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Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird

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Abstract We studied: (1) concentrations and fatty acid compositions of plasma non-esterified fatty acids, neutral lipids, and phospholipids, and (2) fatty acid composition of flight muscle phospholipids in wintering, premigratory, and spring and fall migrating western sandpipers (*Calidris mauri*). Plasma neutral lipid and phospholipid levels were elevated in migrants, reflecting high rates of fat deposition. An important role of phospholipids in fattening is suggested by the fact that the amount of fatty acids in plasma phospholipids was similar to, or in spring as much as twice, that of neutral lipids. Changes in the ratio of plasma neutral lipids to phospholipids may indicate seasonal changes in triacylglycerol stores of invertebrate prey. Monounsaturation and total unsaturation of plasma neutral lipids and phospholipids increased during migration. Muscle phospholipids were more monounsaturated in spring and fall, but total unsaturation was reduced in fall. Arachidonic acid [20:4(n-6)] was especially abundant in muscle phospholipids in winter (29%) and declined during migration (19–22%), contributing to a decline in the ratio of n-6 to n-3 fatty acids. The abundance of plasma phospholipids and variability of neutral lipid to phospholipid ratio indicates that measurement of plasma phospholipids will improve methods for assessment of fattening rates of birds. The functional significance of changes in muscle phospholipids is unclear, but may relate to depletion of essential n-6 fatty acids during exercise.

Keywords Bird · Exercise · Lipid · Nutrition · Substrate metabolism

Abbreviations *FA* fatty acid(s) · *FID* flame ionization detector · *GC* gas chromatograph · *NEFA* non-esterified fatty acid(s) · *NL* neutral lipid(s) · *PL* phospholipid(s)

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Introduction

Fatty acid (FA) oxidation provides most of the fuel energy for avian flight muscles during migration, and fat stores can reach as much as 50% of body mass in some bird species (Ramenofsky 1990; Jenni and Jenni-Eiermann 1998; Piersma and Gill 1998). To support the very high metabolic rates necessary for flight, migrating birds must substantially increase their overall capacity to metabolize FA (Butler and Woakes 1990; Weber 1992). They do so by up-regulating many of the biochemical mechanisms responsible for FA transport and catabolism in flight muscles (Marsh 1981; Lundgren and Kiessling 1985, 1986; Driedzic et al. 1993; Pelsers et al. 1999; Guglielmo et al. 2002a), as well as by altering the FA composition of adipose stores (Blem 1990; Egeler and Williams 2000). Generally, migrants store a higher proportion of unsaturated fatty acids, particularly oleic acid (18:1; Blem 1990; Egeler and Williams 2000), which are thought to be more readily mobilized from adipocytes and preferentially used by working muscles (Leyton et al. 1987; Raclot and Groscolas 1993, 1995). Fatty acids are transported through the circulation as non-esterified FA (NEFA) bound to albumin, neutral lipids (NL, mainly triacylglycerol) and phospholipids (PL). Previous studies of migratory bird energetics have focused on plasma NEFA and NL as routes of FA

transport for exercise and fat deposition, yet the potential role of circulating PL in these processes has not been investigated (Jenni-Eiermann and Jenni 1991, 1992, 1994; Williams et al. 1999).

Between migratory flights, birds replenish fat and protein stores at stopover sites (Lindström and Piersma 1993; Karasov and Pinshow 1998), and the rate of refueling can be a major determinant of migration speed and flight strategy (Alerstam and Lindström 1990). Rapid fattening is associated with elevated plasma triacylglycerol concentration, a blood parameter recognized as a good indicator of individual mass deposition rate and stopover habitat quality (Jenni-Eiermann and Jenni 1994; Jenni and Jenni-Eiermann 1996; Williams et al. 1999; Guglielmo et al. 2002b). However, some fatty acids are absorbed from the gut lumen as PL and transported as such in lipoproteins along with triacylglycerol through the circulation (Homan and Jain 2001; Phan and Tso 2001). The usefulness of plasma PL concentration as an alternative index of fattening rate has not been evaluated.

Phospholipids are the major structural lipid component of biological membranes and variation in their physico-chemical characteristics has a major impact on membrane function (Hazel and Williams 1990). The FA composition of muscle PL is dramatically influenced by dietary FA intake (Ayre and Hulbert 1996), and in rats (*Rattus norvegicus*), increased endurance is correlated with high concentrations of n-6 polyunsaturated fatty acids in muscle PL (Ayre and Hulbert 1997). Similarly, endurance training itself influences the FA composition of muscle PL (Andersson et al. 1998; Helge et al. 1999; Andersson et al. 2000; Helge et al. 2001). The mechanisms responsible for these effects are unknown, but their physiological significance could be evaluated in a natural context by studying seasonal variation in muscle PL in relation to the capacity for long-distance flight in migratory birds.

