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Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping

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Summary

During their fall migration from the Arctic to South America, semipalmated sandpipers *Calidris pusilla* stop in the Bay of Fundy (east coast of Canada) before flying non-stop for ~4500 km across the ocean. Refueling birds double their body mass by feeding on *Corophium volutator*, an amphipod containing high amounts of n-3 polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic (20:5) and docosahexaenoic acid (22:6). In mammals, high dietary intake of n-3 PUFA is known to increase capacity for oxidative metabolism. Therefore, we hypothesized that tissue incorporation of n-3 PUFA would be associated with increases in the activity of key muscle enzymes to upregulate energy metabolism for prolonged exercise.

Birds were collected at various stages of fat loading to monitor changes in lipid composition and flight muscle enzymes simultaneously. Enzymes were measured to assess oxidative capacity [citrate synthase (CS)], β-oxidation [carnitine palmitoyl transferase (CPT) and 3-hydroxyacyl dehydrogenase (HOAD)] and glycolytic capacity [lactate dehydrogenase (LDH)]. Changes in the fatty acid composition of muscle membranes (phospholipids) and fuel reserves (neutral lipids) were measured separately to distinguish between membrane-related and systemic effects of n-3 PUFA. Results show that muscle CS and HOAD are stimulated during refueling and that their activities are correlated with n-3 PUFA content in phospholipids (22:6 for CS, 20:5 for HOAD) and in neutral lipids (20:5 for CS). This suggests that 20:5 and 22:6 have different effects on energy metabolism and that they act *via* changes in membrane structure and systemic mechanisms. CPT and LDH did not change during refueling, but LDH activity was significantly related to the n-3 PUFA content of fuel reserves. This study shows that oxidative capacity increases rapidly during refueling and supports the idea that dietary n-3 PUFA are used as molecular signals to prime flight muscles of some long-distance migrants for extreme exercise.

Key words: dietary n-3 fatty acid, eicosapentaenoic acid, docosahexaenoic acid, long-distance migrant bird, semipalmated sandpiper, *Calidris pusilla*, muscle enzyme, endurance exercise.

Introduction

Metabolic studies of birds preparing for migration have focused on the building of adequate energy reserves, but whether flight muscles are also primed for endurance exercise has received less attention. For semipalmated sandpipers *Calidris pusilla* L., dietary lipids are not simply an essential energy source, but a metabolic signal that improves muscle performance (Maillet and Weber, 2006). During their fall migration from the Arctic to South America, these shorebirds refuel in the Bay of Fundy (east coast of Canada) before the longest non-stop flight of their entire migration: a 3-day, ~4500 km, trans-oceanic journey (Hicklin, 1987). During a 2-week stopover, 86% of their diet consists of *Corophium volutator* Pallas (Napolitano and Ackman, 1990; Napolitano et al., 1992), an amphipod containing unusually high amounts of the n-3 polyunsaturated fatty acids (n-3 PUFA) eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6). Together, these two n-3 PUFA account for ~45% of total *Corophium* fatty acids (Ackman et al., 1979; Maillet and Weber, 2006). This diet is intriguing because changes in the n-3 PUFA content of phospholipids affect membrane fluidity (Daveloose et al., 1993; Ernst, 1994; Stillwell and Wassal, 2003), thereby modifying muscle performance. We have recently shown that semipalmated sandpipers refueling on *Corophium* greatly increase the n-3 PUFA content of their tissues (Maillet and Weber, 2006). During the stopover, dietary n-3 PUFA are rapidly incorporated in flight muscle membranes and this physiological change is consistent with improved capacity for fat intake and oxidation.

Numerous mammalian studies have also shown that n-3 PUFA regulate the expression of genes coding for key enzymes of energy metabolism, in part by acting on peroxisome proliferator-activated receptors (PPARs) (Jump, 2002b; Jump and Clarke, 1999; Lapillonne, 2004). A large number of *in vitro*

studies in fish, birds and mammals have shown that n-3 PUFA stimulate cellular capacity for lipid oxidation in hepatocytes, cardiomyocytes and adipocytes. Dietary intake of n-3 PUFA causes mitochondrial and peroxisomal proliferation (Froyland et al., 1997; Totland et al., 2000). Some studies show an increased capacity for these isolated organelles to oxidize fatty acids (Guo et al., 2005; Moya-Falcon, 2004; Yamazaki et al., 1987), others report elevated cellular activities for carnitine palmitoyl transferase (CPT) (Guo et al., 2005; Sanz et al., 2000; Totland et al., 2000), and 3-hydroxyacyl dehydrogenase (HOAD) (Sanz et al., 2000) or fatty acyl oxidase (FAO) (Froyland et al., 1997; Kim and Choi, 2005; Totland et al., 2000; Yamazaki et al., 1987). Unfortunately, the effects of n-3 PUFA on skeletal muscle cells have not been investigated.

The semipalmated sandpiper provides a unique natural model to investigate the potential role of dietary n-3 PUFA in priming the metabolic machinery of a long-distance migrant for endurance exercise. In this study, birds were collected at various stages of fat loading to assess possible changes in the activity of flight muscle enzymes. CPT and HOAD were selected to assess capacity for β-oxidation, whereas citrate synthase (CS) was measured as an index of mitochondrial density/oxidative capacity (Moyes, 2003). Glycolytic capacity was also assessed by monitoring lactate dehydrogenase (LDH). Changes in the fatty acid composition of muscle membranes (phospholipids, PL) and fuel reserves (neutral lipids, NL) were also measured during the stopover in an attempt to distinguish between membrane-related and generalized, systemic effects of dietary PUFA on enzyme activities.

The goals of this study were therefore to determine: (1) whether the capacity for energy metabolism of flight muscle changes during refueling, and (2) whether changes in enzyme activities are associated with tissue incorporation of individual fatty acids, particularly n-3 PUFA. Potential associations between enzyme activities and the percent contribution of individual fatty acids were assessed separately for muscle membranes (PL) and fuel reserves (NL). We hypothesized that dietary PUFA are used to prepare pectoral muscles of semipalmated sandpipers for migration. During refuelling, it was predicted that capacity for oxidative metabolism would increase and that enzyme activities would be positively related to changes in the abundance of n-3 PUFA, either specifically in muscle membranes or in neutral lipid reserves in general.

