FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Dominique Maillet
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Biology)
GRADE / DEGREE

Department of Biology
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Preparing For Long-Distance Migration: Effects of Dietary Fatty Acids on Metabolism of Semipalmated Sandpipers

TITRE DE LA THÈSE / TITLE OF THESIS

Dr. J-M. Weber
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Dr. K. Gilmour

Dr. T.W. Moon

Dr. G. Gilchrist

Gary W. Slater
Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

PREPARING FOR LONG-DISTANCE MIGRATION: EFFECTS OF DIETARY FATTY ACIDS ON METABOLISM OF SEMIPALMATED SANDPIPERS

Dominique Maillet B.Sc.

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
University of Ottawa
in partial fulfillment of the requirements for the M.Sc. degree at the
Ottawa-Carleton Institute of Biology

Thèse soumise à la
Faculté des études supérieures et postdoctorales
Université d'Ottawa
en vue de satisfaire aux exigences nécessaires pour l'obtention de la M.Sc. à
l'Institut de Biologie d'Ottawa-Carleton

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-18443-1
Our file Notre référence
ISBN: 978-0-494-18443-1

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

Master of Science (2006)

(Biology)

Title: Preparing for Long-Distance Migration: Effects of Dietary Fatty Acids on Metabolism of Semipalmated Sandpipers

Author: Dominique Maillet

Supervisor:

Dr. Jean-Michel Weber – Professor, Department of Biology, University of Ottawa

PREPARING FOR LONG-DISTANCE MIGRATION: EFFECTS OF DIETARY LIPIDS ON FATTY ACID COMPOSITION AND FLIGHT MUSCLE METABOLISM OF SEMIPALMATED SANDPIPERS

SUMMARY

At the end of every summer, the semipalmated sandpiper (*Calidris pusilla*) stops in the Bay of Fundy (east coast of Canada) to build large fat reserves before its non-stop flight to wintering grounds in South America. During this two-week stopover, body mass is doubled by feeding on a burrowing amphipod, *Corophium volutator*, containing extremely high levels of n-3 polyunsaturated fatty acids (n-3 PUFA), most notably 31% eicosapentaenoic acid (EPA). Several lines of evidence link high n-3 PUFA content in membranes with elevated muscle and exercise performance. Furthermore, high PUFA content in muscle phospholipids have been shown to increase membrane fluidity, thereby accelerating cellular lipid uptake and, possibly, improving endurance. In mammals, dietary n-3 PUFA are known to increase mitochondrial and peroxisomal content, as well as the activities of oxidative enzymes. I hypothesized that dietary EPA could be used by semipalmated sandpipers as a natural "performance enhancing" substance to prime their flight muscles for migration. Birds were collected at various stages of fat loading throughout the stopover to measure changes in percent fat and fatty acid composition of muscle and lipid reserves (in both neutral lipids and phospholipids). Activities of several enzymes were also measured in flight muscle: namely citrate synthase (CS, Krebs cycle enzyme used as a marker for mitochondrial density), carnitine palmitoyl transferase, 3-hydroxyacyl dehydrogenase (respectively CPT and HOAD, markers for β-oxidation) and lactate dehydrogenase (LDH, index of glycolytic metabolism). Results show that EPA and docosahexaenoic acid (DHA) were rapidly incorporated in muscle phospholipids and lipid reserve neutral lipids. Over the course of the stopover, the fatty acid profiles of these tissues converged with that of the birds' diet. However, data indicate that a significant proportion of dietary EPA and DHA are converted before storage (particularly into 18:1). Stored oleate (18:1) may offer a compromise between high energy density and ease of

mobilization/oxidation of lipid reserves. In addition, CS and HOAD activities in pectoral muscle increased during the stopover. Unexpectedly, LDH activity was strongly related to n-3 PUFA content in the sandpipers' tissues. LDH may be used to convert lactate into pyruvate, which may serve as an important fuel for aerobic metabolism. Observed physiological changes prior to migration in the semipalmated sandpiper are consistent with the stimulatory effects of n-3 PUFA on endurance capacity previously observed in other vertebrates. Dietary PUFAs may therefore be used as pharmacological agents to prepare the flight muscles of long-distance migrant shorebirds for extreme endurance exercise.

SOMMAIRE

À la fin de chaque été, le bécasseau semipalmé (*Calidris pusilla*) s'arrête dans la Baie de Fundy (côte est du Canada) afin d'accumuler des réserves lipidiques avant le vol sans-arrêt aux sites d'hivernage en Amérique du Sud. Pendant cet escale de deux semaines, les oiseaux doublent leur masse corporelle en se nourrissant d'un amphipode, *Corophium volutator*, qui contient des quantités élevées d'acides gras polyinsaturés de type n-3 (n-3 PUFA), notamment 31% d'acide eicosapentaenoïque (EPA). Plusieurs études ont démontré un lien entre le contenu membranaire en n-3 PUFA et une performance musculaire et d'endurance accrue. De plus, un contenu élevé de PUFA dans les phospholipides du muscle augmente la fluidité membranaire qui, à son tour, accélère l'entrée d'acides gras et améliore l'endurance physique. Chez les mammifères, les n-3 PUFA diététiques semblent augmenter la densité mitochondriale et peroxisomale, ainsi que l'activité de certaines enzymes aérobiques. L'hypothèse principale de cette thèse est que les bécasseaux semipalmés utilisent EPA afin d'améliorer la performance physique de leurs muscles pectoraux avant la migration. Des oiseaux de diverses masses ont été récoltés tout au long de l'escale afin de mesurer les changements dans le pourcentage de lipides et la composition en acides gras de leurs muscles et de leurs réserves lipidiques (lipides neutres et phospholipides.) Les activités des enzymes suivantes ont été mesurées dans le muscle pectoral : citrate synthase (CS, enzyme du cycle de Krebs index de la densité mitochondriale), carnitine palmitoyl transférase, 3-hydroxyacyl-déshydrogénase (CPT et HOAD respectivement, index de la β-oxidation) et lactate déshydrogénase (LDH, enzyme glycolique). Mes résultats démontrent que EPA et acide docosahexaenoïque (DHA) ont été rapidement incorporés dans les phospholipides du muscle et dans les lipides neutres des réserves de gras. Pendant l'escale dans la Baie de Fundy, la composition en acide gras des tissus a convergé avec celle de la diète. Cependant, les

données indiquent qu'une portion significative de EPA et DHA diététiques est modifiée avant le stockage (converti surtout en 18:1.) Le stockage d'oléate (18 :1) permet, à la fois, une densité d'énergie élevée ainsi qu'une facilité dans la mobilization et l'oxidation des réserves lipidiques. De plus, les activités de CS et HOAD dans le muscle pectoral ont augmenté pendant l'escale. L'activité de LDH est fortement reliée avec le contenu de n-3 PUFA dans les tissus des bécasseaux. LDH peut être utilisé pour convertir le lactate en pyruvate, qui, par la suite, peut être utilisé dans le métabolisme aérobique. Les changements physiologiques observés pendant la période pré-migratoire chez le bécasseau semipalmé sont conformes aux effets stimulatoires des n-3 PUFA sur la capacité d'endurance observés chez d'autres vertébrés. Ainsi, les PUFA diététiques semblent être utilisés comme des agents pharmacologiques servant à préparer les muscles pectoraux des oiseaux de rivage migratoires pour l'exercice d'endurance.

ACKNOWLEDGEMENTS

I'd like to express my deepest gratitude to my supervisor, Jean-Michel Weber for accepting me into his lab and agreeing to let me study these birds. Thank you for your guidance, patience and the numerous hours you spent working on this project. I am also appreciative of the advice and help I obtained from the members of my thesis committee: Tom Moon, Grant Gilchrist and Jaroslav Picman.

Thank you to the people at the Canadian Wildlife Service in Sackville, NB. Colin MacKinnon supported my idea to study the sandpipers and encouraged me to pursue my Master's even though it left him short-handed. A big thanks to Nev Garrity and Peter Hicklin. You never let me down or gave up on this study no matter how "unpopular" it was. This project could not have been completed without your help.

I'd also like to thank my lab mates/friends who made my experience at the University of Ottawa a memorable one. Thanks Shoshanah for always having the time to go over whatever problems I encountered in my studies and for being a great mentor. Thank you Leo for your patience and for letting me learn from your vast knowledge. Thanks Phil, fellow "bird bastard", for coming to New Brunswick with me and helping me through a very tumultuous field season. I am grateful to Eric, who was busily trying to complete his own thesis by the time I arrived in the lab, but rarely hesitated to lend a helping hand. Thanks also to François who gave me valuable advice, particularly at the beginning stages of my Master's.

Thank you to two special professors I had the pleasure of knowing during my studies in biology at the Université de Moncton: Stéphan Reebs, for introducing me to the world of research, and Céline Bérubé who forced me to write like mad in the four courses she taught me. Though it may not be evident, my writing improved immensely under her guidance.

I'd like to thank my parents who, throughout my life, always made me feel that I'm smarter than I actually am, which gave me the confidence to pursue things when I had absolutely no clue how. Thanks to my brother and sister, Alexandre and Marie-Josée, and my godmother, Carmen, for their support. I'd also like to thank my close friends: Gabrielle, Monique, Lisa and Marie-Hélène for supporting me and reminding me to make time for dinner and drinks every now and then. Merci beaucoup!!

Last but not least, thanks Dave for being my best friend, for following me to Ottawa and for always believing in me. I am lucky to have you in my life.

TABLE OF CONTENTS

LIST OF FIGURES

Figure 2.5: % Contribution of individual fatty acids in pectoral muscle, carcass (including all tissues except for pectoral muscles, liver and distinct fat depots) and total fat depots in neutral lipids (NL) of semipalmated sandpipers in lean, medium and fat birds. Values are mean ± S.E.M. (*N* = 10- 14). * or a,b,c only indicate 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.

Figure 2.6: Δ Predator-Prey Index in pectoral muscle PL and lipid reserves NL in lean, medium and fat birds. Significant differences between groups are shown by * or a,b. Values are mean ± S.E.M. *N*= 13 in lean and fat birds and 14 in medium birds.

Figure 2.7: Differences between observed and theoretical fatty acid composition of lipid reserve 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).

Figure 3.1: Pectoral muscle enzyme activities (μmol/min*g) versus % Body fat in semipalmated sandpipers during the stopover in the Bay of Fundy.

Figure 3.2: Pectoral muscle CS and LDH activities (μmol/min*g) versus % EPA in total body neutral lipids of semipalmated sandpipers during the stopover in the Bay of Fundy.

LIST OF TABLES

LIST OF ACRONYMS

PUFA= Polyunsaturated Fatty Acid
MUFA= Monounsaturated Fatty Acids
SFA= Saturated Fatty Acid
NL= Neutral Lipids
PL= Phospholipids
DBI= Double Bond Index
Δ PPI= Δ Predator-Prey Index
EPA= Eicosapentaenoic Acid
DHA= Docosahexaenoic Acid
CS= Citrate synthase
CPT= Carnitine palmitoyltransferase
HOAD= 3-Hydroxyacyl CoA dehydrogenase
LDH= Lactate dehydrogenase

FATTY ACID TERMINOLOGY

20:5[1]= Eicosapentaenoic Acid
22:6= Docosahexaenoic Acid
16:0= Palmitic Acid
16:1= Palmitoleic Acid
18:0= Stearic Acid
18:1= Oleic Acid
18:2= Linoleic Acid
20:4= Arachidonic Acid

---

[1] Number of carbon atoms: Number of double bonds

CHAPTER 1. GENERAL INTRODUCTION

Migration

Birds respond to extreme environmental conditions in a variety of ways including migration. This spatial movement helps to ensure a sufficient food supply and nesting sites for survival and/or reproduction (Blem, 1990). Migratory birds exemplify some of the most extreme aerobic capacities observed in all species of vertebrates. During flight, these birds are often able to sustain metabolic rates equalling 5 to 16 times basal metabolic rate (i.e. the rate observed during rest) (Dawson et al., 1983).

Avian migrant species demonstrate a wide range of physiological adaptations that have allowed them to achieve these unparalleled energetic feats. For example, migrants have shown a great ability to attain and utilize oxygen; an indispensable component in the breakdown of lipid reserves (Blem, 1990). In addition, and perhaps most importantly, these birds demonstrate an impressive phenotypic flexibility, allowing them to accumulate large energy stores in relatively short periods of time (Lindstrom et al., 1999).

The latter adaptation is of particular significance in long-distance migrants. Contrary to their short-distance counterparts, these birds must undergo extreme preparation involving numerous physiological changes. This preparation is essential to their relocation from nesting to wintering grounds (and vice-versa) and allows the birds to rely solely on energy reserves (accumulated prior to migration) during non-stop flights of considerable distance and duration. Short-distance migrants relocate by combining short-flight bouts with periodic stopovers allowing them to replenish their fuel reserves. In this case, extensive metabolic and energetic specializations need not have taken place (Blem, 1990).

The semipalmated sandpiper (*Calidris pusilla*) is an example of a long-distance migrant that doubles its body mass by accruing fat in preparation for the last bout of the fall migration. Every July and August, hundreds of thousands of semipalmated sandpipers (up to

74% of the world population) stop in the Bay of Fundy en route to their wintering grounds in northern South America (Hicklin, 1987). For most of these birds, this will be their last stopover before embarking on a 72-hour non-stop transoceanic flight. During this last part of the migration, the birds will cover approximately 4,500 kilometres and will fly at speeds of up to 90 km/h (McNeil, 1969).

The Bay of Fundy offers vast mudflats containing high densities of *Corophium volutator*, a burrowing amphipod accounting for 86% of the shorebirds' diet (Napolitano et al., 1992). In North America, *Corophium* is found exclusively in the Bay of Fundy and in the nearby Gulf of Maine (Gratto, 1979). This prey contains unusually large amounts of n-3 polyunsaturated fatty acids (n-3 PUFA), particularly eicosapentaenoic acid (EPA), which has been shown to enhance fatty acid utilization during endurance exercise in other vertebrates (see section on the role of dietary fatty acids in migration).

To date, studies on the physiology of birds at migratory stopovers have focused almost entirely on the accumulation of energy reserves. This thesis offers a new approach by investigating the effects of dietary fatty acids on changes in lipid content and composition as well as the capacity of flight muscles to mobilize and oxidize these fat reserves in semipalmated sandpipers during their stopover in the Bay of Fundy.

