1976; Blem, 1980; Conway et al., 1994; Pierce et al., 2004). The amount of unsaturated FA has been of particular interest, as these fats are mobilized more readily from adipocytes (Johnston, 1973; Groscolas, 1990; Raclot, 2003; Price et al., 2008). Previous authors have suggested that birds increase the proportion of unsaturated FA during migration to provide readily mobilized fuel for endurance flight (Conway et al., 1994; McWilliams et al., 2002; Pierce et al., 2004; Pierce and McWilliams, 2005). This observation has not been consistent, however, and given it is based on a limited sample size (only six studies have examined seasonal changes in wild migrants) the evidence remains equivocal (Pierce and McWilliams, 2005). Our results show that migrant white-throated sparrows carried a smaller proportion of their fat load (in adipose triglyceride) as saturates than wintering birds. This was offset by an increase in PUFA in the migrants, a result also mirrored in the intramuscular triglyceride. This increase in PUFA resulted in an elevated proportion of

unsaturated FA and elevelated DBI in both adipose tissue and intramuscular triglyceride of migrants.

Adipose triglyceride composition is greatly affected by diet (Pierce and McWilliams, 2005; Pierce et al., 2005; Pierce et al., 2004; Price and Guglielmo, 2009), although selective deposition, selective metabolism, and *de novo* synthesis and modification can also play a role (Blem, 1990; Egeler et al., 2003; Else and Hulbert, 2003; Pierce et al., 2004; Pierce and McWilliams, 2005; Price et al., 2008). Thus, we suspect that the seasonal differences in adipose stores we observed are primarily a result of seasonal diet changes. Birds are known to switch diets during migration (Bairlein and Gwinner, 1994), and the major increase in unsaturated FA occurred *via* an increase in PUFA, which cannot be synthesized *de novo*. Of special interest are the elevated levels of 18:2n-6 in migrant fat stores, indicating a high dietary component. Previous work has shown that high dietary levels of this essential FA can increase exercise performance in birds (Pierce et al., 2005; McWilliams and Pierce, 2006; Price and Guglielmo, 2009). High levels of stored 18:2n-6, but not n-3 FA (e.g. 22:6n3), in the migrants also demonstrate that any possible natural doping by ingestion of n-3 FA during migration (Weber, 2009) is probably limited to species (such as shorebirds) that would have access to these FA in the wild. The diet of many shorebirds feeding in marine intertidal zones consists mainly of invertebrates high in 22:6n-3 (Napolitano and Ackman, 1990; Maillet and Weber 2006; Maillet and Weber 2007). In contrast, the diet of white-throated sparrows is composed mainly of seeds high in 18:2n-6 (Falls and Kopachena, 1994; Fietz et al., 2005) and, therefore, improved migratory flight performance could occur by ingestion of 18:2n-6 in a large number of seed-eating migrants.

Seasonal compositional differences in adipose triglyceride were generally mirrored by similar changes in intramuscular triglyceride composition, particularly for major FA 16:0 and 18:2n-6. Diet therefore likely plays a role in intramuscular triglyceride composition. Andersson and colleagues (Andersson et al., 2002) found that changes in intramuscular triglyceride composition paralleled the composition of the subjects' diet. Previous work by Andersson and colleagues has shown that exercise can also affect intramuscular triglyceride composition. A significant decrease in 16:0 was observed during migration in the current study. A similar decrease in 16:0 was observed by Andersson and colleagues (Andersson et al., 2000) in the muscle of trained humans, compared with untrained subjects. However, a previous study by Andersson and coworkers (Andersson et al., 1998) found no changes in intramuscular triglyceride in trained *versus* untrained humans. The relative contributions of diet and exercise to the observed changes in FA composition of intramuscular triglyceride remain unclear.

In summary, we observed changes in the seasonal FA profile of white-throated sparrow muscle membrane PL, adipose triglyceride and intramuscular triglyceride. An increase in 18:2n-6 during the migratory seasons (spring and autumn) was the cause of an overall increase in the n-6:n-3 ratio in the muscle membrane PL, and was a contributing factor to the increase in the per cent of PUFA in both the adipose triglyceride and intramuscular triglyceride stores. These results oppose a previous observation showing a decrease in membrane 18:2n-6 (Guglielmo et al., 2002) during the migratory season; because there are high dietary levels of 18:2n-6 in white-throated sparrows, this suggests that changes in both adipose and muscle triglyceride and PL are driven more by diet than by other factors such as exercise. Furthermore, these results demonstrate a limitation to the general applicability of natural doping by ingestion of n-3 FA; instead, we suggest that migratory performance may be enhanced by ingestion of 18:2n-6 in many overland migrants.

LIST OF SYMBOLS AND ABBREVIATIONS

BHT	butylated hydroxytoluene
DBI	double bond index
FA	fatty acid
MUFA	monounsaturated fatty acid
NL	neutral lipid
PL	phospholipids
PUFA	polyunsaturated fatty acid
SAT	saturated fatty acid

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