FRACTIONATION OF CARBON ISOTOPES DURING FATTY ACID METABOLISM IN ATLANTIC POLLOCK *(POLLACHIUS VIRENS)*

by

Lacey R AuCoin

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
September 2011

DALHOUSIE UNIVERSITY

DEPARTMENT OF BIOLOGY

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "FRACTIONATION OF CARBON ISOTOPES DURING FATTY ACID METABOLISM IN ATLANTIC POLLOCK *(POLLACHIUS VIRENS)*" by Lacey R AuCoin in partial fulfilment of the requirements for the degree of Master of Science.

Dated: September 2, 2011

Supervisor: _____

Readers: _____

_____

DALHOUSIE UNIVERSITY

DATE: September 2, 2011

AUTHOR: Lacey R AuCoin

TITLE: FRACTIONATION OF CARBON ISOTOPES DURING FATTY ACID METABOLISM IN ATLANTIC POLLOCK *(POLLACHIUS VIRENS)*

DEPARTMENT OR SCHOOL: Department of Biology

DEGREE: MSc CONVOCATION: May YEAR: 2012

_____

Signature of Author

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Feeding experiments were conducted on Atlantic pollock (*Pollachius virens*) to examine the variability in tissue fatty acid (FA) composition and stable carbon isotope fractionation of FA during digestion, assimilation and mobilization of lipids. The FA profiles and compound-specific carbon isotopes of chylomicrons, liver, muscle and fasted serum were compared to diet. FA analysis demonstrated similarity among tissue groups despite differences in feeding states. The FA results indicate the blood of post-prandial fish may serve as an alternative to tissue biopsies for the estimation of marine fish diets with compound-specific isotope analysis (CSIA). Despite similarity among FA profiles, the carbon isotope discrimination factors of FA varied independently, which suggests that fractionation is influenced by the degree to which individual FA are oxidized. These results provide preliminary information that is necessary in order to use CSIA to estimate the effects of fish diets.

LIST OF ABBREVIATIONS USED

AA	Amino acid
ANOSIM	Analysis of similarities
ATP	Adenosine triphosphate
BHT	Butylated hydroxytoluene
EAA	Essential amino acid
ER	Endoplasmic reticulum
CO	Canola oil
CSIA	Compound-specific stable isotope analysis
EFA	Essential fatty acid
FA	Fatty acid
FAME	Fatty acid methyl esters
FID	Flame ionization detector
FFA	Free fatty acid
FO	Fish oil
GC	Gas chromatography
GC-C-IRMS	Gas chromatography-combustion-Isotope ratio mass spectrometer
HPLC	High-performance liquid chromatography
LPL	Lysoglycerophospholipid
MAG	Monoacylglycerol
MANOVA	Multivariate analysis of variance
MDS	Multi-dimensional scaling
MUFA	Monounsaturated fatty acid
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
QFASA	Quantitative Fatty Acid Signature Analysis
RER	Rough endoplasmic reticulum
SER	Smooth endoplasmic reticulum
SFA	Saturated fatty acid
SI	Stable isotope
SIMPER	Similarity percentage
SNARE	Soluble NSF Attachment Protein Receptor
TAG	Triacylglycerol
TLC	Thin Layer Chromatography
VLDL	Very low density lipoprotein
VPDB	Vienna Peedee Belemnite
WE	Wax ester

ACKNOWLEDGEMENTS

This research was funded by the National Research Council of Canada and performed at Dalhousie University.

I would like to express by deepest thanks to my supervisor, Dr. Suzanne Budge. Your knowledge, guidance and excitement have inspired me throughout this project. Thank you for your patience and advice; you are a truly wonderful teacher. Many thanks to Anne Timmins for her countless tips and help with the laboratory analysis- your energy, enthusiasm and stories always brightened my day. I would also like to thank Drs. Santosh Lall and Susan Ziegler for their advice and resources during my analyses. Dr. Geert Van Bisen, your knowledge and dedication with the GC-C-IRMS is truly appreciated, thank you so much. Many thanks to the girls in the marine oils lab for sharing laughs; time always passed quickly when working with you.

I am ever grateful for the people who have surrounded me throughout my time in university; especially my parents. Your unconditional love and support throughout the years made this thesis possible. Dad, thank you for your wisdom when things got difficult. Mom, thank you for being the best listener in the world. Last but not least, I would also like to thank David, for your love, encouragement, and humour.

CHAPTER 1.0. INTRODUCTION

1.1. TRACERS IN ECOLOGY

Understanding animal diets can provide insight into important aspects of an ecosystem, such as shifts in food quality or availability (Kirsch et al., 2000; Copeman et al., 2009), migratory movement and subsequent diet changes (Iverson et al., 1997) and seasonal variations of plankton (Napolitano et al., 1997; Ruess et al., 2002; Budge et al., 2008a). Knowledge of food webs is also useful in developing guidelines for ecosystem-based management and conservation (Hooker et al., 2002). Traditional methods of studying diet include stomach content and fecal analysis (e.g., MacInnis et al., 1983) but these methods can be inconclusive and biased towards certain diet items (Yonezaki et al., 2003). The examination of an animal's tissues for diet tracers can reveal information that would otherwise be inaccessible by direct observation. Fatty acid (FA) analysis enables one to trace food webs and determine the contribution of specific items to an animal's diet by identifying the presence of particular FAs (Pond et al., 1997; Hooker et al., 2001; Budge et al., 2002; Iverson et al., 2004). Likewise, the natural abundance of stable carbon isotopes in the organic material that comprises animal tissues can be used to infer diets by distinguishing between the spatial origin of the dietary items, such as marine and terrestrial sources. The use of FA and stable carbon isotopes as diet tracers has provided researchers with methods that do not have the difficulties associated with radiolabelling techniques and can be done with more convenience (Michener et al., 1994). Furthermore, new technology that allows for accurate compound-specific isotope analysis (CSIA) has expanded the biochemical methods of studying diet, particularly in the last ten years (Evershed et al., 2007), and several studies have used CSIA to reveal information on the

origin of individual biomarkers in an animal's diet (Pond et al., 1998; Howland et al., 2003; Budge et al., 2008b).

1.2. FATTY ACIDS

Animal FA typically consist of a straight chain of 4 to 24 carbon atoms and can be saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA), defined by the number of double bonds. FA are expressed as *A:Bn-x*, where *A* represents the number of carbon atoms, *B* represents the number of double bonds and *x* indicates the position of the first double bond from the methyl end of the carbon chain. Double bonds are normally separated by a single methylene group (Figure 1.1). Up to 70 FA structures can be identified in marine organisms and differences in metabolism, particularly desaturation and chain elongation mechanisms in animals, may limit the array of FA that can be synthesized *de novo* (Ackman, 1989). Due to the variety of FA present in marine life (Ackman, 1989), the lipid composition of an animal's tissues is dependent on both diet and a genetically determined ability to synthesize FA from precursors.

All animals contain enzymes that can modify FA by desaturation and elongation; however, the ability and extent of FA modification varies among taxa. For instance, FA have diverse structures and are plentiful in zooplankton communities, and for this reason marine fish have evolved to depend on the FA of their diet rather than *de novo* synthesis to fulfill their nutritional needs (Sargent et al., 1989). All animals, including fish, contain Δ9-desaturase enzymes that can carry out desaturation and elongation processes that include, for example, 16:0 and 18:0 into 16:1n-7 and 18:1n-9, and the extent to which these FA precursors are elongated varies across species (Figure 1.2; Sargent et al., 1993; Tocher, 2003). Linoleic and linolenic acid, 18:2n-6 and 18:3n-3 respectively, are

considered essential FA (EFA) for animals as they effectively treat the symptoms of essential FA deficiency (Cunnane 2003) and cannot be synthesized *de novo*. In theory, these essential FA can be desaturated and elongated to satisfy all other FA requirements, specifically requirements for 20:5n-3 and 22:6n-3; however animals, including fish, have low Δ6-desaturase activity (Tocher 2003). Δ6-desaturase is necessary to synthesize 20:5n-3 and 22:6n-3 from the 18 carbon atom precursors. Marine fish ultimately rely on phytoplankton in their diet for sufficient 20:5n-3 and 22:6n-3 (Falk-Petersen et al., 2000; Budge et al., 2002; Dahl et al., 2003; Sargent et al., 1993). FA obtained through the diet are transferred to a consumer's tissues in a conservative manner (St. John et al., 1996; Dahl et al., 2003), making their analysis useful in ecology.

FA biomarkers have been successfully used to study the trophic dynamics of marine ecosystems. FA biomarkers cannot necessarily quantify the dietary components of a consumer, but FA that are exclusive to diet can confirm dietary contribution For example, FA were used to identify the trophic levels of Antarctic and Arctic krill (Falk-Petersen et al., 2000), and to identify subtle distinctions in pinniped feeding niches (Budge et al., 2007). FA profiles can identify subtle patterns in an ecosystem that may not be apparent from physical data (Iverson et al., 2007; Springer et al., 2007, Cooper et al., 2009). Multivariate analyses of FA have been used to examine subtleties in the diets of polar bears (Thiemann et al., 2007), and to detect "bottom-up" changes in the environment through seabird diets (Iverson et al., 2007).

More complex methods have been proposed to use predator FA profiles to quantify prey species in diets (Iverson et al., 2004). This technique, coined Quantitative Fatty Acid Signature Analysis (QFASA), uses a statistical model to calculate the most

probable components of a diet profile, while correcting for predator metabolism (Iverson et al., 2004). QFASA has been shown useful when comparing the diets of large predators (Iverson et al., 2006; Nordstrom et al., 2008) and for identifying differences in seabird feeding niches (Iverson et al., 2007). QFASA can provide a relatively long-term estimate of animal diets and attempts to identify the relative proportion of individual prey items in a diet (Iverson et al., 2004). This is a complex technique requiring a prey database and detailed knowledge of predator metabolism that usually require expensive captive feeding studies; for these reasons its application is not always feasible.

1.3. STABLE CARBON ISOTOPES

Like dietary FA, stable carbon isotopes vary in a fixed and predictable manner within a food web (Deniro and Epstein, 1978) and can be used to infer the environmental origin of biological specimens based on their $^{13}C/^{12}C$ ratio (Smith et al., 1996; Pond et al., 1998; Oczkowski et al., 2010). The ^{13}C isotope is naturally much less abundant, and the $^{13}C/^{12}C$ is normally expressed relative to the $^{13}C/^{12}C$ of Vienna Pee Dee Belemnite (VPDB), a standard material. In chemical and physical reactions, ^{12}C is incorporated into products at a faster rate than ^{13}C, which results in a fractionated $^{13}C/^{12}C$ relative to the standard. The $^{13}C/^{12}C$ is expressed as $\delta^{13}C$ with unit of parts per mill, or ‰. The degree of fractionation is often dependent on the organism's physiology and metabolism, and on environmental factors (Libes, 1992; Sulzman, 2007; Bowling et al., 2008). Fractionation, however, can remain relatively consistent within some groups of organisms such that differences in the C input (or diet composition) can be identified from their tissues. This concept is often referred to an isotope "signature" in animal ecology. For instance, photosynthesis results in kinetic fractionation that is associated with CO_2 uptake and its

subsequent C reaction pathways in the plant cell (O'Leary 1988). Atmospheric CO_2 has a $\delta^{13}C$ of -8‰, and carbon incorporated into plant tissues exhibits a net fractionation of 20‰ in C_3 plants during photosynthesis, resulting in a depleted $\delta^{13}C$ of -28‰. Similarly, the photosynthetic pathway that characterizes C_4 plants involves less fractionation (~5 ‰) and results in a $\delta^{13}C$ of -13‰ (O'Leary 1988; Farquhar et al., 1989; Marshall et al., 2007, Fry, 2008). CO_2 uptake as dissolved inorganic carbon (DIC) in the surface layer of the ocean involves little fractionation (~1‰); however, photosynthetic activity in algae results in a fractionation that results in $\delta^{13}C$ values that range between -19‰ and -24‰ (Peterson et al., 1987).

The $\delta^{13}C$ that result from various states in the C cycle can be used to estimate animal diets, and identify areas that an animal routinely occupies. The $\delta^{13}C$ of animal tissues are thought to reflect a mixture of $\delta^{13}C$ from all diet items, which creates opportunities to study food webs and migration patterns of wildlife (Hobson, 1999; Maruyama et al., 2001; Voight et al., 2008). Stable carbon isotopes have been used to identify the diet items in species ranging from zooplankton to marine mammals (Schell et al., 1998; Hobson et al., 1998; Crawford et al., 2008; Kurle, 2009; Mellbrand et al., 2010;). Past studies using carbon isotopes were mostly based on techniques that analyzed the $\delta^{13}C$ values from the protein and carbohydrate content of bulk tissue samples. Bulk analysis can be problematic when inferring an animal's diet, as C values will represent both endogenous and exogenous C sources (Gannes et al., 1997). The $\delta^{13}C$ of lipids, proteins and carbohydrates are not equivalent, and thus the products of biosynthesis will consequently differ from the dietary components. This discrepancy is an important problem associated with bulk analysis (Hobson, 1995). Standard analytical processes for

bulk analysis involve removing lipids from tissue, which may result in further deviations in $\delta^{13}C$ (Sweeting et al., 2006) and eliminate the opportunity to examine potential biomarkers. A tissue biopsy taken from larger animals may also carry biased information if dietary components (proteins and carbohydrates) are not equally deposited across tissues (Jim et al., 2003).

The ambiguity associated with the C sources of bulk tissue analysis can be overcome by the examination of $\delta^{13}C$ in specific biomarkers, referred to as compound-specific isotope analysis (CSIA), to determine environmental sources (Lichtfouse, 2000; Meier-Augenstein, 2002) and capitalize on diet information. CSIA of essential compounds removes uncertainty regarding the origin of C in the animal, as the fractionation that may be associated with *de novo* synthesis is eliminated. CSIA of FA and amino acids (AA) has been recently applied in several studies to examine the diet composition, foraging location, and food web interactions of both marine and terrestrial consumers (Howland et al. 2003; Pond et al., 1997, Hammer et al., 1998; Chamberlain et al. 2004; Ruess et al., 2005; Budge et al., 2008b).

1.4. IMPACT OF LIPID METABOLISM ON THE INTERPRETATION OF FA DATA

CSIA of FA is a relatively recent development and has created a new window of opportunity in wildlife ecology. However, dietary FA go through a number of enzyme catalyzed biochemical processes in the consumer and the associated alternations to the $\delta^{13}C$ are currently unknown. The FA with lighter C isotopes may be preferentially incorporated during enzymatic reactions by a kinetic isotope effect, which can fractionate the ^{13}C of FA even in instances where the chemical structures of FA are not changed. For instance, FA are activated by an acyl CoA enzyme prior to entry into the mitochondria or

peroxisomes to complete oxidation. Acyl CoA may be more likely to selectively activate a FA that has a low $^{13}C/^{12}C$ if a pool of FA is available. The $\delta^{13}C$ of the consumer is assumed to be equivalent to the weighted proportions of the $\delta^{13}C$ of all dietary components (Gannes et al., 1997; Hobson, 1999; Budge et al., 2008b), but fractionation associated with biochemical reactions of FA can complicate interpretation of diet from FA $\delta^{13}C$ data. These assumptions are made in order to continue on with the current research possibilities that CSIA provides. Experimental data, however, is required to accurately draw conclusions on diet composition based on the $\delta^{13}C$ of FA in animal tissues. Individual FA were recently shown to independently vary in their degree of isotopic fractionation from diet to tissue (Budge et al., 2011 in review). AA were also shown to vary individually, and to a greater extent for non-essential AA (McMahon et al., 2010). Essential compounds are likely to provide a far more accurate representation of diet origin due to the absence of modification; however, fractionation can still potentially occur in these compounds during catabolism.

1.5. OBJECTIVES

The overall goal of my research was to provide some insight into the current ability to estimate diet based on the FA composition of tissue samples. More specifically, my research objectives were to determine variation in FA profiles and the associated variation in $\delta^{13}C$ of individual dietary FA that can be attributed to fish metabolism. This study began by focusing on multivariate FA analysis of fish tissues to determine how FA proportions change with diet. Second, the $\delta^{13}C$ of individual FA in diet and tissue were examined to determine the extent to which stable carbon isotopes fractionate as a result of lipid metabolism in Atlantic pollock (*Pollachius virens*), a lean fish. CSIA may become

widely applied in ecological research alongside FA analysis to acquire diet information (Gannes et al., 1997; Evershed et al., 2007; Budge et al., 2008b); however, the challenges with predator metabolism that present themselves in FA research must equally be addressed in the $\delta^{13}C$ of individual FA.

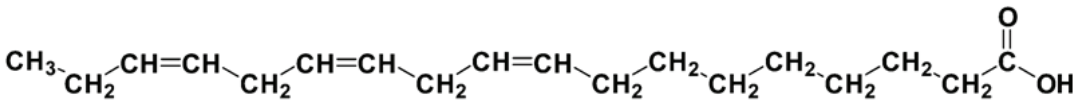

Figure 1.1 Fatty acid structure (18:3n-3).

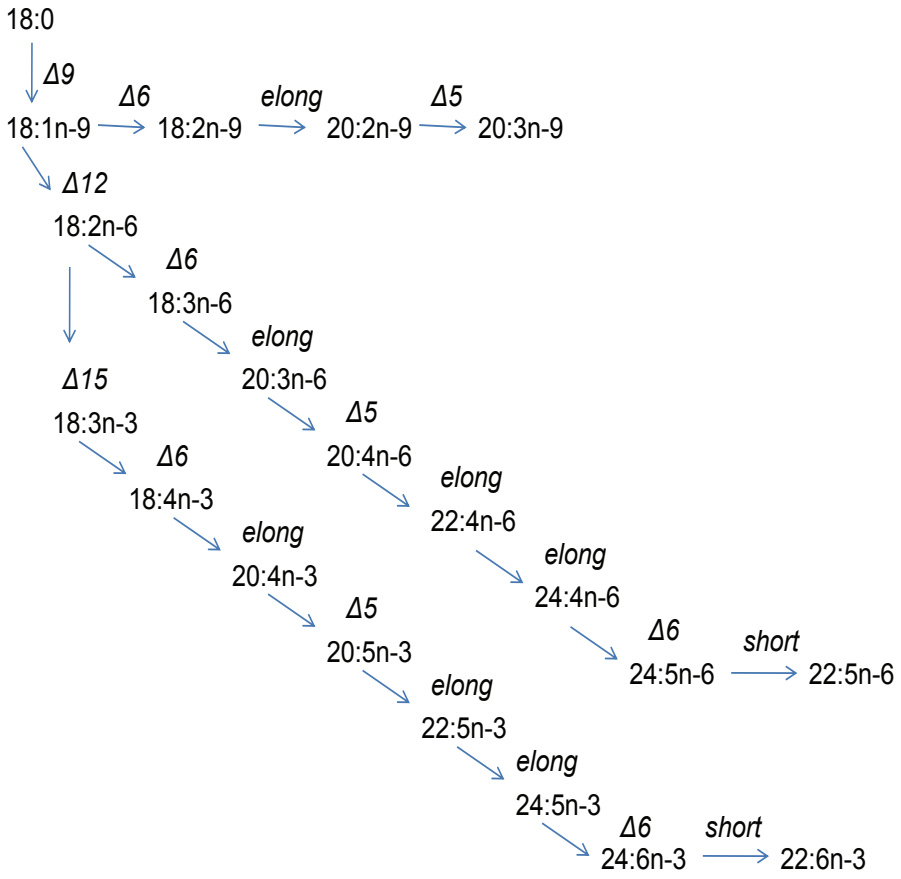

Figure 1.2. Fatty acid metabolic pathway showing the synthesis of C_{20} and C_{22} fatty acids from C_{18} precursors, and demonstrating the need for dietary input of fatty acids in animals. The Δ6-desaturase activity is insufficient in animals, and Δ12- and Δ15-desaturase are only present in plants. Figure adapted from Tocher, 2003.