Pre-migratory lipid deposition

Long-distance migration is primarily fuelled by lipid reserves, which are generally accumulated during a relatively short period preceding migration departure (Blem, 1990). During this time, several mechanisms that apparently prepare the metabolic pathways for sustained flight are triggered, causing increases in nutrient absorption and assimilation efficiency, and simultaneous decreases in energy expenditure. The latter effect could result from a number of mechanisms such as the selection of less extreme thermal environments, a lowered metabolic rate and/or a decrease in activity (Ramenofsky, 1990; Scott et al., 1994). Hyperlipogenesis, an increase in fat synthesis from non-fatty precursors, also occurs (Ramenofsky, 1990). Hyperphagia, an absolute increase in food intake, is probably the most important of the fattening mechanisms in most migratory species (Blem, 1990; Dawson et al., 1983; Scott et al., 1994). The energy intake of birds prior to migration is extremely elevated and can reach levels of 2273 kJ/kg$^{0.72}$ per day in the thrush nightingale (*Luscinia luscinia*) (Lindstrom et al., 1999). This value even surpasses Kirkwood's estimate (1983) of the maximum daily metabolizable energy intake in homeotherms (2200 kJ/kg$^{0.72}$).

The degree of fat deposition varies widely between species of migratory birds. There is evidence that the amount of lipids deposited prior to migration is a function of the distance over which the bird must fly without refueling. This relationship is almost linear (Klassen, 1996). In the case of the semipalmated sandpiper, lipid depots totalling 41% of total body mass are accumulated prior to flying from the Bay of Fundy to wintering grounds (Driedzic et al., 1993; Napolitano and Ackman, 1990).

Fuel selection during migration

As mentioned, lipids accumulated at stopovers are the predominant fuels used during migratory flight (Blem, 1990). However, migrant birds may also utilize other fuels, namely proteins and carbohydrates, which have been shown to affect lipid metabolism. These energetic fuels are essential to the production of energy during flight and are key determinants of the birds' migratory capacity (i.e. its ability to sustain flight for extended periods). Here, I examine the specific and relative roles of each substrate used by migrant birds.

Role of proteins and carbohydrates

The use of protein as a fuel for migratory flight appears unlikely since this substrate yields far less energy (per unit mass) than do lipids (Klassen, 1996). Protein is not readily mobilized and its degradation produces toxic by-products (such as ammonia). In addition, unlike carbohydrates and lipids, proteins are part of functional organs such as muscles and digestive tissues and are not stored as energy reserves. The functioning of these organs could be diminished as a result of protein use during migration (Jenni and Jenni-Eiermann, 1998). Hence, protein is usually only used as an energy substrate after lipid and carbohydrate stores have almost been completely depleted (Blem, 1990). Nevertheless, it has been shown that protein is deposited in tissues during the pre-migratory phase (Driedzic et al., 1993; Gaunt et al., 1990; Piersma et al., 1999) and consists of 9 to 18% of the total mass deposited (Klassen, 1996; Klassen et al., 2000). In addition, protein reserves appear to be catabolized during migratory flight indicating a non-negligible utilization of this substrate (Battley et al., 2001; Jenni-Eiermann et al., 2002; Klassen et al., 2000).

Among possible functions, deposited protein matter in the pectoral muscle may be required due to increased power demands imposed by a significant gain in body mass prior to migration (Marsh, 1983). During flight, as lipid reserves are depleted, protein may correspondingly be degraded due to a reduced need for enhanced power output by flight muscles.

One of the most important functions of protein during migration may be related to the actual maintenance of the biochemical processes that derive energy from lipid precursors. Fatty acids are oxidized via the Krebs cycle, which necessitates certain intermediates often found in the form of protein (Dohm, 1986). In addition, prior to migration, concentrations of fatty acid binding protein increase. These proteins play an important role in the intracellular transport of lipids and represent 14% of the total proteins found in the flight muscle and 21% of those observed in the heart (Guglielmo et al., 1998). Thus, at least a portion of the increase in total protein content occurring during the pre-migratory period serves to enhance the efficiency of lipid metabolism.

The role of carbohydrates in fueling long-distance flight is more poorly known than that of protein. As opposed to lipids, carbohydrates must be stored with extensive amounts of water (as much as three to five grams per gram of substrate) and thus, may substantially increase costs of transport (Schmidt-Nielsen, 1972). It is still unclear whether carbohydrates are stored prior to migration and conclusions differ greatly among studies. For example, Marsh (1983) found no correlation between glycogen and fat stores in migratory gray catbirds (*Dumetella carolinensis*). Conversely, King et al. (1963) observed high glycogen levels in fall migrants of the white-crowned sparrow (*Zonotrichia leucophrys gambelii*).

In bird metabolism, carbohydrates are used as a primary fuel only during very high exercise intensities, such as hovering, burst or short flights and take-off (Suarez et al., 1990). Thus, extensive carbohydrate storage prior to migration is probably unnecessary. Possibly the most important role of carbohydrates in migration may be tied to refueling periods at staging areas along the migratory trajectory. To spare the growing lipid stores essential for migration fueling, carbohydrates may be used in significant proportions during foraging (Suarez and Gass, 2002; Suarez et al., 1990). However, this hypothesis must be examined more closely since it seems that even the maximal levels of glycogen reserves found in birds could only support very short durations of exercise (Dawson et al., 1983).

Role of lipids

Lipids play the most crucial role in migration energetics and far surpass proteins and carbohydrates with regards to energy density and consequently, storage capacity. Adipose fat yields 8 to 10 times more ATP per unit mass than do proteins or carbohydrates (Bairlein, 2002). Additionally, lipids are particularly compact and since they can be stored with very little water, are tremendously efficient at minimizing added weight, and thus, wing-loading in migrant birds (Suarez et al., 1986).

As opposed to other forms of fuel, lipids appear to support lower rates of exercise intensities, such as those observed during migratory flight, which may not require a rate of ATP turnover as high as that needed for burst flights (e.g. predator evasion, take-off, etc.) (Suarez et al., 1986). One could argue that flight is a relatively energy-demanding activity that most likely requires elevated metabolic rates. However, long-distance migrants have evolved ways to partly circumvent these limitations to maximize the use of their fat reserves.

In mammals, the peak of fatty acid utilization occurs at approximately 50% of VO_2 max (the maximal volume of oxygen that can be acquired and utilized during exercise) after which carbohydrates take over as the primary fuel (Brooks, 1998). This pattern of fuel selection appears to be altered in birds. If birds utilized energetic substrates in the same manner as do mammals, we could conclude that at such exercise intensities, carbohydrates may play a relatively important role in ATP synthesis throughout migration. However, this is not the case. During migration, 85 to 90% of substrates used for fuel are lipids (Blem, 1990). Schmidt-Nielsen (1972) found that flying birds consumed oxygen at rates of 60 to 85% of VO_2 max. However, Klassen et al. (2000) observed that power requirements for migratory flight were lower than those measured during non-migratory flight. Consequently, the percentage of VO_2 max observed during migration may not be as elevated as previously thought. Migrant birds appear to have developed several adaptations allowing them to maximize the selection of lipids during migration. In recent years, diet has been revealed to be a key determinant of fat selectivity and utilization in a number of species. The effect of dietary fatty acids on the use of lipids will be discussed in the following section.

The role of dietary fatty acids in migration

The amount of fat deposited during the pre-migratory period is not the only determinant of a bird's capacity for long-distance flight. The fatty acid composition of lipid reserves also appears to play a vital role in various factors that could affect fat mobilization and oxidation during sustained exercise. Specific fatty acids are deposited depending on the composition of the bird's diet prior to migration, and on selective metabolism. The latter consists of a physiological selection or conversion (through an elongation/reduction and/or a saturation/desaturation of carbon chains) of fatty acids for storage and utilization purposes (Pierce and McWilliams, 2005). Little is known about selective metabolism of lipids in birds, however, it has been shown that adipose tissue composition is primarily dictated by the fatty acid composition of the diet (Pierce and McWilliams, 2005). "Direct deposit" of fatty acids probably improves the efficiency of the use of metabolizable energy since it eliminates the energetic costs of intestinal breakdown and transport to and synthesis within the liver.

A study by Pierce et al. (2005) showed that exercise performance of red-eyed vireos (*Vireo olivaceus)* was affected by fatty acid composition of their diet. Stored lipids (obtained directly from diet or modified by metabolism) may affect performance because not all fatty acids have the same energy content or mobilization / oxidation capacity. Energy density increases with saturation and chain length (Blem, 1990). In contrast, mobilization from adipose tissue decreases with saturation and chain length (Raclot, 2003; Raclot and Groscolas, 1995). Short-chain and unsaturated fatty acids are more polar than their long-chain saturated counterparts. The higher mobilization of more polar fats may be due to their lower solubility in a non-polar phase (e.g. triacylglycerol contained in a lipid droplet) than in a relatively polar phase (e.g. the aqueous cytoplasm in which they are released). Similarly to mobilization, oxidation capacity also decreases with saturation and chain length (DeLany et al., 2000; Henderson and Sargent, 1985). Ease of mobilization and oxidation could explain why shorter-chained and unsaturated fatty acids appear to be selectively deposited, in spite of having a lower energy content than saturated and longer-chained fatty acids, in birds' fat reserves prior to migration. In fact, up to 90% of lipid reserves of migrant birds consist of oleate (18:1), linoleate (18:2) and palmitate (16:0) (Bairlein, 2002; Pierce and McWilliams, 2005).

Fatty acids obtained from the diet are not only used as fuel but also as components of cellular membranes. Several studies have shown that the fatty acid profile of membrane phospholipids is relatively resistant to changes in dietary fats (Awad and Spector, 1976; Kaduce et al., 1977). However, more recent studies have found that membrane phospholipids can be influenced to varying degrees by certain dietary fatty acids. For instance, diets high in polyunsaturated fatty acids (PUFAs) have led to increases in membrane phospholipid PUFA content (Awad, 1986). Animals fed diets rich in the n-3 PUFA docosahexaenoic acid (DHA) have shown increases in cellular membrane fluidity (Ernst, 1994) whereas PUFA-deficient diets have led to decreased fluidity (Daveloose et al., 1993). In addition, numerous studies have linked diets containing elevated levels of DHA to increased membrane permeability (Stillwell and Wassal, 2003). Enhanced membrane fluidity and permeability may play an important role in increasing fatty acid movement through cellular membranes. Consistent with this observation, highly aerobic muscles tend to display high concentrations of n-3 PUFA (Infante et al., 2001).

In addition to increasing fatty acid mobilization and oxidation, dietary PUFAs have been linked to several other important determinants of lipid catabolism capacity. In mammalian cells, dietary eicosapentaenoic acid (EPA, 20:5 n-3) has led to mitrochondrial and peroxisomal proliferation (Froyland et al., 1997; Totland et al., 2000). Elevated n-3 PUFA doses have also been shown to increase the specific activities of carnitine palmitoyltransferase I (CPT I: responsible for the transportation of fatty acids through the mitochondrial membrane) and of 3-hydroxyacyl-CoA dehydrogenase (HOAD: β-oxidation enzyme) in chickens (Sanz et al., 2000). If these effects of n-3 PUFA also occur in migrant birds, we can easily expect semipalmated sandpipers to utilize their *Corophium* diet to enhance the use of their lipid reserves and prime muscles for long-distance migration.

Objectives of the study

Semipalmated sandpipers refueling in the Bay of Fundy provide a unique model to not only observe the physiological changes taking place during preparation for migration, but also to investigate how a natural diet rich in n-3 PUFA may affect lipid metabolism in a highly aerobic species. Here, I hypothesize that semipalmated sandpipers use dietary fatty acids (obtained from *Corophium*) to prime their metabolism for long-distance migration. The objectives of chapter 2 are to determine 1) the anatomical distribution of fat stores, 2) the effect of a high n-3 PUFA diet on muscle phospholipids, and 3) changes in storage lipid composition throughout the stopover. I predict that storage and membrane lipids will be affected by dietary n-3 PUFA and that the composition of these tissues will converge with that of *Corophium*.

In chapter 3, I will examine changes in the activities of the following enzymes in flight muscles throughout the stopover: citrate synthase (CS, Krebs cycle enzyme used as a marker for mitochondrial density), carnitine palmitoyl transferase, 3-hydroxyacyl dehydrogenase (respectively CPT and HOAD, markers for β-oxidation) and lactate dehydrogenase (LDH, index of glycolytic metabolism). Because sandpipers preparing for migration would benefit from an enhanced capacity to metabolize lipids, I predict that the activities of CS, CPT and HOAD will increase throughout the stopover. In contrast, I don't expect to see changes in LDH activity because this enzyme is used in carbohydrate metabolism, which, in comparison to lipids, contributes little to endurance exercise metabolism. In addition, I will correlate enzymatic activities with the percent contributions of various fatty acids in several tissues of the sandpipers to examine whether fatty acid composition of the birds affects aerobic and anaerobic metabolism. Based on previous studies of other vertebrates showing a link between ingested n-3 PUFA and aerobic enzyme activities, I anticipate that CS, CPT and HOAD activities will be related to n-3 PUFA content in tissues.

CHAPTER 2. PERFORMANCE-ENHANCING ROLE OF DIETARY FATTY ACIDS IN A LONG-DISTANCE MIGRANT SHOREBIRD: THE SEMIPALMATED SANDPIPER

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 (making up ~75% of their world population) load large fat reserves before the longest non-stop flight of their entire migration: a 4,500 km transoceanic trip lasting ~3 days (Hicklin, 1987). During a two-week stopover, the birds double body mass by feeding on *Corophium volutator*, a small amphipod responsible for up to 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). Such 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), probably 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 muscle and rattlesnake shaker muscle (Infante et al., 2001). Moreover, 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). Paradoxically, feeding rats a diet rich in n-3 PUFA affects membrane PL composition (Turner et al., 2004), but decreases endurance capacity *in vivo* (Ayre and Hulbert, 1997), although isolated muscle function seems to remain normal (Ayre and Hulbert, 1996).

EPA and DHA are also known to have numerous metabolic effects unrelated to changes in membrane composition. They trigger mitochondrial and peroxisomal proliferation (Froyland et al., 1997; Jump, 2002a; Jump, 2002b; Totland et al., 2000; Yamazaki et al., 1987), and increase the activities of key β-oxidation and Krebs cycle enzymes (Froyland et al., 1997; Guo et al., 2005; Jump and Clarke, 1999; Sanz et al., 2000; Yamazaki et al., 1987). A particularly interesting study in the present context shows that the aerobic capacity of a migrant bird species, the red-eyed vireo (*Vireo olivaceous*), can be influenced by the fatty acid composition of its diet (Pierce et al., 2005).

