The Journal of Experimental Biology 209, 2686-2695
Published by The Company of Biologists 2006
doi:10.1242/jeb.02299

Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper

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Accepted 25 April 2006

Summary

At the end of summer, semipalmated sandpipers (*Calidris pusilla*) traveling from the Arctic stop in the Bay of Fundy (east coast of Canada) to build large fat reserves before a non-stop flight to South America. During a 2-week stopover, the body mass of this small shorebird is doubled (~20 g to 40 g) by feeding on a burrowing amphipod, *Corophium volutator*, that contains unusually high levels of n-3 polyunsaturated fatty acids (PUFA). In mammals, high n-3 PUFA content of membrane phospholipids (PL) is linked to improved exercise performance due to increased membrane fluidity that accelerates transmembrane lipid transport. We hypothesized that dietary n-3 PUFA could be used as a natural 'performance-enhancing substance' by semipalmated sandpipers to prepare their flight muscles for migration. Also, PUFA stored as fuel in neutral lipids (NL) can be mobilized more quickly than saturated fatty acids, but they contain less energy per unit mass. It is therefore unclear whether dietary fatty acids are modified before storage. Birds were collected at various stages of fat loading to examine changes in the composition of tissue PL (membranes) and NL (fuel stores). Results show that dietary n-3 PUFA are incorporated in tissue lipids in less than 2 weeks. During the stopover, the double bond index of muscle PL increases by 25% and the fatty acid profiles of both muscle PL and adipose NL converge with that of the diet. However, >50% of dietary n-3 PUFA are converted to other fatty acids before storage, mainly to oleate (18:1), possibly because monounsaturates offer a compromise between high energy density and ease of mobilization. This study shows that long-distance migrant birds can (1) use natural diets rich in specific lipids to prime flight muscles for endurance exercise, and (2) modify dietary fatty acids before storing them as fuel.

Key words: membrane phospholipids, fat stores, bird migration, nutrition, endurance exercise, fatty acid metabolism, sandpiper, *Calidris pusilla*, *Corophium volutator*.

Introduction

In late summer, semipalmated sandpipers (*Calidris pusilla*) stop on the mudflats of the Bay of Fundy (east coast of Canada) during their migration from nesting areas in the Arctic to wintering grounds in South America. About one million individuals (or 75% of their world population) load large fat reserves before the longest non-stop flight of their entire migration: a 4500 km transoceanic trip lasting 3 days (Hicklin, 1987). During the 2-week stopover, the birds double body mass by feeding on *Corophium volutator*, a small amphipod responsible for 86% of their diet (Napolitano and Ackman, 1990; Napolitano et al., 1992). This small mud shrimp is an extremely rich source of the n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) (Ackman et al., 1979). Rapid fattening on this unique diet is rather intriguing because n-3 PUFA are known to improve aerobic performance in other vertebrates and have many beneficial health effects in humans (Ruxton et al., 2004).

Multiple lines of evidence show that muscle performance can be affected by the n-3 PUFA content of membrane phospholipids (PL) *via* changes in fluidity and permeability (Daveloose et al., 1993; Ernst, 1994; Stillwell and Wassal, 2003). For example, unusually high levels of n-3 PUFA have been found in the membrane PL of highly aerobic muscles such as hummingbird flight muscles (Infante et al., 2001). Moreover, endurance training in rats and humans increases the n-3 PUFA content of their muscle PL (Andersson et al., 2000; Helge et al., 2001; Turner et al., 2004). A particularly relevant laboratory study (Pierce et al., 2005) shows that the aerobic capacity of a migrant bird species, the red-eyed vireo (*Vireo olivaceous*), is influenced by the fatty acid composition of its diet.

Successful completion of a long migration does not only depend on intrinsic characteristics of muscles, but also on the nature of the oxidative fuels available. For example, saturated fatty acids provide an advantage because of their higher energy content per unit mass compared to PUFA (Blem, 1990).

Selectivity has been demonstrated for key steps of lipid metabolism including storage, mobilization and oxidation. Diets high in monounsaturated fatty acids (MUFA) are normally preferred (McWilliams et al., 2002; Pierce et al., 2004) and long-distance migrants commonly store large amounts of oleate (18:1) (Blem, 1990; Caldwell, 1973). However, fasting studies reveal that PUFA (and EPA in particular) are more easily mobilized than MUFA or saturated fatty acids (Herzberg and Farrell, 2003; Raclot and Groscolas, 1995). At the β-oxidation step, preference for MUFA (Henderson and Sargent, 1985) or PUFA (DeLany et al., 2000) have both been reported.

Semipalmated sandpipers stopping in the Bay of Fundy provide ideal conditions to uncover potential effects of nutrition on lipid metabolism of long-distance migrants and, ultimately, on their capacity for endurance exercise. In this 'natural experiment', large amounts of n-3 PUFA are consumed and it is unclear whether cell membranes are affected or if dietary fatty acids are modified before storage. Therefore, the goals of this study were to investigate semipalmated sandpipers during rapid fattening to determine: (1) the anatomical distribution of their lipid reserves, (2) the effect of a natural diet high in n-3 PUFA on the fatty acid composition of cell membranes, and (3) changes in the composition of storage lipids and whether the fatty acid profiles of the bird's fat reserves and of the diet converge during refueling. We anticipated that changes in lipid metabolism might not only provide sufficient energy for migration, but increased muscle capacity for endurance exercise.