CHAPTER 2.0 FATTY ACID PROFILES OF ATLANTIC POLLOCK (*POLLACHIUS VIRENS*) TISSUES

2.1. INTRODUCTION

2.1.1. FATTY ACIDS

FA in the form of lipids are ideal for long-term energy storage in animal tissue, as they are lighter in weight than carbohydrate or protein and more efficient in energy production. For instance, 1 mole of stearic acid, 18:0, yields 122 moles of (adenosine triphosphate) ATP, whereas only 38 moles of ATP are produced per 1 mole of glucose (Campbell et al., 2009). For this reason, animals store lipid to fulfill metabolic needs during fasting, and lipid composition can reveal information on an animal's physiology and environment.

2.1.2. LIPID TRANSPORT

Marine fish receive the majority of their dietary lipids in the form of triacylglycerols (TAG), wax esters (WE) and phospholipids (PL). TAG consist of three FA esterified to a glycerol backbone, and are the main source of lipid in commercial fish feeds (Sargent et al., 1993; Tocher, 2003). TAG molecules become hydrolyzed by pancreatic enzymes in the small intestine during digestion, where the TAG molecules are cleaved at the *sn-1* and *sn-3* positions to produce a pool of free fatty acids (FFA) and 2-monoacylglycerols (MAG) (Tocher, 2003). WE are similarly hydrolyzed in the intestine to yield FFA and fatty alcohols. Dietary PL are cleaved by lipolytic enzymes in the pancreas at the *sn-2* position on the molecule to form a pool of FFA and lysoglycerophospholipids (LPL; Sargent et al., 1993). Dietary FA that result from hydrolyzed TAG, WE and PL, as well as 2-MAG and LPL, are transported across the cell membranes in the intestinal mucosa,

and are re-esterified to form TAG and PL in the endoplasmic reticulum (ER). TAG and PL then enter a pool of both endogenous and dietary lipids in the ER (Mansbach et al., 1998; Tocher, 2003). Dietary TAG are shown to be preferentially transferred from the ER to the Golgi apparatus for export to the lymph in the form of chylomicrons (Mansbach et al., 1998, Shen et al., 2001). Although the phospholipid content is much lower than TAG in the chylomicrons, pooled FFA that originate from dietary PL can be esterified to TAG following the absorption in the intestinal cells (Karmen et al., 1963; Whyte et al., 1963).

The lipid composition of chylomicrons in teleost fish are similar to other vertebrate chylomicrons and mainly consist of a TAG core contained within a phospholipid (PL) monolayer (Chapman, 1985). The TAG in chylomicrons have been shown to consist almost exclusively of dietary FA, while the PL monolayer is mainly formed from endogenous sources (Mansbach et al., 1982). Mechanisms of lipid deposition and mobilization in fish are thought to follow similar pathways to mammals (Tocher 2003). Mammalian chylomicrons are delivered to lipoprotein receptors on capillaries where they are hydrolyzed by lipoprotein lipase to FFA and taken up by adipose cells, re-esterified into TAG and stored in the tissue (Figure 2.1). Fish are thought to have similar lipid transport pathways, and only differ in their storage sites. Lean marine fish, such as cod (*Gadus morhua*) and pollock (*Pollachius virens*) store their excess lipid in the liver, whereas oily fish such as Atlantic salmon (*Salmo salar*) store the majority of their lipid in the muscle and belly flap tissues.

After ingestion of a meal, chylomicrons provide TAG necessary for immediate energy catabolism and ATP synthesis needs; during periods of food deprivation, TAG can also be mobilized from energy reserves in order to maintain homeostasis. In mammals, TAG

in adipose cells are hydrolyzed by lipolytic enzymes to generate FFA for transport in the blood to other tissues for oxidation to generate energy and for the synthesis of essential compounds, such as eicosanoids (Plisetskaya, 1980). As a result, blood FFA levels are typically elevated during fasting in many animals (Karpe et al., 1998; Zimmerman et al., 2004). This system has also been noted in several species of teleost fish such as rainbow trout (*Onchorynus mykiss*) and European eel (*Anguilla anguilla*) (Black et al., 1986a; Larsson et al., 1973; Kjer et al., 2009); however, cod have shown stable FFA levels despite being subjected to periods of fasting, which is likely related to their fatty livers providing the majority of their energy supply (Black et al., 1986b; Alkanani et al., 2005; Kjer et al., 2009). Instead, TAG molecules in the form of very-low density lipoproteins (VLDL) are mobilized from the liver of gadoids and other lean marine fish rather than FFA. VLDL are then transported from the liver into the blood to the peripheral tissues (Tocher, 2003; Gruffat et al., 1996).

2.1.3. TISSUE SAMPLES FOR FATTY ACID ANALYSIS

The FA proportions identified in a lipid sample taken from animal tissue, often referred to as the "FA profile," are dynamic and change according to an animal's prandial state. FA serve individual purposes, and some FA may be retained for a longer timeframe in the storage tissues, or catabolized quickly upon assimilation. FA analysis relies on the FA composition of animal tissues for the advantageous reason that they can provide dietary information over a long timeframe; however, selective catabolism and modification to meet FA requirements of the body result in FA profiles that are not identical to the diet. Dietary FA may also be desaturated and elongated in the storage tissue, which can further change the proportions of FA in tissues as compared to the FA

composition of diet. Storage tissues, such as the liver of lean fish, should give a more accurate representation of diet than muscle or brain tissue which have specific functions and a more refractory FA profile (Yehuda, 2003); however, this is dependent on the extent of fasting and FA mobilization in animal tissues. It may be possible to obtain samples of dietary FA prior to their modification and oxidation in the animal tissues by analyzing the chylomicron TAG of an animal. Chylomicron TAG-FA have been demonstrated by QFASA to provide an accurate representation of diet FA profiles of marine mammals when metabolism is taken into account (Cooper et al., 2005), and for this reason, chylomicrons may provide a reliable and convenient method or means to study an animal's tissues in a non-lethal manner. The majority of biochemical changes to FA are thought to occur after the chylomicron TAG has been deposited in the tissues, where FA are hydrolyzed and re-esterified. These biochemical demands will affect the FA proportions in the stored lipid (Sargent et al., 1989). With every hydrolysis or re-esterification reaction, FA that comprise the lipid composition in the tissues will have the opportunity to change and ultimately vary the FA proportions with respect to the diet FA profile. Only one study to date investigated chylomicron FA as a method of estimating the diets of pinnipeds (Cooper et al., 2005), and the extent to which chylomicron FA can be used for estimating the diets of marine fish is currently unknown.

The analysis of VLDL in fasted animal serum could also serve as a tool for analyzing dietary FA in animals. Although the lipid in the VLDL is likely to have experienced some degree of modification from the dietary FA profile, the FA in transport have yet to become oxidized at peripheral tissues and may therefore also serve as a reliable window of the FA profile of the tissue from which it was mobilized and therefore, provide long-

term dietary information. In conjunction with the analysis of chylomicron FA, the VLDL from animal serum may serve as an alternative to collecting lethal tissue biopsiesprovide an opportunity to study dietary FA when post-prandial chylomicrons are not available. In order to confirm the reliability of blood lipids for estimating diet composition, their FA profiles must be examined and compared to the FA profiles of tissue samples to determine if individual FA are mobilized in equal proportions. FA profiles of blood lipids must also be compared to the diet FA to determine their accuracy. Plasma FA signatures from captive herring gulls were recently examined to explore the idea of using blood samples in diet estimations, but results indicated little concordance with diet FA (Käkelä et al., 2009). However, the FA chosen for analysis of the samples may not have been necessarily indicative of diet, and the application of biomarkers to this sampling method may still be promising.

2.1.4. OBJECTIVES

FA proportions were examined across post-prandial chylomicrons, liver TAG and muscle PL, along with the fasted serum TAG from VLDL, liver TAG and muscle PL; these were compared to the original diet FA profiles to observe the degree to which FA proportions change as a result of animal metabolism. By comparing the FA profiles of routinely sampled fish tissues under different feeding conditions, I determined the similarity of each tissue FA profile with its original diet, and identified the more suitable tissue to analyze for diet information. Chylomicrons and VLDL from blood samples could provide information on a fish's current diet and stored lipid; however, these sampling methods are not routinely used in fish due to a lack of experimental evidence regarding their ability to reflect dietary FA profiles. Feeding experiments were conducted

using captive wild pollock *(Pollachius virens)*, a lean marine fish that stores the majority of its lipid in the liver (Nanton et al., 2001). Feeding trials were followed by fasting trials, to determine the variance that may arise from lipid mobilization during periods where food is scarce in the wild. I wanted to investigate specifically the variation in tissue FA composition relative to diet for the following processes; 1) dietary lipid absorption via chylomicrons, 2) FA deposition in the liver, 3) FA mobilization from the liver in VLDL particles, 4) and the FA variation of the muscle tissue in both fed and fasted states. By examining the FA profiles that correspond to these processes, I aim to determine the effects of lipid metabolism on FA profiles in several different tissue types.

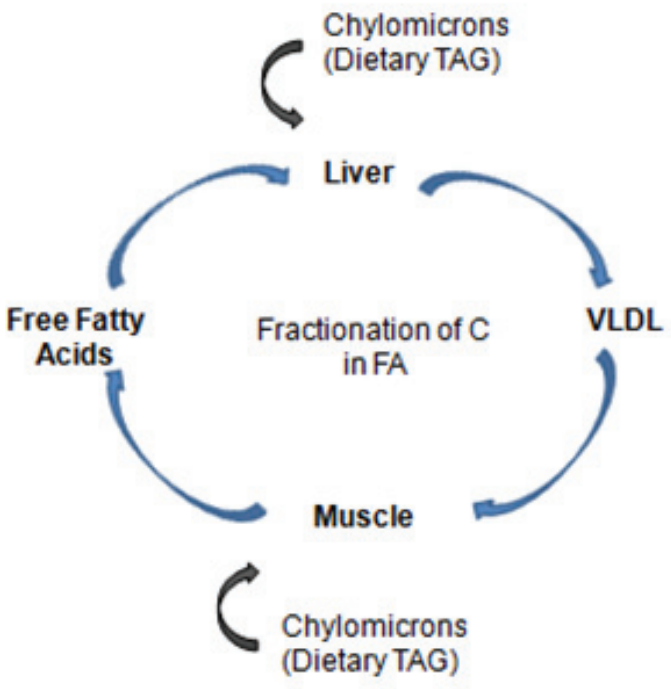

Figure 2.1. Diagram of major steps in lipid metabolism of lean marine fish which may result in the fractionation of C in fatty acids; steps where fractionation may occur include: the mobilization of fatty acids from the muscle (in free fatty acid form) and liver (in the form of very low density lipoproteins) into the bloodstream; as well as the assimilation of fatty acids (in the form of chylomicrons) into the muscle and liver tissue.

2.2 MATERIALS AND METHODS

2.2.1. EXPERIMENTAL FISH

Wild Atlantic pollock were caught near Duncan's Cove, Nova Scotia on July 15th 2009 using a commercial long line baited with mackerel. Adult pollock were kept in a 1,000 L aerated holding tank aboard the vessel and transferred to the Aquatron Facility at Dalhousie University, Halifax, Nova Scotia. Pollock (n=64) were kept in a 684,000 L aerated pool tank with flow-through seawater at ambient temperature from the Northwest Arm of Halifax Harbour. From July-December the temperature of the tank varied according to natural water temperatures of the inlet (6-13˚C), and the water was heated to 8˚C upon entry into the facility from January to May. The fish were anaesthetized (by immersion, MS220, 60 mg/L), weighed, measured and tagged using a Passive Integrated Transponder (PIT) tag near the dorsal fin and separated into two groups (n=24 in group 1, n=42 in group 2) by large net pens (4 m depth and 2.5 m diameter) suspended in the pool tank for the duration of the experiment.

2.2.2. EXPERIMENTAL DESIGN

All the pollock (Group 1 and 2, n=64 in total) were maintained on a marine oil-based diet consisting of herring protein and a mixture of herring and anchovy fish oil (FO diet, 18% lipid wet weight; 18:2n-6: -26.4 ± 0.1‰, 18:3n-3: -30.8 ± 0.1‰, 20:5n-3: -29.6 ± 0.1‰, 22:6n-3: -28.1 ± 0.2‰) and were fed to satiation once daily for a period of 15 weeks prior to sampling, a time that was estimated to achieve turnover of the initial lipid in the tissues of haddock (*Melanogrammus aeglefinus*) (Penney 2010, unpublished results). Twelve fish (n=12, Group 1) were sampled following the 15 week feeding period for post-prandial blood chylomicrons, muscle, and liver tissue on December 14th,

2009. Post prandial tissue collection occurred 12 h after a meal, a time which was previously determined to be an ideal sampling time to obtain blood chylomicrons in fish (see below). Twelve (n=12, Group 1) of the remaining fish were then fasted for 3 weeks and sampled on January 9th, 2010. The remaining fish (n=42, Group 2) were switched to a feed formulated using canola oil (CO, 12% lipid wet weight; 18:2n-6: -32.2 ± 0.1‰, 18:3n-3: -36.3 ± 0.2‰, 20:5n-3: -27.5 ± 0.1‰, 22:6n-3: -29.6 ± 0.2‰) for 12 weeks. Twenty-one (n=21, Group 2) fish were sampled following the 12-week CO diet feeding period on May 3rd, 2010, while the remaining 21 fish (Group 2) were fasted for 3 weeks prior to sampling on May 24th, 2010.

2.2.3. EXPERIMENTAL DIETS

The CO diet was formulated and produced at the Marine Research Station, National Research Council, Institute for Marine Biosciences (Table 2.1.). The diet was prepared according to the information available on the nutritional requirements of adult gadoids (Lall et al., 2003) and contained 12% lipid on a wet weight basis. Pollock nutritional requirements were assumed to be similar to other adult gadoid fish, such as cod and haddock, because of their similar physiology and body composition (Scott et al., 1988). Soybean and corn gluten meal were finely ground and mixed with herring meal, vitamin and mineral premixes, and krill hydrozylate using a Hobart mixer (model H600T, Rapids Machinery Co., Troy, OH, USA) for 30 min. Canola oil was added for an additional 10 min of mixing. The diet mixture was steam pelleted (California Pellet Mill Co., San Francisco, CA, USA) into 5.5 mm pellets and baked for 50 min at 80˚C.

The FO diet was obtained commercially, and was composed of the following ingredients (in order of % contribution): fish meal (herring and anchovy), corn gluten

meal, whole wheat, poultry by-product meal, fish oil (herring and anchovy), soybean meal, poultry fat, salt premix, brewer's yeast, vitamin/mineral premix, astaxanthin, vitamin C, choline chloride, vitamin E (Corey Aquabrood, Corey Feed Mills, Fredericton, NB, Canada). The lipid content was approximately 18% of the wet weight. Both experimental diets were stored at -20˚C throughout the study prior to feeding.

Table 2.1 Formulation of the canola oil experimental diet[1].

Ingredient	**Percentage**
Herring meal[a]	36.5
Soybean meal[b]	12
Wheat middlings[c]	21.7
CPSP-G[d]	3.5
Corn gluten meal[b]	14
Krill Meal[e]	2
Choline chloride[g]	0.3
Canola oil[h]	8
Vitamin premix[i]	1
Mineral premix[j]	1
TOTAL	100

[a] Corey Feed Mills (Fredericton, NB, Canada)

[b]Northeast Nutrition (Truro, NS, Canada)

[c] Dover Mills (Halifax, NS, Canada)

[d]Fish protein hydrolysate, Sopropêche, France.
[e]Aqion (Colorado Springs, CO, USA)

[g]USB Corporation (Cleveland, OH, USA)

[h]Bioriginal Food and Science Corp., Saskatoon, Saskatchewan.
[a]USB Corporation (Cleveland, OH, USA)

[j] Vitamin mix (IU per kg); Vitamin A, 8000 IU; Vitamin D_3, 4500 IU; Vitamin E 300 IU, Vitamin K_3, 40 mg kg^{-1};

Thiamin 50 mg kg^{-1}, Riboflavin, 70 mg kg^{-1}, Pantothenate 200 mg kg^{-1}, Biotin, 1.5 mg kg^{-1}, Folic acid, 20 mg kg^{-1};

Vitamin B_{12} 0.15 mg kg^{-1}; Niacin, 300 mg kg^{-1}; Pyridoxine, 20 mg kg^{-1}; Ascorbic acid, 300 mg kg^{-1}; Inositol, 400 mg kg^{-1}; Butylated hydroxy toluene, 15 mg kg^{-1}; Butylated hydroxy anisole, 15 mg kg^{-1}

[k]Mineral mix (per kg); Manganous sulfate, 40 mg kg^{-1} ; ferrous sulfate, 30 mg kg^{-1}; copper sulfate, mg kg^{-1}, zinc sulfate, 75 mg kg^{-1}, sodium selenite 1 mg kg^{-1}, cobalt chloride 2.5 mg kg^{-1}, sodium fluoride 4 mg kg^{-1}

[l]Estimated protein and lipid content of the diet after extraction was 48 and 12.8 % respectively

2.2.4. SAMPLING

Pollock were anaesthetized (MS220, 60 mg/L) for blood collection, and were euthanized (MS220, 150 mg/L) immediately afterwards. Blood (5 ml) was retrieved by severing the caudal vein and fresh blood was transferred to a 7 ml BD vacutainer without anticoagulant. The more commonly used method of obtaining blood by needle and syringe was avoided to prevent the capillary pressure of the needle from damaging the chylomicrons (Mills et al., 1984). Fresh blood was chilled and centrifuged at 1000 RPM for 20 min to separate the serum within 2 h of blood retrieval. Whole liver and muscle tissue were homogenized in a food processor, and all tissues were kept in a 2:1 chloroform: methanol ($CHCl_3$: MeOH) solution with 0.01% butylated hydroxytoluene (BHT) and stored at -20˚C prior to lipid extraction.