Successful completion of a long migration does not only depend on intrinsic characteristics of muscles, but 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), although several other criteria could be used to compare the usefulness of different fatty acids for migration. Selectivity has been demonstrated for all the main 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 much more easily mobilized than MUFA or saturated fatty acids (Herzberg and Farrell, 2003; Raclot and Groscolas, 1995). At the 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 examine 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 diet high in n-3 PUFA on the fatty acid composition of cell membranes, 3) changes in the composition of storage lipids, and whether the fatty acid profiles of the birds' fat reserves and of the diet converge during refueling. This study is among the first to examine alternative performance-enhancing roles of natural diets in migrant birds. I anticipated that observed changes in lipid metabolism would not only provide enough energy for migration, but increased functional capacity for endurance exercise.

Materials and methods

Animals and field methodology

Semipalmated sandpipers (*Calidris pusilla*) were used to monitor lipid accumulation before a ~4,500 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. 2.1). 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*) were collected on the sandpipers' feeding grounds by sieving patches of mud using a 0.85 mm mesh (McCurdy et al., 2000). Sieved amphipods were extracted from the mesh and placed in 500 ml jars containing seawater until transportation to the laboratory. Samples of Corophium were then removed from seawater, placed in clean Eppendorf vials and frozen at -20°C.

Lipid extraction

Birds were thawed and carefully dissected into 6 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, 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, potassium chloride (0.25%) was added and the mixture was 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, Switzerland). Lipids from the diet samples of (*Corophium volutator*) were extracted using the same procedure.

Separation of neutral lipids and phospholipids

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 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). 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).

Calculations and statistical analyses

The relationships between body mass and % body fat (Fig. 2.1), and between lean pectoral muscle mass and body fat mass (Fig. 2.2b) 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. 2.2a). For all analyses of lipid composition (Figs. 2.3-2.5), the birds were divided in 3 groups of equal size. Birds were ranked according to % body fat and divided them in 3 equal groups containing "lean", "medium" and "fat" birds (N=15 for each group)[2]. By capturing and re-capturing the same individual semipalmated sandpipers, White (1985) determined that body mass is an accurate predictor of feeding time in the Bay of Fundy. Here, I show that % body fat is closely correlated with body mass (Fig. 2.1), and have therefore used % fat as an indirect measure of refueling time. 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

---

[2] One-way ANOVAs were used to verify that groups did not differ in the size of structural body features. There was no significant difference in tarsus, bill or wing length among groups (P> 0.05).

index was used to quantify the level of fatty acid unsaturation in each lipid class and was calculated as follows (expressing percentages as ratios):

$$DBI = \frac{\text{average number of double bonds}}{\%\ \text{saturated FA}}$$

The average number of double bonds (also called degree of unsaturation) was calculated as:
$\text{Average number of double bonds} = (1 \times \%\ \text{monoenes}) + (2 \times \%\ \text{dienes}) + (3 \times \%\ \text{trienes}) + \ldots + (n \times \%\ \text{FA with } n \text{ double bonds})$

A Δ predator-prey index (Δ PPI) 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:

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

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 deviation. Probabilities below 0.05 were considered significant.

Results

Distribution of fat reserves

Figure 2.1 shows the relationship between percent body fat and body mass. The slope of the linear regression between the 2 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. 2.2a. For this analysis, ventral, tracheal and pelvic fat depots were pooled (hereafter referred to as 'fat depots') because no significant differences among them were 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 mass of the carcass, liver and fat depots does not change during the stopover. However, the lean mass of pectoral muscle increases while fat is being deposited ($P<0.001$, $R^2= 0.24$; Fig. 2.2b).

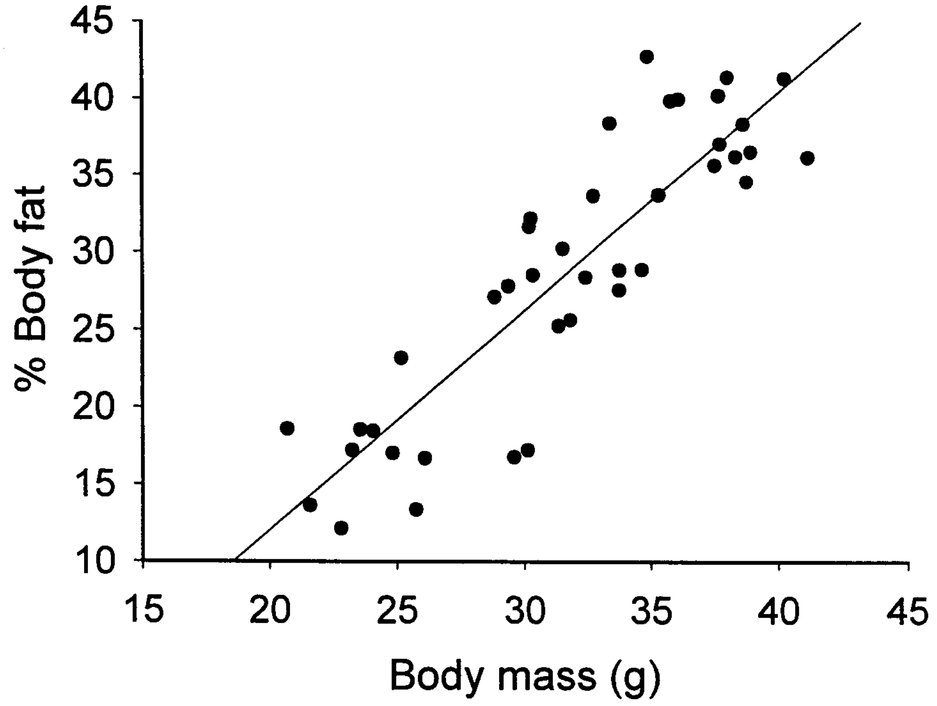

Figure 2.1: % Body fat in relation to body mass of semipalmated sandpipers during the stopover period in the Bay of Fundy. Each data point represents one individual (*N*= 40, $P<0.0001$, $R^2= 0.78$).

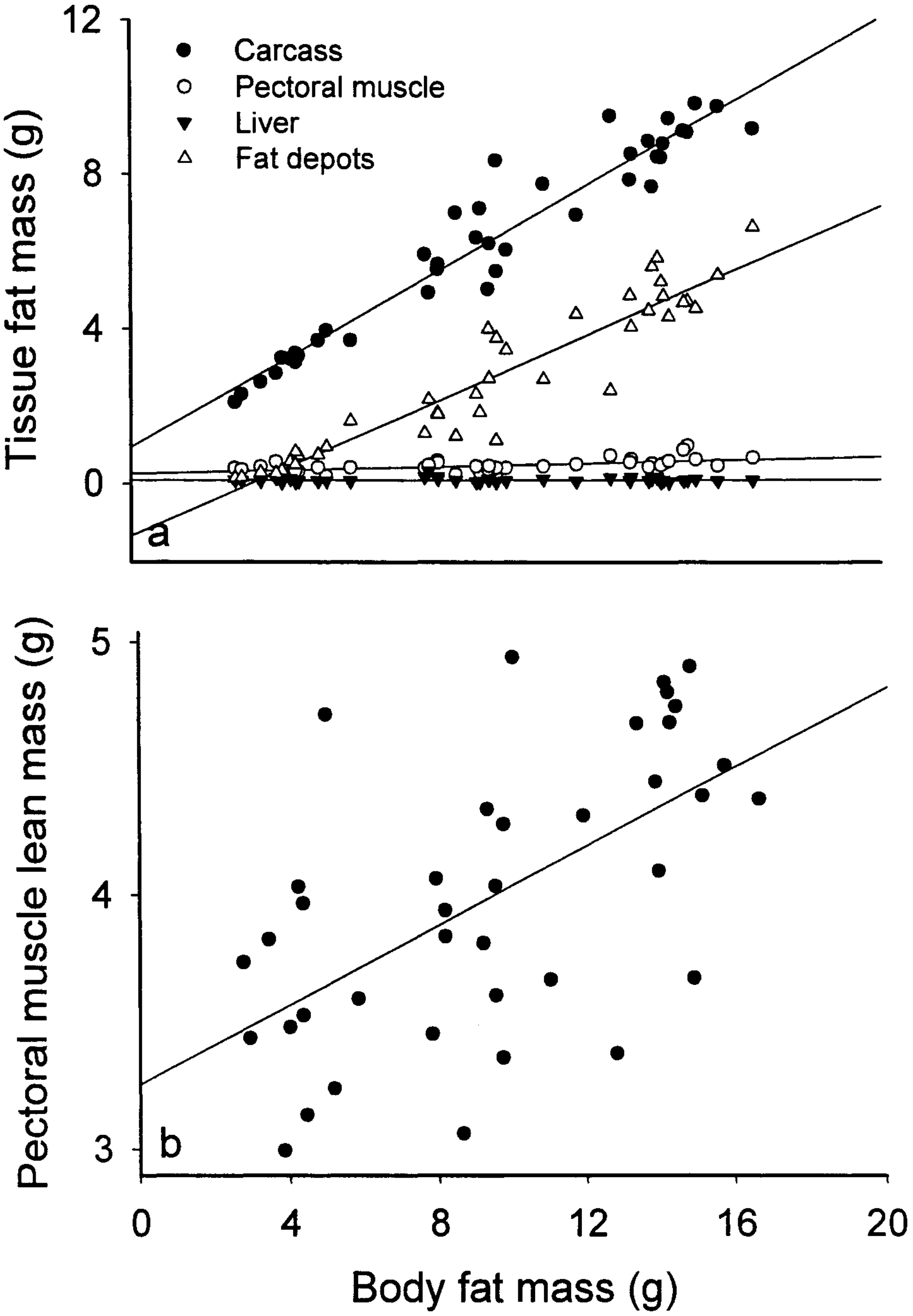

Figure 2.2: Fat masses of various tissues (a) and pectoral muscle lean mass (b) in relation to body fat mass in the semipalmated sandpiper during the stopover period in the Bay of Fundy. Each data point represents one individual (a; *N*=40, b; *N*= 39).

Double bond index

To analyze changes in tissue lipid composition during refueling, the sandpipers were divided in 3 groups: lean, medium and fat (see methods). Figure 2.3 summarizes changes in double bond index (DBI) in storage lipids (NL) and in membrane lipids (PL) of the 3 tissues that show significant fat accumulation: pectoral muscle (top panel), carcass (middle panel) and fat depots (bottom panel). 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. 2.3). For fat depots, no differences in DBI were observed between lean, medium and fat birds ($P>0.05$).

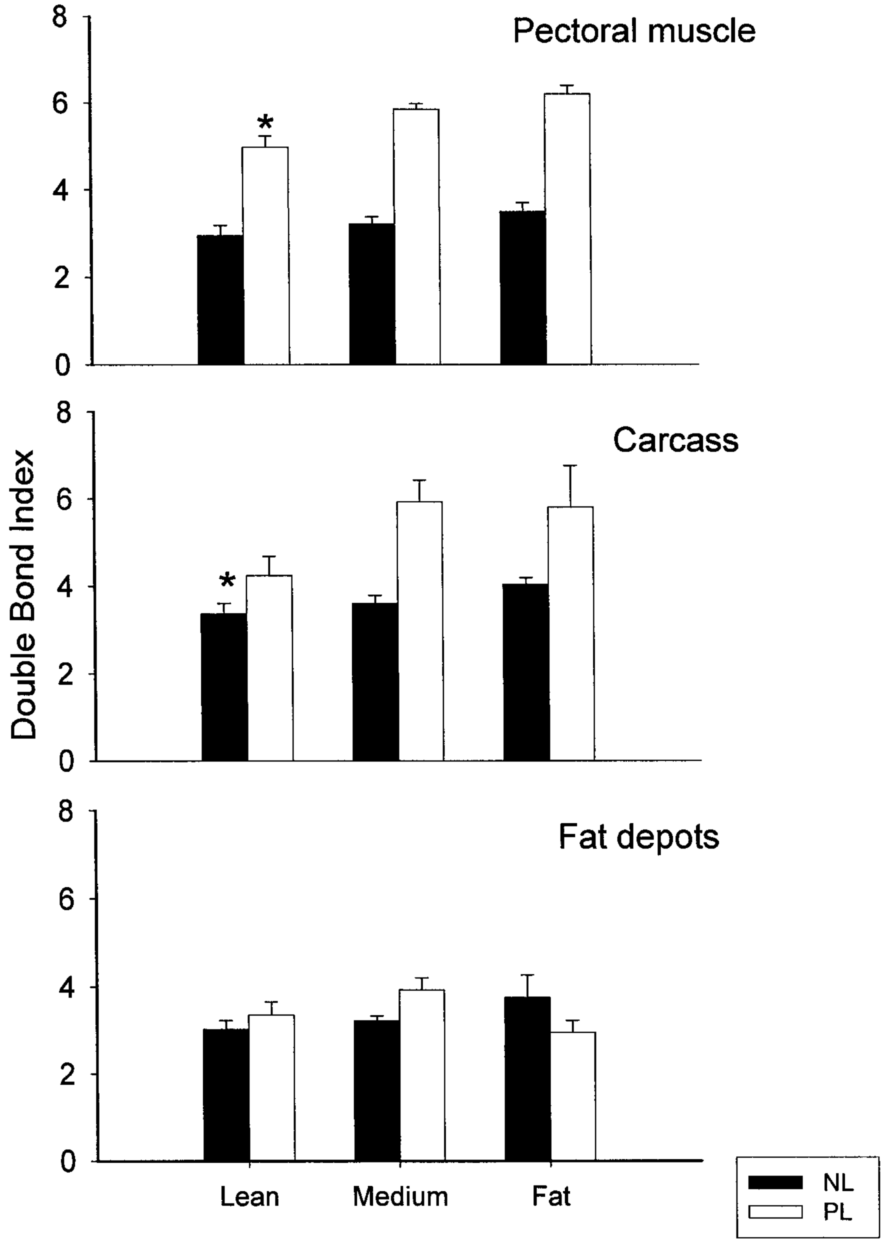

Figure 2.3: Double bond indices (DBI) of pectoral muscle, carcass (including all tissues except for pectoral muscles, liver and distinct fat depots) and total fat depots in neutral lipids (NL) and phospholipids (PL) of semipalmated sandpipers in lean, medium and fat birds. Significant differences *between groups within lipid fraction* are shown by *. Values are mean ± S.E.M. *N* varies between 11 and 14 per group.

Tissue fatty acid composition

Changes in the fatty acid composition of tissues during refueling are presented in Table 2.1. To start analyzing the mechanisms responsible for the observed changes in DBI, Figs. 2.4 and 2.5 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. 2.4. 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 ($P<0.001$; top Fig. 2.4). In carcass PL, DBI remains unchanged because the increase in % 20:5 is offset by a decrease in % 20:4 ($P<0.01$; middle Fig. 2.4). In the PL of fat depots, % 18:2 and % 20:4 both decrease during the stopover ($P<0.01$; bottom Fig. 2.4).