In this study, we have investigated seasonal changes in selected plasma and muscle lipids of the western sandpiper (*Calidris mauri*), a migrant shorebird traveling up to 20,000 km each year between arctic breeding sites and temperate/tropical wintering areas (Wilson 1994). We wanted to relate the migration state of the animal (non-migratory, premigratory and spring/fall migrating states) to the concentration and FA composition of plasma NEFA, NL, and PL, as well as to the FA composition of flight muscle PL. Our major goals were: (1) to assess the relative importance of the three plasma lipid transport pathways during migratory fat deposition, and (2) to determine whether the modulation of membrane PL composition is a component of the physiological response to long-distance migration.

Materials and methods

Adult female western sandpipers were sampled in four seasons under permits from the Canadian Wildlife Service and INRENARE (Panama), and in accordance with Canadian Committee for Animal Care guidelines. Wintering (non-migratory) and premigratory birds were sampled in December 1995–January 1996 and March 1996, respectively, at Chitre, Panama (8°N, 79°W). Migrating sandpipers were sampled during stopover refueling in spring (30 April–7 May) and fall (16–25 July) 1996 at the Fraser estuary, British Columbia, Canada (49°10′N, 123°05′W). Sandpipers were captured in mist nets (Avinet, Dryden, N.Y., USA) either while returning to a mudflat from upland roosting/feeding in shrimp and salt production areas (Panama), or after 6–10 h mudflat feeding (Fraser estuary). Birds were removed immediately from nets, anesthetized (Guglielmo et al. 1998), and bled from a jugular incision into Pasteur pipettes (rinsed with 1000 IU/ml porcine sodium heparin). Blood was centrifuged 10 min at 6000 rpm (2000 *g*) and plasma stored at –20 °C. Pectoralis muscle (0.3–1 g) was sampled and stored in liquid N_2 (–196 °C).

Plasma (300 µl) was extracted twice with 15 ml chloroform:methanol (2:1, v/v; Folch et al. 1957). Pectoralis muscle (80–300 mg) was extracted similarly, but was first homogenized 3×10 s in 10 ml chloroform:methanol (2:1) with a high-speed stainless homogenizer. The extract was filtered (Whatman no. 1), and aqueous solutes removed by partitioning with 7.5 ml 0.25% KCl. The organic phase was evaporated (Rotovapor, Buchi, Switzerland) and lipids resuspended in 100 µl chloroform for loading onto Supelclean solid phase extraction tubes (Supelco, LC-NH2, 100 mg). NL were eluted with 1.8 ml chloroform:isopropanol (2:1 v/v). NEFA were then eluted with 1.6 ml isopropyl ether:acetic acid (49:1 v/v), and PL were eluted with 2 ml methanol. Heptadecanoic acid (17:0; 200 µl, 30 mg/100 ml in hexane) was added as an internal standard, and each lipid fraction was evaporated to dryness under N_2. NEFA were methylated at room temperature for 30 min after addition of 100 µl methanol, 1 ml dimethoxypropane, and 40 µl concentrated HCL. Pyridine (20 µl) was added, the sample was dried under N_2, and extracted with isooctane (750 µl) after addition of water (500 µl). The isooctane phase was isolated, the aqueous phase re-extracted with 750 µl isooctane, and the combined isooctane extracts dried under N_2. NL and PL fractions were transesterified as previously described (Anderton et al. 1981). Fatty acid esters were resuspended in 50 µl isooctane for injection onto the gas chromatograph (GC) column.

Plasma lipid FA were separated on a Chrompack CP 9000 GC (Delft, The Netherlands) with an Omegawax 250 column (Supelco 24136) and flame ionization detector (FID). The carrier gas was N_2 and the temperature program was: 4 min 160 °C, 30 min 2 °C/min to 220 °C, 16 min 220 °C, 10 min 10 °C/min to 240 °C, 2 min 240 °C. Fatty acids from muscle PL were separated on a Hewlett-Packard 5890 Series II GC (Mississauga, Canada) with a Supelco-2330 fused silica capillary column and FID. The carrier gas was He and column temperature was held constant at 180 °C for 40 min. Fatty acids were identified by comparison to standard mixtures (Sigma 189–19, Supelco PUFA-3), and mass percents determined from peak areas correcting for recovered 17:0. Within each lipid class, only FA above 0.1% of the total in any season were considered in the analysis. Fatty acids with less than 16 carbons were excluded from statistical analysis because precautions to minimize volitization were not taken.

To summarize FA composition, mass percents of total monounsaturated and total unsaturated (monounsaturated + polyunsaturated) FA were calculated. Proportions were arcsine transformed for statistical analysis. Plasma concentration (mmol/l), monounsaturation and total unsaturation of NEFA, NL, and PL were compared across seasons by one-way ANOVA and the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ). While body mass was found previously to affect plasma NL concentration (Guglielmo et al. 2002b), analysis of covariance of NL controlling for body mass did not qualitatively change the interpretation of the data, and so we report results of standard ANOVA. Within seasons, paired (where possible) and unpaired *t*-tests were used to compare total FA concentration, monounsaturation, and total unsaturation among plasma NEFA, NL, and PL fractions. Seasonal changes in FA composition, monounsaturation, total unsaturation, and (n-6)/(n-3) of muscle PL were detected by ANOVA and REGWQ. Differences were considered significant at $\alpha = 0.05$ and analyses were conducted in SAS version 6.0.