2.2.5. CHYLOMICRON PILOT EXPERIMENT

Chylomicrons were shown to appear in marine mammal blood samples after ~ 1 h following a meal, and reach a maximum in the bloodstream 6- 8 h following a meal (Cooper et al., 2005). It has been suggested that chylomicrons peak at a similar time in the blood of sea bream, while their clearance rate is slower (Arnold-Reed et al., 1996). After a number of unsuccessful attempts to retrieve chylomicrons from post-prandium salmon blood at 8 h, I postulate that chylomicrons will peak in the bloodstream of fish at a much slower rate than warm blooded animals (Cooper et al., 2005). I conducted a pilot experiment on pollock to determine the time at which chylomicrons can be obtained from the blood following a meal. To accomplish this task, I varied the time at which fish were sampled and compared the masses of chylomicron TAG retrieved. Three pollock (n=3) were anaesthetized and sampled for each of 6 h, 12 h, 18 h and 24 h post-prandium

intervals and blood was transferred to a 7ml BD vacutainer for serum and subsequent chylomicron isolation. Sampling intervals were separated by 2 d in order to avoid any interference from incomplete digestion of the meals in the fish. The chylomicron TAG masses were recorded to determine the optimal time for obtaining a chylomicron peak in the bloodstream following a meal. The presence of the digesta and its distance travelled in the gut was also noted.

2.2.6. CHYLOMICRON ISOLATION

Serum was carefully overlain with 0.169M sodium chloride with 0.01% ethylenediaminetetraacetic acid (EDTA), maintaining a 1:1 volume of salt:serum. Chylomicrons were separated using a Beckman L-2 ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA, USA) and an SW 50L rotor (Beckman Instruments Inc., Palo Alto, CA, USA) at 18,000 rpm at 15˚C for 30 min. Mammalian chylomicrons have been reported to range from 35-250 nm in diameter (Davis, 1991), and fish chylomicrons are thought to have a corresponding size range (Tocher et al., 2003); therefore, a separation density of 1.006 g/ml that is typically used to separate mammalian chylomicrons in the serum was employed, isolating chylomicrons that are roughly 100 nm in size. VLDL have a typical diameter range of 30-110 nm, therefore the density target range minimized VLDL contamination (Mills et al., 1984). Chylomicrons appeared as a white cloudy layer in the top portion of the NaCl solution, and were carefully extracted using a 1.5 ml Samco fine-tip polyethylene transfer pipet. The chylomicrons were transferred to a 10 ml heavy duty glass test tube and stored in 2:1 $CHCl_3$:MeOH with 0.01% BHT at -20˚C until lipid extraction.

2.2.7. LIPID EXTRACTION

Total lipid was extracted from all the diets, tissues and chylomicron samples by a modified Folch et al. (1957) procedure (Budge et al., 2006) using 2:1 chloroform: methanol solution. Lipid extracts were washed with a salt solution (0.088% NaCl for tissues, 0.7% NaCl for chylomicrons and diet) and dehydrated with anhydrous sodium sulfate (Na_2SO_4) before the solvent was evaporated under a nitrogen bath. Thin-layer chromatography (TLC) used to separate the lipid classes of liver, muscle and chylomicrons, thus partitioning the lipid samples into TAG and PL. Lipid samples were distributed onto active silica plates and placed in a developing tank containing a mixture of 85:15:1 hexane: diethyl ether: glacial acetic acid to separate the lipids based on polarity. TAG was retrieved from liver and chylomicrons and PL was retrieved from muscle. Lipids were extracted from the silica particles using a 1:1 hexane:chloroform mixture for neutral lipids, or a 2:1 methanol:chloroform mixture for PL. The lipid of diet samples was not partitioned into lipid classes during its extraction.

2.2.8. FATTY ACID ANALYSIS

Lipids from the diets and the lipid classes of interest in the tissue samples were transesterified to fatty acid methyl esters (FAME) using methanol and H_2SO_4 as an acid catalyst. FAME were analyzed using a Perkin Elmer Autosystem II capillary gas chromatograph (GC) with a flame ionization defector (FID) and a polar column (30 m x 0.25 mm ID Agilent Technologies, DB-23; Palo Alto, CA, USA). Helium was used as the carrier gas, and FAME were analyzed with splitless injection mode at a concentration of 0.5mg/ml hexane. Samples were analyzed in duplicate and 60 FA structures were identified. FAME chromatograms were edited using Star Chromatography Workstation

software (Agilent Technologies, Santa Clara, CA, USA) and menhaden FAME was used as a standard for purposes of identification.

2.2.9. STATISTICAL ANALYSIS

FA proportions were normalized to 100% and transformed using a *log (x+1)* function. Bray-Curtis similarity matrices were generated for the entire data set using PRIMER-E (Primer-E Ltd, Plymouth Marine Laboratory, UK), which rank the data based on similarity in the FA proportions of groups. Analyses of similarity (ANOSIM) were used to determine whether the variance between multivariate groups was higher than the variance within groups (Clark et al., 1994). ANOSIM reports a global R value, which has a range of 0 to 1, to determine the amount of variance across groups. R-values near 1 indicate that the variability among all groups can be discriminated from the variability within groups. ANOSIM generates also a p- value, which indicates the level of confidence in the R-value ($\alpha = 0.05$), thus a low R-value (ex., $R = 0.45$) can still be significant (in other words, the R-value that is reported is unlikely to occur by chance). Because the global R-value only provides indication that variability exists, it does not specify which groups contribute to the most substantial variability in the data. To solve for this ambiguity, pairwise ANOSIM tests are used to generate several test statistics for each group to determine whether a particular tissue comparison is responsible for the variation. The transformed data was also used to calculate the similarity percentages (SIMPER) of FA using PRIMER-E, which rank the FA that are most likely responsible for the highest dissimilarity within the data.

2.3. FATTY ACID RESULTS

2.3.1. GAS CHROMATOGRAPHY

FA were successfully extracted from 90 tissue samples, 6 FO diet and 5 CO diet samples, and were identified in all samples from their FAME analogs by gas chromatography. The FA that were consistently present in amounts greater than 0.1% were reported as weight percent of total FA (Appendix I). FAME of TAG samples generally contained higher proportions of saturated and monounsaturated FA, such as 14:0, 16:0, 16:1n-7, 18:1n-9 and 18:2n-6, while PL samples contained relatively higher proportions of long-chain polyunsaturated FA such as 20:4n-6, 20:5n-3 and 22:6n-3.

2.3.2. EXPERIMENTAL DIETS

Abundant FA in the diets included 16:0 (18% in FO, 8% in CO), 18:1n-9 (17% in FO, 38% in CO), 18:2n-6 (9% in FO, 19% in CO), 20:5n-3 (11% in FO, 2% in CO), and 22:6n-3 (5% in FO, 3% in CO). Only a small amount of 18:3n-3 was identified in the FO diet (less than 1%) while higher concentrations were found in the CO diet (5%).

The proportions of SFA, MUFA, and PUFA differed significantly between FO and CO diets (Figure 2.2; ANOSIM: $R=1$, $p< 0.008$); the proportion of MUFA was higher in the CO diet for all FA that were C_{18} or longer, whereas only C_{16} MUFA were found at higher proportions in the FO diet. C_{14}, C_{16} and C_{18} SFA, along with all major PUFA, were more abundant in the FO diet than the CO diet. The levels of 20:5n-3 were higher than 22:6n-3 in the FO diet, and the reverse was true in the CO diet. SIMPER revealed that the FA most likely driving the difference in dietary lipid were 18:1n-9, 18:2n-6, 16:0, 18:3n-3, 22:1n-11, 20:1n-9, 22:6n-3, 18:1n-7, 20:5n-3, and 18:0, in order of decreasing contribution.

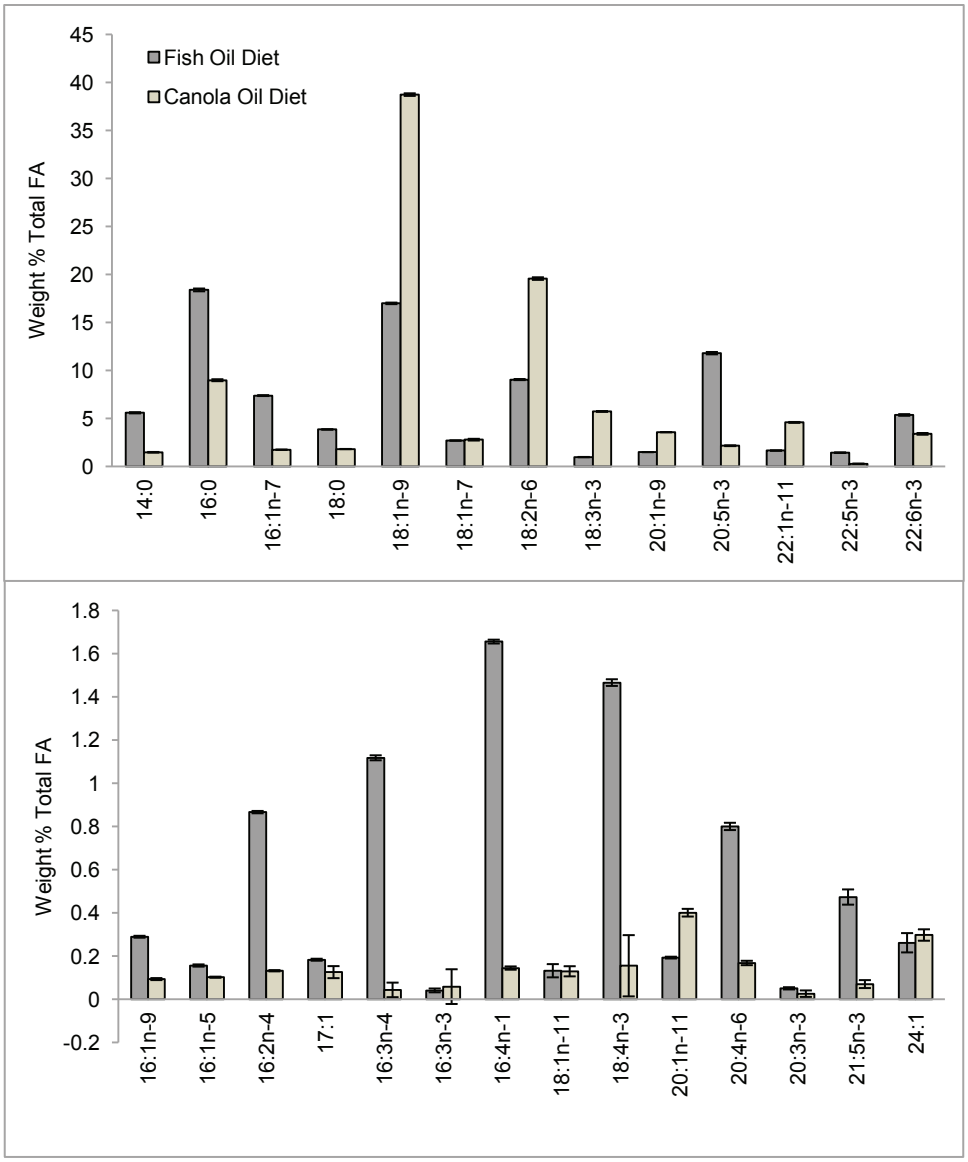

Figure 2.2. Mean proportions and standard deviation of weight % fatty acid from fish oil (n=5) and canola oil (n=5) diets. Fatty acids chosen for display were those that were identified as most important in differentiating between fish oil and canola oil diets using similarity percentages.

2.3.3. DIET AND POST-PRANDIAL TISSUE COMPARISONS

2.3.3.1. FISH OIL EXPERIMENT

The FA profiles of post-prandial liver and muscle of pollock fed the FO diet were compared to their diet to determine the extent of FA modification upon deposition in tissues. The liver TAG FA proportions resembled the diet to a greater extent than muscle PL. Higher C_{18}, C_{20} and C_{22} MUFA proportions in the liver TAG of FO-fed fish relative to the FO diet. PUFA, particularly 20:5n-3 and 22:6n-3, were substantially higher in the FO post-prandial muscle PL relative to the FO diet (% 22:6n-3: muscle= 27.8 ± 0.4, and diet= 5.4 ± .01), while only subtle differences existed between the 22:6n-3 of FO post-prandial liver TAG relative to its diet (% 22:6n-3: liver= 4.4 ± 0.1). The FO diet had a higher proportion of 20:5n-3 than the liver TAG (% 20:5n-3: liver= 6.2 ± 0.2, diet= 11.8 ± 0.02). Significant differences were found for the comparison of FA in the FO diet and the muscle and liver tissues (Figure 2.3; ANOSIM: $R= 1$, $p<0.001$), and pairwise tests indicated significant differences existed between all pairwise comparisons ($R= 1$ for all). SIMPER results are presented in Table 2.2. Blood samples were obtained for FO-fed post prandial pollock; however I was unsuccessful at recovering chylomicrons in sufficient amounts for FAME analysis from those fish.

Table 2.2. Similarity percentages for the fish oil diet, post-prandial liver triacylglycerols and muscle phospholipids of fish following a 10-week feeding trial.

Diet & Post-prandial Liver TAG (R=1, p<0.002)		Diet & Post-prandial Muscle PL (R=1, p<0.001)		Post-prandial Liver TAG & Post-prandial Muscle PL (R=1, p<0.002)	
FA	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**
14:0	7.62	22:6n-3	10.20	22:6n-3	11.11
16:4n-1	7.01	14:0	9.60	22:1n-11	7.95
20:5n-3	6.42	16:1n-7	8.92	16:1n-7	7.49
20:1n-9	5.75	22:1n-11	5.91	18:1n-9	6.69
18:1n-9	5.23	16:3n-4	4.88	20:1n-9	6.06
20:1n-11	4.51	20:4n-6	4.78	20:4n-6	5.92
16:3n-4	4.49	18:4n-3	4.68	20:5n-3	5.73
22:1n-11	3.54	16:4n-1	4.55	14:0	4.76
18:4n-3	3.46	18:1n-9	3.53	18:1n-7	3.01
18:1n-7	3.22	16:2n-4	3.46	18:2n-6	2.79

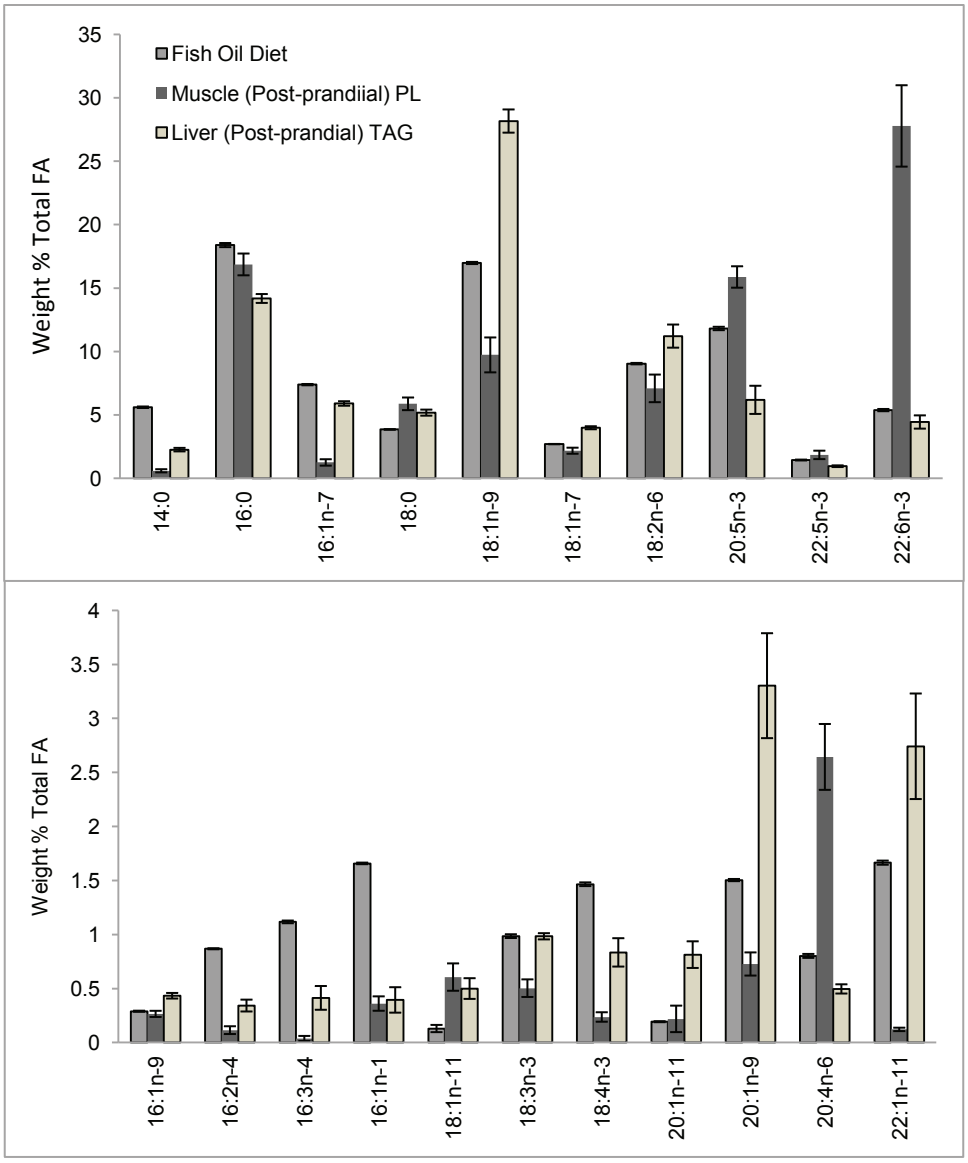

Figure 2.3. Mean proportions and standard deviation of fatty acids that were responsible for the highest variance between the fish oil diet (n=5), and post-prandial pollock liver triacylglycerols (n=6) and muscle phospholipids (n=7) after a 10 week feeding period. Fatty acids that contributed to the most variance in similarity percentages were chosen for display.