Table 2.1: Relative contribution of individual fatty acids to total tissue fatty acids (%) in main tissues of lean, medium and fat semipalmated sandpipers. Separate values are given for neutral lipids (NL) and phospholipids (PL). 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).

	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

▨ NL
□ PL

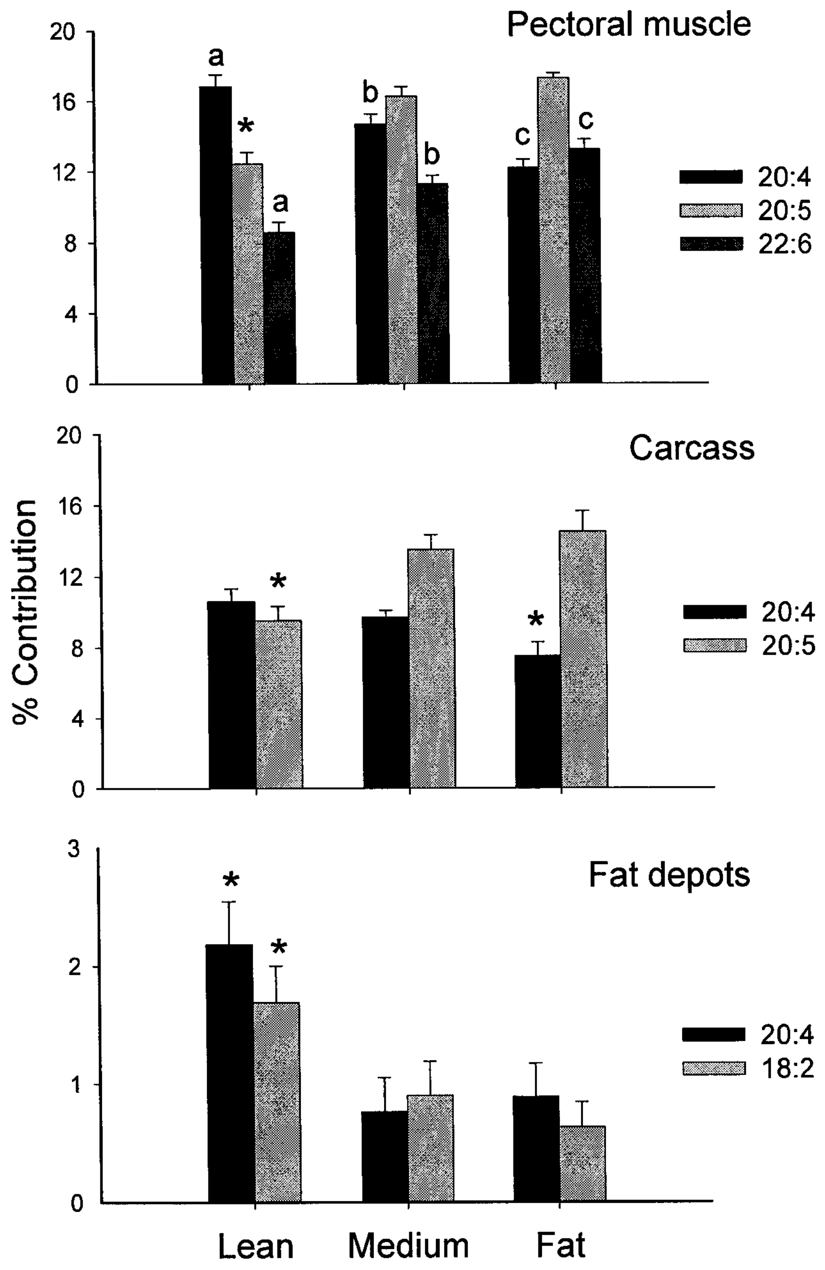

Figure 2.4: % Contribution of individual fatty acids in pectoral muscle, carcass (including all tissues except for pectoral muscles, liver and distinct fat depots) and total fat depots in phospholipids (PL) of semipalmated sandpipers in lean, medium and fat birds. Values are mean ± S.E.M.(*N*=11 -14). * or a,b,c only indicate 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.

Significant changes in the fatty acid composition of tissue NL are presented in Fig. 2.5. In pectoral muscle NL, % 20:5 increases while % 18:0 decreases as lipid stores are being deposited ($P<0.05$; top Fig. 2.5). In carcass NL, % 20:5 and % 22:6 show increases while % 20:4 decreases ($P<0.005$; middle Fig. 2.5). In the NL of fat depots, DBI remains unchanged because increases in % 20:5 and % 22:6 are compensated by decreases in % 20:4 and % 18:2 as the birds accumulate fat reserves ($P<0.05$; bottom Fig. 2.5).

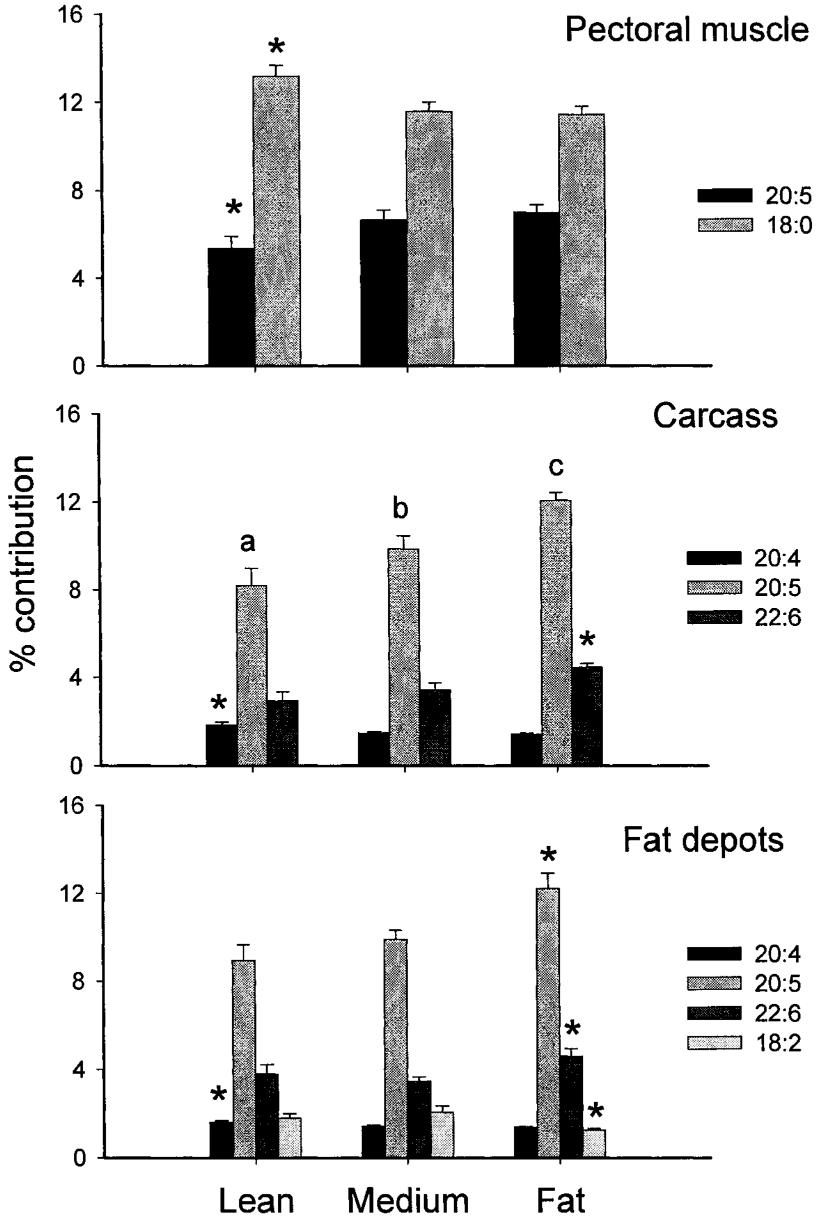

Figure 2.5: % Contribution of individual fatty acids in pectoral muscle, carcass (including all tissues except for pectoral muscles, liver and distinct fat depots) and total fat depots in neutral lipids (NL) of semipalmated sandpipers in lean, medium and fat birds. Values are mean ± S.E.M. (*N* = 10- 14). * or a,b,c only indicate 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.

*Fatty acid composition of the food (*Corophium volutator*) 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 2.2. *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 Δ PPI (a single value representing the sum of differences for all fatty acids; see Methods). Changes in Δ PPI are summarized in Fig. 2.6. During refueling, convergence between the fatty acid compositions of predator and prey was observed for pectoral muscle PL (top Fig. 2.6) and lipid reserve NL (bottom Fig. 2.6) that showed a significant decrease in Δ PPI ($P< 0.005$).

Table 2.2: % Contribution of individual, saturated, monounsaturated and polyunsaturated fatty acids and total fatty acid concentration (μmol/g) in *Corophium volutator*. Values are for total fat content.

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
[Total fatty acids]	*12.3*

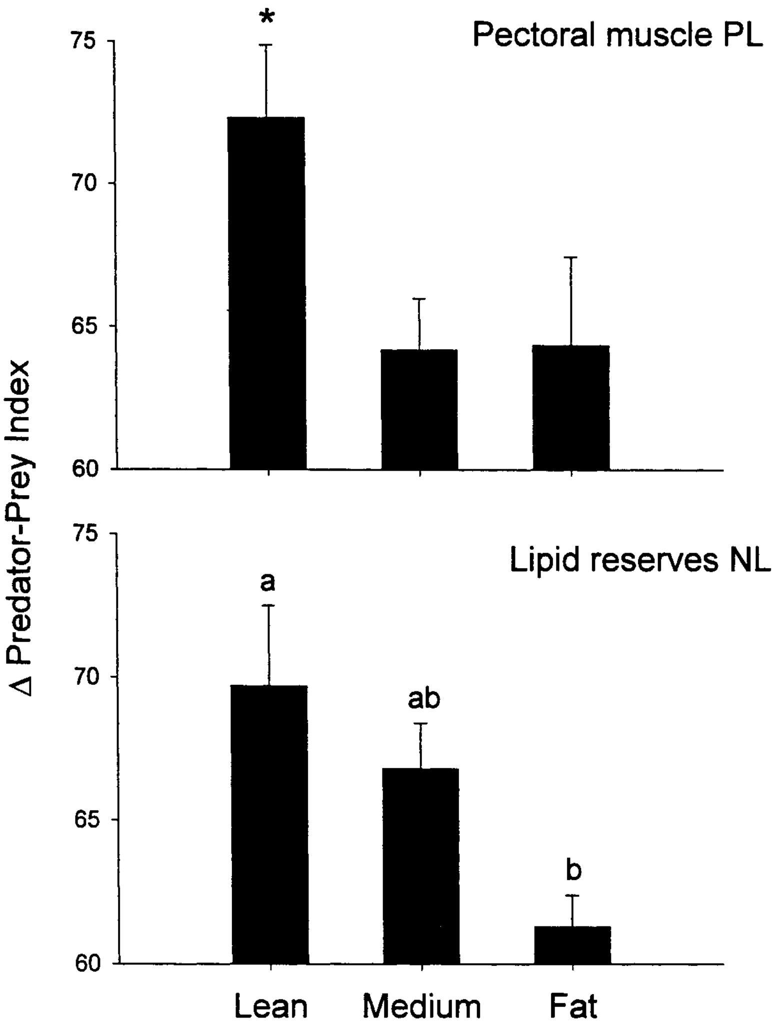

Figure 2.6: Δ Predator-Prey Index in pectoral muscle PL and lipid reserves NL in lean, medium and fat birds. Significant differences between groups are shown by * or a,b. Values are mean ± S.E.M. *N*= 13 in lean and fat birds and 14 in medium birds.

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. 2.6). This study shows that the massive consumption of n-3 PUFA causes an increase in the unsaturation levels of muscle PL and adipose tissue NL. However, a large fraction of the dietary n-3 PUFA consumed during the stopover is modified before storage to maintain high MUFA levels in fuel reserves used for migration.

Flight muscle membranes are modified

Phospholipids are the main structural lipids of cellular 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. 2.3), and this change is achieved by incorporating dietary EPA and DHA (Table 2.1; Fig. 2.4). Recent experiments on rats (Turner et al., 2004) and humans (Andersson et al., 2002) reveal that mammals respond similarly after eating a high n-3 PUFA diet, but for periods of 2-3 months. Our study is the first to show that 1) nutrition may also modify avian muscle membranes, 2) natural diets can have such an effect in wild birds, and 3) muscle membranes of long-distance migrants can respond very rapidly (1-2 weeks).

Information presently available is insufficient to identify a detailed mechanism linking membrane fatty acid composition with muscle performance. However, several lines of evidence suggest that these parameters may be dependent. 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), and endurance training increases the n-3 PUFA content of muscle PL in humans (Andersson et al., 2000; Helge et al., 2001). In addition, controlled-diet studies in mammals (Ayre and Hulbert, 1997), fish (Wagner et al., 2004), and birds (Pierce et al., 2005) show that changing the fatty acid composition of the food can alter exercise performance. Unfortunately, few studies have tried to link the consumption of specific fatty acids with increased endurance and they provide conflicting results. In rats, Ayre and Hulbert (1997) report that dietary n-6 PUFA cause the greatest increase in endurance. For salmon, Wagner et al (2004) suggest that n-3 PUFA increase swimming performance, whereas McKenzie et al. (1998) come to the opposite conclusion. It is therefore not yet possible to conclude that either 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 high PUFA diets (Ernst, 1994) or decreased by low PUFA diets (Daveloose et al., 1993). Here, we show that semipalmated sandpipers may increase the fluidity / permeability of their muscle membranes by feeding on *Corophium* as they prepare for long-distance migration. This functional change is consistent with increased capacity for fatty acid transport across the sarcolemma, and contributes to support the very high lipid fluxes that these amazing athletes need for migration.

EPA and DHA increase in neutral lipids

The composition of lipid reserves will affect the capacity to migrate because various fatty acids have a different energy content per unit mass and they are not all mobilized or oxidized equally well. Results show that feeding on *Corophium* affects the composition of the sandpipers' lipid reserves, which, throughout the stopover converges with that of the diet (Fig. 2.6, bottom panel). Percent EPA (20:5) and DHA (22:6) increase significantly in carcass and fat depots NL that account for >95% of total body lipids (Fig. 2.5). Determining what fatty acid composition of fuel reserves is "ideal" for migration is a very difficult task because stores with the highest energy content and stores most easily metabolized would have different compositions. The energy content of fatty acids increases with saturation and chain length (Blem, 1990), whereas rates of mobilization and oxidation decrease with saturation and chain length (Raclot, 2003; Raclot and Groscolas, 1995). Studies on humans and rainbow trout show that MUFA and PUFA are more readily oxidized than saturated fatty acids, with the exception of 12:0 (lauric acid: a fatty acid that we did not detect in the tissues of semipalmated sandpipers) (DeLany et al., 2000; Henderson and Sargent, 1985). Selectivity for mobilization and oxidation is based on molecular structure and not on the relative abundance of individual fatty acids in the reserves.