Results

Plasma concentrations of FA differed among the NEFA, NL and PL fractions and varied substantially through the year (Fig. 1). NEFA concentration did not vary seasonally ($F_{3,14}=1.0$, $P=0.44$), but NL and PL levels changed in relation to migration ($F_{3,20}=8.4$, $P=0.0008$; $F_{3,19}=36.3$, $P=0.0001$, respectively). NL concentration was higher in fall migrants than in wintering, premigrant and spring migrating sandpipers, which did not differ. PL levels were higher in spring and fall migrants than in wintering and premigrant birds, and were 75% higher in spring than in fall. Substantially more FA were present in NL and PL than in NEFA in every season. Fatty acid concentrations were similar between NL and PL in all seasons except spring migration when FA were more than twice as abundant in PL than in NL. Summed PL and NL varied seasonally ($F_{3,19}=21.41$, $P=0.0001$), and

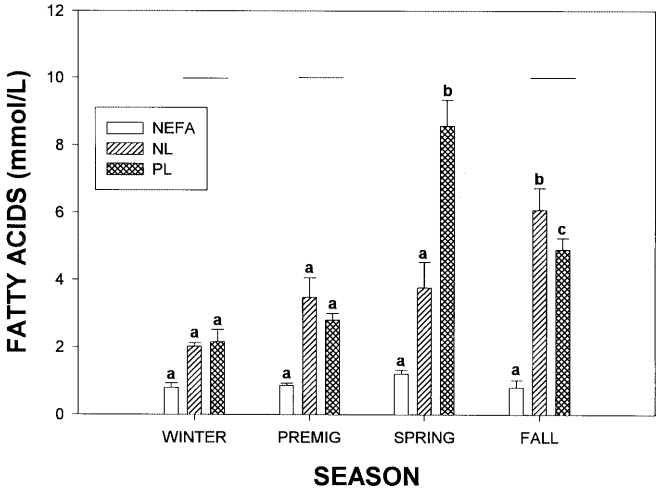

Fig. 1. Seasonal variation in concentrations of fatty acids (FA) in non-esterified FA (*NEFA*; $n=18$), neutral lipid (*NL*; $n=23$), and phospholipid (*PL*; $n=24$) fractions in plasma of western sandpipers. For each lipid fraction, *shared letters* indicate no significant difference between seasons. Within each season, *lines* connect lipid fractions that are not significantly different

was lower in winter and premigration (which did not differ) than during migration stages. The high PL levels in spring migrants were offset by lower NL levels such that the combined amount of FA in NL and PL was not different between spring and fall migration.

Fatty acid composition varied among plasma NEFA, NL, and PL fractions and changed in relation to migration (Tables 1,2, 3). Generally, 16:0, 18:0, and 18:1 were the most abundant FA, but several long-chain, polyunsaturated FA (e.g., 20:4, 20:5, 22:6) were important in most seasons. Although monounsaturation and total unsaturation of NEFA varied significantly among seasons ($F_{3,14}=7.2$, $P=0.004$; $F_{3,14}=4.3$, $P=0.01$, respectively), there was no consistent pattern in relation to migration (Fig. 2). Monounsaturation of NL and PL fractions increased during premigration and remained higher than winter levels during spring and fall migration ($F_{3,20}=8.2$, $P=0.001$; $F_{3,19}=7.4$, $P=0.002$, repectively; Fig. 2A), owing mainly to greater amounts of 16:1 and 18:1. Total unsaturation of NL varied among seasons ($F_{3,20}=12.9$, $P=0.0001$), but was only significantly different between spring migration and the three other seasons (Fig. 2B). In contrast to NL, total unsaturation of plasma PL increased substantially during spring and fall migration compared to winter ($F_{3,19}=13.5$, $P=0.0001$), with premigrants having intermediate total unsaturation levels (Fig. 2B). Changes in the relative amounts of 20:4, 20:5, and 22:6 contributed to variation in total unsaturation of all plasma lipid fractions. Monounsaturation and total unsaturation levels of NEFA did not differ systematically from those of NL and PL (Fig. 2). However, in all seasons PL were lower than NL in both monounsaturation and total unsaturation (Fig. 2). Plasma PL were particularly rich in 16:0 and 18:0.

While not fluctuating as dramatically as the FA composition of plasma lipid fractions, the FA composition of pectoralis muscle PL changed systematically in relation to migration (Table 4). Arachidonic acid (20:4), 18:0, 16:0, 20:5, and 18:1 were the most abundant FA in muscle PL. 18:2 and 18:3 levels did not vary significantly. Monounsaturation was higher during spring and fall migration than during winter and premigration ($F_{3,20}=5.1$,

Table 1. Fatty acid composition of plasma non-esterified fatty acids (NEFA) of adult female western sandpipers sampled at a wintering area in Panama (winter, premigration) and during migratory stopover in British Columbia (spring, fall). Data are mean mass percent ± SE. Sample size in *parentheses*; *nd* not detectable