2.3.3.2. CANOLA OIL EXPERIMENT

The CO diet had relatively higher proportions of 18:2n-6 and 18:3n-3 than the liver and muscle, and the proportion of polyunsaturated FA in the liver of CO-fed fish was relatively similar to the CO diet proportions; however, the proportions were again greater than the muscle tissue (for instance, %18:2n-6: diet= 19.6 ± 0.03, liver= 13.7 ± 0.1, muscle= 10 ± 0.01). Similar to the FO experiment, significant differences were also found for the post-prandial tissues of CO-fed fish and their diet, (Figure 2.4; ANOSIM: $R= 1$, $p<0.001$) and pairwise tests confirmed that significant variance was present in all comparisons ($R= 1$ for all). The FA that were estimated by SIMPER to contribute to the variance found are listed in Table 2.3.

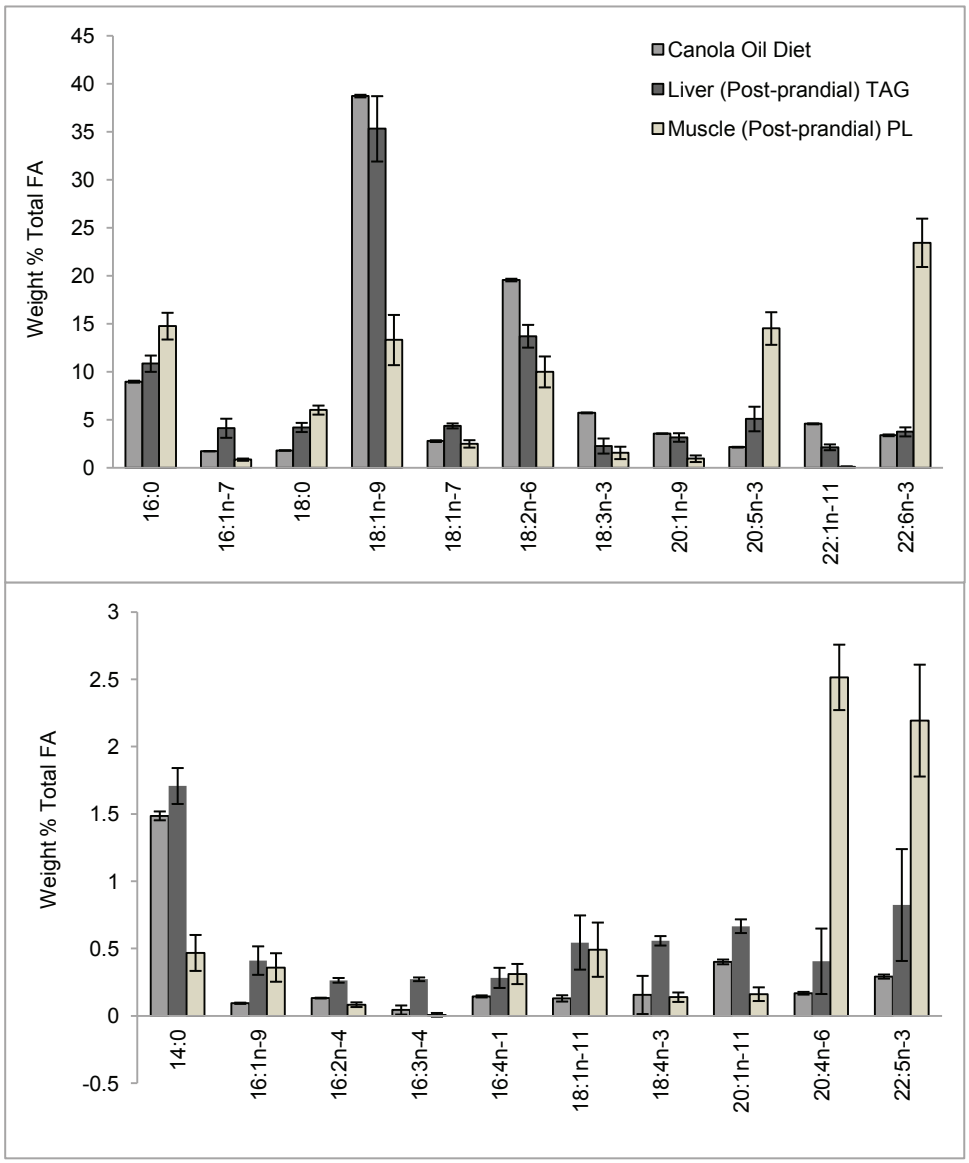

Figure 2.4. Mean fatty acid proportions and standard deviation of the canola oil diet (n=5), and post-prandial liver triacylglycerols (n=9) and muscle phospholipid (n=11). Fatty acids that were considered most important from the similarity percentage calculations were chosen for display.

Table 2.3. Similarity percentages of the post-prandial tissue fatty acids of fish that were fed the canola oil diet.

Diet & Post-prandial Liver TAG (R= 0.965, p<0.002)		Diet & Post-prandial Muscle PL (1, p<0.001)		Post-prandial Liver TAG & Muscle PL (1, p<0.002)	
FA	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**
18:3n-3	7.97	22:6n-3	9.60	22:6n-3	11.19
20:5n-3	6.96	22:1n-11	8.91	16:1n-7	7.29
16:1n-7	6.63	20:5n-3	8.90	22:1n-11	7.09
18:0	6.31	20:4n-6	6.11	18:1n-9	6.56
22:1n-11	5.76	18:1n-9	5.95	20:5n-3	6.24
22:5n-3	3.68	18:3n-3	5.76	20:4n-6	6.15
18:1n-7	3.61	22:5n-3	5.15	20:1n-9	5.49
18:2n-6	3.55	18:0	5.07	14:0	4.52
18:4n-3	3.28	20:1n-9	4.94	22:5n-3	3.85
18:1n-11	3.07	18:2n-6	3.66	18:1n-7	3.15
20:0	2.69	14:0	3.05	20:1n-11	2.62

2.3.4 CHYLOMICRONS

2.3.4.1. CHYLOMICRON PILOT EXPERIMENT

Chylomicron TAG samples were obtained at 4 different time intervals post-prandial (Table 2.4), and it appeared that pollock blood chylomicrons reached a maximum in the serum at approximately 18 h post-prandial, where the chylomicron TAG concentration was an average of 1.88 mg/ml in the serum (Table 2.4). The digesta remained mostly in the gut with a small amount in the small intestine at 6 hours post-prandial, while the digesta present in pollock sampled at 12 h and 18 h post-prandial was concentrated in the upper small intestine. The pollock sampled at 24 h post-prandial showed little presence of digesta in the stomach, and more presence throughout the entire length of the intestine.

Table 2.4. Chylomicron triacylglycerol masses obtained at 6 h, 12 h, 18 h, and 24 h post-prandial.

TAG mass (mg)	6 h	12 h	18 h	24 h
Replicate 1	0.79	0.23	4	1.32
Replicate 2	1.39	0.35	1.3	0
Replicate 3	0	0	0.34	0.79
Average	0.73±0.69	0.19±0.18	1.88±1.90	0.71±0.66

2.3.4.2.CHYLOMICRON-DIET COMPARISON

Chylomicron TAG obtained from the fish fed the CO diet had FA proportions that were relatively similar to CO diet FA (for instance, % 20:5n-3 diet= 3.4 ± 0.01, chylomicrons= 2.69 ± 0.06), and the major differences that existed appeared to be due to a few individual FA proportions (i.e., 18:2n-6, 18:3n-3, 20:1n-11; Figure 2.5) rather than the entire FA profile. However, the variance between chylomicron FA and diet was statistically significant (ANOSIM: $R= 0.912$, $p< 0.002$), and the FA that were calculated to contribute to the most variance were 18:0, 18:3n-3, 18:1n-11, 18:2n-6, 22:6n-3, 20:1n-7, 20:1n-11, 18:4n-3, 22:5n-3, 20:2n-6, in order of decreasing contribution.

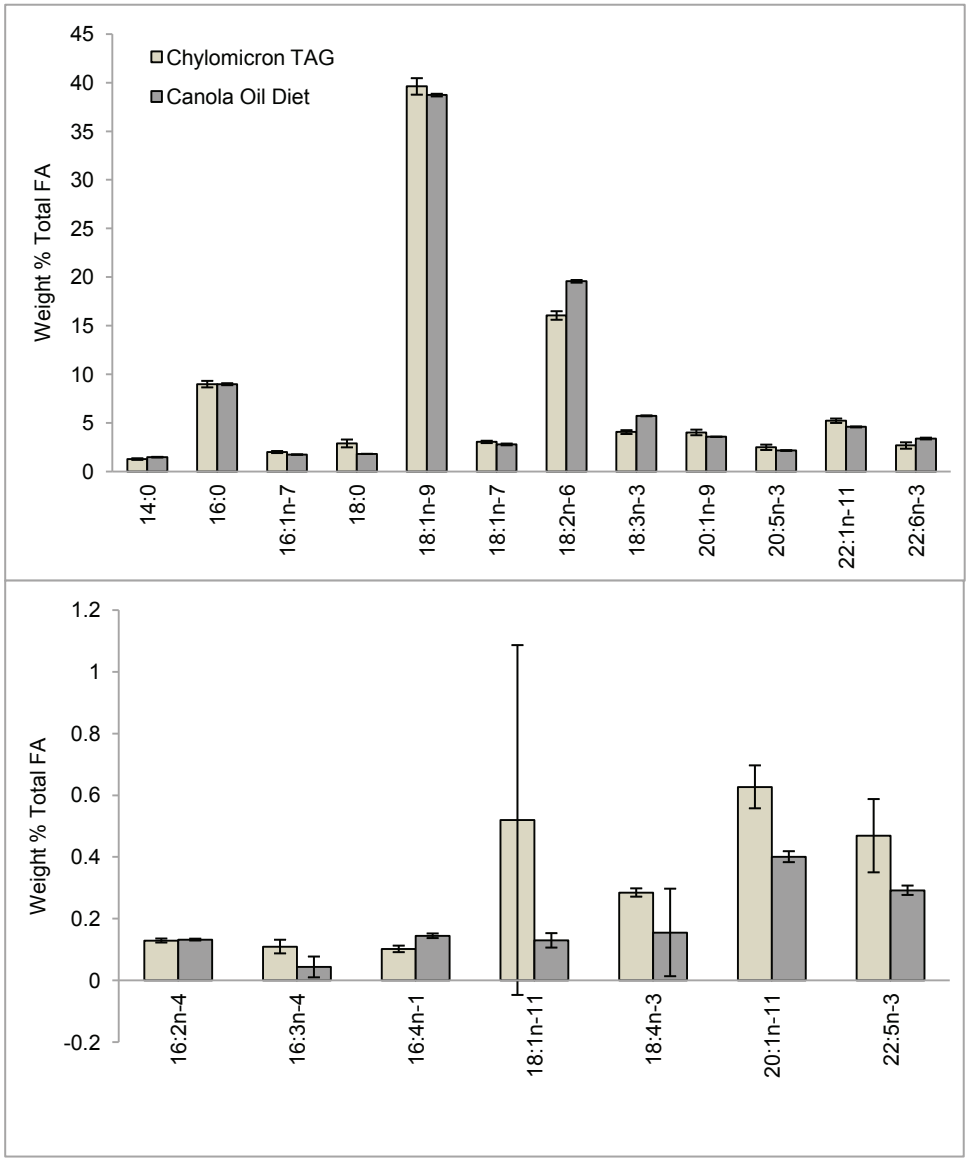

Figure 2.5. Mean fatty acid proportions and standard deviation of the canola diet (n=5) and post-prandial chylomicrons (n=6). Fatty acids that were chosen for display were identified as the most important in differentiating between sample groups using similarity percentage calculations.

2.3.5. POST-PRANDIAL AND FASTED TISSUE COMPARISONS

Comparisons were made across fish tissues from the post-prandial and fasted states of each diet experiment to demonstrate the changes that may occur to tissue FA compositions as a result of food deprivation. Little difference was found between the muscle tissue of the post-prandial and fasted fish from both diet groups (Figure 2.6, 2.7; ANOSIM: $R=0.235$, $p<0.009$ for fish from the FO experiments; and $R=0.155$, $p<0.005$ for fish from the CO experiments); the low R- values suggest that the tissues are not significantly different despite the low p- value. The post-prandial and fasted liver TAG FA of fish from the CO experiment were also not significantly different (Figure 2.9, ANOSIM: $R=0.047$), although significant differences were found in the liver samples of fish from the FO experiment (Figure 2.8, ANOSIM: $R= 1$, $p< 0.001$). The FA that contributed to the highest variance between post-prandial and fasted tissues of each experiment are presented in Table 2.5 for comparison.

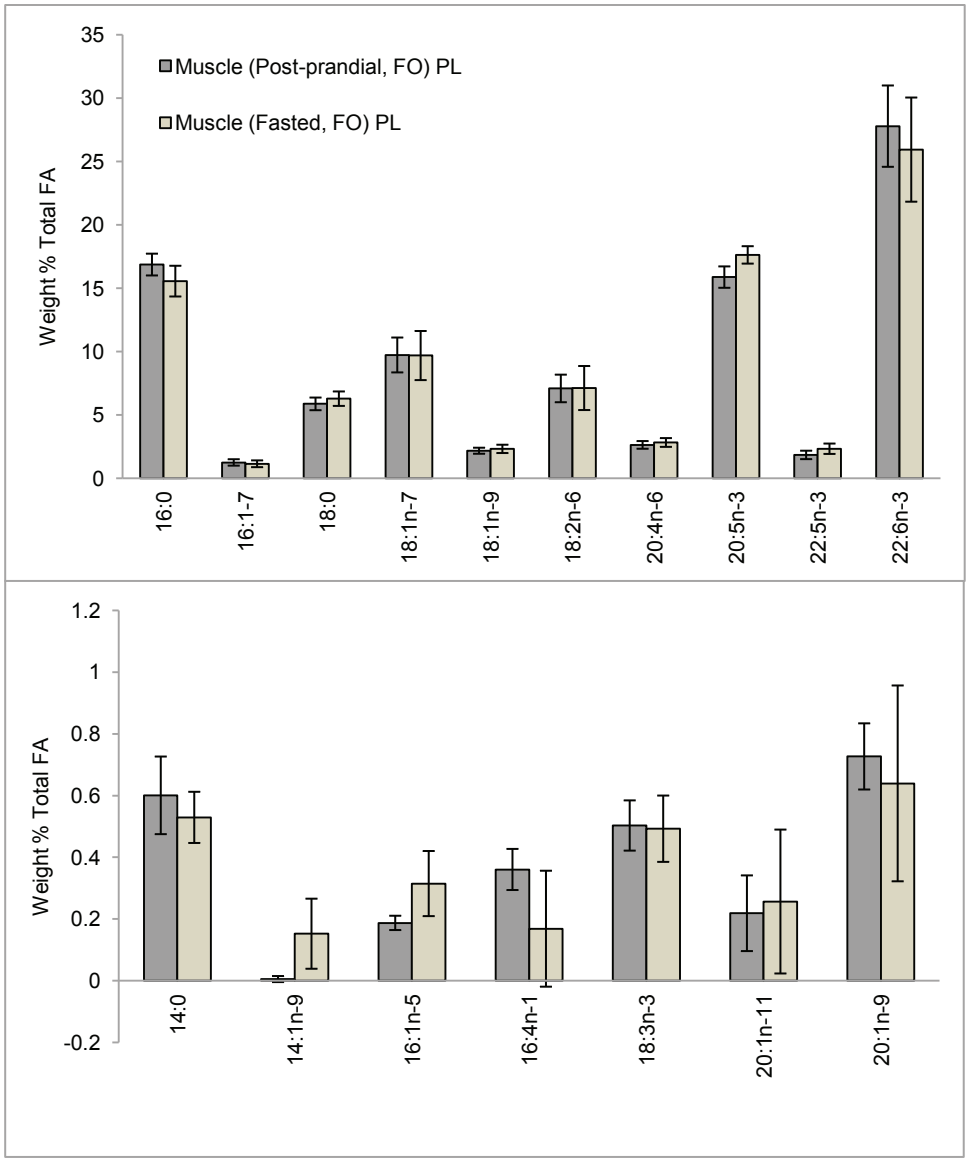

Figure 2.6. Mean proportions and standard deviation of fatty acids that contributed to the highest variance between post-prandial muscle phospholipids (n=7) and fasted muscle phospholipids (n=8) of pollock from the fish oil feeding trial.