Materials and methods

Animals and field methodology

Semipalmated sandpipers *Calidris pusilla* L. were used to monitor lipid accumulation before a ~4500 km migration from the Bay of Fundy (Canada) to South America. A previous study on the same species had shown that body mass is an accurate predictor of time spent refueling in the Bay of Fundy (White, 1985). Therefore, changes in body mass were used as an indirect measure of feeding time at the last stopover before their long-distance flight to wintering grounds. Wild sandpipers were caught with a pull trap (Hicklin et al., 1989) at Dorchester Cape, New Brunswick, Canada (65°10′N, 77°27′W; August 10 and 11, 2004; Canadian Wildlife Service permit SC2354). After weighing, 45 adults were selected to obtain the widest possible range of body masses (20–41 g) and percent body fat (12–43%) (see Fig. 1). These animals were euthanized by cervical dislocation, immediately frozen, and stored at –20°C for up to 3 months before analyses. Mud shrimps (*Corophium volutator* Pallas) were collected on the sandpipers' feeding grounds as described previously (McCurdy et al., 2000), and were immediately frozen at –20°C.

Lipid extraction

Birds were thawed and carefully dissected into six parts for separate lipid analyses: ventral, tracheal and pelvic fat depots, as well as pectoral muscle, liver and the rest of the carcass (that included skin, bones, feathers and other remaining tissues and organs). The carcass was pre-blended with an industrial grade food processor (Robot Coupe, Newark, NJ, USA). Then, 0.5–1 g of each tissue was homogenized with a Polytron (Luzern, Switzerland) and lipids were extracted twice in chloroform-methanol (2:1 v/v) (Folch et al., 1957). After filtration, KCl (0.25%) was added and the mixture centrifuged to separate aqueous and organic phases. The aqueous phase was discarded and the organic phase containing the lipids was dried on a rotating evaporator (Büchi Rotavapor, Flawil, Switzerland). Lipids from the diet samples of (*Corophium volutator*) were extracted using the same procedure.

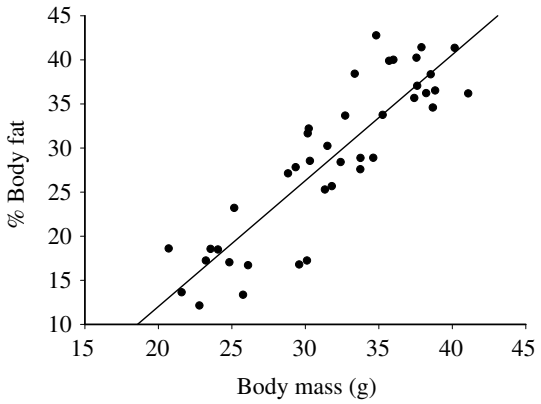

Fig. 1. Relationship between % body fat and body mass of semipalmated sandpipers refueling in the Bay of Fundy. Each point represents one individual. Line was obtained by linear regression: $P<0.0001$, $R^2=0.78$, $N=40$.

Separation of neutral lipids (energy stores) and phospholipids (membranes)

Following extraction and drying, total tissue lipids were resuspended in chloroform. Neutral lipids (NL), nonesterified fatty acids (NEFA), and phospholipids (PL) were separated by filtration on Supelclean solid-phase extraction tubes (3 ml LC-NH_2; Sigma, St Louis, MO, USA) as described previously (Bernard et al., 1999). Briefly, NL were eluted from the column with chloroform:isopropanol (2:1 v/v), NEFA with isopropyl ether:acetic acid (98:2 v/v) and PL with methanol. The NL and PL fractions were then used for analysis of their fatty acid composition. The detailed fatty acid composition of NEFA is not reported here because they only accounted for less than 1% of total tissue lipids.

Fatty acid composition

Heptadecanoic acid was added to each sample as an internal standard. The fatty acid compositions of NL and PL were measured by gas chromatography (McClelland et al., 1999) after acid transesterification with acetyl chloride in methanol (Abdul-Malak et al., 1989). Individual fatty acid methyl esters were separated and quantified on a Hewlett-Packard gas

chromatograph (5890 series II with 7673 autosampler) equipped with flame-ionization detector and a 30 m fused silica column (Supelco 2330; Sigma, St Louis, MO, USA). Helium was the carrier gas. The injector port was at 220°C and the detector at 240°C. Column temperature was kept at 185°C for 35 min, raised to 210°C at a rate of 5°C min^{-1}, and maintained at 210°C for 10 min. Exact retention times of individual fatty acids were determined with pure standards (Sigma-Aldrich, St Louis, MO, USA).

Calculations and statistical analyses

The relationships between body mass and % body fat (Fig. 1), and between lean pectoral muscle mass and body fat mass (Fig. 2B) were assessed by simple linear regression, whereas the relationships between fat mass of different tissues (carcass, pectoral muscle, liver and fat depots) and body fat mass were assessed by multiple regression (Fig. 2A). For all analyses of lipid composition (Figs 3–5), the birds were divided into three groups of equal size. Because % body fat is an accurate index of time spent refueling in the Bay of Fundy (White, 1985); Fig. 1), we ranked the birds according to % body fat and divided them in three equal groups containing 'lean', 'medium' and 'fat' birds (N=15 for each group). Lean birds had <25% fat and were assumed to have recently arrived in the Bay of Fundy. Medium birds had 25–35% fat, whereas fat birds had stored >35% lipids and were ready to start their long migratory flight.