Materials and methods

Animals and tissue collection

To minimize the number of wild shorebirds to be sacrificed for our experiments, all measurements were made on the pectoral muscles of the same animals used in a previous study (Maillet and Weber, 2006). Semipalmated sandpipers *Calidris pusilla* L. were selected to monitor changes in flight muscle enzyme activities before a ~4500 km migration from the Bay of Fundy (Canada) to South America. In this species, it was established that percent body fat is an accurate predictor of time spent refueling in the Bay of Fundy (Maillet and Weber, 2006; White, 1985). Therefore, changes in percent body fat were used as an indirect measure of feeding time at the last stopover before this long-distance flight. Wild semipalmated 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%). These animals were euthanized by cervical dislocation and the right pectoral muscle was immediately dissected, freeze-clamped in liquid nitrogen and stored at–80°C until enzyme analyses. The rest of the carcass was stored at –20°C.

Muscle homogenates

Subsamples of frozen pectoral muscle (~200 mg) were combined with ice-cold homogenization buffer [20 mmol l^{-1} Na_2HPO_4, 5 mmol l^{-1} EDTA (ethylenediaminetetraacetic acid), 0.1% Triton X-100, 0.2% fatty acid-free BSA (bovine serum albumin), 50 μg ml^{-1} aprotinin and 50% glycerol, pH 7.4] at a 9:1 ratio (volume/mass). Samples were homogenized on ice using a ground-glass homogenizer. Homogenates were then centrifuged at 13 750 ***g*** for 10 min at 4°C, and the supernatant was frozen at –80°C.

Enzyme assays

The activities of the following enzymes were measured at 39°C using a Beckman DU 640 spectrophotometer (Fullerton, CA, USA): citrate synthase (CS; E.C. 2.3.3.1), total carnitine palmitoyl transferase (CPT I + CPT II; E.C. 2.3.1.21), 3-hydroxyacyl CoA dehydrogenase (HOAD; E.C. 1.1.1.35) and lactate dehydrogenase (LDH; E.C. 1.1.1.27) (Guglielmo et al., 2002; Hansen and Sidell, 1983). Activities were determined by changes in absorbance at 412 nm (CS and CPT) or 340 nm (HOAD and LDH). Preliminary measurements were performed to determine homogenate concentrations yielding maximum reaction velocities. For CS, assay conditions were 0.15 mmol l^{-1} DTNB (5,5′-dithiobis 2-nitrobenzoic acid), 0.15 mmol l^{-1} acetyl CoA, 0.5 mmol l^{-1} oxaloacetate (substrate) and diluted homogenate (1:10) in Tris buffer (50 mmol l^{-1}, pH 8.0). For CPT, assay conditions were 0.15 mmol l^{-1} DTNB, 0.035 mmol l^{-1} palmitoyl CoA, 5 mmol l^{-1} carnitine (substrate) and diluted homogenate (1:2) in tris buffer (50 mmol l^{-1}, pH 8.0). For HOAD, assay conditions were 1 mmol l^{-1} EDTA, 0.2 mmol l^{-1} NADH (β-nicotinamide adenine dinucleotide, reduced form), 0.1 mmol l^{-1} acetoacetyl CoA (substrate) and diluted homogenate (1:2) in imidazole buffer (50 mmol l^{-1}, pH 7.4). For LDH, we used 0.15 mmol l^{-1} NADH, 1 mmol l^{-1} KCN, 10 mmol l^{-1} sodium pyruvate (substrate) and diluted homogenate (1:10) in imidazole buffer (50 mmol l^{-1}, pH 7.5).

Lipid analyses

Pectoral muscle and other body lipids were extracted twice in chloroform–methanol (2:1 v/v) (Folch et al., 1957) as detailed previously (Maillet and Weber, 2006). After

resuspension in chloroform, different lipid classes [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) (Bernard et al., 1999). This was done to distinguish potential effects of changes in whole-body storage lipids (NL) and muscle membrane lipids (PL) on enzyme activities. In this study, therefore, relationships between enzyme activities and individual fatty acid concentrations in whole-body NL and in pectoral muscle PL were assessed separately. 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 quantified on a Hewlett-Packard gas chromatograph (5890 series II with 7673 autosampler; Mississauga, ON, Canada) 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). Detailed fatty acid composition of semipalmated sandpiper tissues has been reported previously (Maillet and Weber, 2006).