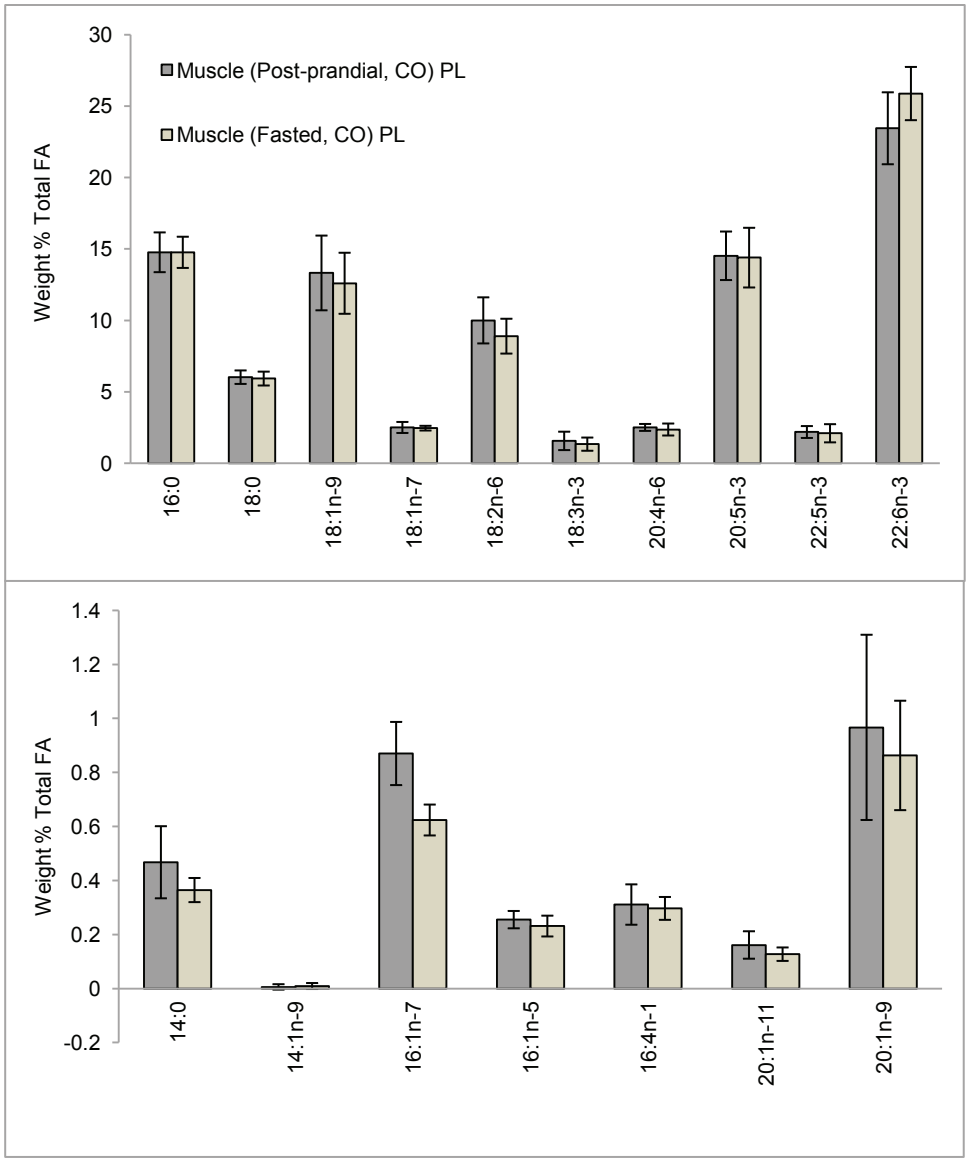

Figure 2.7. Mean proportions and standard deviation of fatty acids that contributed to the highest variance between post-prandial muscle phospholipids (n=11) and fasted muscle phospholipids (n=11) of pollock following the canola oil diet experiment.

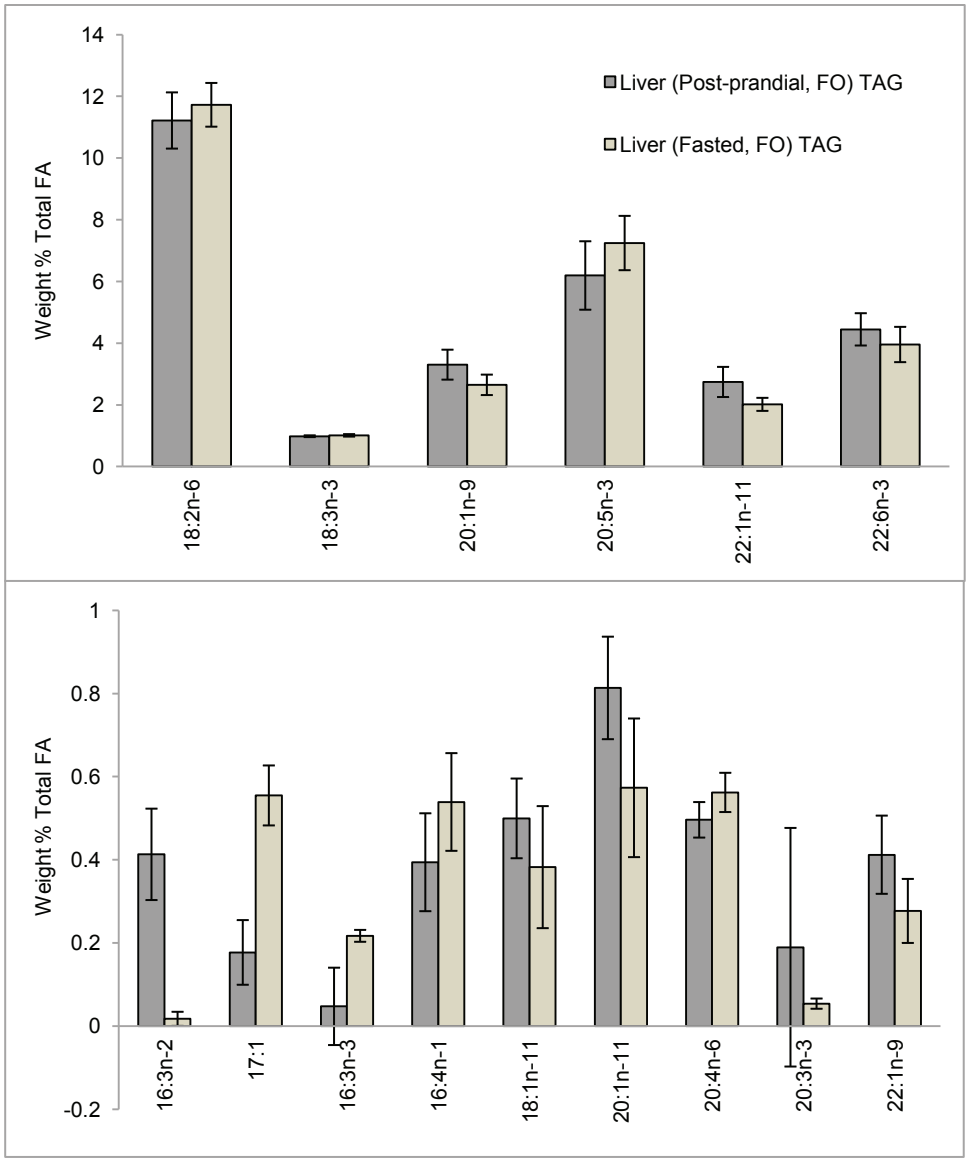

Figure 2.8. Mean proportions and standard deviation of fatty acids that contributed to the highest variance between post-prandial liver triacylglycerols (n=6) and fasted liver triacylglycerols (n=5) of pollock from the fish oil feeding trial.

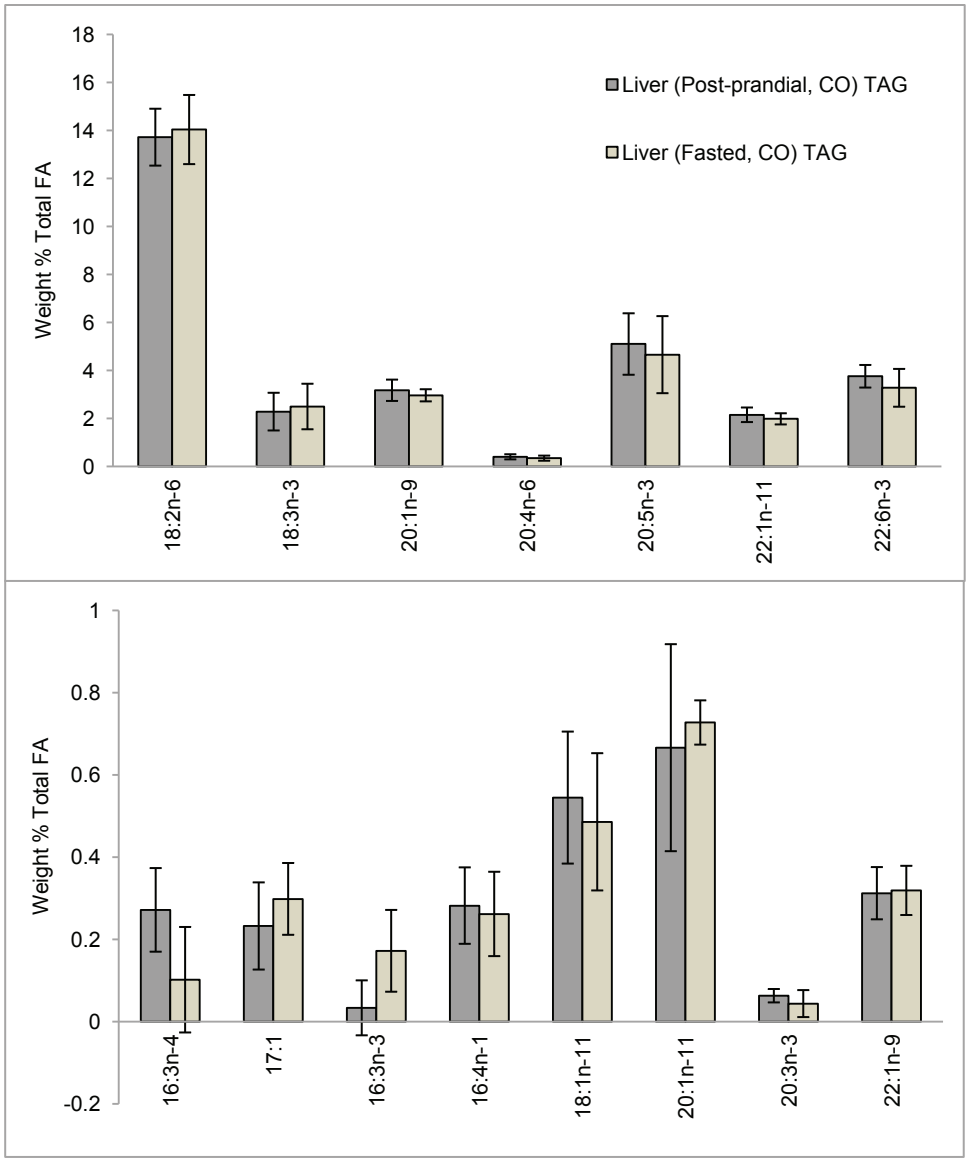

Figure 2.9. Mean fatty acid proportions and standard deviation for post-prandial liver triacylglycerols (n= 9) and fasted liver triacylglycerols (n=11) from the canola oil diet.

Table 2.5. Similarity percentages for the comparison of post-prandial and fasted tissue types from each experiment.

Liver TAG (Post-prandium vs. Fasted) of the FO experiment		Muscle PL (Post-prandium vs. Fasted) of the FO experiment		Muscle PL (Post-prandium vs. Fasted) of the CO experiment	
FA	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**
18:1n-9	11.02	22:5n-3	4.70	18:1n-11	4.16
16:0	8.88	18:2n-6	4.55	18:3n-3	4.03
18:2n-6	8.07	16:4n-1	4.44	20:1n-7	3.93
16:1n-7	6.30	18:1n-9	3.93	22:5n-3	3.82
20:5n-3	6.15	20:1n-9	3.50	21:5n-3	3.68
18:0	5.90	22:6n-3	3.30	22:2n-6	3.62
22:6n-3	5.38	16:1n-7	3.14	16:1n-7	3.21
18:1n-7	5.24	14:1n-9	3.00	18:2n-6	3.09
20:1n-9	4.55	24:1	2.95	18:1n-9	2.84

2.3.6. FASTED TISSUE-SERUM COMPARISONS

2.3.6.1. FISH OIL FASTING EXPERIMENT

Liver, muscle and serum from the fasted fish were compared to determine the degree to which FA profiles vary as a result of lipid mobilization. FA proportions in the serum TAG, liver TAG, and muscle PL from a 3- week fast from the FO diet were significantly different (Figure 2.10; ANOSIM: $R= 0.924$, $p< 0.001$), and pairwise tests revealed that variation was significant for all tissue comparisons ($R=1$ for all). Differences can be seen in the proportions of 20:5n-3 and 22:6n-3, which are lower in the liver tissue relative to the serum; 18:2n-6 and 18:3n-3 contrarily had higher proportions in the liver. Similar to the post-prandial FA profiles, the muscle PL had higher proportions of PUFA and did not resemble the FA profiles of liver TAG and serum TAG. The FA that are most likely contributing to the variance of the pairwise tests, as calculated by SIMPER, are listed for each group in Table 2.6.

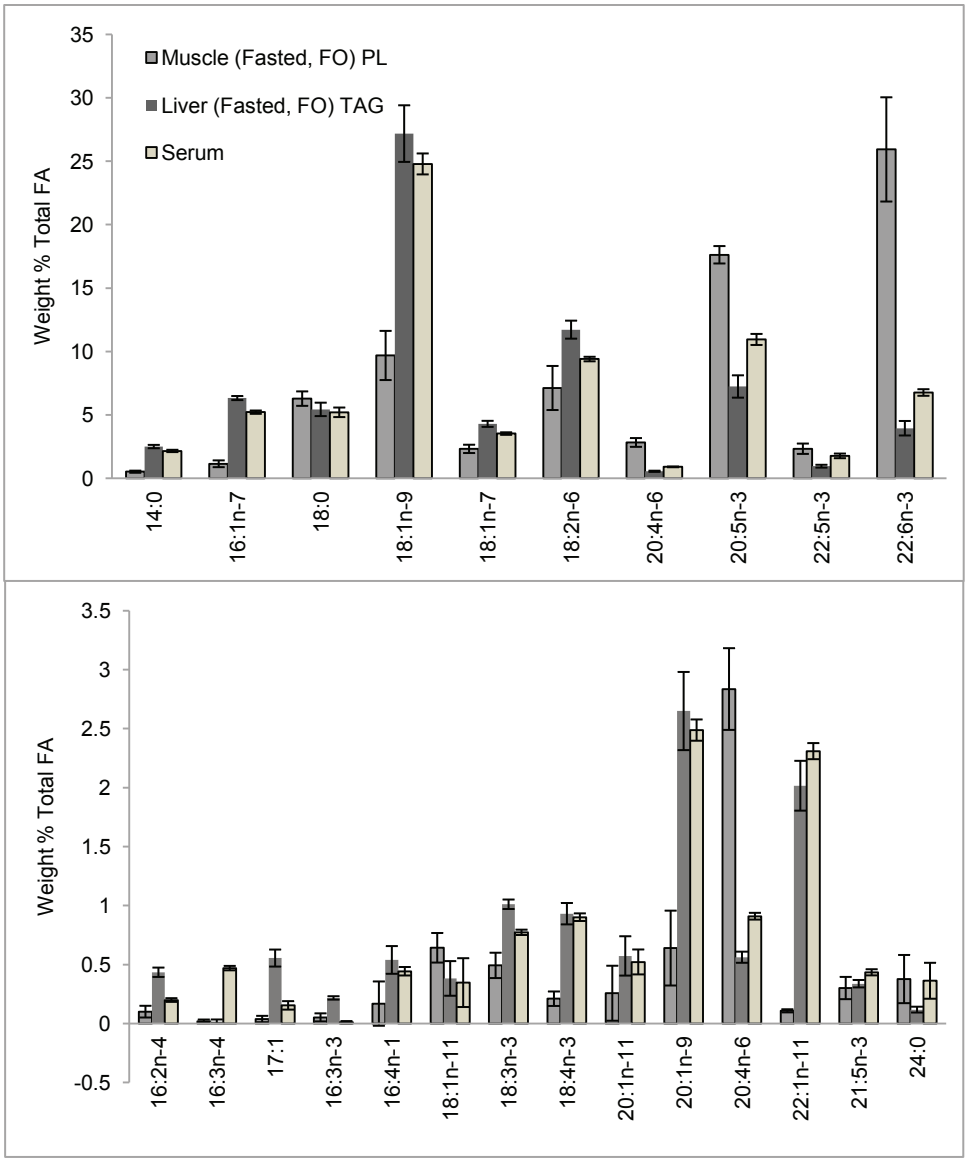

Figure 2.10. Mean proportions and standard deviation of fatty acids that are responsible for the highest variance in pollock serum triacylglycerols (n=5), liver triacylglycerols (n=5) and muscle phospholipids (n=8) following a 3-week fasting period from the fish oil diet.

Table 2.6. Similarity percentages for pollock serum triacylglycerol fatty acids, liver triacylglycerol fatty acids, and muscle phospholipid fatty acids following a 3-week fasting period from the fish oil diet.

Fasted Liver TAG Fasted Serum TAG (R=1, p<0.008)		Fasted Liver TAG Fasted Muscle PL (R=1, p<0.003)		Fasted Serum TAG Fasted Muscle PL (R=1, p< 0.001)	
FA	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**
22:6n-3	7.55	22:6n-3	10.53	22:6n-3	9.02
20:5n-3	6.30	16:1n-7	7.75	22:1n-11	8.00
16:3n-4	6.22	22:1n-11	6.20	16:1n-7	7.83
22:5n-3	5.91	18:1n-9	6.16	18:1n-9	6.51
17:1	4.98	20:4n-6	5.60	20:1n-9	5.67
20:4n-6	3.35	14:0	5.17	14:0	5.29
18:2n-6	3.35	20:1n-9	5.10	20:4n-6	5.08
16:3n-3	3.19	20:5n-3	5.09	18:4n-3	3.30
16:2n-4	3.11	22:5n-3	3.29	20:5n-3	3.22
24:1	3.10	18:2n-6	2.99	16:3n-4	2.65

2.3.6.2 CANOLA OIL FASTING EXPERIMENT

Fasted tissues and serum were also compared for fish that were fasted following the CO diet, and a significant difference was again found between fasted serum TAG and liver TAG (Figure 2.11; ANOSIM: R= 0.816, $p<0.001$). Minimal variation was detected in liver and serum by pairwise testing (R=0.495), while muscle was significantly different relative to serum and liver TAG (R=1 for both). Similar to the pollock that were fasted from the FO diet, the proportion of 20:5n-3 and 22:6n-3 was higher in the serum TAG relative to the liver TAG of the fish that were fasted from the CO diet, while the proportion of 18:2n-6 was higher in the liver TAG relative to the serum TAG. The FA that most likely contributed to the variance in the tissues and serum, as calculated by SIMPER, are listed for each group in Table 2.7.