One-way ANOVA and *post-hoc* Tukey tests were used to compare group means (lean, medium and fat) for double bond index (DBI) and % contribution of individual fatty acids to total fatty acids within each lipid class (NL or PL). Fatty acids accounting for less than 1% of total fatty acids within each lipid class were not included in our analysis. The double bond index was used to quantify the level of fatty acid unsaturation in each lipid class and was calculated as follows (expressing percentages as ratios):

DBI = average number of double bonds / % saturated FA .

The average number of double bonds (also called degree of unsaturation) was calculated as:

Average number of double bonds =
(1× % monoenes) + (2× % dienes) + (3× % trienes) + …
+ (n× % FA with n double bonds) .

A Δ predator–prey index (ΔPPI) was used to measure the difference between the fatty acid composition of pectoral muscle or lipid reserve (combining contributions from fat depots and carcass) and that of *Corophium volutator*. It was calculated as follows:

$$\Delta \text{ PPI} = \Sigma_{i=1}^{n} \left| \%\ \text{FA}i \text{ in predator} - \%\ \text{FA}i \text{ in prey} \right| .$$

Statistical analyses were performed using SYSTAT version 8.0. All variables were tested for normality and homogeneity of variances. Percentages were transformed to the arcsine of their square root before analysis, and all values given are means ± standard error of the mean (s.e.m.). P<0.05 was considered significant.

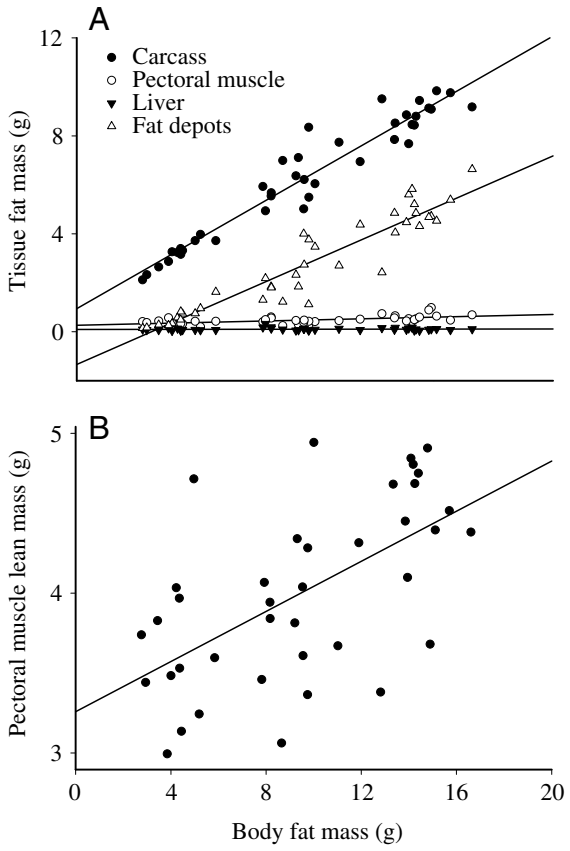

Fig. 2. Fat mass of various tissues (A) and pectoral muscle lean mass (B) in relation to total body fat mass in semipalmated sandpipers refueling in the Bay of Fundy. Each point represents one individual (A: N=40, multiple regression, see results; B: N=39; P<0.001, R^2=0.24).

Results

Distribution of fat reserves

The relationship between percent body fat and body mass is shown in Fig. 1. The slope of the linear regression between the two parameters was significantly different from 0 (P<0.0001). The contribution of individual tissues to the increase in total body fat is presented in Fig. 2A. For this analysis, ventral, tracheal and pelvic fat depots were pooled (hereafter referred to as 'fat depots') because no significant differences between them was detected (P>0.05). Multiple regression reveals that significant fat accumulation takes place in the carcass, in fat depots and to a lesser extent in pectoral muscle (P<0.001), whereas no fat is deposited in the liver during the stopover (P=0.55). Fat reserves are almost entirely deposited in two tissues: carcass (54% of total body stores) and fat depots (42%), whereas pectoral muscle only stores minor amounts (4%). The lean masses (calculated by subtracting lipid mass from total mass) of the carcass, liver and fat depots do not change during the stopover. However, the lean mass of pectoral

muscle increases while fat is being deposited (P<0.001; Fig. 2B).

Double bond index

To analyze changes in tissue lipid composition during refueling, the sandpipers were divided into three groups: lean, medium and fat (see Materials and methods). Fig. 3 summarizes changes in double bond index (DBI) in storage lipids (NL) and in membrane lipids (PL) of the three tissues that show significant fat accumulation: pectoral muscle (Fig. 3A), carcass (Fig. 3B) and fat depots (Fig. 3C). Phospholipids have a higher DBI than NL in the pectoral muscle of all birds (P<0.001). However, only medium birds show a higher DBI in PL than NL for carcass (P<0.001) and fat depots (P<0.05). During refueling, increases in DBI were observed in the membranes of pectoral muscle and in the storage lipids of the carcass. Pectoral muscle PL and carcass NL have a higher DBI in medium and fat birds than in lean birds (P<0.05; Fig. 3). For fat depots, no differences in DBI were observed between lean, medium and fat birds (P>0.05).

Tissue fatty acid composition

Changes in the fatty acid composition of tissues during refueling are presented in Table 1. To start analyzing the mechanisms responsible for the observed changes in DBI, Figs 4 and 5 only focus on the fatty acids showing significant differences between lean, medium and fat birds. Changes in the fatty acid composition of tissue PL are presented in Fig. 4. In pectoral muscle PL, the increase in DBI observed during the stopover is explained by increases in %20:5 and %22:6 that overcompensate a small decrease in %20:4 (P<0.001; Fig. 4A). In carcass PL, DBI remains unchanged because the increase in %20:5 is offset by a decrease in %20:4 (P<0.01; Fig. 4B). In the PL of fat depots, %18:2 and %20:4 both decrease during the stopover (P<0.01; Fig. 4C).