Fatty acid	Season			
	Winter (3)	Premigration (6)	Spring migration (3)	Fall migration (6)
16:0	16.5 ± 2.2	4.5 ± 1.7	21.1 ± 2.8	3.8 ± 1.2
16:1(n-7)	3.3 ± 0.8	0.9 ± 0.4	4.4 ± 0.9	1.2 ± 0.8
16:2(n-4)	1.1 ± 0.1	0.1 ± 0.1	1.4 ± 0.3	nd
18:0	24.0 ± 1.9	21.3 ± 1.7	19.7 ± 1.4	22.8 ± 1.5
18:1(n-9)	28.1 ± 4.9	16.0 ± 2.1	26.2 ± 1.3	15.8 ± 1.5
18:1(n-7)	4.2 ± 0.2	4.1 ± 0.9	5.0 ± 0.3	4.4 ± 0.4
18:2(n-6)	2.3 ± 0.3	2.7 ± 0.3	2.9 ± 0.7	2.4 ± 0.4
18:3(n-3)	3.0 ± 0.5	2.7 ± 0.4	1.7 ± 0.7	2.2 ± 0.3
20:0	1.5 ± 0.3	3.0 ± 0.5	1.5 ± 0.6	2.7 ± 0.4
20:1(n-11)	1.1 ± 0.2	2.8 ± 0.3	1.0 ± 0.5	2.2 ± 0.3
20:4(n-6)	5.4 ± 1.2	9.3 ± 1.0	2.9 ± 0.4	10.3 ± 1.3
20:5(n-3)	4.5 ± 2.4	15.0 ± 3.1	7.4 ± 1.7	14.9 ± 1.6
22:5(n-3)	1.9 ± 0.8	4.1 ± 0.7	1.6 ± 0.4	3.8 ± 0.6
22:6(n-3)	3.2 ± 0.8	13.6 ± 2.3	3.2 ± 0.8	13.5 ± 1.9

Table 2. Fatty acid composition of plasma neutral lipids of adult female western sandpipers sampled at a wintering area in Panama (winter, premigration) and during migratory stopover in British Columbia (spring, fall). Data are mean mass percent ± SE. Sample size in parentheses; *nd* not detectable

Fatty acid	Season			
	Winter (6)	Premigration (6)	Spring migration (6)	Fall migration (6)
16:0	13.9 ± 1.0	21.7 ± 2.0	15.7 ± 0.5	21.8 ± 1.1
16:1(n-7)	2.6 ± 0.2	3.2 ± 0.7	4.1 ± 0.2	5.6 ± 0.6
18:0	9.8 ± 0.5	11.8 ± 0.8	8.3 ± 0.8	11.9 ± 0.8
18:1(n-9)	20.7 ± 0.7	23.4 ± 2.2	27.8 ± 2.1	27.0 ± 1.8
18:1(n-7)	2.6 ± 0.3	4.7 ± 0.5	4.8 ± 0.4	4.3 ± 0.9
18:2(n-6)	1.9 ± 0.1	1.9 ± 0.4	3.1 ± 0.8	3.8 ± 1.4
18:3(n-3)	1.9 ± 0.2	1.9 ± 0.4	1.4 ± 0.3	2.5 ± 0.7
18:3(n-6)	0.5 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
18:4(n-3)	0.7 ± 0.1	0.5 ± 0.3	0.7 ± 0.2	0.6 ± 0.1
20:0	3.6 ± 0.2	1.8 ± 0.4	0.8 ± 0.8	0.4 ± 0.2
20:1(n-11)	1.1 ± 0.4	2.1 ± 0.5	1.0 ± 0.6	1.0 ± 0.1
20:2(n-6)	11.1 ± 0.8	0.7 ± 0.1	4.5 ± 0.4	0.4 ± 0.1
20:3(n-6)	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
20:4(n-3)	5.9 ± 0.4	1.3 ± 0.4	0.3 ± 0.2	0.7 ± 0.1
20:4(n-6)	6.0 ± 0.6	6.2 ± 0.8	4.4 ± 0.5	4.8 ± 0.9
20:5(n-3)	5.8 ± 0.7	8.9 ± 1.5	14.8 ± 2.3	9.4 ± 1.3
22:0	3.1 ± 0.2	0.7 ± 0.1	1.0 ± 0.2	0.5 ± 0.1
22:1(n-9)	1.0 ± 0.1	3.9 ± 2.3	0.4 ± 0.1	0.9 ± 0.1
22:2(n-6)	2.5 ± 0.1	nd	0.5 ± 0.2	nd
22:5(n-3)	1.2 ± 0.2	1.8 ± 0.3	1.7 ± 0.2	1.3 ± 0.1
22:6(n-3)	1.8 ± 0.4	2.3 ± 0.3	3.5 ± 1.0	1.6 ± 0.3
24:0	1.7 ± 0.2	nd	nd	nd

Table 3. Fatty acid composition of plasma phospholipids of adult female western sandpipers sampled at a wintering area in Panama (winter, premigration) and during migratory stopover in British Columbia (spring, fall). Data are mean mass percent ± SE. Sample size in parentheses; *nd* not detectable