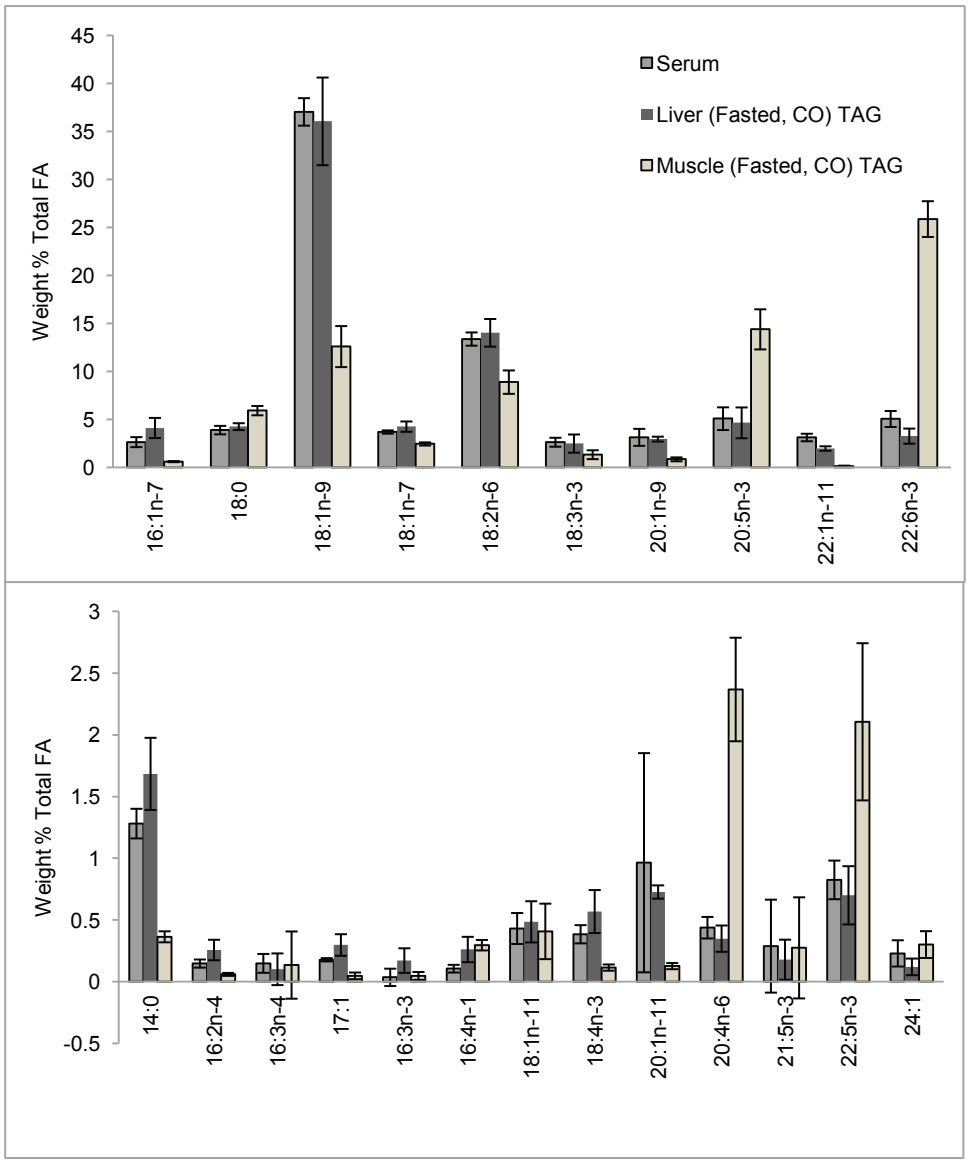

Figure 2.11. Mean fatty acid proportions and standard deviation for pollock serum triacylglycerols, liver triacylglycerols and muscle phospholipids following a 3-week fasting period. Pollock were fasted following a 10-week diet of canola oil diet.

Table 2.7. Similarity percentages for pollock serum triacylglycerol fatty acids, liver triacylglycerols fatty acids and muscle phospholipid fatty acids following a 3-week fast from the canola oil diet.

Serum vs. Muscle (R=1, p< 0.001)		Liver vs. Muscle (R=1, p<0.001)		Serum vs. Liver (R=0.495, p<0.001)	
FA	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**	**FA**	**Contribution to variance (%)**
22:6n-3	9.89	22:6n-3	11.71	16:1n-7	6.59
22:1n-11	8.55	16:1n-7	7.06	22:6n-3	6.19
18:1n-9	6.88	20:5n-3	6.67	22:1n-11	5.60
20:5n-3	6.24	18:1n-9	6.37	20:5n-3	5.06
20:4n-6	5.57	22:1n-11	6.09	18:3n-3	3.57
16:1n-7	5.32	20:4n-6	5.73	20:1n-9	3.28
20:1n-9	5.28	20:1n-9	4.81	14:0	3.00
22:5n-3	3.47	14:0	4.19	21:5n-3	2.96
14:0	3.41	22:5n-3	3.83	18:4n-3	2.90
20:1n-11	3.30	20:1n-11	2.73	16:4n-1	2.59

2.4 FATTY ACID DISCUSSION

2.4.1 DIET COMPOSITION

The CO diet agreed with expectations, with high levels of MUFA that contrasted with the characteristically high proportion of PUFA in the FO diet. The CO diet contained a small amount of fish oil (derived from the fish meal) to maintain EFA requirements, as was apparent by the presence of 20:5n-3, 22:6n-3 and 16:4n-1; however, the CO diet profile remained distinct from the FO diet that showed relatively higher proportions of these FA.

Animals cannot synthesize 18:2n-6 and 18:3n-3, and have only limited ability to form 20:5n-3 and 22:6n-3 from precursors; therefore, these FA that originate from diet were of greater interest for my experiment. The FA proportions present in both the FO and CO diet profiles were the result of mixed dietary inputs. Poultry fat was incorporated in the commercial FO feed produced by Corey Feed Mills, (Fredericton, New Brunswick) and was likely responsible for the high proportion of 18:2n-6 in the diet, while the 18:2n-6 and 18:3n-3 in the CO diet originated from the canola oil, with some input from the supplemented fish meal. Trace amounts of lipid present in the diets could have also been derived from the soy and corn meal that were added to each diet, and may contribute to 18:2n-6 and 18:3n-3 proportions.

2.4.2 POST-PRANDIAL TISSUE REFLECTION OF DIET

It was assumed that the initial storage lipid in liver and muscle derived from the natural diet of pollock had completely turned over in the tissues upon sampling for the FO experiment after 15 weeks of feeding (Penney, 2010 unpublished). The fat content of the FO diet was 18% of the wet weight and is probably much higher than a typical diet

for wild pollock, which feed mostly on juvenile fish, copepods and euphausiids (Scott et al., 1988), that likely have lipid contents < 10% of the wet weight (Saether et al., 1986). Often this is the case with captive feeding studies involving cod (*Gadus morhua*), such that the typical commercial feeds that are given to cod are substantially higher in lipid content than the natural diet and can result in fatty livers (Jobling, 1988). Unfortunately samples of pollock tissues were not available prior to the start of the experiment to confirm lipid turnover. Pollock were also quite active in relatively high ambient water temperature of the aquarium (11-16˚C), supporting a high metabolic rate. For both of these reasons, it appears that the lipid present in the post-prandial tissues of pollock from the FO experiment largely reflected the FO diet, and any lipid from the natural diet was highly diluted if present.

The degree of confidence held for wild dietary lipid turnover in the FO post-prandial tissues was not comparable for the post-prandial tissues of the fish fed the CO diet. This is because the overall lipid content of the CO diet was 12%, or 6% less than the FO diet, so under identical conditions it is not surprising that some lipid from the FO diet would still remain in post-prandial tissues of fish fed the CO diet. This is demonstrated in the liver FA profiles of the CO-fed fish, which show great similarity to the liver of the FO-fed fish. Aside from the different lipid contents of the diets, the conditions that the post-prandial and fasted fish from the CO experiments experienced were not identical to the FO experimental conditions, which would likely further influence the turnover rate of the FO diet in the CO fed post-prandial fish. For instance, ambient water temperature dropped slightly (1-2˚C) during the CO experiment and despite efforts to heat the facility, the temperatures experienced by the fish were reduced (8˚C from 13˚C) from the FO

experiments and could have lower the metabolic activity and appetite (Podoliak, 1961). Wild pollock were also not as attracted to the CO diet and did not reach the same degree of satiation as the fish fed the FO diet. The failure of lipid turnover can be seen in the unusual increase in the proportion of 22:5n-3 in the CO post-prandial muscle, which was characteristic of the FO diet rather than the CO diet. PL have been shown to have a slower rate of turnover than TAG, as a higher percentage are used for cell structure rather than ATP synthesis (Truschenski et al., 2008), which could further impede muscle PL turnover. These findings were not in agreement with previous studies that suggested that the turnover rate of diet in cod tissues was approximately 12 weeks (Penney, 2010 unpublished).

A decrease in appetite prior to spawning has been reported in fish (Brawn, 1961), and pollock assigned to the CO experiment were sampled at 12 weeks rather than 15 weeks of feeding to avoid a transfer of energy to the gonads. The pollock gonads were carefully inspected and it was confirmed that sizes had not changed from the fish fed the FO diet; therefore, the reduced appetite was likely a result of less interest in the CO diet.

Unlike the fish that were only fed the FO diet before sampling, the CO-fed fish experienced a change in diet which appeared to affect the lipid composition of their tissues to a greater extent than the fish that were only subjected to the FO diet. The fish that were sampled following a CO diet had been previously exposed to the high-fat FO diet, whereas the fish that were sampled following only the FO diet were only exposed to the initial diet in the wild prior to capture. The presence of lipid from a previous diet source is depleted from the tissues by both the rate of diet turnover, and dilution by the addition of new lipid and animal growth. Lipid turnover refers to the efficiency at which

FA are oxidized from the stored lipid and replaced by FA from a new diet. Lipid dilution implies that the FA from a previous diet may still remain in the stored tissues of the fish, but the presence of these FA from the previous diet is diluted by the abundance of FA that are received from the new diet (Jobling, 1988). The FA profiles of the fish tissues are likely a result of these two processes, and would depend on the composition of both the previous and more recent diets. The FA that were present in small proportions are likely to completely turnover from the previous diet as a result of oxidation for energy purposes. For instance, because 18:3n-3 was only present in trace amounts in the FO diet while more abundant in the CO diet, this FA is likely to have a complete turnover in the CO post-prandial tissues. In cases where the FA input from the initial diet was high and the animal has not completely depleted its lipid stores, a new diet source will likely dilute the FA that remain from the previous diet as the fish grow in size. Because several marine FA including 20:5n-3 and 22:6n-3 were abundant in the FO diet relative to the CO, these FA are likely diluted in the post-prandial tissues of the CO diet, rather than a complete turnover. Because the fish from post-prandial experiments were fed on a daily basis, the tissue FA composition of the CO-fed fish will likely still contain a FO lipid background, despite the FA oxidation activity that would deplete the lipid stores.

Due to the slow rate of FO FA turnover in the CO experiment, I focused on the results obtained from the post-prandial tissues of fish fed the FO diet rather than those that were fed the CO diet to examine the variation associated with FA deposition in the tissues after feeding. Blood collections from the CO experiment were more successful, and for this reason the CO experiment focused on variation associated with FA assimilation into blood chylomicrons, and mobilization of FA in the serum VLDL. These

comparisons were possible because they were not linked to the diet composition. It should be noted, however, that some absorption of lipid from CO diet occurred in the post-prandial tissues as indicated by an increase in 18:3n-3 in the post-prandial tissues CO-fed fish, which was only present at trace levels in the FO diet.

The liver TAG of the FO-fed fish reflected the FA proportions of diet better than the muscle PL. The liver serves as the lipid storage site in lean marine gadoids (Nanton et al., 2001), whereas the muscle phospholipids play a larger role in the maintenance of cell membranes (Jump, 2002). The FA composition of the liver and muscle varies according to three major biochemical processes following assimilation; 1) elongation and desaturation of dietary FA, 2) *de novo* synthesis of FA, and 3) β-oxidation of FA. The liver serves as the major site for *de novo* FA synthesis in lean marine fish (Sargent et al., 1993), however *de novo* synthesis in the liver is thought to be low for marine fish due to the wide array of FA already available from diet.It is thought that marine fish rely more on modification of dietary FA by desaturation and elongation (Tocher, 2003). Elongation and desaturation occurs in the cytoplasm of liver, adipose tissue and nervous system of vertebrates, along with the mammary glands of mammals (Havel et al., 1970; Carey et al., 1972; Innis et al., 2003) to produce an array of FA structures. FA created by *de novo* synthesis can also be used as substrates for desaturation and elongation. There is evidence for the dominance of elongation/desaturation over *de novo* synthesis in the post liver of fish fed the FO diet, which deviate from the dietary FA in such a way that indicates some modification of 16:1n-7 and 18:0 occurred to produce 18:1n-7 and 18:1n-9 by elongation and Δ9 desaturase, respectively (Fig. 2.4). FA proportions can also vary due to β-oxidation in the tissues and the differences found here in the liver and muscle FA relative

to the diet are likely attributed to a combination of elongation, desaturation, and β-oxidation; however, the extent to which each process is affecting the FA composition of these samples cannot be determined.

β-oxidation of FA occurs throughout the body for ATP generation, and occurs to a greater extent in the peripheral tissues that expend substantial amounts of energy on contractile movement (Sidell et al., 2005). Lipoproteins and chylomicrons supply lipid to the liver and muscle tissue for β-oxidation, which occurs in the mitochondria, and to a lesser extent in the peroxisomes of the cell. There seems to be a general agreement that FA are selectively oxidized, depending on both the dietary input (Hendersen et al., 1985; Neat et al., 1981; Osmundsen, 1981; Torstensen et al., 2000) and substrate specificity (Torstensen et al., 2000; Eaton et al., 1996), factors which might need to be considered for FA analysis of individual biomarkers and in obtaining tissue samples from the wild fish. Both the feeding state of the animal and the fat content of the diet (Henderson et al., 1985; Neat et al., 1981; Turchini et al., 2003) may influence whether β-oxidation occurs in the peroxisomes or the mitochondria. Peroxisomes and mitochondria activity were shown to be tissue-specific (Froyland et al., 1998; Froyland et al., 2000). Muscle tissue will oxidize FA to a greater extent than the liver, however variation may still arise in liver FA of wild fish that are not receiving a consistent diet.

Substrate specificity, and perhaps tissue specificity of FA oxidation likely contributed to the higher proportions of 22:6n-3 in the muscle of pollock fed the FO diet despite its moderate proportions in the FO diet. Similar results were found in tuna, which selectively catabolized 20:5n-3 and accumulated 22:6n-3 in their muscle tissue, regardless of dietary input (Satio et al., 1996). Such may be the case with the fish that

were fed the canola oil diet, as the proportions of 20:5n-6 was maintained at a relatively high % proportions, despite its decrease in the CO diet. Similarly, MUFA were selectively oxidized over SFA in the heart and muscle tissue of Antarctic fishes (Sidell et al., 2005). In both studies by Sidell et al. (1996; 2005), evidence for FA selectivity was found, even when alternate FA substrates were abundant, indicating that these results could apply to the experiments here.

2.4.3 CHYLOMICRONS

The masses of TAG that were obtained from chylomicron samples of the pilot experiment were highly variable and sample size was limited (n=3 per time interval). TAG masses at 18 hours after feeding were among the highest obtained (Fig. 2.8), and plasma samples at this hour appeared as a cloudy opaque color. Some of the blood samples at 12 h after feeding also yielded chylomicrons , and fish chylomicron concentrations likely peak in the blood at some time between these two time intervals, depending on the size of the meal. Due to the variation in the TAG masses (Table 2.4) it was not possible to establish an optimal time to sample blood for chylomicrons; however, digesta was found in the small intestine at both 12 h and 18 h post-prandial, which suggests there is a relatively large window to obtain chylomicrons and supports a peak in chylomicron concentration within this time frame. A better strategy seemed to be to feed fish continuously if possible, before chylomicron retrieval to achieve an overlap in digestion times and to maximize chylomicron concentration.

Chylomicron sampling for the FO experiment was unsuccessful, as the fish only received 1 meal in the 24 h prior to sampling, and led to a smaller window of opportunity to collect chylomicrons in the blood. Chylomicrons were retrieved more successfully in

the CO diet experiment, as fish ate more frequently before sampling. Due to an overlap in the chylomicron and VLDL diameters (Davis, 1991), ultracentrifugation resulted in separation of a layer that appears as a small cloudy ring at the top of the centrifuge tube that fades into the lower layers, rather than a clear separation. For this reason, it was difficult to judge where the top chylomicron layer ended and the lower VLDL layer began; recovery of the top layer by pipet was done conservatively to minimize VLDL contamination but could have influenced the TAG masses obtained. TAG mass is also lost when separating lipid classes by TLC due to the amount of care required to avoid contamination, and in the future it will be useful to use high-performance liquid chromatography (HPLC) to conserve samples.

Despite the significant differences detected by ANOSIM, the FA profiles of chylomicrons and diet were strikingly similar (Fig. 2.9). These statistical differences were unexpected, as FA modification does not occur in the intestinal mucosa during digestion, limiting the biochemical activity of FA to TAG hydrolysis and re-esterification (Tocher et al., 2003). A number of factors may have contributed to this significant difference between the FA profiles of chylomicron TAG and diet; the general steps where variation can arise are 1) the digestibility of the diet, and 2) the absorption of the FA into the bloodstream. Fish have been noted to have a digestibility spectrum for lipids that is dependent on the melting point, such that the degree of saturation can affect how efficient a FA is absorbed in the gut. In general, the digestibility of PUFA is higher than MUFA and SFA in teleost fish (Austreng et al., 1980, Lie et al., 1986 Olsen et al., 1998), and the chain length of FA further affects their digestibility. SFA that were between C_{20}-C_{22} in length were also more efficiently absorbed than C_{18} in rainbow trout *(Oncorhynchus*

mykiss) (Austreng et al., 1980). Because only the pollock from the CO diet regime were used for chylomicron comparisons, the chylomicrons examined in our study were derived from fish that were fed high levels of dietary MUFA (specifically, 18:1n-9) than the pollock would have been accustomed to in the wild and may have impeded digestibility. These possible factors for digestion could affect the degree to which certain FA are assimilated into the chylomicrons of the fish.

In addition to the digestibility of diet, the uptake of FA in the bloodstream is thought to be further affected by two processes: the rate at which FFA are released from the glycerol backbone, and the rate at which these FFA are absorbed by the intestine. PUFA in Arctic charr (*Salvelinus alpinus*) were shown to be almost completely absorbed by the intestine, marked by low levels present in the digesta, whereas MUFA were noted to have a particularly slow rate of release from TAG molecules (Olsen et al., 1998). These differences in hydrolysis and absorption can ultimately lead to differences in lipid class absorption if a FA structure is more frequently esterified to a particular lipid class.