Significant changes in the fatty acid composition of tissue NL are presented in Fig. 5. In pectoral muscle NL, %20:5 increases while %18:0 decreases as lipid stores are being deposited (P<0.05; Fig. 5A). In carcass NL, %20:5 and %22:6 show increases while %20:4 decreases (P<0.005; Fig. 5B). In the NL of fat depots, DBI remains unchanged because increases in

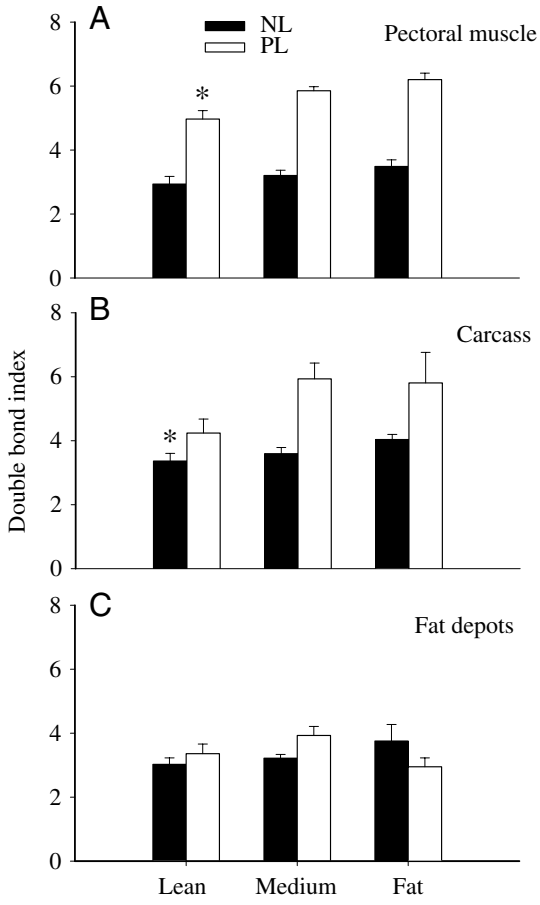

Fig. 3. Double bond indices (DBI) of pectoral muscle, carcass and total fat depots in neutral lipids (NL) and phospholipids (PL) of lean, medium and fat semipalmated sandpipers. Values are means ± s.e.m. (N=11–14). *Significant differences between bird groups within each lipid fraction (P<0.05).

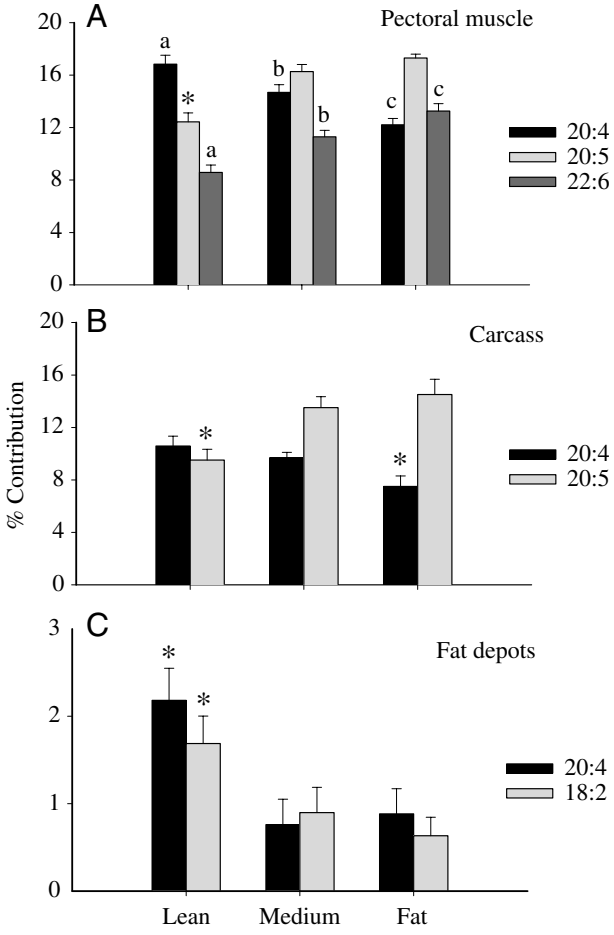

Fig. 4. Contribution (%) of individual fatty acids in the tissue phospholipids (PL) of semipalmated sandpipers. Values are means ± s.e.m. (N=11–14). * or a,b,c only: significant differences between lean, medium or fat birds (P<0.05). No statistical comparisons were made between the different fatty acids within each group of birds.