Fatty acid	Season			
	Winter (5)	Premigration (6)	Spring migration (6)	Fall migration (6)
16:0	20.8 ± 3.7	33.0 ± 2.7	21.0 ± 1.4	32.2 ± 2.2
16:1(n-7)	0.2 ± 0.1	0.8 ± 0.2	1.1 ± 0.2	2.0 ± 0.8
18:0	54.8 ± 1.1	28.8 ± 3.9	18.1 ± 2.1	21.8 ± 1.7
18:1(n-9)	6.9 ± 1.3	13.7 ± 2.3	15.0 ± 1.5	16.9 ± 2.2
18:1(n-11)	1.4 ± 0.2	1.8 ± 0.6	3.4 ± 0.2	4.4 ± 0.7
18:2(n-6)	0.4 ± 0.1	0.8 ± 0.1	1.7 ± 0.7	1.4 ± 0.5
18:3(n-3)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
18:3(n-6)	0.5 ± 0.1	nd	0.2 ± 0.1	0.1 ± 0.1
20:0	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
20:1(n-11)	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
20:2(n-6)	nd	0.4 ± 0.1	nd	0.3 ± 0.1
20:3(n-6)	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
20:4(n-3)	1.5 ± 0.1	0.6 ± 0.3	nd	0.7 ± 0.4
20:4(n-6)	7.4 ± 1.3	8.3 ± 1.3	12.3 ± 0.9	7.6 ± 0.9
20:5(n-3)	2.0 ± 0.8	5.6 ± 1.7	16.4 ± 1.4	7.7 ± 1.3
22:0	0.8 ± 0.1	1.4 ± 0.9	0.3 ± 0.1	0.3 ± 0.1
22:5(n-3)	0.8 ± 0.3	1.0 ± 0.3	2.8 ± 0.4	0.9 ± 0.1
22:6(n-3)	0.7 ± 0.2	1.5 ± 0.3	5.9 ± 1.2	2.1 ± 0.6

$P = 0.009$), due to increased 18:1. Total unsaturation varied among seasons ($F_{3,20} = 9.3$, $P = 0.0005$), but in contrast to monounsaturation, total unsaturation was lower during fall migration than in all other seasons, which did not differ. Decreased total unsaturation in migrants was related to an increase in the relative amount of 18:0 and a substantial decline in 20:4. However, the decrease in 20:4 between winter and migration seasons was compensated somewhat by increases in other long-chain polyunsaturated n-3 FA to maintain total unsaturation nearly constant. This shift in FA composition is reflected in a significant decrease in the n-6/n-3 ratio between winter and fall migration. It is notable that muscle PL contained detectable quantities of an odd-chain fatty acid (15:0; 1.6–3.0%) in all seasons.

Discussion

Concentrations and FA compositions of plasma lipids varied dramatically through the year in the long-distance migrant shorebird we studied. NL and PL appeared to be of greater importance than NEFA for transporting lipids in fattening birds. Our most novel findings were the high plasma concentrations and large changes in FA composition of plasma PL during migration, which suggest that plasma PL may be useful as an indicator of fattening rate. The FA profile of flight muscle PL changed in relation to migratory state, but was not a simple reflection of changing plasma PL or other plasma lipid fractions. Our results have important implications