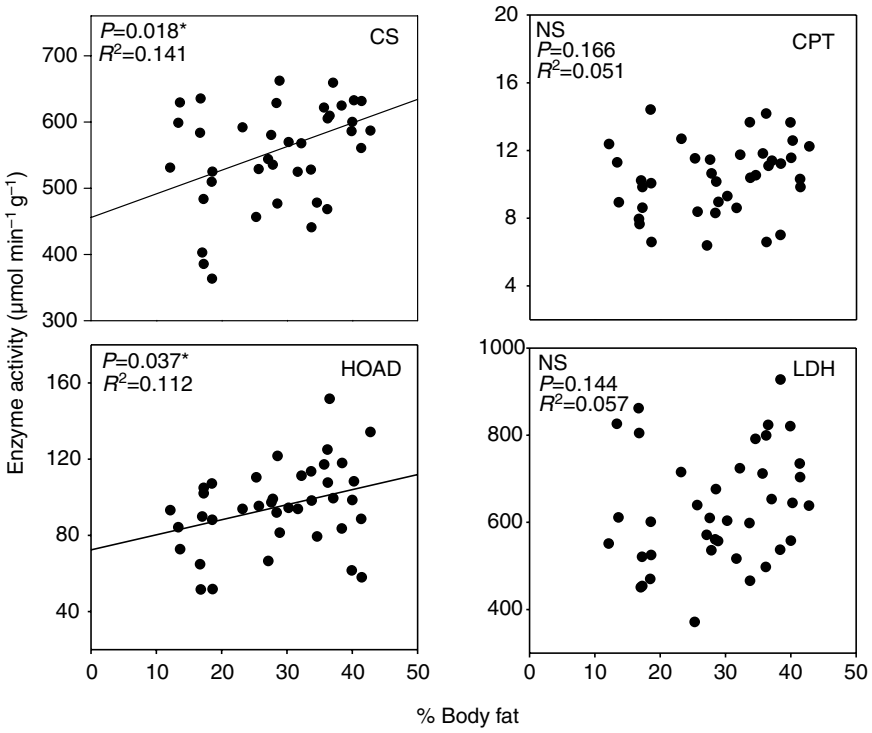

Fig. 1. Pectoral muscle enzyme activities (μmol min^{-1} g^{-1} lean muscle mass) *versus* percent body fat in semipalmated sandpipers refueling in the Bay of Fundy. CS, citrate synthase (*N*=38 animals); CPT, carnitine palmitoyl transferase (*N*=39); HOAD, 3-hydroxyacyl dehydrogenase (*N*=37); LDH, lactate dehydrogenase (*N*=39). Lines were fitted by linear regression (CS: y=455.93+3.56x; HOAD: y=72.29+0.79x). *P<0.05; NS, not significant.

Calculations and statistics

Enzyme assays were run in triplicate and mean absorbance was used for calculations and statistical analyses. Enzyme activities (in μmol min^{-1} g^{-1}) were calculated as follows:

$$\text{Activity} = [(\Delta\text{Absorbance}/\Delta t \times V_f) / (\epsilon \times V_h)] \times D ,$$

where Δt is reaction time in min, V_f is final cuvette volume in μl, V_h is volume of added homogenate in μl, ϵ is the extinction coefficient (13.6 for DTNB and 6.22 for NADH) and D is the dilution factor for the homogenate. Enzyme activities are expressed per g lean muscle mass (i.e. activities were divided by lean mass of pectoral muscle) to eliminate artefacts caused by differences in lipid content between lean and fat birds. Statistical analyses were performed using SigmaStat (version 3.1). Relationships between enzyme activities and percent body fat or percent contribution of individual fatty acids were assessed by linear regression analysis (see Hulbert et al., 2002; Turner et al., 2006). All variables were normally distributed and all variances were homogeneous. Percentages were transformed to the arcsine of their square root before analysis. Probabilities <0.05 were considered significant.

Results

Changes in enzyme activities during the refueling stopover

The relationships between enzyme activities in the pectoral muscle of semipalmated sandpipers and percent body fat (an indirect measure of time spent refueling in the Bay of Fundy) are presented in Fig. 1. CS and HOAD activities showed a significant increase while the animals were building their large fat reserves during the stopover (positive slopes for linear regressions; P<0.05), but CPT and LDH activities did not (slopes not different from 0; P>0.05). However, it is worth noting that three lean birds had particularly high LDH values (see Fig. 1) and that ignoring data points for these three individuals from our analysis would have made the regression slope significantly different from 0 (N=36, P=0.002, R^2= 0.245).

Relationships between enzyme activities and individual fatty acids

The relationships between muscle enzyme activities measured in this study and the percent contribution of the main individual fatty acids present in whole-body storage lipids (NL) or in muscle membrane lipids (PL) (see Maillet and Weber, 2006) were assessed by linear regression. This analysis was performed for all combinations of four enzymes of energy metabolism (CS, CPT, HOAD and LDH) and the percent contribution of eight tissue fatty acids (16:0, 16:1, 18:0, 18:1,

18:2, 20:4, 20:5 and 22:6). Only n-3 PUFA (20:5 and 22:6) and oleate (18:1) showed significant correlations with measured enzyme activities that were physiologically relevant. Therefore, only data involving 20:5, 22:6 and 18:1 are reported in detail in this paper.

Citrate synthase (CS)

Relationships between CS activity of pectoral muscle and percent contribution of n-3 PUFA are presented in Fig. 2. CS activity was positively related to %20:5 in whole-body storage lipids (R^2=0.19; slope of regression different from 0 at P<0.01) and with %22:6 in muscle membrane PL (R^2=0.11; P<0.05). However no relationship was found between CS activity and %20:5 in muscle membranes (R^2=0.03; P>0.05) or %22:6 in whole-body storage lipids (R^2=0.07; P>0.05). In addition, there was no significant relationship between CS activity and %18:1 in muscle PL or total body NL (P>0.05).

Carnitine palmitoyl transferase (CPT)

No significant relationship between CPT activity and any specific fatty acid from NL or PL was uncovered (P>0.05).

3-hydroxyacyl dehydrogenase (HOAD)

The relationships between HOAD activity and the percent contribution of 20:5, 22:6 and 18:1 are presented in Fig. 3. In total body NL, HOAD activity was not related to any fatty acid (regression slopes not different from 0, P>0.05). For muscle membrane PL, however, HOAD activity was positively related to %20:5 (R^2=0.14; P<0.05) and negatively related to %18:1 (R^2=0.22; P<0.005). There was no relationship between HOAD activity and %22:6 in muscle PL (P>0.05).

Lactate dehydrogenase (LDH)

Fig. 4 shows the relationships between LDH activity and %20:5 or %22:6 for total body NL and pectoral muscle PL. Although LDH activity did not increase significantly during the stopover (see Fig. 1), it was positively related to %20:5 (R^2=0.25; P<0.005) and %22:6 (R^2=0.2; P<0.005) in total body NL. In muscle PL, however, no relationship was found between LDH activity and %20:5 or %22:6 (slopes not different from 0, P>0.05). In addition, there was no significant relationship between LDH activity and %18:1 in muscle PL or total body NL (P>0.05).