Table 1. *Relative contribution of individual fatty acids to total tissue fatty acids (%) in main tissues of lean, medium and fat semipalmated sandpipers*

	Pectoral muscle			Carcass			Fat depots		
Fatty acids	Lean	Medium	Fat	Lean	Medium	Fat	Lean	Medium	Fat
16:0	21.7±0.5	22.2±0.8	22±1.2	22.5±0.9	22±0.6	23.1±0.9	25.7±0.5	25.1±0.6	27.1±0.7
	12.6±0.6	12.4±0.5	11.9±0.8	18.2±0.2	13.8±0.2	14.9±0.3	20.3±1.7	18.7±1.4	20.3±2.2
16:1	7.7±1.1	7.4±0.5	6.6±1.9	9.8±1.2	8.4±0.6	7.8±0.5	8.5±1.0	7.9±0.7	8.8±0.6
	1.4±0.2	1.3±0.2	1.1±0.1	3.1±0.1	2.1±0.1	2.1±0.1	4.6±0.8	3.9±0.7	4.8±0.6
18:0	13.2±0.5	11.6±0.4	11.5±0.4	11.3±0.4	10.1±0.4	10.5±0.4	13.1±0.6	11.7±0.5	11.6±0.4
	29.1±1.0	27.9±0.6	28.2±0.6	23.8±0.9	22.8±0.8	22.2±0.8	20.3±1.2	21±1.4	19.7±1.1
18:1	39.5±0.9	40.1±0.9	40.6±0.6	35.8±1.1	35.2±0.7	34.3±0.7	31.9±0.9	32.9±0.6	31.7±0.9
	14.1±0.7	12.4±0.5	13.4±0.4	24.5±0.8	23.3±0.8	25.4±1.0	33.8±0.9	33±0.9	30.8±0.8
18:2	2.7±0.3	3.1±0.4	2.1±0.2	2.8±0.3	3.1±0.4	2±0.2	1.8±0.2	2.1±0.3	1.3±0.1
	1.8±0.2	1.3±0.2	1.2±0.1	1.8±0.3	1.9±0.3	1.3±0.2	1.7±0.3	0.9±0.3	0.6±0.2
20:4	1.7±0.2	1.4±0.1	1.3±0.1	1.8±0.1	1.5±0.1	1.4±0.1	1.1±0.1	1.4±0.1	1.4±0.1
	16.8±0.7	14.7±0.6	12.2±0.5	10.6±0.8	9.7±0.4	7.5±0.8	2.2±0.4	0.8±0.3	0.9±0.3
20:5	5.4±0.6	6.7±0.4	7±0.4	8.2±0.8	9.9±0.6	12.1±0.4	9±0.7	9.9±0.4	12.2±0.7
	12.4±0.7	16.7±0.6	17.3±0.3	9.5±0.8	13.5±0.8	14.5±1.2	14.5±1.5	18.7±1.5	14.8±1.6
22:6	3.2±0.4	3.3±0.2	3.5±0.2	2.9±0.4	3.4±0.3	4.5±0.2	3.8±0.4	3.5±0.2	4.6±0.3
	8.6±0.6	11.3±0.5	13.3±0.6	7.2±1.4	11.5±1.3	10.6±2.1	1.2±0.6	1±0.5	2±0.7
SFA	35.6±0.9	34.6±0.8	35.4±0.7	35.4±1.1	34.5±0.7	34.6±0.7	40.3±0.7	37.9±0.7	39.3±0.8
	41.9±1.1	40.2±0.6	40.5±0.8	42±1.9	36.5±1.2	38.1±2.5	40.7±1.5	39.6±1.0	43.5±1.5
MUFA	49.6±0.9	50.2±0.7	48.7±0.4	47.6±1.0	46.2±0.6	44.1±0.4	41.8±0.6	43.4±0.5	42±0.7
	15.9±0.9	13.9±0.7	14.7±0.5	28±1.3	25.6±1.1	28.3±1.7	39.8±1.4	37.9±1.3	36.3±1.3
PUFA	14.5±1.1	16.5±0.8	15.4±0.9	17.1±1.3	19.4±0.8	21.2±0.6	17.8±0.9	18.7±0.5	20.9±1.1
	42±1.0	46±0.6	45.8±0.5	32±2.8	37.9±1.9	33.6±3.8	19.6±1.9	24±1.9	19.4±1.7

Separate values are given for neutral lipids (NL; grey) and phospholipids (PL; white). Subcutaneous fat accounts for most of the carcass NL. Fat depots is the sum of ventral, tracheal and pelvic adipose reserves.

Values are mean ± s.e.m. (N=10–14).

%20:5 and %22:6 are compensated by decreases in %20:4 and %18:2 as the birds accumulate fat reserves (P<0.05; Fig. 5C).

Fatty acid composition of food and differences between prey and predator

The fatty acid composition of *Corophium volutator*, the invertebrate prey item accounting for 86% of the birds' diet (Napolitano et al., 1992), is presented in Table 2. *Corophium* is particularly rich in the n-3 polyunsaturated fatty acids 20:5 and 22:6 that, together, account for 45% of all fatty acids consumed by the sandpipers. The difference in fatty acid composition between *Corophium* and tissue lipids of the sandpipers were calculated as an index: the delta predator-prey index or ΔPPI (a single value representing the sum of differences for all fatty