Despite its statistically significant variation, the similarity in FA profiles of chylomicrons and diet were much greater than those of liver and diet. Some caution must be taken when drawing conclusions based solely on statistical data, as a practical relationship can easily be overlooked when relying on a binary significant / non-significant classification system for relationships. The 5% confidence interval that is applied to most data is arbitrary, making it difficult to determine which relationships are most important (Gelman et al., 2006). The subtle differences in FA quantities may not be as important as the degree of similarity among major FA proportions. As with most of the comparisons made between the FA proportions of pollock tissues throughout this study,

FA that are present in small proportions (for instance, 20:1n-11 differs by 0.2% in the diet and chylomicron FA) may appear substantially different, however it may be more important to focus on the degree of similarity that exists among the FA that are present in larger proportions in the tissues (such as 22:6n-3, which differs by 0.7%). Although the FA that exist in smaller proportions (i.e., 16:2n-4, 18:1n-11, 16:2n-4) will contribute to overall statistical significance, these differences may draw attention away from the similarities in the data (i.e., 20:5n-3, 20:1n-11, 22:6n-3).

2.4.4 FATTY ACID COMPOSTION OF TISSUES OF FISH AFTER FASTING

Fatty acid profiles of tissues in fasted fish were very similar to their corresponding post-prandial tissues in both experiments. FA variance among the post-prandial and fasted muscle tissues was considered less important, as most of the lipids present in the muscle tissue are linked to cellular function and structure so would not be expected to be mobilized for oxidation. The similarities that existed between the post-prandial and fasted liver FA are more interesting and demonstrated that a 3-week fast does not drastically change the FA profiles of pollock. The liver is the primary lipid storage site for lean marine fish and subtle variation in the FA profiles may be difficult to detect due to the amount of lipid present; changes in FA concentrations due to differential mobilization may be too small compared to the total lipid concentration to be noticeable after only a 3- week fast. The similarities between the liver of the post-prandium and fasted fish from both diets are further exemplified by the SIMPER (Fig. 2.13 and 2.14) results; SIMPER identifies the FA that are responsible for variance, but even those differences were subtle. These similarities indicate that over short time periods the

feeding state of the fish will have little effect on the FA profiles of liver samples taken from the wild.

The post-prandial and fasted tissues of fish appeared very similar, and these results were found in both the fish oil and canola experiments. Nevertheless, explanation must be given to clarify the deviations that were found between the tissues of fish from each feeding state in order to apply these findings to fish from the wild that will undoubtedly have a lower fat content in their natural diet. There are two main processes that can result in differences between the tissues of post-prandial and fasted fish. First, the fish will deplete stored lipid by β-oxidation of FA in all tissues as a source of energy; second, the FA may be selectively mobilized from the liver into the serum to supply energy to the peripheral tissues. β-oxidation may not occur consistently across all FA structures, and there is evidence that certain FA, including 20:5n-3 such as seen in the data, are spared from oxidation according to their biochemical importance (Rea et al., 1997; Nieminen et al., 2006; Raclot, 2003).

Previous studies have found that FA structure influences whether β-oxidation occurs primarily in the mitochondria or peroxisomes of a specific tissue; the majority of FA in fish, including 20:5n-3, are thought to be readily oxidized by the mitochondria (Sargent et al., 1989; Henderson et al., 1985). Alternatively, 22:6n-3 is oxidized in the peroxisomes as it does not serve as a suitable substrate for mitochondrial β-oxidation (Froyland et al., 2000, Crockett et al., 1993; Tocher, 2003). Although the published literature on this concept is limited, it is likely that the rates of β-oxidation for mitochondria and peroxisomes differ and could result in certain FA being oxidized to a greater amount over time. These processes would apply across all the feeding

experiments conducted on pollock, but would be more apparent in the fasting experiments. Greater differences were found in the 20:5n-3 proportions of post-prandial and fasted fish from the FO experiments (a difference of 1.1%) when compared to the proportions of 22:6n-3 (a difference of 0.5%), which may be explained by the different rates at which these FA are oxidized.

Selectivity during β-oxidation may not be present in cases where the fish has experienced an extensive fasting period and have a more depleted lipid reserve. Interestingly, the increase in 20:5n-3 was not noted for the fish that were fasted from the CO diet, which may be explained by the lower dietary lipid content that the fish received from the CO diet. The fish that were fed the CO diet had a lower percent lipid in the liver relative to the fish that were fed the FO diet (61.0 ±1% lipid in the CO fed fish; 70.9 ±10.1% lipid in the FO fed fish). It is possible that selectivity of FA is dependent on the lipid available for oxidation, such that animals could spare the oxidation of certain FA structures in situations where the FA substrates available for energy are in excess.

In addition to selective oxidation of FA in the tissues, the amount of lipid available in the storage tissues may also result in selective mobilization of FA in fish during a fasting period. Despite some differences in the FA proportions, the overall FA profiles of liver and serum looked very similar. Because the diets of wild fish will likely contain a lower percentage of lipid than the experimental fish, the apparent selective oxidation and mobilization of FA in the liver of fish that were fasted from high-fat diets may be less complicated in nature.

2.4.5 IMPLICATIONS

Some variation did exist in the FA proportions of chylomicrons and diet; however these deviations were far less than those between diet and post-prandial tissues. Chylomicron FA samples may be more convenient and less intrusive than tissue biopsies, although one must use caution when relying on chylomicrons, as it becomes more difficult to obtain chylomicrons from wild animals when the time of their last meal is unknown. When the probability that an animal is foraging (usually dependent on season) is high, or when large sample sizes are available, the risk of not acquiring chylomicron samples would be low. Chylomicron could be convenient when studying animals that are feeding during a phytoplankton bloom, or preparing to hibernate as it could be assumed that there are short time intervals between meals.

Additionally, serum TAG may also serve as a reliable alternative to tissue biopsies for fasting animals when chylomicrons are not obtainable. Although not indicative of the most recent meal, VLDL can provide an integrative signature of the FA that are key to the fish over longer periods of time. In some circumstances, the analysis of serum FA may serve as a more important resource when one is interested in FA that are metabolically important to the animal. Mobilized lipid may provide more practical and current information by providing insight into the specific FA that a fish relies during periods of stress or spawning. Furthermore, in situations where the feeding state of an animal is unknown ahead of time, chylomicron TAG and serum TAG samples can be isolated within the same blood sample by the same general procedure of zonal centrifugation (Bachorik, 1982; Babin, 1987), in order to achieve either a chylomicron or

VLDL layer in instances where the dominant lipid composition in the serum of wild fish is unknown.

Because marine fish can obtain an assortment of FA in the diets, the extent to which FA synthesized or modified has evolved to be limited, and details of these biochemical limitations is currently uncertain (Henderson, 1996; Tocher et al., 1999; Tocher, 2003). As a consequence, the tissues of the animal reflect their diet, but the degree of change associated with the animal's metabolism remains a problem when estimating diet by FA analysis and QFASA. The resulting variation from diet that was found in post-prandial tissue FA, and fasted tissue FA gives insight into the amount of change that occurs with FA assimilation and mobilization in fish. These results can be used to refine other studies using FA analysis on lean marine fish and used to develop calibration coefficients for QFASA for other marine fish species of similar biology. Cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) are two commercially important lean marine fish from the Gadoid family that are physiologically similar to pollock with lipid storage in the liver, and numerous studies have been conducted on these fish in order to assess their condition, status and ecology (Buckley, 1987; Trippel, 1999; Smedbol et al., 2001). The results I obtained for variations in pollock FA associated with metabolism will be useful for research on other gadoid fish.

CHAPTER 3.0 STABLE CARBON ISOTOPE ANALYSIS OF FATTY ACIDS IN ATLANTIC POLLOCK (*POLLACHIUS VIRENS*)

3.1 INTRODUCTION

3.1.1. COMPOUND-SPECIFIC STABLE ISOTOPE ANALYSIS

Natural abundance isotope ratios of carbon and nitrogen are becoming increasingly studied at the compound-level in biological material and used to infer food web relationships. While $\delta^{15}N$ fractionates at a relatively large degree for consumers relative to their diet (DeNiro et al., 1981), little fractionation occurs in $\delta^{13}C$ of consumer bulk tissues (~1‰ enrichment) relative to the diet, allowing $\delta^{13}C$ to be used as a tool to reconstruct predator diets (DeNiro et al., 1978). Fractionation of C isotopes is the basis for isotope ecology (Hobson et al., 1998), and CSIA of EFA and essential AA (EAA) are thought to be more informative analytical methods than stable isotopic bulk analysis for studying predator diets (Boshker et al., 2002, Post, 2002, Jim et al., 2003; Chamberlain et al., 2004). Unlike bulk material, the essential compounds examined by CSIA undergo no structural modification during their metabolism, thus eliminating the contribution of endogenous C sources, along with the kinetic fractionation that is associated with biosynthesis (Ruess et al., 2005; Howland et al., 2003). The $\delta^{13}C$ values of individual FA have thus been used to gain information on an animal's environment and diet components (Hammer et al., 1998; Pond et al., 1998; MacAvoy et al., 2003; Budge et al., 2008).

3.1.2. STABLE ISOTOPE FRACTIONATION

The ^{12}C isotope requires less potential energy than the heavy ^{13}C isotope due to differences that exist in the isotope bond strengths (Bigeleisen and Mayer 1947; Bigeleisen and Wolfsberg 1958). For this reason, ^{12}C reacts at a faster rate than ^{13}C in

chemical kinetics because its bonds are more easily broken. Substrate material that is left behind following the enzymatic reactions will be composed of a higher fraction of ^{13}C than the products due to the effects of fractionation, a process termed isotopic enrichment. Thus, a reactant molecule is said to be isotopically enriched (or "heavier") if the ^{13}C present is greater than the amount of ^{13}C in the product. These mechanisms could also be functional in animal metabolism, such that substrate-selectivity of enzymes could fractionate the C isotopes (Osmundsen, 1981, Henderson et al., 1985); however no experiments have been done to address this potential aspect of fractionation. Considering the biochemical pathways of EFA from diet in a consumer's FA metabolism, carbon fractionation may arise due to two general processes: 1) FA digestion and its assimilation into tissues; and 2) mobilization of dietary FA from the storage tissues into the circulation system. Despite an increasing number of studies that have used CSIA of dietary compounds to study natural ecosystems, very little is known regarding the impact of metabolism of individual compounds on their stable isotopic composition.

CSIA could be a sophisticated and informative method of studying diets, and despite its increased application in ecology, only a few controlled feeding experiments have been conducted to examine molecular fractionation that is associated with consumer metabolism (Fantle et al. 1999; Howland et al. 2003, Jim et al. 2003, McMahon et al. 2010; Budge et al., 2011 in review). There is currently only one controlled feeding experiment involving fish, which focused on the compound-specific analysis of amino acids (McMahon et al., 2010). The C isotopes of EAA were found to have minimal fractionation, although non-EAA had substantial fractionation that varied across individual compounds. As suggested by McMahon et al. (2010), it can be assumed that

these differing $\delta^{13}C$ across compounds form an average $\delta^{13}C$ value that is routinely reported for bulk tissues, and much of the information on compound-specific variance is omitted when diet predictions are based on bulk tissue. EFA are routinely used due to their reliability for diet estimation; however, the isotopic composition of individual EFA in the animal tissues must be experimentally compared to the animal's diet in order to continue to rely on these compounds for interpretation of dietary composition or source.

EFA are generally assumed to be unmodified by the consumer, and fractionation of $\delta^{13}C$ in EFA could therefore not be a result of structural modification of the FA. Because various stages in lipid metabolism involve enzymatic activities, the purpose of my experiment was to examine $\delta^{13}C$ of selected FA in fish tissues to determine whether these compounds are fractionated from the $\delta^{13}C$ of diet during digestion, assimilation or mobilization. Four EFA, 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3, were examined in pollock liver, muscle and blood from fish of both the CO and FO experiments. The 18:2n-6 and 18:3n-3 originated primarily from terrestrial FA sources, while 20:5n-3 and 22:6n-3 are not efficiently synthesized in fish, and largely originate from marine algal FA sources. By examining these isotopically distinct FA sources of C (O'Leary, 1988; Bowling et al., 2008) for dietary biomarkers across each diet group, two opportunities were provided to examine the relative fractionation of $\delta^{13}C$ from different dietary sources. The use of two diets in my study also provides the opportunity to examine the effectiveness of CSIA at detecting a change in the animals' diet, and whether the tissue turnover of lipids correlates to a change in isotopic signal.

3.1.3. OBJECTIVES

The major objective of my experiment was to determine the reliability of CSIA in the estimation of diets of wild marine fish, which was accomplished by conducting captive feeding experiments on Atlantic pollock. The purpose of this study, along with the analysis of FA (Chapter 1), was not to estimate diet composition in pollock, but rather to fine-tune the current understanding of the application of isotopic analysis of individual FA by quantifying fractionation associated with animal biochemistry for a particular species, and provide a foundation for further understanding of $\delta^{13}C$ fractionation in consumers. In order to accomplish this objective, I focused on fractionation occurring in 1) the assimilation of diet in chylomicrons, 2) the deposition of FA in post-prandial liver and muscle, 3) the mobilization of FA from fasted liver to serum VLDL.

3.2. MATERIALS AND METHODS

3.2.1. PREPARATION OF FAME FOR ISOTOPE ANALYSIS

Fatty acid methyl esters (FAME) of the dietary FA were analyzed by GC-FID, and peak areas were measured in order to estimate the mass of each FA present in the samples. Concentrations were adjusted such that each peak that would have a concentration ranging from 4.0 to10.0 x 10^{-6} µg/ml hexane for isotopic analysis.

3.2.2. GC-COMBUSTION-ISOTOPE RATIO MASS SPECTROMETRY

The $\delta^{13}C$ values of 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3were analyzed by GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) at the Core Research Equipment and Instrument Training Network (CREAIT Network) at Memorial University of Newfoundland in October 2010. FA were separated by GC (Agilent 6890) and combusted to CO_2 by a CuO/NiO/Pt oxidation reactor in a Thermo Finnigan GC

combustion III interface. Water molecules were removed, and CO_2 was carried to an IR-MS (Finnigan MAT252), for analysis of the C isotopes relative to a reference CO_2 gas. Precision and accuracy of the $\delta^{13}C$ PLFA analyses were assessed by repeated sample injections and comparison to a commercially available bacterial acid methyl ester standard mixture (ester mixture "F8") supplied by Indiana University, respectively. The F8 standard comprised of a mixture of 14:0, 16:0, 18:0 and 20:0 ethyl and methyl esters. All $\delta^{13}C$ values are reported relative to the Vienna Peedee Belemnite (VPDB) standard using standard delta notation presented below:

$$\delta‰ = \left[\frac{\frac{^{13}C}{^{12}C}\text{sample}}{\frac{^{13}C}{^{12}C}\text{standard}}\right] \text{x}1000$$

Further, the methanol used for methylation of FA was collected for each major batch of samples (May, June and July methanol samples) and isotopically analyzed to ensure that the thermodynamic isotope effects were minimal in the methanol over the course of the extraction stage of the experiment. A small aliquot of the methanol used during methylation was analyzed for its $\delta^{13}C$ composition on an Aurora 1020 TOC analyzer (O.I. Analytical) coupled to a Delta Vplus isotope ratio mass spectrometer (Thermo Scientific). The average $\delta^{13}C$ of methanol was then used to correct for the additional methyl group added to FA during transesterification, by subtracting the proportional contribution of methanol to the $\delta^{13}C$ of FAME with the following equation:

$$\delta^{13}C = (n+1)\left[\delta^{13}C_{FAME}\right] - n[\delta^{13}C_{FFA}]$$

where *n* is equal to the number of C atoms in the FFA. FFA were independently corrected using this equation, as the methyl group varies in its contribution to the FA structure.

3.2.3. DISCRIMINATION FACTORS

Discrimination factors (Δ) were calculated by subtracting the $\delta^{13}C$ of diet (D) from the FA of each tissue (T) sample, such that $\Delta_{T\text{-}D} = T\text{-}D$. Discrimination factors were determined for the following groups relative to diet: 1) chylomicrons ($\Delta_{C\text{-}D}$), post-prandial muscle ($\Delta_{M\text{-}D}$), post-prandial liver ($\Delta_{L\text{-}D}$), and 3) serum-liver ($\Delta_{S\text{-}F}$) from the fasted fish.

3.2.4. STATISTICAL ANALYSIS OF C ISOTOPE RATIOS

$\delta^{13}C$ values were statistically tested using SPSS 15.0 for Windows (IBM Corporation, Somers, NY). Multivariate analysis of variance (MANOVA) was calculated and Wilks' λ from MANOVA statistics was used to represent the variance within all the data groups. MANOVA is particularly useful for acquiring information of the global differences between groups, rather than the differences between individual FA. Post-hoc univariate tests were used to generate tissue pairwise tests of individual FA that are responsible for the variance reported in Wilks' λ. Due to the repetitive use of data sets to generate post-hoc tests, Bonferroni corrections are used to adjust the confidence interval to a more conservative level, and reduce the probability of statistical error.

$$\alpha = \frac{p - value}{n}$$

where *n* is the number of tests performed in the data set. For example, n=12 for a post-hoc test on 3 tissue groups that each contain 4 FA values.

3.3. ISOTOPE RESULTS

3.3.1. DIET DISCRIMMINATION

The $\delta^{13}C$ values of 18:2n-6 and 18:3n-3 of the CO diet were isotopically lighter relative to the FO diet, while the $\delta^{13}C$ of 20:5n-3 and 22:6n-3 were more enriched in both diets and reflected the $\delta^{13}C$ values of typical marine FA sources (Budge et al., 2008a; Figure 3.1.). There was a significant difference in the overall $\delta^{13}C$ of FAs of the FO and CO based diets (MANOVA: Wilks' $\lambda = 0.023$, $p < 0.001$), and univariate tests showed significant differences for all FA ($p < 0.001$). Discrimination factors were calculated for all fish groups that were analyzed, and are presented in Table 3.1.