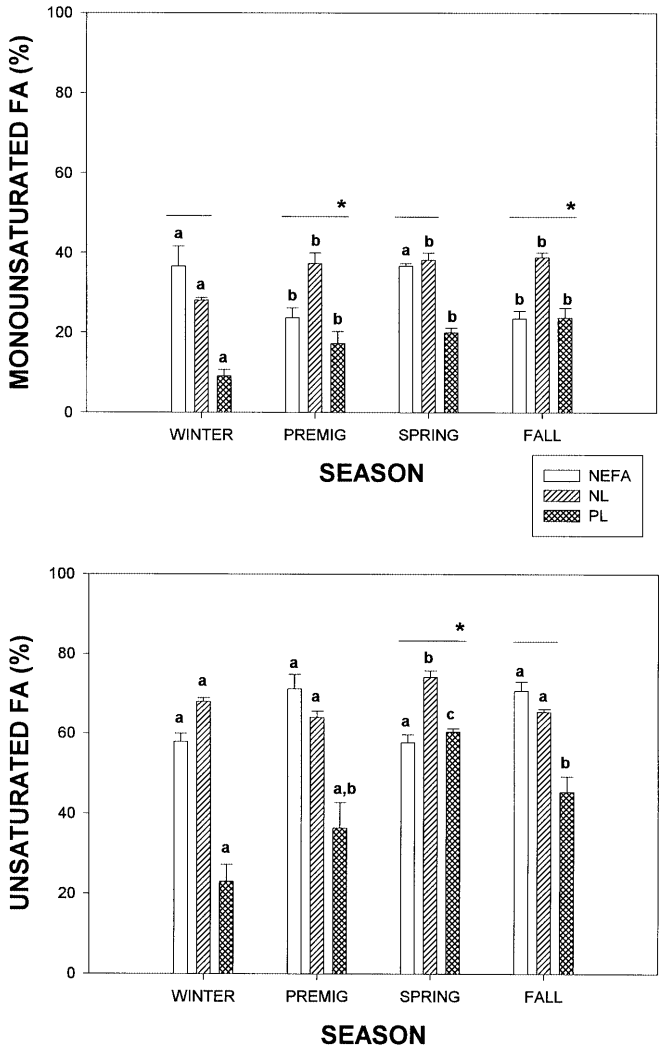

Fig. 2. Seasonal variation in total monounsaturation (**A**) and total unsaturation (**B**) of fatty acids in NEFA ($n = 18$), NL ($n = 23$), and PL ($n = 24$) fractions in plasma of western sandpipers. For each lipid fraction, *shared letters* indicate no significant difference between seasons. Within each season, *lines* connect lipid fractions that are not significantly different. The *asterisk* indicates no significant difference between NEFA and PL

for: (1) understanding the mechanisms of fat deposition in migratory birds, (2) applying plasma lipid analyses to assess bird fattening rates, and (3) testing the hypothesis that variation in FA composition of muscle PL is related to endurance exercise performance.

Plasma lipids

The birds we sampled were not captured during endurance flight, nor were they in reproductive condition. Thus, the observed variation in plasma lipids was most likely caused by changes in feeding behavior, and possibly by endogenous seasonal modulation of lipid metabolism. In Panama, western sandpipers reach a low and stable body mass in winter (P.D. O'Hara, unpublished data). During February and March they become premigratory and deposit fat slowly over several weeks before initiating northward migration. The rate of premigratory fat deposition (0.09 g/day; P.D. O'Hara, unpublished data) is four to ten times lower than the rate attained during spring and fall migratory stopover in the Fraser estuary (0.4–1 g/day; Butler et al. 1997). Most of the lipid energy in plasma was present in NL and PL. Although plasma NEFA appears to be a significant route of transport of absorbed dietary FA in chickens (*Gallus gallus*; Sklan et al. 1984), NEFA concentration was relatively low in all seasons in feeding sandpipers. NL (mainly triacylglycerol) concentration tended to be elevated during premigration and spring, but only increased significantly during fall migration. On the other hand, plasma PL concentration increased nearly threefold during spring migration compared to winter and premigration, and remained high during fall migration. The quantity of FA in plasma PL was as high as in NL during most seasons and was twice as high as in the NL fraction during spring migration, highlighting the importance of this previously overlooked pathway of dietary FA transport for fat deposition in birds. With a larger sample of western sandpipers collected from the same locations used in the present study, Guglielmo et al. (2002b) found triacylglycerol concentrations to be similar between spring and fall migrants, and higher in premigrants than in winter birds. In the latter study, a significant interaction between sampling location and body mass precluded direct comparison of Fraser estuary migrants to sandpipers from Panama. However, when the two studies are considered together, it seems apparent that changes in plasma triacylglycerol alone do not perfectly follow known seasonal differences in fattening rates (zero in winter, low during premigration and high during migration), and are made difficult to interpret by covariation with body mass. Our new data suggest that combining information on plasma PL levels with triacylglycerol measurement may substantially improve techniques to assess rates of mass change in birds (Jenni-Eiermann and Jenni 1994; Williams et al. 1999). For example, summed NL and PL was twice as high in migrants as in wintering and premigrant birds, reflecting the expected high rates of mass gain during migratory stopover. Although a multiple comparison test of summed NL and PL (including migrants) did not show a difference between winter and premigrant birds, a simple ANOVA comparing these two groups was significant ($P = 0.05$), indicating that with a larger sample of birds even the moderate fattening rates of premigratory sandpipers could be detected.

The distribution of FA differed among the three plasma lipid fractions, and seasonal changes were most apparent in plasma PL. Saturated FA appeared to be preferentially transported in PL rather than NL, perhaps exploiting the greater polarity of PL to compensate for the lower aqueous solubility of saturated FA. Plasma NL had a high concentration of unsaturated FA in all seasons, and total unsaturation of NL was further ele-

Table 4. Fatty acid composition, total monounsaturates (*MONO*), total unsaturates (*UNSAT*) and the n-6/n-3 ratio of pectoralis muscle phospholipids of adult female western sandpipers sampled at a wintering area in Panama (winter, premigration) and during migratory stopover in British Columbia (spring, fall). *Shared symbols* within a row are not significantly different. Data are mean mass percent ± SE. Sample size in parentheses; *nd* not detectable