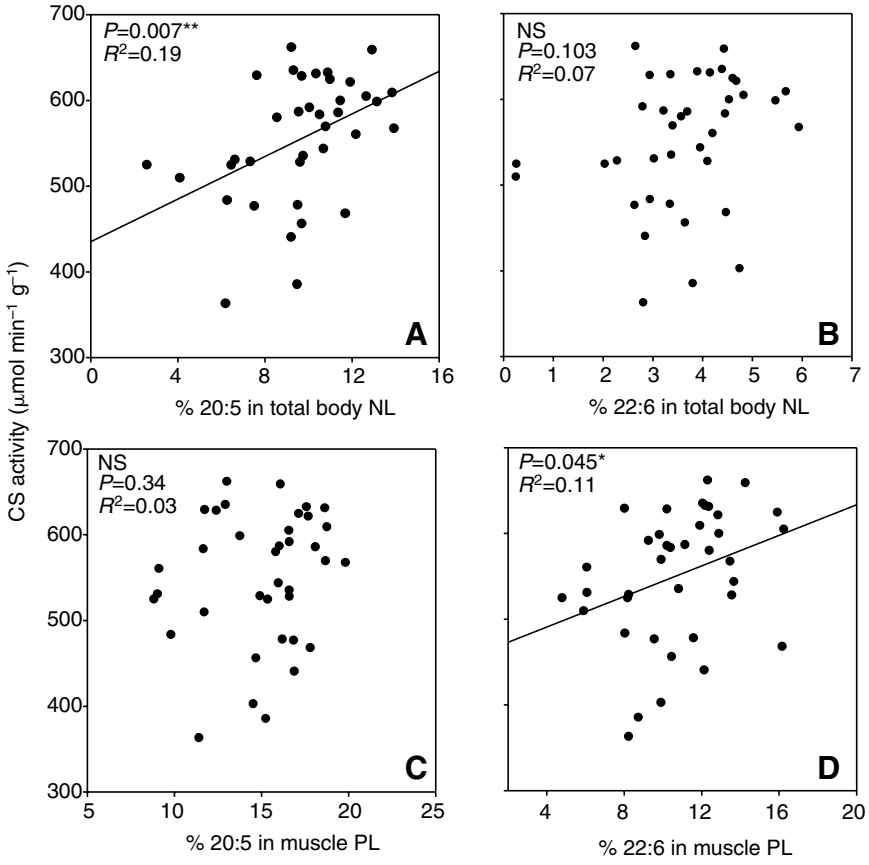

Fig. 2. Citrate synthase (CS) activity (μmol min^{-1} g^{-1} lean muscle mass) in pectoral muscle of refueling semipalmated sandpipers *vs* %20:5 (A,C) and %22:6 (B,D) in fuel reserves (total body neutral lipids, NL; A,B) and muscle phospholipids (PL; C,D). 20:5 is eicosapentaenoic acid and 22:6 is docosahexaenoic acid. Lines were fitted by linear regression (%20:5 NL: y=435.32+12.42x; %22:6 PL: y=455.30+8.90x). N=38. *P<0.05; **P<0.01; NS, not significant.

Discussion

This study shows that the activities of the flight muscle enzymes CS and HOAD increase during preparation for long-distance migration (Fig. 1) and that these changes in oxidative capacity take place very rapidly (<2 weeks). CS activities measured here in semipalmated sandpipers are the highest reported for vertebrates, suggesting that the mitochondrial density of their pectoral muscle could even exceed that of hummingbirds (see Suarez et al., 1991). Furthermore, the observed increments in CS and HOAD of refueling sandpipers are positively correlated with the changes in tissue n-3 PUFA content caused by feeding on *Corophium*. The results also show that 20:5 and 22:6 are associated with different responses and that the mechanisms responsible for stimulating CS and HOAD probably involve membrane-related as well as general, systemic effects (Figs 2 and 3).

Changes in enzyme activities during refueling

Our goal was to determine whether oxidative capacity (Krebs cycle, β-oxidation) and glycolytic capacity of semipalmated sandpipers are modified during the most critical refueling stopover of their migration. We predicted that oxidative enzymes (namely CS, CPT and HOAD) would be positively related to