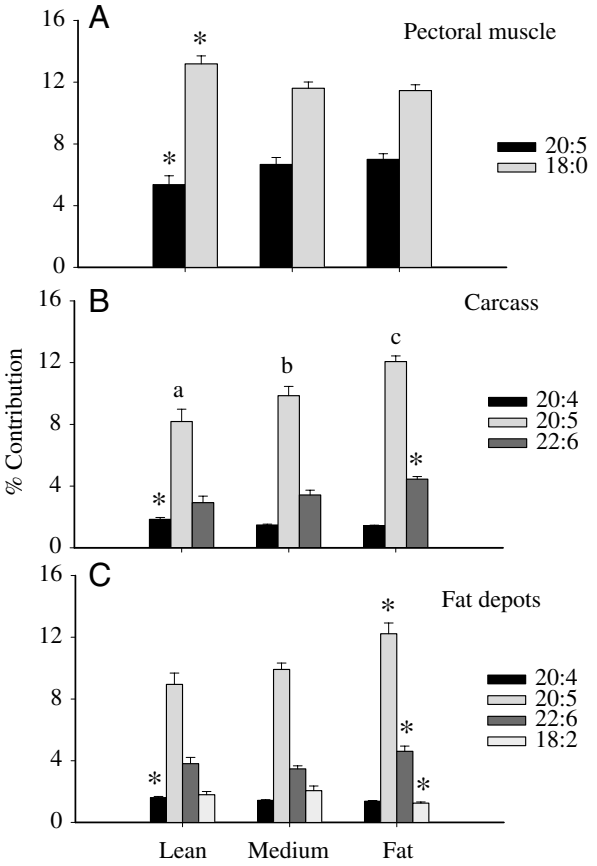

Fig. 5. Contribution (%) of individual fatty acids in pectoral muscle, carcass and total fat depots in neutral lipids (NL) of semipalmated sandpipers. Values are means ± s.e.m. (N=10–14). * or a,b,c only: significant differences between lean, medium or fat birds (P<0.05). No statistical comparisons were made between the different fatty acids within each group of birds.

acids; see Materials and methods). Changes in ΔPPI are summarized in Fig. 6. During refueling, convergence between the fatty acid compositions of predator and prey was observed for pectoral muscle PL (Fig. 6A) and lipid reserves NL (Fig. 6B) that showed a significant decrease in ΔPPI (P<0.005).

Table 2. *Relative contribution of different fatty acids to total fatty acids (%) of Corophium volutator*

Fatty acids	% Contribution
16:0	18.1
16:1	7.3
18:0	7.5
18:1	16.8
18:2	1.7
20:4	3.9
20:5	31.1
22:6	13.6
SFA	25.6
MUFA	24.1
PUFA	50.3

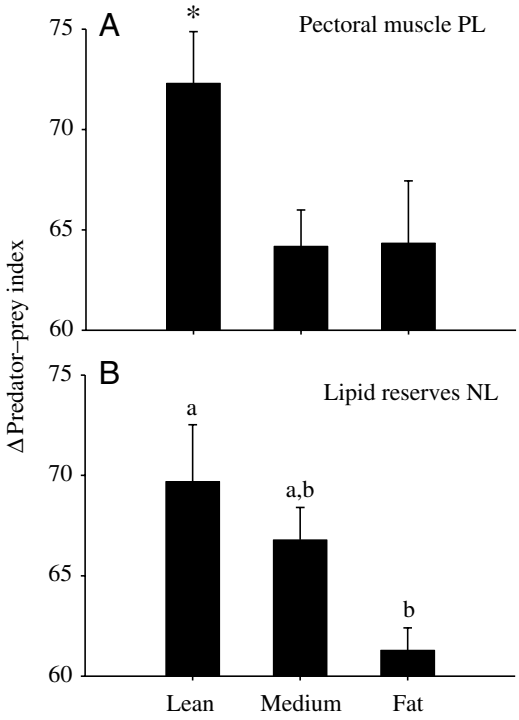

Fig. 6. Δ Predator–Prey index in pectoral muscle PL and lipid reserves NL in lean, medium and fat birds. Values are means ± s.e.m. (N=13 in lean and fat birds and 14 in medium birds). * or a,b: significant differences between lean, medium or fat birds (P<0.05). Values sharing the same letter are not different from each other.

Discussion

During refueling, the natural diet of semipalmated sandpipers is responsible for modifying the lipid composition of their tissues, and these nutritional effects may improve capacity for endurance exercise. The fatty acid composition of flight muscle membranes and adipose tissue reserves become more similar to that of *Corophium volutator* while the shorebirds prepare for long-distance migration (Fig. 6). In addition to membrane-related effects, EPA and DHA are also known to trigger mitochondrial and peroxisomal proliferation (Froyland et al., 1997; Jump, 2002a; Jump, 2002b; Totland et al., 2000; Yamazaki et al., 1987), and to increase the activities of key Krebs Cycle and β-oxidation enzymes (Froyland et al., 1997; Guo et al., 2005; Jump and Clarke, 1999; Sanz et al., 2000; Yamazaki et al., 1987). This study shows that massive consumption of n-3 PUFA causes a rapid increase in the unsaturation levels of muscle PL and adipose tissue NL. However, a significant fraction of the dietary n-3 PUFA consumed is modified before storage to maintain high MUFA levels in the fuel reserves used for migration.

Flight muscle membranes are modified

Phospholipids are the main structural lipids of cell membranes. In semipalmated sandpipers, the rapid consumption of n-3 PUFA over a short stopover period is sufficient to cause a 25% increase in the double bond index of

flight muscle phospholipids (Fig. 3), a change achieved by incorporating dietary EPA and DHA (Table 1; Fig. 4). Recent experiments on rats (Turner et al., 2004) and humans (Andersson et al., 2002) reveal that mammals respond similarly after feeding on a high n-3-PUFA diet for periods of 2–3 months. Our study is the first to show: (1) that nutrition can also modify avian muscle membranes, (2) that natural diets can have this effect in wild birds, and (3) that muscle membranes of some long-distance migrants respond very rapidly (~1 week).