Migrant birds may therefore modify dietary fatty acids before storage to achieve a compromise between high energy density and ease of mobilization / oxidation. Like semipalmated sandpipers, most migrant birds' fat stores display higher contributions of oleate (18:1) and palmitate (16:0) than all other fatty acids (Blem, 1976; Blem, 1990; Caldwell, 1973). In the present study, we observed a convergence of the fatty acid profiles of semipalmated sandpipers and *Corophium*, but not to as great an extent as we had initially expected. Though lipid reserve NL appear to be influenced by the sandpipers' *Corophium* diet, endogenous lipid metabolism appears to play a highly significant role. To address this possibility, we have calculated the composition of a theoretical fat bird from the measured composition of lean birds (Table 2.1) and of their food (Table 2.2), assuming that dietary fatty acids are stored without modification. Then, we have compared this theoretical fatty acid composition with that actually observed in fat birds. This analysis is presented in Fig. 2.7 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. 2.7). 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, 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 monounsaturated fatty acids may offer optimal characteristics by providing higher energy density than PUFA, together with higher mobilization and oxidation rates than saturated fatty acids.

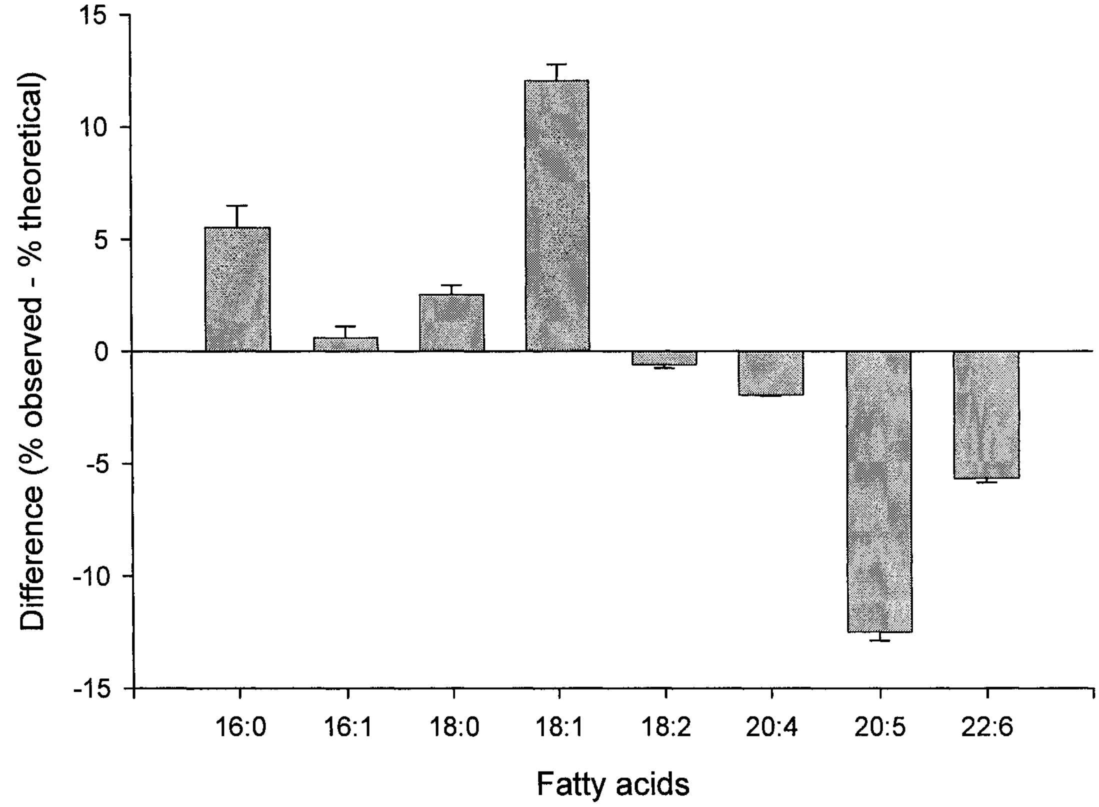

Figure 2.7: Differences between observed and theoretical fatty acid composition of lipid reserve NL in fat semipalmated sandpipers. Theoretical values were calculated from measured compositions in lean birds and in the *Corophium* diet, assuming that dietary fatty acids are stored without modifying chain 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).

Changes in lipid reserves and pectoral muscle lean mass

In migratory birds, lipid reserves are highly dynamic and vary greatly between tissues and seasons. Many studies have examined seasonal fluctuations in fat content at different stages of complete migrations (Marsh, 1983; Piersma et al., 1999; Scott et al., 1994). My study is the first to examine rapid changes in fat reserves during a single refuelling stopover, as migrants prepare for a long flight.

As shown in Figure 2.2a, most of the mass accumulated during the stopover period was deposited in adipose tissue and in carcass. As expected, fat mass did not vary in the liver and only slightly increased in pectoral muscles. In heavy birds, carcass was responsible for 54%, fat depots for 42% and pectoral muscles for 4% of the increase in total fat mass. The largest relative increase in tissue fat mass was observed in fat depots, where fat mass increased by approximately six fold. Though carcass contains the largest absolute mass of fat (close to 10 grams in the heaviest birds), the relative increase (about 4-fold) was less important than that measured in fat depots. This suggests a possible anatomical selection for the distinct fat depots over subdermal adipose tissue during lipid deposition.

Formerly, researchers thought that mass increases observed during pre-migratory periods could be attributed solely to fat deposition. More recent studies on pre-migrant and staging birds have shown that lean mass also fluctuates in various organs such as pectoral and leg muscles, heart, kidneys, liver and gut (Driedzic et al., 1993; Jehl, 1997; Marsh, 1983; Piersma and Gill, 1998; Piersma et al., 1999). We found that pectoralis lean mass in the semipalmated sandpiper increased by about 40% (close to 2 g) during the refueling period in the Bay of Fundy. Similar trends in increased pectoralis lean mass have been observed in staging red knots (*Calidris canutus islandica*) (Piersma et al., 1999), bartailed godwits

(*Limosa lapponica*) (Piersma and Gill, 1998) and eared grebes (*Podiceps nigricollis*) (Jehl, 1997). Increased protein matter during pre-migration may be used to supplement poor feeding conditions and/or for reproductive purposes (such as egg formation) at the arrival site (Evans et al., 1992). One study also explored the possibility that protein deposition occurs to recuperate protein matter that has been catabolized during extensively long flights, which provided no real opportunity for food and water uptake (Baucheinger and Biebach, 2005).

In contrast with the above-mentioned cases, the semipalmated sandpiper's stopover in the Bay of Fundy is not usually preceded by particularly strenuous flights over large ecological barriers. Birds usually make their way to this specific staging area from the nesting grounds using short-distance flights (Hicklin, 1987). In addition, at this stage, the breeding season has passed and thus protein deposition for reproductive purposes is not required. The Bay of Fundy stopover period is followed by the longest non-stop flight of the semipalmated sandpiper's entire migration and presumably entails certain anatomical alterations that would facilitate the completion of this arduous task. Subsequently, the increase in semipalmated sandpipers' pectoral muscle lean mass is most likely an adaptive response to increased power requirements brought on by considerable weight gain prior to a long non-stop migratory flight.

Limitations of study

The experimental design of this study only allowed to provide indirect support for the idea that dietary PUFA are being used to improve capacity for endurance exercise. However, my 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. see 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 "flight wheel" protocol developed by Chappell et al. (1999) may be ideal for this purpose.

Conclusions

Refueling on a diet high in n-3 PUFA led to increases in the percent contribution of these fatty acids in the flight muscle PL of semipalmated sandpipers. These results are consistent with increases in membrane fluidity and permeability, which may enhance capacity for fatty acid movement during migration (Daveloose et al., 1993; Ernst, 1994; Stillwell and Wassal, 2003). Improved muscle performance due to an increase in n-3 PUFA in cell membranes has been demonstrated in several highly aerobic species, including the flight muscles of hummingbirds (Infante et al., 2001). As such, semipalmated sandpipers feeding in the Bay of Fundy may use their diet to alter the fatty acid composition of their membranes and, subsequently, prime muscles for the upcoming long-distance migration.

Dietary n-3 PUFA also led to increases of EPA and DHA in lipid reserves NL, however, the impact of these fatty acids was smaller than expected. Data indicates that over half of ingested n-3 PUFA is converted to other fatty acids (mostly 18:1) before storage. Oleate (18:1) is highly abundant in the fat stores of numerous species, including migrant birds (Caldwell, 1973). This fatty acid displays intermediate degrees of saturation and chain length and thus, may offer a compromise between high energy density and elevated fatty acid mobilization and oxidation.

CHAPTER 3. NATURAL DOPING OF A LONG-DISTANCE MIGRANT: DIETARY PUFA AND FLIGHT MUSCLE ENZYMES IN SEMIPALMATED SANDPIPERS

Introduction

Long-distance migrants are faced with the considerable challenge of obtaining and then utilizing large amounts of fat for energy (Blem, 1990). During flights that may last several days without the prospect of refueling, energy requirements soar to 4 to 10 times those required to fuel average resting metabolism (Odum et al., 1961; Pennycuick, 1975). In addition to significant pre-migratory fattening, migrants such as the semipalmated sandpiper may also undergo a series of physiological changes aimed at increasing the capacity to mobilize and catabolize lipid reserves.

Increased capacity for lipid metabolism during the pre-migratory period has been observed in several species, particularly at the flight muscle level. Several enzymes involved in lipid oxidation, such as carnitine plamitoyl transferase (CPT), 3-hydroxyacyl dehydrogenase (HOAD) and ultimately, citrate synthase (CS) are often used as indicators of the capacity for aerobic metabolism. In pre-migrant gray catbirds (*Dumetella carolinensis*), HOAD activity nearly doubles, whereas CS activity remains constant during the fattening period (though aerobic capacity is enhanced in proportion with the increase in flight muscle mass) (Marsh, 1981). In contrast, CS, CPT and HOAD activities did not change during the premigratory period of western sandpipers (*Calidris mauri*) but did increase throughout migration (Guglielmo et al., 2002). During entire migration, increases in CS and HOAD activities take place in the pectoral muscles of reed warblers (*Acrocephalus stentoreus*) and sedge warblers (*Acrocephalus schoenobaenus*) (Lundgren and Kiessling, 1985). Reduced capacity for anaerobic metabolism often occurs in combination with improved potential for lipid oxidation (Lundgren and Kiessling, 1985; Marsh, 1981). The relative activities of aerobic and anaerobic enzymes may reflect the rate of muscle use and/or the type of substrate used for energy production (Alp et al., 1976). Fat is largely selected as fuel during sustained flight, and as a result, an increase in the capacity to utilize this fuel in preparation for migration may reflect an indispensable physiological adaptation. In contrast, carbohydrates are used very little as fuel for endurance exercise, and as such, changes in the activities of glycolytic enzymes (such as lactate dehydrogenase: LDH) in prepration for migration may not be necessary.

Unlike many other long-distance migrant birds, semipalmated sandpipers refueling in the Bay of Fundy feed on a diet containing unusually large amounts of n-3 polyunsaturated fatty acids (Ackman et al., 1979). These lipids have been shown to regulate the expression of genes involved in lipid metabolism, possibly via peroxisome proliferator-activated receptors (PPAR) (Jump, 2002b; Jump and Clarke, 1999; Lapillonne, 2004; Price, 2000; Sampath and Ntambi, 2005; Wolfrum and Spener, 2000). PUFAs serve as "signalling molecules" and may interact either directly with enzymes to modify their activity, or with nuclear transcription to increase the expression of specific genes (Wolfrum and Spener, 2000). Evidence of enhanced aerobic capacity induced by n-3 PUFA supplementation has been revealed in a number of species and cell types (see Table 3.1 for a summary of known effects). In general, dietary n-3 PUFA enhance mitochondrial and peroxisomal densities and increase lipid oxidation within these organelles.

In addition to increasing oxidative metabolism prior to migration, refueling birds must also maximize fat gain. Exactly how dietary fatty acids affect lipid deposition remains uncertain. At first glance, n-3 PUFA appear to reduce fatty acid synthesis and triacylglycerol levels, though these effects may simply be a product of a parallel increase in fat catabolism (Froyland et al., 1997; Sanz et al., 2000; Totland et al., 2000; Yamazaki et al., 1987). Consequently, two important physiological features are necessary for successful long-distance migration: 1) capacity for high lipid deposition rates during the pre-migratory period, and 2) increased capacity for fat oxidation during migratory flight.

Table 3.1: Effects of n-3 PUFA on lipid metabolism.

n-3 PUFA effect	**Species/tissue**	**References**
Effects on mitochondria		
↑ mitochondrial density	Rat hepatocytes	Froyland et al., 1997; Totland et al., 2000
↑ mitochondrial β-oxidation	Human adipocytes Salmon hepatocytes	Guo et al., 2005; Moya-Falcon, 2004
↑ CPT activity	Human adipocytes Chicken hepatocytes and cardiac myocytes Rat hepatocytes and myocytes	Guo et al., 2005; Sanz et al., 2000; Totland et al., 2000
↑ HOAD activity	Chicken hepatocytes	Sanz et al., 2000
Effects on peroxisomes		
↑ peroxisomal density	Rat hepatocytes	Froyland et al., 1997
↑ peroxisomal β-oxidation	Rat hepatocytes	Yamazaki et al., 1987
↑ Fatty acyl-CoA oxidase	Rat hepatocytes	Froyland et al., 1997; Kim and Choi, 2005; Totland et al., 2000; Yamazaki et al., 1987

The amount of fat deposited in semipalmated sandpipers during their two-week stopover was measured in Chapter 2. The goals of the present chapter are to determine if indicators of aerobic and anaerobic capacities vary during the birds' refueling period in the Bay of Fundy and if these changes are related to changes in n-3 PUFA contribution in the sandpipers' tissues.