Fatty acid	Season			
	Winter (6)	Premigration (6)	Spring migration (6)	Fall migration (6)
16:0	[a]16.4 ± 0.8	[a]17.0 ± 0.5	[a]13.8 ± 0.4	[a]16.7 ± 1.1
16:1(n-7)	[a]0.2 ± 0.1	[a,b]0.3 ± 0.1	[c]0.6 ± 0.1	[b,c]0.5 ± 0.1
16:2(n-4)	[a]0.7 ± 0.1	[a]0.9 ± 0.1	[a,b]0.6 ± 0.1	[b]0.3 ± 0.1
18:0	[a]21.4 ± 0.8	[a]21.4 ± 0.6	[b]25.7 ± 0.4	[b]24.8 ± 0.8
18:1(n-9)	[a]10.4 ± 0.5	[a]10.1 ± 0.4	[b]11.9 ± 0.5	[b]12.0 ± 0.5
18:2(n-6)	[a]1.2 ± 0.1	[a]1.6 ± 0.3	[a]1.9 ± 0.6	[a]1.8 ± 0.3
18:3(n-3)	[a]0.5 ± 0.1	[a]0.5 ± 0.1	[a]0.3 ± 0.1	[a]0.6 ± 0.1
20:0	[a]0.1 ± 0.1	[a]0.1 ± 0.1	[a]0.1 ± 0.1	nd
20:3(n-6)	[a]1.7 ± 0.3	[a,b]1.1 ± 0.2	[b]0.5 ± 0.1	[b]0.5 ± 0.2
20:4(n-6)	[a]29.2 ± 1.5	[a,b]25.3 ± 0.9	[b,c]22.3 ± 1.9	[c]19.4 ± 0.9
20:5(n-3)	[a]9.8 ± 1.5	[a]13.0 ± 0.8	[a]13.4 ± 1.9	[a]13.6 ± 0.2
22:3(n-6)	[a]2.4 ± 0.1	[b]1.9 ± 0.1	[c]1.2 ± 0.1	[c]0.9 ± 0.1
22:5(n-3)	[a,b]1.4 ± 0.2	[a]1.2 ± 0.1	[b,c]1.7 ± 0.1	[c]1.9 ± 0.1
22:6(n-3)	[a]3.4 ± 0.1	[a,b]4.4 ± 0.4	[a,b]5.2 ± 1.1	[b]6.3 ± 0.1
24:0	[a]1.1 ± 0.1	[a,b]1.0 ± 0.1	[a,b]0.8 ± 0.2	[b]0.6 ± 0.1
MONO	[a]10.6 ± 0.6	[a]10.5 ± 0.4	[b]12.4 ± 0.6	[b]12.5 ± 0.5
UNSAT	[a]61.0 ± 0.3	[a]60.4 ± 0.2	[a]59.6 ± 0.1	[b]57.8 ± 0.8
n-6/n-3	[a]2.4 ± 0.3	[a,b]1.5 ± 0.1	[a,b]1.6 ± 0.6	[b]1.0 ± 0.1

vated only in spring migrants. Total unsaturation of plasma PL was low in winter, increased three-fold during spring migration and remained high in fall migrants. In an analysis of tissues taken from the same western sandpipers used in our study, Egeler and Williams (2000) found that monounsaturation and total unsaturation of triacylglycerol from adipose tissue increased from winter to premigration, peaked during spring migration, and returned to premigration levels during fall. Increased adipose triacylglycerol unsaturation was related to increased concentrations of 16:1 and 18:1, and decreased 18:0. Surprisingly, these seasonal changes in adipose tissue triacylglycerol were most similar to the changes we found in plasma PL. Thus, our results suggest that qualitative changes in FA composition of fat stores may be reflected better in plasma PL than NL.

The seasonal changes in concentrations and FA compositions of plasma lipids we observed are probably best explained by changes in feeding rate and diet lipid composition. Daily food intake in refueling migrants is greatly elevated (Bairlein 1990), and sandpiper-sized migrants can deposit fat at up to 7% or more of lean body mass each day (Lindström 1991). During fat deposition, plasma lipoprotein-associated FA esters (e.g., NL and PL) rise due to intestinal absorption and processing of dietary fats into portomicrons, and by hepatic de novo production of very low density lipoproteins (Ramenofsky 1990; Jenni-Eiermann and Jenni 1994; Williams et al. 1999). In the gut, the action of pancreatic lipase and phoshpholipase A2 produces monoacylglycerol, lysophospholipids and FA which are rapidly absorbed across the brush-border membrane and reesterified into triacylglycerol and PL (Homan and Jain 2001; Mansbach 2001; Phan and Tso 2001). Therefore, plasma concentration and FA composition of NL and PL should reflect the characteristics of lipids in the diet. In the benthic invertebrates fed upon by western sandpipers, total body PL is related to body size, while NL content is determined by the amount of lipid storage (Hentschel 1998). Thus, the ratio of NL to PL is often used as a measure of the nutritional condition of the invertebrate, which may vary among species, and due to factors like age, reproductive state or environmental stress (Hill et al. 1992; Graeve et al. 1997; Hentschel 1998). In northern oceans and lakes, the nutritional condition of benthic invertebrates may decline over the winter, resulting in a low NL/PL ratio in spring, followed by recovery as feeding conditions improve over the summer (Hill et al. 1992). The high PL concentration and low NL/PL ratio in plasma in spring migrants indicates that the available invertebrates had low NL stores, but that sandpipers compensated by eating more (or larger) individuals. Conversely, fall migrants may have consumed fewer individuals (lower total PL) of higher quality. The result was that total FA energy in plasma was similar between spring and fall, suggesting that fattening rates may have been similar in the two seasons.