Information presently available on various animal models is not sufficient to identify a clear mechanism linking a specific fatty acid composition of membranes with increased muscle performance. However, several lines of evidence show that muscle PL composition and capacity for endurance exercise are related. Membranes of high-performance muscles like hummingbird flight muscle, rattlesnake shaker muscle and hare locomotory muscle, all contain unusually high amounts of n-3 PUFA (Infante et al., 2001; Valencak et al., 2003). In addition, endurance training increases the n-3 PUFA content of muscle PL in rats and humans (Andersson et al., 2000; Helge et al., 2001; Turner et al., 2004). Paradoxically, feeding rats a diet rich in n-3 PUFA affects their membrane PL composition (Turner et al., 2004), but decreases endurance *in vivo* (Ayre and Hulbert, 1997), though isolated muscle function seems to remain normal (Ayre and Hulbert, 1996). Controlled-diet studies in fish (Wagner et al., 2004) and birds (Pierce et al., 2005) also show that changing the fatty acid composition of food can affect their performance. Unfortunately, very few studies have attempted to characterize a direct link between the consumption of specific fatty acids with improved endurance, and they provide conflicting results. In rats, dietary n-6 PUFA have been reported to cause the greatest increase in endurance (Ayre and Hulbert, 1997). For salmon, Wagner et al. suggest that n-3 PUFA increase swimming performance (Wagner et al., 2004), whereas McKenzie et al. come to the opposite conclusion (McKenzie et al., 1998). It is therefore premature to determine whether n-3 or n-6 PUFA are most beneficial, or to generalize to all animals. However, it is well established that membrane fluidity and permeability are significantly increased by all high PUFA diets (Ernst, 1994) and decreased by diets poor in PUFA (Daveloose et al., 1993). In this study, we show that semipalmated sandpipers modify their muscle membranes by feeding on *Corophium* as they prepare for long-distance flight. This functional change is consistent with changes in membrane fluidity and permeability that would increase capacity for fatty acid transport. Therefore, this response may contribute to support the high lipid fluxes that these impressive athletes need for migration.

Percent n-3 PUFA increases in lipid reserves

The composition of neutral lipids can affect the capacity to migrate because various fatty acids have a different energy content per unit mass and cannot all be metabolized at the same rate. Results show that fatty acid composition in sandpiper lipid reserves and in the diet converge during the stopover (Fig. 6B). Percent EPA and DHA increase significantly in the NL of carcass and fat depots that, together, account for >95% of lipid reserves in the whole organism (Figs 2, 5). Determining what fatty acid composition of fuel reserves would be 'ideal' for migration is difficult because stores with the highest energy content are not the most easily metabolized. Increasing fatty acid saturation and chain length increases energy content (Blem, 1990), but decreases the rates of mobilization and oxidation (DeLany et al., 2000; Raclot, 2003; Raclot and Groscolas, 1995). Therefore, migrant birds may modify dietary fatty acids before storage to achieve a compromise between high energy density and ease of mobilization/oxidation. To address this possibility, we calculated the composition of a theoretical fat bird from the measured composition of lean birds (Table 1) and of their food (Table 2), assuming that dietary fatty acids are stored without modification. Then, we compared this theoretical fatty acid composition with that actually observed in fat birds. This analysis is presented in Fig. 7 where stored fatty acids with a higher observed abundance than expected have a positive value whereas those with a lower abundance have a negative value. We can estimate that over 50% of total dietary EPA and DHA are modified before storage, and show that they are mainly converted to oleate and palmitate (Fig. 7). One possible reason for this conversion is to decrease the overall vulnerability of lipid reserves to degradation, PUFA being particularly sensitive to peroxidation (Gutierrez et al., 2006). Like semipalmated sandpipers, other migrant birds (Blem, 1990), some mammals (Florant et al., 1990) and fish (Lund and Sidell,

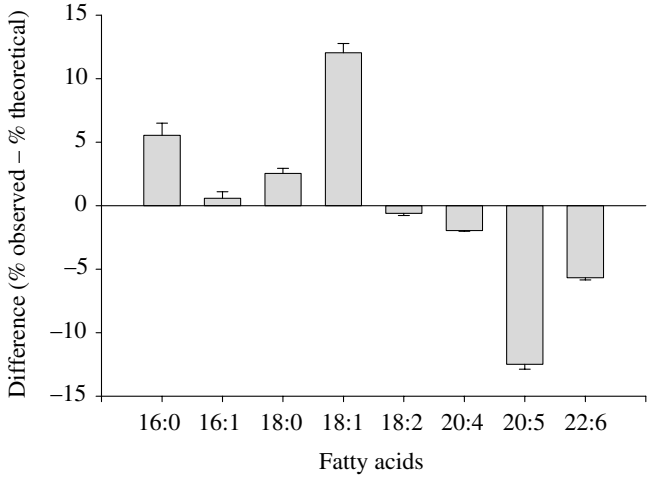

Fig. 7. Differences between observed and theoretical fatty acid composition of lipid reserves NL in fat semipalmated sandpipers. Theoretical values were calculated from measured compositions in lean birds and in the *Corophium* diet, assuming that dietary fatty acids are stored without modifying chain length or number of double bonds. In this comparison, stored fatty acids with a higher observed abundance than expected have a positive value whereas those with a lower abundance have a negative value. Values are means ± s.e.m. (N=13).

1992) also store most of their lipids as oleate, and to a lesser extent as palmitate. This remarkably ubiquitous preference for storing monounsaturates has never been explained (Weber et al., 2003). The above analysis suggests that MUFA may offer optimal characteristics by providing higher energy density than PUFA, together with higher mobilization and oxidation rates than saturated fatty acids.