The activities of CPT (I and II, both involved in fatty acid transportation through the mitochondrial membrane) and HOAD in pectoral muscle were measured as an index of lipid oxidation capacity, whereas CS activity was used as an indicator of mitochondrial density (Moyes, 2003). Glycolytic enzymes have been studied very little in migrant birds and their role in migration is still unclear. As a result, LDH activity measurements were used to assess anaerobic metabolism and verify whether n-3 PUFA also affect the activity of a glycolytic enzyme. Changes in enzymatic activities during the refueling period were estimated using % body fat as an indirect measure of time spent refueling (see Chapter 2). Regressions were also performed to determine the relationship between flight muscle enzymatic activity and % contribution of individual, saturated, monounsaturated and polyunsaturated fatty acids in the sandpipers' tissues. Enzyme activities measured in pectoral muscle were correlated with % contribution of fatty acids in various tissues because during flight, fatty acids are mobilized from lipid stores located at different anatomical sites (and not only directly from the exercising muscle). I predict that the activities of enzymes related to aerobic capacity will increase with % body fat whereas those related to anaerobic capacity will not because, contrary to lipids, carbohydrates are rarely used during migration. In addition, based on the known effects discussed above, I reason that CS, CPT and HOAD activities will be related to increases in n-3 PUFA contributions.

Materials and methods

Animals and tissue collection

Semipalmated sandpipers were collected in August 2004 and euthanised as described in Chapter 2. The right pectoral muscle was immediately extracted following euthanasia, freeze-clamped in liquid nitrogen and stored at -80°C until analysis (up to three months later).

Fatty acid composition

Lipids were extracted and fatty acid composition was measured as described in Chapter 2.

Homogenate preparation

Approximately 200 mg of frozen pectoral muscle samples were combined with an ice-cold homogenization buffer (20 mM Na_2HPO_4, 5 mM EDTA, 0.1% Triton X-100, 0.2% fatty acid-free BSA, 50 µg/ml aprotinin and 50% glycerol, pH 7.4) at a 9:1 ratio (10% mass/volume). Samples were homogenized on ice using a ground-glass homogenizer. Homogenate was centrifuged at 12,400 rpm for 10 minutes at 4°C. The supernatant was then frozen at -80°C until later analysis (up to 2 weeks).

Enzyme assays

The activities of the following enzymes were measured using a Beckman DU 640 spectrophotometer (USA): citrate synthase (CS), total carnitine palmitoyl transferase (CPT I and II), 3-hydroxacyl CoA dehydrogenase (HOAD) (Guglielmo et al., 2002) and lactate dehydrogenase (LDH) (Hansen and Sidell, 1983). During activity measurements, cuvettes were maintained at 39 °C using a Brinkmann Lauda RC 3 circulating water bath and cuvettes were pre-heated to attain this temperature. Activities were determined by increases or decreases in absorbance at 412 nm (for CS and CPT) and 340 nm (for HOAD and LDH). Preliminary measurements were performed to determine the optimal concentrations of homogenates needed to yield maximum reaction velocities.

For CS, assay conditions were 0.15 mM DTNB and 0.15 mM acetyl CoA, 0.5 mM oxaloacetate (substrate) and 1:9 diluted homogenate in tris buffer (50 mM at pH 8.0). For CPT, 0.15 mM DTNB, 0.035 mM palmitoyl CoA, 5 mM carnitine (substrate) and 1:2 diluted homogenate were added to tris buffer (50 mM at pH 8.0). For HOAD, assay conditions were 1 mM EDTA, 0.2 mM NADH, 0.1 mM acetoacetyl CoA (substrate) and 1:2 diluted homogenate in imidazole buffer (50 mM at pH 7.4). For LDH, I used 0.15 mM NADH, 1 mM KCN, 10 mM pyruvate Na (substrate) and 1:9 diluted homogenate in imidazole buffer (50 mM at pH 7.5). Substrates and homogenates were omitted in controls, and background activity was subtracted from measurements in the presence of substrate and homogenate. The reaction was started by addition of substrate.

Calculations and statistics

All assays were run in triplicate and mean absorbance was used for calculations and statistical analyses. Enzyme activities were calculated as follows:

$$\text{Activity} = [(\Delta \text{ Absorbance/minute} * 1000\ \mu\text{l})/(\varepsilon * 10\ \mu\text{l})] * D$$

where ε is the extinction coefficient (13.6 for DTNB and 6.22 for NADH) and D is the dilution factor. Because of variability in % fat of birds (see chapter 2), enzyme activities were divided by lean mass of assay tissue (and not total mass), thus activities presented are in μmol*min^{-1}/g of lean pectoral muscle.

All statistical analyses were performed using SigmaStat (version 3.1). Linear regressions were used to verify the relationships between enzyme activities and % body fat and % fatty acid contribution. All variables were tested for normality and homogeneity of variances. Percentages were transformed to the arcsine of their square root before analysis. P values below 0.05 were considered significant.

Results

Changes in enzymatic activity during the stopover

Figure 3.1 shows the relationships between pectoralis enzymatic activities (for CS, CPT, HOAD and LDH) and % body fat. % body fat is used as an index of time spent refueling in the Bay of Fundy (see Chapter 2). Although all enzymes measured showed some increase in activity in relation to % body fat, only CS and HOAD were significantly related ($P< 0.05$, with $R^2= 0.141$ and 0.112 respectively).

Relationships between enzymatic activities and % contributions of fatty acids

Regression results for enzyme activities and % contributions of individual, saturated, monounsaturated and polyunsaturated fatty acids are shown in Table 3.2 (for neutral lipids) and 3.3 (for phospholipids). +/- Symbols indicate the direction of the relationship (positive or negative). In neutral lipids (Table 3.2), CS and LDH activities show positive relationships with % contribution of polyunsaturated fatty acids EPA and DHA (particularly in carcass and total body- see Fig. 3.2 for regressions between CS and LDH activities and % EPA contribution in total body). In contrast, these enzymes appear to be negatively related to saturated and monounsaturated fatty acid content (specifically 16:0 and 16:1). CPT activity showed no positive relationship with % contribution of n-3 PUFA, but exhibited strong negative relationships with n-6 PUFA 18:2 in almost all tissues. HOAD was only slightly positively related to EPA in fat depots.

Fewer significant relationships between enzyme activities and fatty acid contributions were observed in phospholipids. Once again, LDH activity was positively related to EPA and DHA contributions, particularly in carcass and total body. On the other hand, CS activity was not related to EPA content and was only slightly related to DHA content in pectoral muscle.

As in neutral lipids, CPT activity is negatively related to 18:1 and 18:2 content. Contrary to other enzymes, HOAD showed more significant relationships with fatty acid contributions for phospholipids than for neutral lipids. HOAD was strongly negatively related to 18:1 PL content (particularly in pectoral muscle and carcass: $P< 0.005$), and was positively related to EPA (in pectoral muscle and carcass) and DHA (in total body).

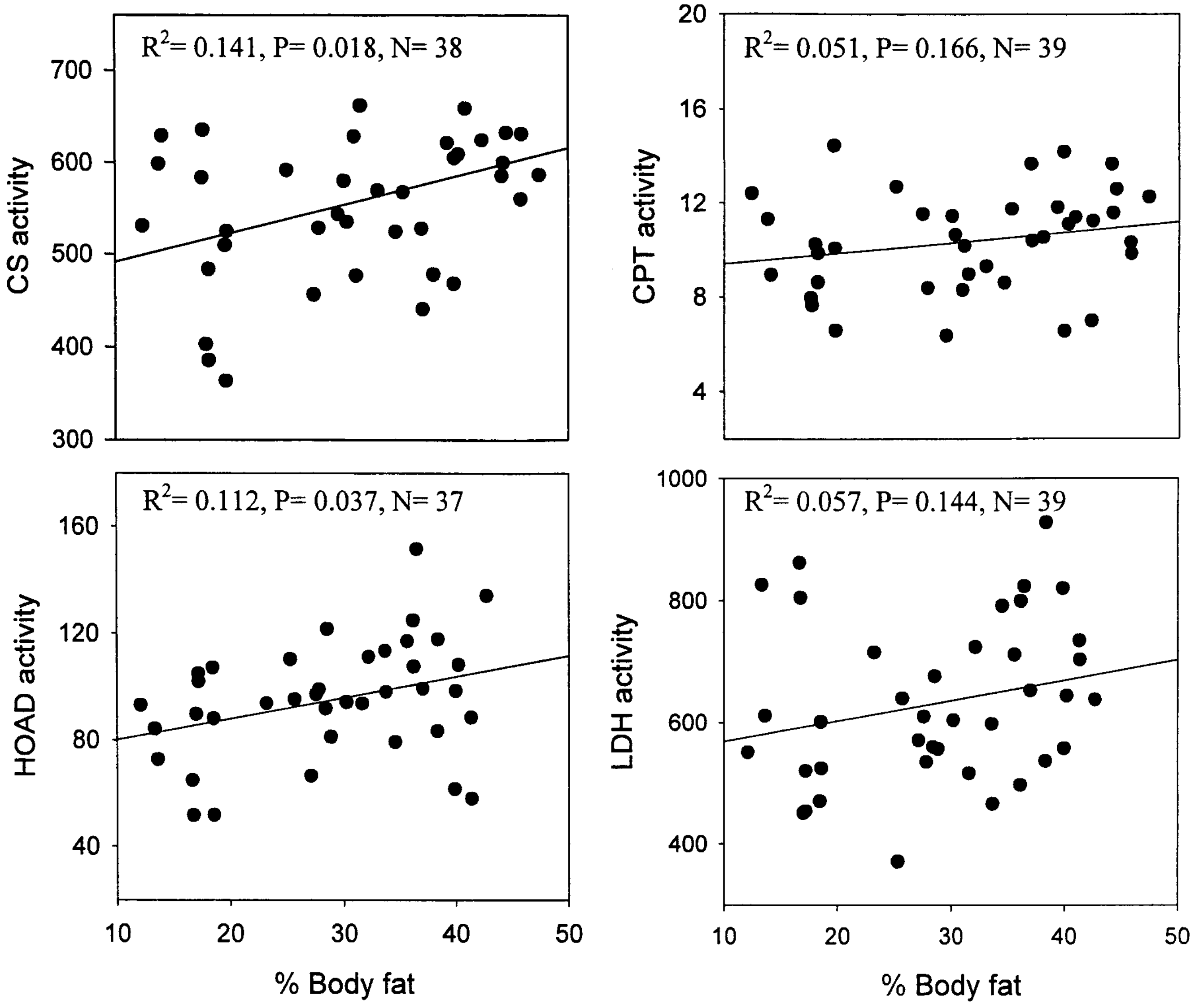

Figure 3.1: Pectoral muscle enzyme activities (μmol/min*g) versus % Body fat in semipalmated sandpipers during the stopover in the Bay of Fundy.

Table 3.2: Results from regressions of enzyme activities (μmol/min*g) versus % contribution of individual, saturated, monounsaturated and polyunsaturated fatty acids in **neutral lipids** of semipalmated sandpipers during the stopover in the Bay of Fundy. N is between 34 and 39.

Enzyme	Fatty acid	Pectoral muscle	Carcass	Fat depots	Total body
CS	*16:0*	-			
	16:1	- -	-		
	18:0				
	18:1	+			
	18:2				
	20:4				
	EPA/20:5		+		+ +
	DHA/22:6		+		
	SFA	-			
	MUFA				
	PUFA		+ +		+ +
CPT	*16:0*				
	16:1				
	18:0				
	18:1			-	
	18:2		- - -	- -	- - -
	20:4				
	EPA/20:5				
	DHA/22:6				
	SFA				
	MUFA	+	- - -		- - -
	PUFA				
HOAD	*16:0*				
	16:1				
	18:0				
	18:1				
	18:2				
	20:4				
	EPA/20:5			+	
	DHA/22:6				
	SFA				
	MUFA				
	PUFA			+	
LDH	*16:0*				-
	16:1		-		
	18:0				
	18:1				
	18:2				
	20:4				
	EPA/20:5		+ + +	+	+ + +
	DHA/22:6	+	+ + +		+ + +
	SFA				
	MUFA				
	PUFA		+ + +		+ + +

+/- 0.05
+ +/- - P<0.01
+ + +/- - - P<0.005

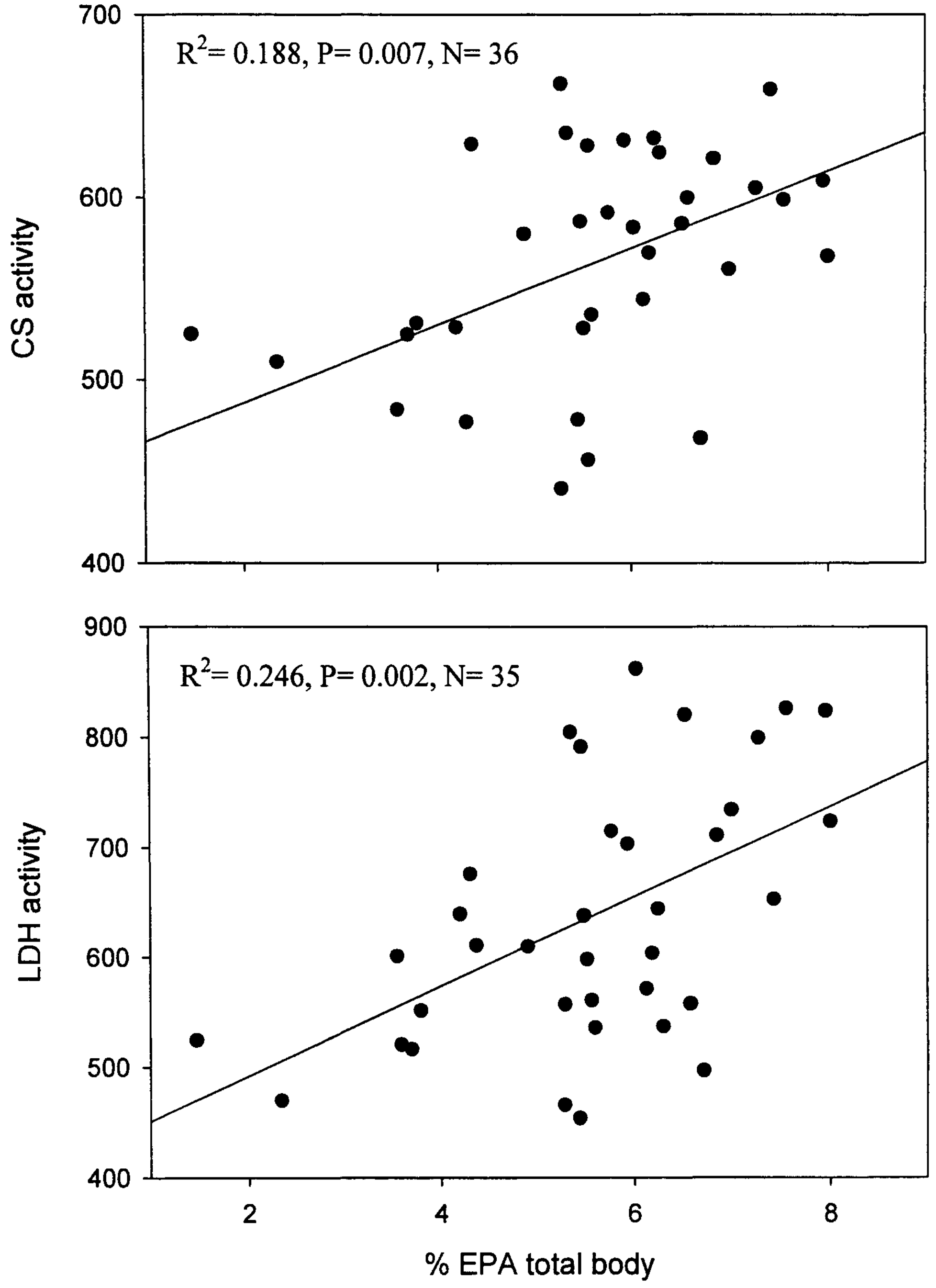

Figure 3.2: Pectoral muscle CS and LDH activities (μmol/min*g) versus % EPA in total body **neutral lipids** of semipalmated sandpipers during the stopover in the Bay of Fundy.