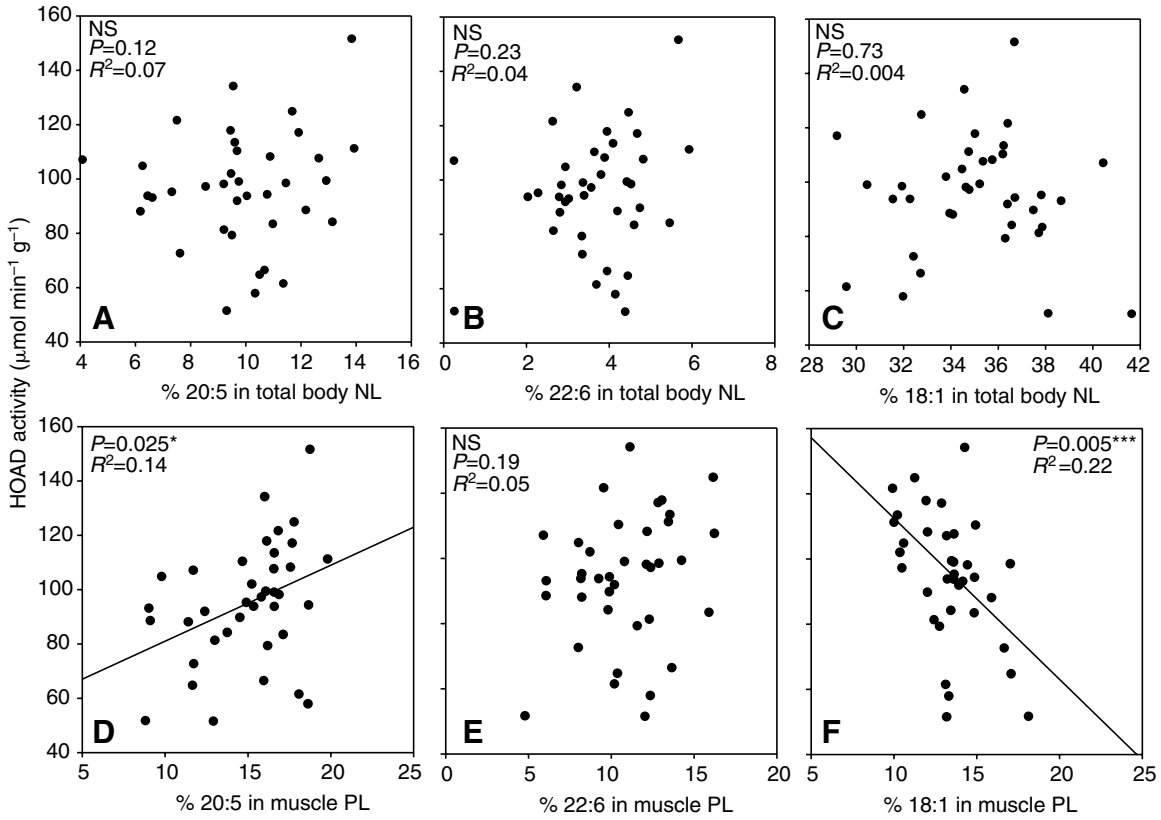

Fig. 3. 3-hydroxyacyl dehydrogenase (HOAD) activity (μmol min^{-1} g^{-1} lean muscle mass) in pectoral muscle of refueling semipalmated sandpipers *vs* %20:5 (A,D), %22:6 (B,E) and %18:1 (C,F) in fuel reserves (total body neutral lipids, NL; A–C) and muscle phospholipids (PL; D–F). 20:5 is eicosapentaenoic acid, 22:6 is docosahexaenoic acid, and 18:1 is oleic acid. Lines were fitted by linear regression (%20:5 PL: y=53.02+2.80x; %18:1 PL: y=161.90–4.94x). N=37. *P<0.05; **P<0.01; ***P<0.005; NS, not significant.

increases in n-3 PUFA, whereas no such relationship would be observed for LDH, a glycolytic enzyme. As anticipated, we found that CS and HOAD activities of flight muscle increase significantly during the 2-week stopover, whereas LDH activity does not (see Fig. 1). Contrary to our expectations, CPT activity was not positively related to n-3 PUFA content in NL or PL. Very few studies have examined short-term metabolic changes of long-distance migrants during single refueling events. In another long-distance migrant, the gray catbird *Dumetella carolinensis*, HOAD and CS activities increased in the supracoracoid muscle during premigration, whereas pectoral muscle only showed an increase in HOAD (Marsh, 1981). It is unclear whether the different results obtained for pectoral muscle CS in gray catbirds and semipalmated sandpipers are due to interspecific differences or to the different ranges of fat accumulation in the two studies. In another study, fat semipalmated sandpipers were reported to have lower CS activities per g muscle than lean birds (Driedzic et al., 1993), but the authors did not discuss this rather surprising result. All other metabolic studies of migrant birds we were able to find report seasonal differences in enzyme activities at various stages of their annual migration cycles (e.g. wintering *vs* migrating animals). Differences in CS, CPT, HOAD and LDH activities were observed between migratory and non-migratory sedge warblers *Acrocephalus schoenobaenus*, reed warblers *Acrocephalus scirpaceus* and western sandpipers *Calidris mauri* (Guglielmo et al., 2002; Lundgren and Kiessling, 1985). However, results from these long-term studies cannot be directly compared with our data on short-term increases during one single refueling event.

CPT was the other β-oxidation enzyme measured (in addition to HOAD) here for semipalmated sandpipers and, contrary to expectation, its activity does not increase during refueling. This is somewhat surprising because HOAD is upregulated, indicating that capacity for flux through β-oxidation is increased. However, only total CPT activity was quantified and it may have been necessary to measure CPT I separately (possibly a more sensitive assay) to detect significant differences. The lack of increase in LDH activity during the stopover was consistent with our prediction, but significant associations between LDH activity and tissue n-3 PUFA content were detected (see next section).

Relationships between fatty acid composition and enzyme activities

A large number of studies show that cells isolated from different species of mammals, fish and birds fed on high n-3 PUFA diets show elevated oxidative capacities. The effects of

these diets on various indices of cellular potential for maximal flux through the Krebs cycle or β-oxidation in mitochondria and peroxisomes are summarized in the Introduction, and led us to investigate whether wild migrant birds would respond similarly and increase the aerobic capacity of their flight muscles by feeding on a natural diet enriched in n-3 PUFA. After demonstrating that tissue n-3 content increases rapidly during the stopover (Maillet and Weber, 2006), we have looked for potential correlations between tissue content in individual fatty acids and the activities of flight muscle enzymes. This approach is clearly inadequate to prove cause and effect, and cannot be used to demonstrate a direct mechanistic link between the presence of particular fatty acids and the induction of enzymes. However, it represents an important first step towards identifying potential mechanisms of action for specific fatty acids on specific enzymes of energy metabolism. This valuable information can then be used to characterize detailed mechanisms of performance enhancement in further experiments.

The FA composition of total body NL and flight muscle PL was measured [see table 1 in Maillet and Weber (Maillet and Weber, 2006)] and regressions between enzyme activities and percent contribution of individual fatty acids were performed. Our results indicate that n-3 PUFA can modify energy metabolism either by direct action on membrane structure/function (PL) or *via* some more general, systemic mechanisms (NL).