Changes in lipid reserves and pectoral muscle lean mass

By capturing and re-capturing the same individual semipalmated sandpipers, White determined that body mass is an accurate predictor of feeding time in the Bay of Fundy (White, 1985). Here, we show that % body fat is closely correlated with body mass (Fig. 1), and have therefore used % fat as an indirect measure of refueling time (i.e. birds were allocated to 'lean', 'medium' and 'fat' groups according to % body fat). Previous studies have reported the anatomical distribution of lipid reserves in migratory birds to assess seasonal differences at different stages of complete migrations (Marsh, 1983; Piersma et al., 1999; Scott et al., 1994). Our study is the first to examine rapid changes in fat reserves during a single refueling stopover, as migrants prepare for a long flight. In semipalmated sandpipers, almost all the fat is deposited in discrete adipose tissue depots placed around flight muscles (42%) and as subcutaneous reserves in the carcass (54%) (Fig. 2). In contrast, minimal amounts of fat are directly stored in pectoral muscles (4%), and the liver is not used for this purpose. Interestingly, lean birds have completely depleted their fat depots, but they maintain significant amounts of subcutaneous fat in the carcass (Fig. 2). Favoring the use of internal adipose tissue over subcutaneous fat may be linked to the thermoregulatory requirements of these animals as they travel from the Arctic.

In migrating birds, seasonal changes in body mass were traditionally attributed to fluctuations in fat reserves. However, recent studies have shown that the lean mass of various organs like pectoral and leg muscle, heart, kidney, liver and gut is also modified (Driedzic et al., 1993; Jehl, 1997; Marsh, 1983; Piersma and Gill, 1998; Piersma et al., 1999). During refueling, we observed that the pectoral muscle lean mass of semipalmated sandpipers increases by ~40% (or 2 g). Flight muscle hypertrophy has also been observed in red knots (*Calidris canutus islandica*) (Piersma et al., 1999), bartailed godwits (*Limosa lapponica*) (Piersma and Gill, 1998) and eared grebes (*Podiceps nigricollis*) (Jehl, 1997) in preparation for migration. In these other studies, it is not clear whether the change is necessary to meet the new power requirements of heavy birds, or if additional proteins are stored for another purpose [e.g. to restore muscle mass after particularly strenuous flights (Baucheinger and Biebach, 2005) or to provide an immediate protein source for reproduction upon landing (Evans et al., 1992)]. Semipalmated sandpipers only use multiple short-distance flights to reach the Bay of Fundy (Hicklin, 1987) and do not reproduce in South America. Therefore, the observed increase in pectoral lean mass of this species is probably needed to transport their large fuel load.

Limitations of study

The experimental design of this study only provides indirect support for the idea that dietary PUFA are being used to improve capacity for endurance exercise. However, our results are an important step towards a more rigorous test of this hypothesis under the controlled laboratory conditions that a 'natural experiment' does not permit. Significant questions remain unanswered. For example, it could be argued that semipalmated sandpipers do not 'prefer' *Corophium* as a diet, but simply eat large quantities of this invertebrate because it happens to be abundant at the stopover site. A diet preference experiment would settle this issue (e.g. McWilliams et al., 2002), but field observations suggest that *Corophium* would be preferred by semipalmated sandpipers. Other food items are readily available in the Bay of Fundy (Hicklin and Smith, 1979), and even though *Corophium* is abundant, it contains rather low levels of lipids and energy (Ackman et al., 1979). Interestingly, the least sandpiper (*Calidris minutilla*) is another species that refuels on a diet consisting of 89% *Corophium* to prepare for a non-stop transoceanic flight (Cooper, 1994), whereas semipalmated plovers (*Charadrius semipalmatus*) only eat 48% *Corophium*, but migrate over much shorter distances (Hicklin and Smith, 1979; Nol and Blanken, 1999). Future laboratory experiments should also test whether the exercise performance of birds is actually improved by consuming large amounts of n-3 PUFA. The 'flying wheel' protocol developed by Chappell et al. may be ideal for this purpose (Chappell et al., 1999).

Conclusions

Before long-distance migration, semipalmated sandpipers modify their muscle membranes and load large fat reserves by feeding on marine invertebrates. In addition to building adequate fuel stores, migrant birds can therefore use natural diets rich in specific lipids to prepare flight muscles for endurance exercise. Rapid consumption of dietary PUFA allows them to prime membranes for high fatty acid fluxes. However, a large fraction of dietary EPA channeled towards storage is initially converted to oleate. This common preference for storing monounsaturates over other fatty acids may provide the required compromise between high energy density and ease of metabolism. No marine invertebrate other than *Corophium volutator* contains such large amounts of PUFA, and, in North America, this species is only found in the Bay of Fundy and in the Gulf of Maine. If human activities were to compromise *Corophium* populations, the migration cycle of semipalmated sandpipers and their survival could be greatly affected. *Corophium* appears to be a non-replaceable component of this shorebirds' diet and its habitat should therefore be protected.

This work could not have been completed without the help from Nev Garrity, Peter Hicklin, Colin MacKinnon and Richard Elliot at the Canadian Wildlife Service (Sackville, New Brunswick). We also thank Philippe Thomas for his assistance in the field, Marc Surette (Université de Moncton)

for allowing us to use his laboratory, and Mark Forbes (Carleton University) for providing samples of *Corophium*. This work was supported by an NSERC discovery grant to J.-M. Weber.

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