Table 3.3: Results from regressions of enzyme activities (μmol/min*g) versus % contribution of individual, saturated, monounsaturated and polyunsaturated fatty acids in **phospholipids** of semipalmated sandpipers during the stopover in the Bay of Fundy. N is between 34 and 39.

Enzyme	Fatty acid	Pectoral muscle	Carcass	Fat depots	Total body
CS	*16:0*				
	16:1				
	18:0				
	18:1				+
	18:2				
	20:4				
	EPA/20:5				
	DHA/22:6	+			
	SFA				
	MUFA				
	PUFA				
CPT	*16:0*				
	16:1				
	18:0		+		
	18:1	-			- -
	18:2	-			
	20:4				
	EPA/20:5				
	DHA/22:6				
	SFA				
	MUFA				
	PUFA				
HOAD	*16:0*				-
	16:1				-
	18:0		-		
	18:1	- - -	- - -	-	
	18:2				
	20:4				
	EPA/20:5	+	+		
	DHA/22:6				+
	SFA				
	MUFA	- - -	- - -	-	-
	PUFA		+		
LDH	*16:0*				
	16:1				
	18:0				
	18:1				
	18:2				
	20:4		-		
	EPA/20:5		+ + +	+	+ + +
	DHA/22:6		+ + +		+ + +
	SFA			+ + +	
	MUFA	+			+ +
	PUFA				

+/- 0.05
+ +/- - P<0.01
+ + +/- - - P<0.005

Discussion

The main objective of this study was to determine whether the activities of Krebs cycle, β-oxidation and glycolytic enzymes change during the stopover period of a long-distance migrant bird, the semipalmated sandpiper. In addition, I investigated the possibility that these activities are related to the semipalmated sandpipers' high n-3 PUFA diet. Migration requires the accumulation of significant lipid reserves because fat is by far the most important fuel for sustained flight (Blem, 1990). Additionally, several metabolic modifications may occur during pre-migration to enhance the birds' ability to utilize these reserves. In Chapter 2, I reported that flight muscle lean mass increases by 40% throughout the stopover, undoubtedly helping to amplify power output in preparation for migratory flight. Correspondingly, we can predict that sandpipers' muscles could undergo physiological changes that allow them to increase capacity for mobilizing and oxidizing fatty acids during the longest non-stop flight of their migration. The key findings of this study are that 1) activities of CS and HOAD increase during the stopover, and 2) CS and LDH activities are strongly related to n-3 PUFA % contribution in several of the sandpipers' tissues.

Changes in enzyme activities during the stopover

As expected, aerobic capacity is improved in preparation for migration through increases in CS and HOAD activities in semipalmated sandpipers' flight muscles along with an increase in % body fat (Fig. 3.1). Very few studies have looked at changes in aerobic enzyme activities in staging birds and results have differed between species. Pectoral muscle CS activity did not change during pre-migration in western sandpipers (Guglielmo et al., 2002) and gray catbirds (Driedzic et al., 1993; Marsh, 1981). Similarly, HOAD and CPT activities remained constant in the pectoral muscles of pre-migrant western sandpipers

(Guglielmo et al., 2002). Though western sandpipers are closely related to semipalmated sandpipers, the former are short-hop migrants; they migrate using a combination of short flights and frequent stopovers (Guglielmo et al., 2001). In contrast, semipalmated sandpipers are long-jump migrants, and may require higher activities in oxidative enzymes in preparation for a long period of sustained flight. Due to this difference in migratory patterns, the flight muscles of semipalmated sandpipers may require increased oxidative capacity to sustain flight for longer periods.

Other studies have shown that enzyme activities may vary, but only when measured over the course of the entire migration (and not during the stopovers). CS and HOAD activities increased in migrating sedge and reed warblers (Lundgren and Kiessling, 1985) and in western sandpipers (Guglielmo et al., 2002). The latter species also showed increases in CPT activity during total migration (Guglielmo et al., 2002). In contrast, LDH activity in the flight muscle of migrant birds decreased significantly during migration (Lundgren and Kiessling, 1985). Thus, studying semipalmated sandpipers over their entire migration might have revealed additional changes in enzymatic activities to those observed during the stopover.

Relationships between n-3 PUFA content in tissues and enzyme activities

Some migrant birds undergo changes in aerobic and anaerobic capacity during pre-migratory and stopover periods (see previous section). In several species of vertebrates, dietary n-3 PUFA are well known to have strong effects on both mitochondria and peroxisomes which most likely enhance lipid oxidation (see Table 3.1). Improved aerobic metabolism undeniably benefits birds preparing for long-distance migration. However, until now, researchers had not looked at whether effects of diet on Krebs cycle and β-oxidation enzymes already observed in other species also take place in migrant birds.

Pre-migrant semipalmated sandpipers may have an added advantage due to the large quantities of n-3 PUFA that they consume during a refueling period preceding the longest flight of their migration. As predicted, CS activity in pectoral muscle was significantly related to total body EPA and DHA and carcass DHA in NL. The relationship between HOAD activity and EPA content in fat depots NL and in pectoral and carcass PL was significanly positive, however, P values were relatively weak (see Tables 3.2 and 3.3). CPT activity was not related to n-3 PUFA content in NL or PL. The lack of change in CPT (and possibly HOAD) activity in relation to EPA content may be due to the duration of the dietary "treatment". Semipalmated sandpipers refuel in the Bay of Fundy for no more than 15 days before embarking on the long-distance migration to their wintering grounds. Animals in which dietary n-3 PUFA supplementation caused increases in CPT and HOAD activities were treated for comparatively longer periods: from 1 to 3 months (Sanz et al., 2000; Totland et al., 2000). In contrast, my results suggest that experiments showing changes in mitochondrial density and/or the activity of Krebs cycle enzymes (such as CS) may require relatively shorter n-3 PUFA treatment periods.

The most surprising finding of this study was the strong positive relationship between LDH activity and EPA and DHA contribution in carcass and total body (NL and PL: Tables 3.2 and 3.3). Though many studies have focused on the effects of n-3 PUFA on several enzymes of lipid metabolism, almost none have looked at their effects on glycolytic enzymes. To the best of my knowledge, only one study has examined the effects of dietary n-3 PUFA on carbohydrate metabolism and found that these fatty acids increased the activities of several enzymes (including LDH) in rat liver (Yilmaz et al., 2004).

Possible roles of LDH in migration

Carbohydrates are not a primary fuel during long-distance migration, mainly because they are not as energetically dense as lipids (see General Introduction). However, migrants are frequently exposed to conditions that may require high power outputs that simply cannot be fueled by lipids. For instance, take-offs generally involve double the maximal power output required for sustained flight (Marden, 1990). Sandpipers preparing for migration double their body mass, and as such may need even higher power outputs than those observed in non-migratory avian species. High-power flights may also be of substantial importance during other situations encountered during migration such as predator evasion or extreme weather (Harrisson and Roberts, 2000). In fact, sprint-trained birds (i.e. birds trained for anaerobic activities) demonstrated much higher levels of LDH activity than endurance-trained birds (Chaplin et al., 1997). Therefore, it is possible that staging semipalmated sandpipers frequently engaging in high-intensity activities such as loaded take-off and predator avoidance may undergo increases in pectoral muscle LDH activity.

Although LDH functions mostly as a catalyst for the conversion of pyruvate into lactate, it may also be used in aerobic metabolism. It was previously thought that lactate was simply a by-product of glycolysis, and one of the main causes of muscle fatigue. However, more recent studies reveal that lactate may serve as an important fuel for aerobic metabolism (Gladden, 2004). The Lactate Shuttle Hypothesis has exposed the high probability of lactate exchanges between production (i.e. glycolytic fibres) and oxidation sites (i.e. oxidative fibres) (Brooks, 2002). For example, among exercising dogs, a significant fraction (up to 75%) of lactate is removed through oxidation whereas only a small proportion (10-25%) is converted to glucose (Depocas et al., 1969). In addition, through the actions of an aerobic LDH isoenzyme (heart type 4: H4), lactate may also be converted into a more readily oxidized substrate, pyruvate (Brooks, 2002). H4 LDH is indeed predominant over all other LDH isoenzymes in the pectoral muscles of endurance-trained pigeons (Chaplin et al., 1997). As such, lactate (produced during anaerobic activities such as take-off) and elevated LDH activity may be favourable for highly aerobic muscles in migrant birds.

Guglielmo et al. (2001) showed that, like other forms of strenuous exercise, sustained flight caused significant flight muscle damage in western sandpipers and bar-tailed godwits (*Limosa lapponica*), both long-distance migrants. Numerous studies have demonstrated that lactate is an intermediate in wound repair and regeneration processes (Gladden, 2004). Therefore, LDH may also play a role in healing muscle damage arising from long-distance flights.

Conclusions

Enzyme activities of both aerobic and anaerobic metabolisms vary during the migratory stopover of semipalmated sandpipers. CS and HOAD activities increase with % body fat and both CS and LDH activities are strongly positively related to n-3 PUFA content in flight muscles. Whether these changes were caused by the diet remains unclear. Several other factors may come into play during migration and as such, it is difficult to speculate as to the actual effects of dietary n-3 PUFA on enzymatic activity in migratory birds' flight muscles. By eliminating effects of hormones or training, laboratory diet studies may help determine if and how n-3 PUFA affect fuel metabolism.

CHAPTER 4. GENERAL CONCLUSIONS

Major conclusions

Semipalmated sandpipers refueling in the Bay of Fundy ingest exceptionally large amounts of n-3 PUFA. Over 30% of their main prey's lipids are composed of these fatty acids, which, in other vertebrates, are well known to prime tissues for lipid metabolism. The key goal of this thesis was to determine the effects of dietary EPA and DHA on the fatty acid composition and flight muscle enzyme activities of sandpipers at a migratory stopover. These birds are preparing for the longest non-stop flight of their entire migration and, as a result, would benefit from any physiological change that would increase their chances of completing the 4,500 km trip to their wintering grounds in South America.

Previous studies on refueling migratory birds have focused almost exclusively on total energy intake. In Chapter 2, I expanded on this idea by examining how dietary fatty acids can impact storage lipids and, for the first time, membrane lipids of migrant birds. My main objectives were 1) to determine if membrane lipids (PL) become enriched with n-3 PUFA, 2) to find out if the n-3 PUFA content increases in storage lipids (NL), and 3) to observe whether the fatty acid profile of the birds converges with that of their diet during the two-week stopover. In addition, I discussed some of the possible roles that individual fatty acids may have on lipid mobilization and oxidation, and thus endurance capacity. As predicted, membrane lipids are affected by high dietary n-3 PUFA. This finding is consistent with increased membrane fluidity (and thus capacity for fatty acid movement) and muscle performance previously observed in other vertebrates (see Chapter 2 discussion). Dietary n-3 PUFA content also increases the contribution of these fatty acids in storage lipids, however, predator (sandpiper) and diet (*Corophium*) fatty acid compositions do not converge as closely as I had predicted. Considering the massive amounts of n-3 PUFA the birds are ingesting and the fact that the birds double in body mass, the changes in fatty acid profiles were smaller than expected. These data suggest that, prior to storage, significant proportions of dietary EPA and DHA are converted to other fatty acids, mostly 18:1. Oleate (18:1) may offer a compromise because it most likely contains a higher energy density than highly unsaturated fatty acids (such as EPA and DHA). In addition, its intermediate chain length and the presence of a single double bond allow for higher mobilization and oxidation capacities than long-chain and/or saturated fatty acids.

The main objectives of Chapter 3 were 1) to measure changes in flight muscle CS, CPT, HOAD and LDH activities during the stopover, 2) to observe the relationship between stored n-3 PUFA content and aerobic enzyme activity, and 3) to determine whether stored n-3 PUFAs are related to glycolytic enzyme activity. As predicted, aerobic enzymes such as CS and HOAD increase in activity during the stopover, and CS is related to n-3 PUFA content in two of the sandpipers' tissues. Unexpectedly, LDH activity appears to be strongly related to n-3 PUFA content in carcass and total body NL and PL. Further investigation of the literature indicated that LDH may play a more significant role in migration than previously thought. High LDH activity may be necessary for migrants, but not to catalyze the forward reaction of pyruvate into lactate. Instead, this enzyme may be used to convert lactate into pyruvate, which can then be used as an oxidative fuel (Brooks, 2002). Lactate may also be used as an intermediate in the repair of muscle damage (Gladden, 2004).

Future work

This study allowed us to determine the possible effects of dietary fatty acids on lipid and flight muscle metabolism of semipalmated sandpipers preparing for the longest non-stop flight of their entire migration. Though diet clearly has an effect on the lipid metabolism of these birds, we cannot ignore the fact that other factors, such as hormonal fluctuations and training, may also come into play in this natural setting. Controlled diet studies could be used to identify the exact effect of EPA/DHA on the fatty acid profile and on aerobic/anaerobic capacity of these birds. By eliminating outside factors, a "simulated stopover" complete with various diets (differing only in n-3 PUFA contribution), could bring us closer to determining whether the observed changes are entirely caused by a high EPA and DHA ingestion. Although diet clearly impacts both storage and membrane lipids, a large fraction of dietary EPA and DHA were converted to shorter, more saturated fatty acids. Further research is needed to determine why 18:1 and 16:0 appear to be preferentially stored and what role they may play in endurance exercise.

This study looked at various enzymes of lipid and glycolytic metabolism to explore the aerobic and anaerobic capacities of semipalmated sandpipers. The activities of enzymes such as CS or CPT are often used as indices of the capacity for aerobic metabolism, but they may not always reflect actual ability to sustain exercise over long periods. Consequently, it could be useful to measure endurance directly by exercising birds in a flight wheel, rather than relying entirely on indicators of aerobic capacity.