The incorporation of n-3 PUFA in membrane PL is known to increase the molecular activity of membrane proteins (Hulbert and Else, 2000; Stillwell and Wassal, 2003), and, therefore, n-3 PUFA act as regulators of membrane-bound enzymes. Highly aerobic muscles have particularly high amounts of 22:6-rich phospholipids in mitochondrial membranes as well as in the sarcoplasmic reticulum (Infante et al., 2001). In mitochondrial PL, elevated levels of 22:6 appear to be associated with high flux capacity through the respiratory chain. In sarcoplasmic reticulum, the presence of additional 22:6 stimulates capacity for calcium pumping and may be responsible for increasing the net Ca^{2+}/ATP coupling ratio (Infante, 1987; Lee et al., 1994). Consequently, extremely high contents of 22:6 have been found in the membranes of high-performance muscles like hummingbird flight muscle and rattlesnake shaker muscle (Infante et al., 2001). In addition, endurance training of rats and humans increases the n-3 PUFA content of muscle PL (Andersson et al., 2000; Helge et al., 2001; Turner et al., 2004). It is therefore particularly interesting to uncover a positive relationship between %22:6 and CS activity in the flight muscle membranes of semipalmated sandpipers (Fig. 2). Similarly, we have observed a significant association between %20:5 in muscle PL and HOAD activity (Fig. 3). Together, these results suggest that the aerobic capacity of sandpiper flight muscles (Krebs cycle and β-oxidation) is stimulated by dietary n-3 PUFA *via* a mechanism involving membranes. Interestingly, a significant positive relationship was also observed between %20:5 in fuel reserves and CS activity (Fig. 2). This suggests that 20:5 also acts *via* an alternative mechanism, independent of its incorporation in membrane PL. For example, n-3 PUFA are well known ligands for peroxisome proliferator-activated receptors (PPARs) that regulate the expression of several genes involved in energy metabolism (Desvergne, 1999). The induction of CS may therefore be caused, at least in part, by the binding of 20:5 to PPARs. Our analysis reveals that HOAD activity increases during refueling (Fig. 1), and that this change is positively correlated with %20:5, but negatively correlated with %18:1 in muscle PL (Fig. 3). Interestingly,

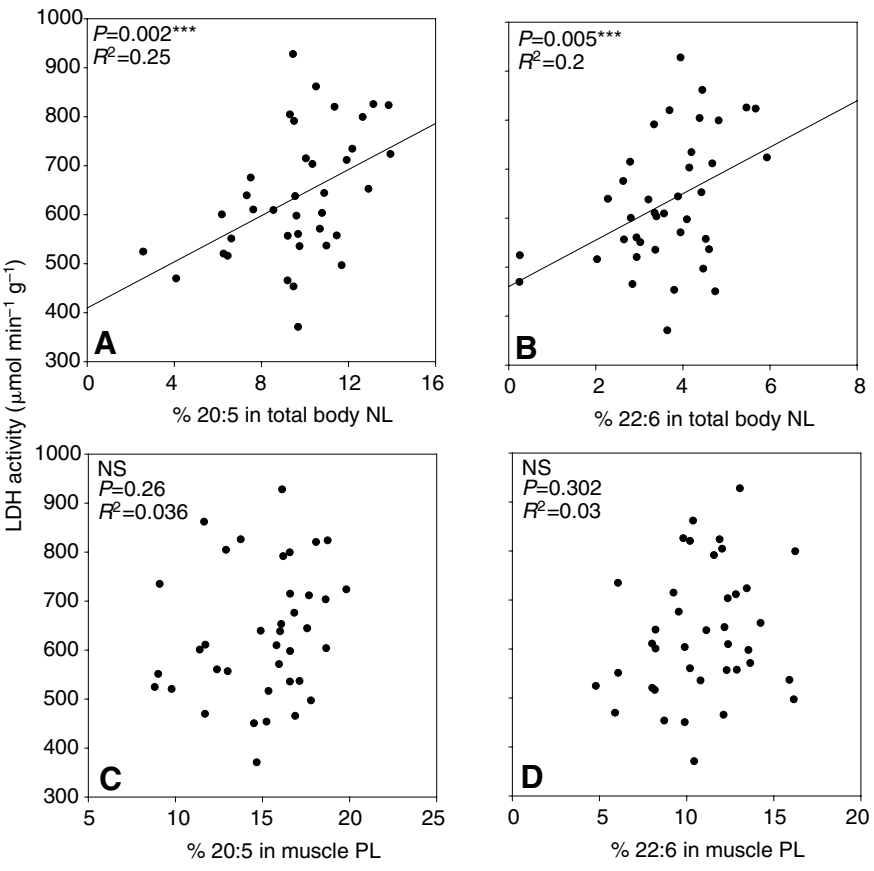

Fig. 4. Lactate dehydrogenase (LDH) activity (μmol min^{-1} g^{-1} lean muscle mass) in pectoral muscle of refueling semipalmated sandpipers *vs* %20:5 (A,C) and %22:6 (B,D) in fuel reserves (total body neutral lipids, NL; A,B) and muscle phospholipids (PL; C,D). 20:5 is eicosapentaenoic acid and 22:6 is docosahexaenoic acid. Lines were fitted by linear regression (%20:5 NL: y=409.86+23.54x; %22:6 NL: y=460.62+47.41x). N=39. ***P<0.005; NS, not significant.

this observed decrease in 18:1 may be due to the known suppressing effect of 20:5 on gene transcription of stearoyl-CoA desaturase (Nakamura and Nara, 2002), a key enzyme in the synthesis of 18:1.

No relationship between n-3 PUFA tissue content and CPT activity could be established in refueling semipalmated sandpipers. This result is somewhat surprising in view of several studies showing increases in CPT activity in cells isolated from mammals and birds fed high n-3 PUFA diets (see Introduction). It may be explained by the short feeding time of our study (2 weeks *vs* 1–3 months), by interspecific differences, or by the fact that we only measured total CPT activity (other studies report CPT I or CPT II specifically).