The relatively high abundance of 20:5 and 22:6 in plasma and muscle lipids was also reported in the semipalmated sandpiper (*Calidris pusilla*; Napolitano and Ackman 1990), and most likely reflects the ingestion of marine invertebrates which are high in these FA (Chapelle 1977; Beninger and Stephan 1985; Graeve et al. 1997). Similarly, the presence of odd-chain FA suggests the ingestion of marine algae, or more likely, consumption of unusual amphipod crustaceans which may accumulate high levels of odd-chain FA (Paradis and Ackman 1976). The latitudinal and seasonal variation in unsaturation of plasma PL of sandpipers may be a result of homeoviscous adaptations of invertebrate membrane PL to water temperature (i.e., more unsaturated in colder waters; Hazel 1995; Williams and Somero 1996; Lahdes et al. 2000). While dietary factors may explain much of the variation in plasma lipid profiles, it is likely that endogenous seasonal modulation of FA metabolism plays an important role as well. For example, hepatic FA synthase and Δ^9-desaturase activity were

elevated four-fold during migration in these sandpipers (Egeler et al. 2000), indicating that the liver is highly active in the biosynthesis and modification of FA in migrants. Careful experimentation is required to partition the effects of diet and liver biochemistry on plasma lipid profiles.

Our findings illustrate how studying plasma PL in addition to NL (or triacylglycerol) may not only provide better information on fattening rates of birds, but also may reveal details of prey identity and quality. Controlled studies of the relationships among diet lipid composition, total energy intake, plasma lipids and fattening rates could lead to much improved methods to investigate the foraging behavior and migration physiology of wild birds.

Muscle phospholipids

Several recent studies suggest a relationship between exercise and the FA composition of skeletal muscle PL. Ayre and Hulbert (1996) manipulated dietary FA profile to alter the FA composition of muscle PL in rats. Subsequently, they found that untrained rats with high muscle PL levels of n-6 polyunsaturated FA (e.g., 20:5, 22:6), and a high ratio of n-6 to n-3 FA, were able to run nearly twice as long and perform more than double the work of rats with either high n-3 polyunsaturated FA or high monounsaturated FA levels (Ayre and Hulbert 1997). They concluded that diets high in n-3 polyunsaturated FA reduce endurance performance, possibly through effects on muscle PL. Taking a different approach, a number of investigations have demonstrated alterations in muscle PL in response to exercise training, yet the results are variable. Holding diet constant, Andersson et al. (1998) found in humans that training caused a reduction in n-6 FA, particularly 18:2 and 20:4, a decrease in the n-6/n-3 ratio, and a increase in 18:1. Two other human studies showed an increase in 18:1 (Helge et al. 2001) and 18:0 (Andersson et al. 2000), and a decrease in the n-6/n-3 ratio (Andersson et al. 2000; Helge et al. 2001) in response to training. In rats, exercise training had no effect on 18:1 of muscle PL, however 20:4, the n-6/n-3 ratio and total unsaturation decreased (Helge et al. 1999).

The FA composition of sandpiper flight muscle PL changed with migration in a manner similar to the mammalian response to training. The most noticeable effects of migration were increases in 18:0 and 18:1 and a decrease in 20:4, which contributed to a rise in monounsaturation and declines in total unsaturation and the n-6/n-3 ratio. These changes could be interpreted as adaptive modulation of membrane biochemistry to enhance endurance flight performance. However, diet and exercise studies in mammals suggest, in fact, that endurance performance is enhanced by high relative amounts of n-6 FA (20:4), and that training causes a selective depletion of these FA from membranes (Helge et al. 1999; Andersson et al. 2000; Helge et al. 2001). Hence, the alteration of muscle PL in sandpipers may in reality represent a physiological cost of migration, leading to the conclusion that an important adaptation for migration may be a very high constitutive level of 20:4 in sandpiper membrane PL. Prior to migration, 20:4 was the most abundant FA in sandpiper muscle PL (25–29%), while in humans and rats, 20:4 ranges from 12% to 19% (Andersson et al. 1998; Helge et al. 1999; Andersson et al. 2000; Helge et al. 2001). The concentration of 20:4 in total body PL of semipalmated sandpipers was only 3.6%, but this may be an underestimate of muscle concentration (Napolitano and Ackman 1990). Exercise activity clearly affects the FA composition of muscle PL, however, it remains to be determined if these changes represent adaptive physiological modulation to enhance performance.

In conclusion, this study shows that plasma and muscle PL undergo major seasonal changes coincident with long-distance migration. Plasma PL may be an important route of FA transport in feeding birds, and as such may provide a new index of fattening rate to be used in concert with triacylglycerol. The large increase in plasma PL concentration observed during migration also suggests that PL may play a significant role in fueling endurance flight in birds, as may be the case for NL (Jenni-Eiermann and Jenni 1992). Therefore, quantifying the oxidation rates of circulating NEFA, PL, and NL in exercising birds will be an important goal for future studies. This area of research will not only advance basic knowledge of avian physiology, but could reveal how long-distance migrants have expanded the standard limits of lipid metabolism to power extreme endurance flight.

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