Study implications

To date, very few studies have looked at changes in fatty acid profiles and flight muscle enzymatic activity throughout the stopover of a migratory bird. This study brought us one step closer to determining the physiological roles that individual fatty acids (specifically EPA and DHA) may play in sustained exercise. Using migratory birds as a model for studying the relative mobilization and oxidation of lipids could be highly useful since these animals exemplify the highest aerobic capacity obtainable in almost all vertebrates. In recent years, much attention has been brought to the effects of n-3 PUFA on lipid metabolism, particularly in the fields of obesity and cardiovascular disease research. In addition, by increasing lipid mobilization and oxidation capacities, n-3 PUFAs may have important implications as natural supplements for endurance athletes.

From an ecological perspective, this study increases our understanding of how migrants select diets containing specific fatty acids in preparation for long periods of sustained flight. Also, by demonstrating that dietary n-3 PUFAs (obtained through a large ingestion of *Corophium*) may enhance aerobic capacity, this thesis provides a new scientific rationale for the protection and conservation of the Bay of Fundy mudflats. In North America, *Corophium* is exclusively found in the Bay of Fundy and the Gulf of Maine. To my knowledge, no other marine invertebrate contains such large concentrations of EPA. If populations of *Corophium* decreased significantly (i.e. due to environmental changes or disruptions) the survival of the birds could be greatly affected. This study shows that *Corophium* may be a non-replaceable component of the shorebirds' diet and, consequently, necessary measures to protect its habitat should be taken.

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.

Alp, P. R., Newsholme, E. A. and Zammit, V. A. (1976). Activities of citrate synthase and NAD+-linked and NADP+-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem. J.* **154**, 689-700.

Andersson, A., Nalsen, C., Tendblad, S. and Vessby, B. (2002). Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. *Am. J. Clin. Nutr.* **76**, 1222-1229.

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. Endocrinol. Metab.* **279**, 744-751.

Awad, A. B. (1986). Effect of dietary fats on membrane lipids and functions. *J. Env. Pathol. Toxicol. Oncol.* **6**, 1-14.

Awad, A. B. and Spector, A. A. (1976). Modification of the fatty acid composition of ehrlich ascites tumor cell plasma membranes. *Biochim. Biophys. Acta* **426**, 723-731.

Ayre, K. J. and Hulbert, A. J. (1996). Effects of changes in dietary fatty acids on isolated skeletal muscle function in rats. *J. Appl. Physiol.* **80**, 464-471.

Ayre, K. J. and Hulbert, A. J. (1997). Dietary Fatty Acid Profile Affects Endurance in Rats. *Lipids* **32**, 1265-1270.

Bairlein, F. (2002). How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. *Naturwissenschaften* **89**, 1-10.

Battley, P. F., Dietz, M. W., Piersma, T., Dekinga, A., Tang, S. and Hulsman, K. (2001). Is long-distance bird flight equivalent to a high-energy fast? Body composition changes in freely migrating and captive fasting great knots. *Physiol. Biochem. Zool.* **74**, 435-449.

Baucheinger, U. and Biebach, H. (2005). Phenotypic flexibility of skeletal muscles during long-distance migration of garden warblers: muscle changes are differentially related to body mass. *Ann. N.Y. Acad. Sci.* **1046**, 271-281.

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.

Blem, C. R. (1976). Patterns of Lipid Storage and Utilization in Birds. *Am. Zool.* **16**, 671-684.

Blem, C. R. (1990). Avian Energy Storage. New York: Plenum Press.

Brooks, G. A. (1998). Review: mammalian fuel utilization during sustained exercise. *Comp. Biochem. Physiol.* **120**, 89-107.

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

Caldwell, L. D. (1973). Fatty acids of migrating birds. *Comp. Biochem. Physiol.* **44B**, 493-497.

Chaplin, S. B., Munson, M. M. and Knuth, S. T. (1997). The effect of exercise and restraint on pectoral muscle metabolism in pigeons. *J. Comp. Physiol.* **167**, 197-203.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Cooper, J. M. (1994). Least Sandpiper (Calidris minutilla). In *Birds N. Am.*, vol. 115 eds. A. Poole and F. Gill). Philadelphia, PA: The Birds of North America, Inc.

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.

Dawson, W. R., Marsh, R. L. and Yacoe, M. E. (1983). Metabolic adjustments of small passerine birds for migration and cold. *Am. J. Physiol.* **14**, 755-767.

DeLany, J. P., Windhauser, M. M., Champagne, C. M. and Bray, G. A. (2000). Differential oxidation of individual dietary fatty acids in humans. *Am. J. Clin. Nutr.* **72**, 905-911.

Depocas, F., Minaire, Y. and Chatonnet, J. (1969). Rates of formation and oxidation of lactic acid in dogs at rest and during moderate exercise. *Can. J. Phys. Pharmacol.* **47**, 603-610.

Dohm, G. L. (1986). Protein as a fuel for endurance exercise. *Ex. & Sport Sci. Rev.* **14**, 143-173.

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. Inter. Med. Res.* **225**, 129-132.

Evans, P. R., Davidson, N. C., Uttley, J. D. and Evans, R. D. (1992). Premigratory hypertrophy of flight muscles: an ultrastructural study. *Orn. Scan.* **23**, 238-243.

Florant, G. L., Nuttle, L. C., Mullinex, D. E. and Rintoul, D. A. (1990). Plasma and white adipose tissue lipid composition in marmots. *Am. J. Physiol.* **258**, R1123-R1131.

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.

Gaunt, A. S., Hikida, R. S., Jehl Jr, J. R. and Fenbert, L. (1990). Rapid atrophy and hypertrophy of an avian flight muscle. *Auk* **107**, 649-659.

Gladden, L. B. (2004). Lactate metabolism: a new paradigm for the third millenium. *J. Physiol.* **558**, 5-30.

Gratto, G. W. (1979). The biology of the amphipod Corophium volutator in the western Minas Basin, Nova Scotia. *Department of Biology*. Wolfville, Nova Scotia.: Acadia University.

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**, 1405-1413.

Guglielmo, C. G., Haunerland, N. H. and Williams, T. D. (1998). Fatty acid binding protein, a major protein in the flight muscle of migrating Western sandpipers. *Comp. Biochem. Physiol.* **119**, 549-555.

Guglielmo, C. G., Piersma, T. and Williams, T. D. (2001). A sport-physiological perspective on bird migration: evidence for flight-induced muscle damage. *J. Exp. Biol.* **204**, 2683-2690.

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.

Gutierrez, A. M., Reboredo, G. R., Mosca, S. M. and Catala, A. (2006). A low degree of fatty acid unsaturation leads to high resistance to lipid peroxidation in mitochondria and microsomes of different organs of quail (Coturnix coturnix japonica). *Mol Cell Biochem* **282**, 109-115.

Hansen, C. A. and Sidell, B. D. (1983). Atlantic hagfish cardiac muscle: metabolic basis of tolerance tc 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.

Henderson, R. J. and Sargent, J. R. (1985). Chain length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in rainbow trout (Salmo gairdneri). *Comp. Biochem. Physiol.* **82B**, 79-85.

Herzberg, G. R. and Farrell, B. (2003). Fasting-induced, selective loss of fatty acids from muscle triacylglycerols. *Nutr. Res.* **23**, 205-213.

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.

Hicklin, P. W. and Smith, P. C. (1979). The diets of five species of migrant shorebirds in the Bay of Fundy. *Proc. N.S. Inst. Sci.* **29**, 483-488.

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.

Jehl, J. R. (1997). Cyclical changes in body composition in the annual cycle and migration of the eared grebe (Podiceps nigricollis). *J. Avian Biol.* **28**, 132-142.

Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindstrom, A., Piersma, T. and Visser, G. H. (2002). Fuel use and metabolic response to endurance exercise: a wind tunnel study of a long-distance migrant shorebird. *J. Exp. Biol.* **205**, 2453-2460.

Jenni, L. and Jenni-Eiermann, S. (1998). Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521-528.

Jump, D. B. (2002a). The biochemistry of n-3 polyunsaturated fatty acids. *J. Biol. Chem.* **277**, 8755-8758.

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.

Kaduce, T. L., Awad, A. B., Fontenelle, L. J. and Spector, A. A. (1977). Effect of fatty acid saturation on alpha-aminoisobutyric acid transport in ehrlich ascites cells. *J. Biol. Chem.* **252**, 6624-6630.

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.

King, J. R., Barker, S. and Farner, D. S. (1963). A comparison of energy reserves during autumnal and vernal migratory periods in the White-crowned Sparrow. *Ecology* **44**, 513-521.

Kirkwood, J. K. (1983). A limit to metabolisable energy intake in mammals and birds. *Comp. Biochem. Physiol.* **75**, 1-3.

Klassen, M. (1996). Metabolic constraints on long-distance migration in birds. *J. Exp. Biol.* **199**, 57-64.

Klassen, M., Kvist, A. and Lindstrom, E. (2000). Flight costs and fuel composition of a bird migrating in a wind tunnel. *Condor* **102**, 444-451.

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

Lindstrom, A., Klassen, M. and Kvist, A. (1999). Variation in energy intake and basal metabolic rate of a bird migrating in a wind tunnel. *Funct. Ecol.* **13**, 352-359.

Lund, E. D. and Sidell, B. D. (1992). Neutral lipid compositions of Antarctic fish tissues may reflect use of fatty acyl substrates by catabolic systems. *Mar. Biol.* **112**, 377-382.

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.

Marden, J. H. (1990). Maximum load-lifting and induced power output of Harris' hawks are general functions of flight muscle mass. *J. Exp. Biol.* **149**, 511-514.

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.

Marsh, R. L. (1983). Adaptations of the gray catbird to long distance migration: energy stores and substarte concentrations in plasma. *Auk* **100**, 170-179.

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.

McCurdy, D. G., Boates, J. S. and Forbes, M. R. (2000). Reproductive synchrony in the intertidal amphipod Corophium volutator. *OIKOS* **88**, 301-308.

McKenzie, D. J., Higgs, D. A., Dosanjh, B. S., Deacon, G. and Randall, D. J. (1998). Dietary fatty acid composition influences swimming performance in atlantic salmon in seawater. *Fish Physiol. Biochem.* **19**.

McNeil, R. (1969). La détermination du contenu lipidique et de la capacité de vol chez quelques espèces d'oiseaux de rivage. *Can. J. Zool.* **47**, 525-536.

McWilliams, S. R., Kearneym, S. B. and Karasov, W. H. (2002). Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. *J. Avian Biol.* **33**, 167-174.

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.

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.

Nol, E. and Blanken, M. S. (1999). Semipalmated Plover (*Charadrius semipalmatus*). In *Birds N. Am.*, vol. 444 eds. A. Poole and F. Gill). Philadelphia, PA: The Birds of North America, Inc.

Odum, E. P., Connell, C. E. and Stoddard, H. L. (1961). Flight energy and estimated flight ranges of some migratory birds. *Auk* **78**, 515-527.

Pennycuick, C. J. (1975). Mechanics of flight. In *Avian Biol.*, vol. 5 eds. F. D.S. and K. J.R.), pp. 1-75. New York: Academic Press.

Pierce, B. J. and McWilliams, S. R. (2005). Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. *Condor* **107**, 271-281.

Pierce, B. J., McWilliams, S. R., O'Connor, T. P., Place, A. R. and Guglielmo, C. G. (2005). Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the red-eye vireo. *J. Exp. Biol.* **208**, 1277-1285.

Pierce, B. J., McWilliams, S. R., Place, A. R. and Huguenin, M. A. (2004). Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyes Vireos (Vireo olivaceous). *Comp. Biochem. Physiol.* **138A**, 503-514.

Piersma, T. and Gill, R. R. (1998). Guts don't fly: small digestive organs in obese bar-tailed godwits. *Auk* **115**, 196-203.

Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. (1999). Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* **72**, 405-415.

Price, P. T., Nelson, C.M. and Clarke, S.D. (2000). Omega-3 polyunsaturated fatty acid regulation of gene expression. *Curr. Opin. Lipidol.* **11**, 3-7.

Raclot, T. (2003). Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Progr. Lipid Res.* **42**, 257-288.

Raclot, T. and Groscolas, R. (1995). Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. *J. Lipid Res.* **36**, 2164-2173.

Ramenofsky, M. (1990). Fat storage and fat metabolism in relation to migration. In *Bird Migration*, (ed. Gwinner). Berlin-Heidelberg: Springer-Verlag.

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A. and Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J. Hum. Nutr. Diet.* **17**, 449-459.

Sampath, H. and Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu. Rev. Nutr.* **25**, 317-340.

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.

Schmidt-Nielsen, K. (1972). Locomotion: energy cost of swimming, flying and running. *Science* **177**, 222-228.

Scott, I., Mitchell, P. I. and Evans, P. R. (1994). Seasonal changes in body mass, body composition and food requirements in wild migratory birds. *Proc. Nutri. Soc.* **53**, 521-531.

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., Brown, G. S. and Hochachka, P. W. (1986). Metabolic sources of energy for hummingbird flight. *Am. J. Physiol.* **20**, 537-542.

Suarez, R. K. and Gass, C. L. (2002). Hummingbird foraging and the relation between bioenergetics and behaviour. *Comp. Biochem. Physiol.* **133**, 335-343.

Suarez, R. K., Lighton, J. R., Moyes, C. D., Brown, G. S., Gass, C. L. and Hochachka, P. W. (1990). Fuel selection in rufous hummingbirds: ecological implications of metabolic biochemistry. *Proc. Natl. Acad. Sci.* **87**, 9207-9210.

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.

Valencak, T. G., Arnold, W., Tataruch, F. and Ruf, T. (2003). High content of polyunsaturated fatty acids in muscle phospholipids of a fast runner, the European Brown Hare (Lepus europaeus). *J. Comp. Physiol.* **173B**, 695-702.

Wagner, G. N., Balfry, S. K., Higgs, D. A., Lall, S. P. and Farrell, A. P. (2004). Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. *Comp. Biochem. Physiol.* **137A**, 567-576.

Weber, J.-M., Brichon, G. and Zwingelstein, G. (2003). Fatty acid metabolism in rainbow trout tissues: differential incorporation of palmitate and oleate. *Can. J. Fish. Aquat. Sci.* **60**, 1281-1288.

White, L. (1985). Body Composition and fat deposition of migrant semipalmated sandpipers during autumn migration in the lower Bay of Fundy. *Department of Biology*. Guelph, Ontario: University of Guelph.

Wolfrum, C. and Spener, F. (2000). Fatty acids as regulators of lipid metabolism. *Eur. J. Lipid Sci. Technol.* **102**, 746-762.

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.