The positive relationship between LDH activity and %n-3 PUFA was unexpected, partly because other long-distance migrant birds (reed and sedge warblers) show lower LDH activities during migratory than non-migratory periods (Lundgren and Kiessling, 1985). In semipalmated sandpipers, both, %20:5 and %22:6 were positively correlated with LDH activity in total body NL (Fig. 4), suggesting that n-3 PUFA may induce this enzyme *via* systemic mechanisms. Interestingly, hepatocytes of rats fed a diet enriched with n-3 PUFA showed increased LDH activity (Yilmaz et al., 2004). It is unclear why semipalmated sandpipers would benefit from a higher glycolytic capacity during migration. However, this may be necessary for take-off when fully loaded with fat reserves, for predator avoidance while feeding, or to cope with extreme weather (Harrisson and Roberts, 2000). For endurance exercise itself, elevated LDH activity may be necessary to catalyze the lactate to pyruvate reaction (reverse reaction from forward glycolysis), thereby allowing the rapid use of lactate as an oxidative fuel (Brooks, 2002).

Conclusions

As they prepare for a non-stop flight from Canada to South America, semipalmated sandpipers increase the aerobic capacity of their flight muscles. During pre-migratory fattening, muscle activities of Krebs cycle (CS) and β-oxidation enzymes (HOAD) are upregulated and these functional changes are correlated with the incorporation of dietary n-3 PUFA. Rapid feeding on *Corophium volutator* is responsible for increasing the bird's tissue content in 20:5 and 22:6 (Maillet and Weber, 2006), and this study demonstrates an association between lipid composition and capacity for energy metabolism. Results also suggest that n-3 PUFA affect enzyme activities through multiple mechanisms involving changes in membrane structure (CS and HOAD) and/or systemic effects (CS and LDH). This study supports the idea that natural, dietary n-3 PUFA are used as molecular signals to prime the flight muscles of a long-distance migrant for endurance exercise. It also allows the design of specific future experiments to characterize mechanisms of action for the induction of particular enzymes by 20:5 and 22:6. The crucial role played by n-3 PUFA in the annual migration cycle of the semipalmated sandpiper is therefore strong justification for protecting the Bay of Fundy mudflats where *Corophium* is abundant.

List of abbreviations

CPT	carnitine palmitoyl transferase
CS	citrate synthase
FA	fatty acid
FAO	fatty acyl oxidase
HOAD	3-hydroxyacyl dehydrogenase
LDH	lactate dehydrogenase
n-3 PUFA	n-3 polyunsaturated fatty acids
NEFA	nonesterified fatty acids
NL	neutral lipids
PL	phospholipids
PPAR	peroxisome proliferator-activated receptors

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References

Abdul-Malak, N., Brichon, G., Meister, R. and Zwingelstein, G. (1989). Environmental temperature and metabolism of molecular species of phosphatidylcholine in the tissues of the rainbow trout. *Lipids* **24**, 318-324.

Ackman, R. G., Nash, D. M. and McLachlan, J. (1979). Lipids and fatty acids of *Corophium volutator* from Minas Basin. *Proc. N. S. Inst. Sci.* **29**, 501-516.

Andersson, A., Sjodin, A., Hedman, A., Olsson, R. and Vessby, B. (2000). Fatty acid profile of skeletal muscle phopsholipids in trained and untrained young men. *Am. J. Physiol.* **279**, E744-E751.

Bernard, S. F., Reidy, S. P., Zwingelstein, G. and Weber, J.-M. (1999). Glycerol and fatty acid kinetics in rainbow trout: effects of endurance swimming. *J. Exp. Biol.* **202**, 279-288.

Brooks, G. A. (2002). Lactate shuttles in nature. *Biochem. Soc. Trans.* **30**, 258-264.

Daveloose, D., Linard, A., Arfi, T., Viret, J. and Christon, R. (1993). Simultaneous changes in lipid composition, fluidity and enzyme activity in piglet intestinal brush border membrane as affected by dietary polyunsaturated fatty acid deficiency. *Biochim. Biophys. Acta* **1166**, 229-237.

Desvergne, W. (1999). Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.* **20**, 649-688.

Driedzic, W. R., Crowe, H. L., Hicklin, P. W. and Sephton, D. H. (1993). Adaptations in pectoralis muscle, hearts mass, and energy metabolism during premigratory fattening in semipalmated sandpipers (*Calidris pusilla*). *Can. J. Zool.* **71**, 1602-1608.

Ernst, E. (1994). Effects of n-3 fatty acids on blood rheology. *J. Int. Med. Res.* **225**, 129-132.

Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Froyland, L., Madsen, L., Vaagenes, H., Totland, G. K., Auwerx, J., Kryvi, H., Staels, B. and Berge, R. K. (1997). Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J. Lipid Res.* **38**, 1851-1858.

Guglielmo, C. G., Haunerland, N. H., Hochachka, P. W. and Williams, T. D. (2002). Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. *Am. J. Physiol.* **282**, R1405-R1413.

Guo, W., Xie, W., Lei, T. and Hamilton, J. (2005). Eicosapentaenoic acid, but not oleic acid, stimulates B-oxidation in adipocytes. *Lipids* **40**, 815-821.

Hansen, C. A. and Sidell, B. D. (1983). Atlantic hagfish cardiac muscle: metabolic basis of tolerance to anoxia. *Am. J. Physiol.* **244**, 356-362.

Harrisson, J. F. and Roberts, S. P. (2000). Flight respiration and energetics. *Annu. Rev. Physiol.* **62**, 179-205.

Helge, J. W., Wu, B. J., Willer, M., Daugaard, J. R., Storlien, L. H. and Kiens, B. (2001). Training affects muscle phospholipid fatty acid composition in humans. *J. Appl. Physiol.* **90**, 670-677.

Hicklin, P. W. (1987). The migration of shorebirds in the Bay of Fundy. *Wilson Bull.* **99**, 540-570.

Hicklin, P. W., Hounsell, R. G. and Finney, G. H. (1989). Fundy pull trap: a new method of capturing shorebirds. *J. Field Ornithol.* **60**, 94-101.

Hulbert, A. J. and Else, P. L. (2000). Mechanisms underlying the cost of living in animals. *Annu. Rev. Physiol.* **62**, 207-235.

Hulbert, A. J., Faulks, S., Buttemer, W. A. and Else, P. L. (2002). Acyl composition of muscle membranes varies with body size in birds. *J. Exp. Biol.* **205**, 3561-3569.

Infante, J. P. (1987). Docosahexaenoate-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in Ca^{2+}-ATPase calcium transport. *Mol. Cell. Biochem.* **74**, 111-116.

Infante, J. P., Kirwan, R. C. and Brenna, J. T. (2001). High levels of docosahexaenoic acid (22:6n-3)-containing phospholipids in high-frequency contraction muscles of hummingbirds and rattlesnakes. *Comp. Biochem. Physiol.* **130B**, 291-298.

Jump, D. B. (2002b). Dietary polyunsaturated fatty acids and regulation of gene transcription. *Curr. Opin. Lipidol.* **13**, 155-164.

Jump, D. B. and Clarke, S. D. (1999). Regulation of gene expression by dietary fat. *Annu. Rev. Nutr.* **19**, 63-90.

Kim, H.-K. and Choi, H. (2005). Stimulation of acyl-CoA oxidase by alpha-linolenic acid-rich perilla oil lowers plasma triacylglycerol level in rats. *Life Sci.* **77**, 1293-1306.

Lapillonne, A., Clarke, S. and Heird, W. C. (2004). Polyunsaturated fatty acids and gene expression. *Curr. Opin. Clin. Nutr. Metab. Care* **7**, 151-156.

Lee, A. G., Starling, A. P., Ding, J., East, J. M. and Wictome, M. (1994). Lipid-protein interactions and Ca^{2+}-ATPase function. *Biochem. Soc. Trans.* **22**, 821-826.

Lundgren, B. O. and Kiessling, K.-H. (1985). Seasonal variation in catabolic enzme activities in breast muscle of some migratory birds. *Oecologia* **66**, 468-471.

Maillet, D. and Weber, J. M. (2006). Performance-enhancing role of dietary fatty acids in a long-distance migrant shorebird: the semipalmated sandpiper. *J. Exp. Biol.* **209**, 2686-2695.

Marsh, R. L. (1981). Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the Gray Catbird (*Dumetella carolinensis*). *J. Comp. Physiol.* **141**, 417-423.

McClelland, G. B., Hochachka, P. W. and Weber, J.-M. (1999). Effect of high-altitude acclimation on NEFA turnover and lipid utilization during exercise in rats. *Am. J. Physiol.* **277**, E1095-E1102.

Moya-Falcon, C., Hvattum, E., Dyroy, E., Skorve, J., Stefansson, S. O., Thomassen, M. S., Jakobsen, J. V., Berge, R. K. and Ruyter, B. (2004). Effects of 3-thia fatty acids on feed intake, growth, tissue fatty acid composition, B-oxidation and Na^+, K^+-ATPase activity in Atlantic salmon. *Comp. Biochem. Physiol.* **139**, 657-668.

Moyes, C. D. (2003). Controlling muscle mitochondrial content. *J. Exp. Biol.* **206**, 1-7.

Nakamura, M. T. and Nara, T. Y. (2002). Gene regulation of mammalian desaturases. *Biochem. Soc. Trans.* **30**, 1076-1079.

Napolitano, A. and Ackman, R. G. (1990). Anatomical distribution of lipids and their fatty acids in the semipalmated sandpiper *Calidris pusilla* L. from Shepody Bay, New Brunswick, Canada. *J. Exp. Mar. Biol. Ecol.* **144**, 113-124.

Napolitano, G. E., Ackman, R. G. and Parrish, C. C. (1992). Lipids and lipophilic pollutants in three species of migratory shorebirds and their food in Shepody Bay (Bay of Fundy, New Brunswick). *Lipids* **27**, 785-790.

Sanz, M., Lopez-Bote, C. J., Menoyo, D. and Bautista, J. M. (2000). Abdominal fat deposition and fatty acid synthesis are lower and B-oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J. Nutr.* **130**, 3034-3037.

Stillwell, W. and Wassal, S. R. (2003). Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem. Phys. Lipids* **126**, 1-27.

Suarez, R. K., Lighton, J. R. B., Brown, G. S. and Mathieu-Costello, O. (1991). Mitochondrial respiration in hummingbird flight muscles. *Proc. Natl. Acad. Sci. USA* **88**, 4870-4873.

Totland, G. K., Madsen, L., Klementsen, B., Vaagenes, H., Kryvi, H., Froyland, L., Hexeberg, S. and Berge, R. K. (2000). Proliferation of mitochondria and gene expression of carnitine palmitoyltransferase and fatty acyl-CoA oxidase in rat skeletal muscle, heart and liver by hypolipidemic fatty acids. *Biol. Cell* **92**, 317-329.

Turner, N., Lee, J. S., Bruce, C. R., Mitchell, T. W., Else, P. L., Hulbert, A. J. and Hawley, J. A. (2004). Greater effect of diet than exercise training on the fatty acid profile of rat skeletal muscle. *J. Appl. Physiol.* **96**, 974-980.

Turner, N., Haga, K. L., Else, P. L. and Hulbert, A. J. (2006). Scaling of Na^+, K^+-ATPase molecular activity and membrane fatty acid composition in mammalian and avian hearts. *Physiol. Biochem. Zool.* **79**, 522-533.

White, L. (1985). Body composition and fat deposition of migrant semipalmated sandpipers during autumn migration in the lower Bay of Fundy. MSc thesis, University of Guelph, Ontario, Canada.

Yamazaki, R. K., Shen, T. and Schade, G. B. (1987). A diet rich in (n-3) fatty acids increases peroxisomal B-oxidation activity and lowers plasma triacylglycerols without inhibiting glutathione-dependent detoxication activities in the rat liver. *Biochim. Biophys. Acta* **920**, 62-67.

Yilmaz, H. R., Songur, A., Ozyurt, B., Zararsiz, I. and Sarsilmaz, M. (2004). The effects of n-3 polyunsaturated fatty acids by gavage on some metabolic enzymes of rat liver. *Prostaglandins Leukot. Essent. Fatty Acids* **71**, 131-135.