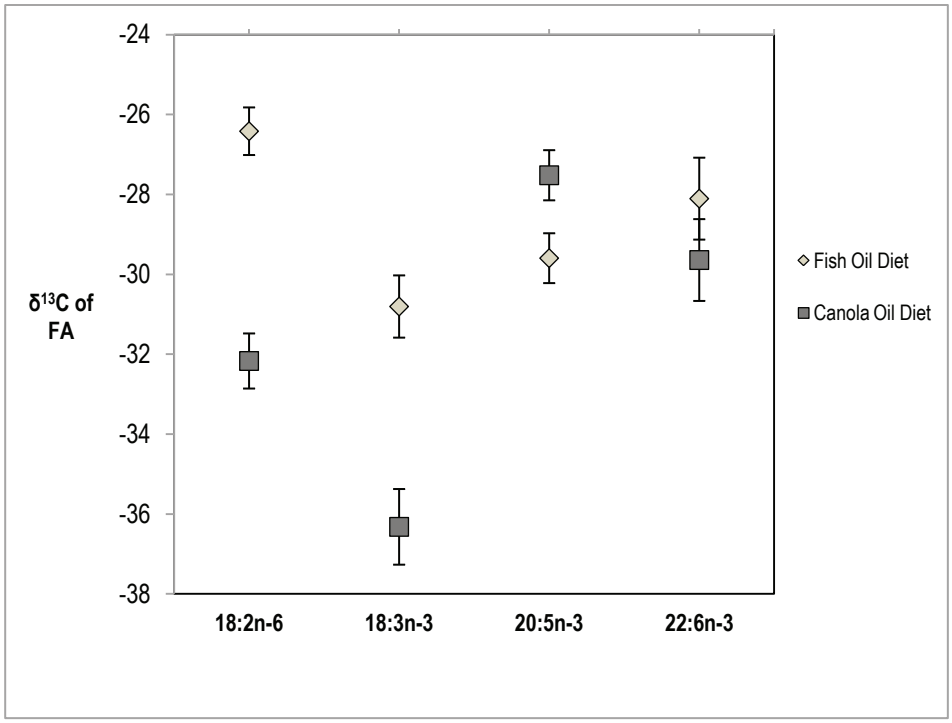

Figure 3.1. Mean $\delta^{13}C$ and standard deviation of fatty acid biomarkers in the fish oil (n=6) and canola oil (n=6) experimental diets.

3.3.2. POST-PRANDIAL EXPERIMENTS

3.3.2.1. $\delta^{13}C$ VARIATION AND TISSUE REFLECTION OF DIET

To better demonstrate the degree to which each tissue reflected diet, the $\delta^{13}C$ of tissue samples from the assigned diets were contrasted (Figure 3.2). Only subtle differences were seen between the $\delta^{13}C$ values of post-prandial tissues from fish fed the FO diet and the CO diet. The most notable difference was in the $\delta^{13}C$ value of 18:2n-6, which was isotopically enriched in the FO tissues relative to the CO tissues, a difference that was more pronounced in the muscle samples. The experiments were designed such that the fish were fed the FO diet prior to the CO diet, and the $\delta^{13}C$ values of 20:5n-3 did not seem to be affected by diet change, although 22:6n-3 showed some subtle changes that were also more pronounced in the muscle rather than the liver. Proportions of 18:3n-3 were too low (< 1%) to allow measurement of $\delta^{13}C$ of that FA in samples from the FO experiment, and were therefore not shown in the comparisons of tissue types.

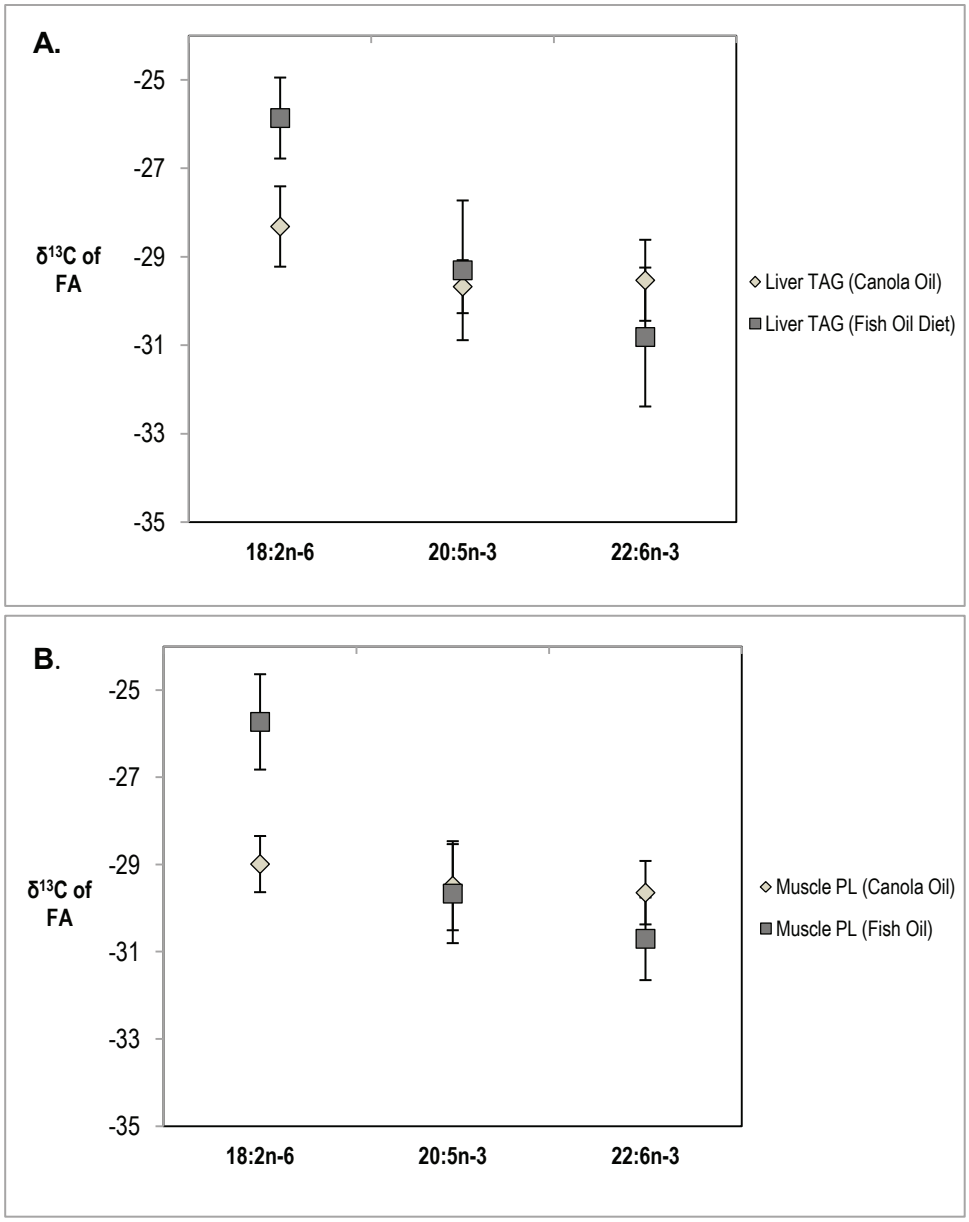

Figure 3.2. The mean $\delta^{13}C$ values and standard deviation of selected fatty acids of post-prandial (A) liver triacylglycerols (from the fish oil experiment, n=8; from the canola oil experiment, n=7), and (B) muscle phospholipids (from fish oil experiment, n=7; from canola oil experiment, n= 6) fed a fish oil or canola oil diet.

3.3.2.2. TISSUE-DIET DISCRIMINATION FROM POST-PRANDIUM FISH

The $\delta^{13}C$ values of 18:2n-6 and 20:5n-3 in the post-prandial liver and muscle of the FO-fed fish reflected the $\delta^{13}C$ of their diet (18:2n-6: $\Delta_{L\text{-}D}$= 0.55, $\Delta_{M\text{-}D}$= 0.70‰; 20:5n-3: $\Delta_{L\text{-}D}$= 0.29‰, $\Delta_{M\text{-}D}$= -0.06‰), while the $\delta^{13}C$ of 22:6n-3 fractionated from diet ($\Delta_{L\text{-}D}$= -2.71‰, and $\Delta_{M\text{-}D}$= -2.59‰), resulting in isotopically depleted $\delta^{13}C$ values in the both the muscle and liver (Fig. 3.3). No significant differences were found between the FA of the FO diet and the FA of the post-prandial tissues of fish that were fed the FO diet (Figure 3.3, MANOVA: Wilks' λ= 0.400, $p< 0.016$). Discrimination factors were calculated for all tissue and diet comparisons for more clarification (Table 3.1).

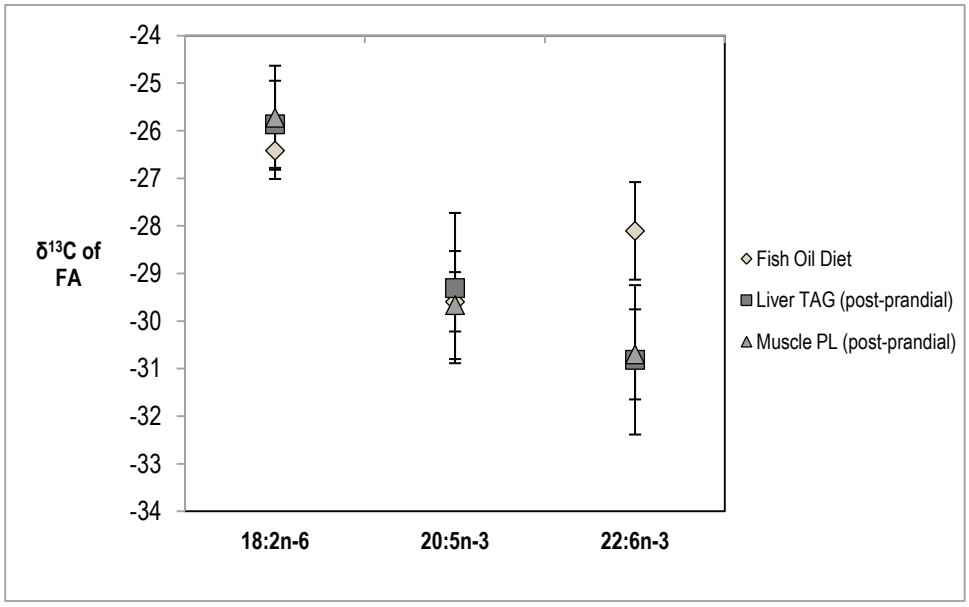

Figure 3.3. Mean $\delta^{13}C$ and standard deviation of fatty acids from the fish oil diet (n=6), and fatty acids of post-prandial liver triacylglycerols (n=8), and muscle phospholipids (n=7) of fish that were fed the fish oil diet.

Table 3.1. Mean isotope discrimination factors and standard error for post-prandial tissue comparisons to fish oil and canola oil diets, chylomicron fractionation from diet, and a fasted tissue comparison to serum.

FO DIET (POST-PRANDIAL)

	Liver & Diet			Muscle & Diet		
	Δ_{L-D}		SE	Δ_{M-D}		SE
18:2n-6	0.55	±	0.32	0.69	±	0.41
20:5n-3	0.29	±	0.56	-0.07	±	0.43
22:6n-3	-2.71	±	0.55	-2.59	±	0.36

CO DIET (POST-PRANDIAL)

	Liver & Diet			Muscle & Diet		
	Δ_{L-D}		SE	Δ_{L-D}		SE
18:2n-6	3.85	±	0.34	3.18	±	0.22
18:3n-3	2.21	±	0.20	3.24	±	0.64
20:5n-3	-2.16	±	0.22	-1.96	±	0.36
22:6n-3	0.11	±	0.35	0.00	±	0.26

	CHYLOMICRONS & DIET			LIVER & SERUM (FASTED)		
	Δ_{C-D}		SE	Δ_{S-L}		SE
18:2n-6	1.04	±	0.46	0.33	±	0.79
18:3n-3	2.21	±	0.29	-0.51	±	0.24
20:5n-3	0.26	±	0.81	1.61	±	0.32
22:6n-3	0.50	±	0.55	1.82	±	0.37

Unlike the FO results, the $\delta^{13}C$ values of 22:6n-3 in the post-prandial tissues of CO-fed fish showed little net fractionation from the CO diet ($\Delta_{L\text{-}D}$ = 0.27‰, $\Delta_{M\text{-}D}$= 0.16‰), while the $\delta^{13}C$ values of 18:2n-6, 18:3n-3, and 20:5n-3 of (Figure 3.4) for the liver and muscle showed substantial fractionation that resulted in isotope enrichment of the liver and muscle relative to the diet (18:2n-6: $\Delta_{L\text{-}D}$ = 3.76‰ and $\Delta_{M\text{-}D}$= 3.09‰; 18:3n-3: $\Delta_{L\text{-}D}$ = 1.96‰ and $\Delta_{M\text{-}D}$= 3.00‰, 20:5n-3: $\Delta_{L\text{-}D}$ = -2.10 ‰ and $\Delta_{M\text{-}D}$= -1.91‰). MANOVA calculated significant differences among the post-prandial tissues and the CO diet (MANOVA: Wilks' λ= 0.113, $p< 0.001$), however the Tukey post-hoc test eliminated 20:5n-3 as a source of variance, and significant differences were only detected in the $\delta^{13}C$ of 18:2n-6 ($p< 0.001$ for diet and liver, $p< 0.001$ for diet and muscle) and 18:3n-3 ($p< 0.001$ for both tissue comparisons to diet).

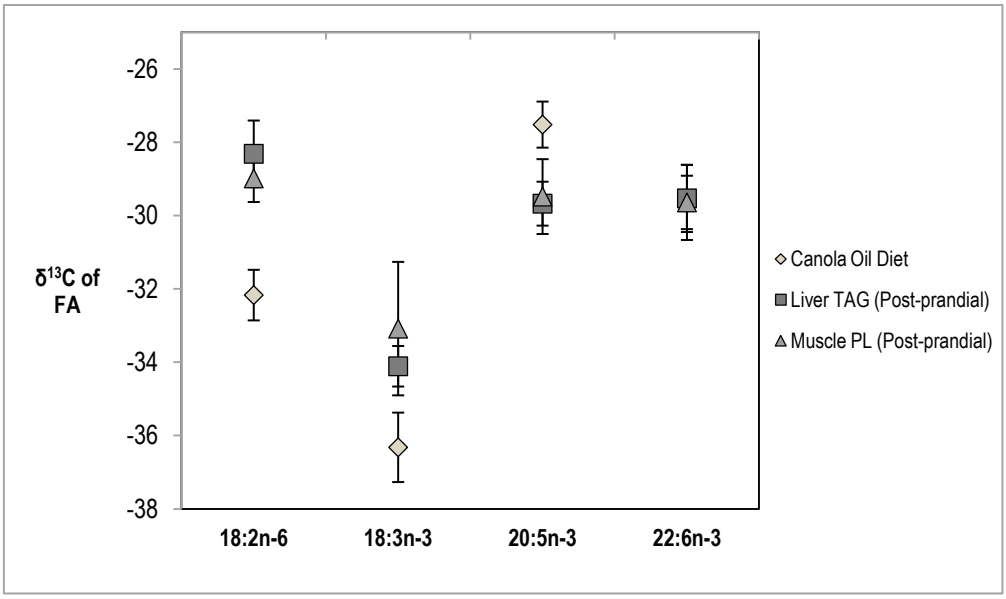

Figure 3.4. Mean $\delta^{13}C$ and standard deviation of the canola oil diet fatty acids (n=6), and the post-prandial liver triacylglycerol fatty acids (n=7) and muscle phospholipid fatty acids (n=8) of canola oil-fed fish.

3.3.3. CHYLOMICRON-DIET DISCRIMINATION

Chylomicron TAG-derived 18:2n-6, 20:5n-3 and 22:6n-3 had $\delta^{13}C$ values that showed very little fractionation from the diet ($\Delta_{C\text{-}D}$= 0.9‰, -0.2‰ and 0.7‰ respectively). The only substantial fractionation that occurred between the chylomicrons and diet was for 18:3n-3, resulting in an enrichment of the $\delta^{13}C$ for chylomicrons ($\Delta_{C\text{-}D}$= 2.2‰). Although a statistically significant difference was calculated between the overall $\delta^{13}C$ of chylomicrons and diet (Figure 3.5, MANOVA: Wilks' λ= 0.302, $p< 0.007$), a univariate test revealed that 18:3n-3 was the only FA responsible for the significant variance ($p< 0.001$).

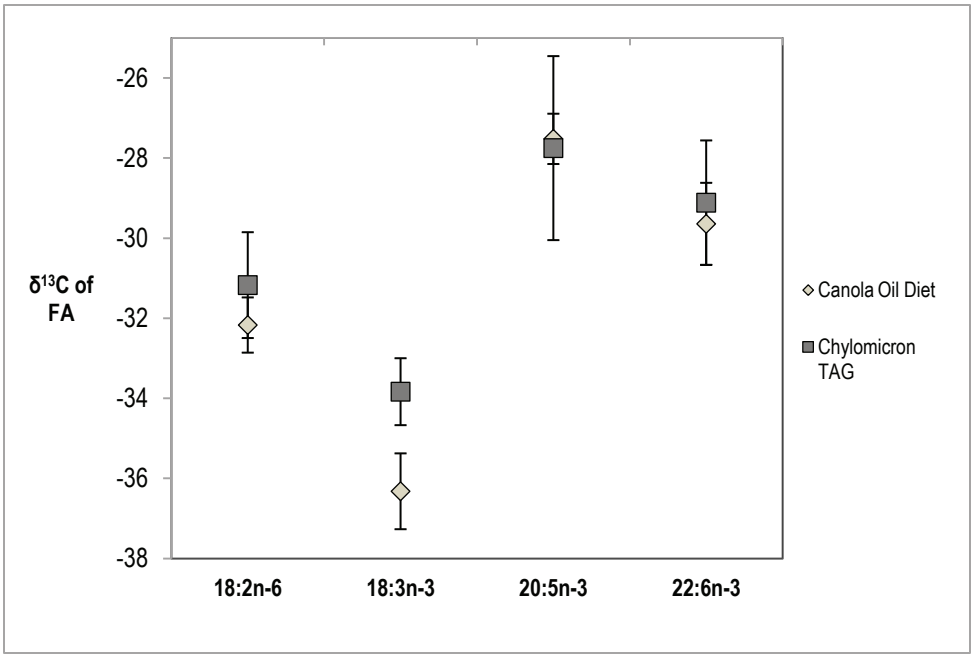

Figure 3.5. Mean $\delta^{13}C$ and standard deviation of canola oil diet fatty acids (n=6) and chylomicron triacylglycerol fatty acids (n=10).

3.3.4. FASTING EXPERIMENT

3.3.4.1. FRACTIONATION ASSOCIATED WITH FASTING

Post-prandial tissues of fish from the CO experiment were compared to their corresponding fasted tissue groups to demonstrate isotopic variance associated with a 3 week fast from the CO diet. The $\delta^{13}C$ of fasted liver FA was significantly different from the liver of the post-prandium state (Figure 3.6, Wilk's $\lambda = 0.268$, $p < 0.002$), and Tukey's post-hoc test revealed that the variance was driven by 18:3n-3 ($p < 0.015$), 20:5n-3 (0.002) and 22:6n-3 ($p < 0.008$), whereas differences were nonsignificant for the $\delta^{13}C$ of 18:2n-6. Alternatively, the muscle tissue FA between each feeding state showed no significant differences.

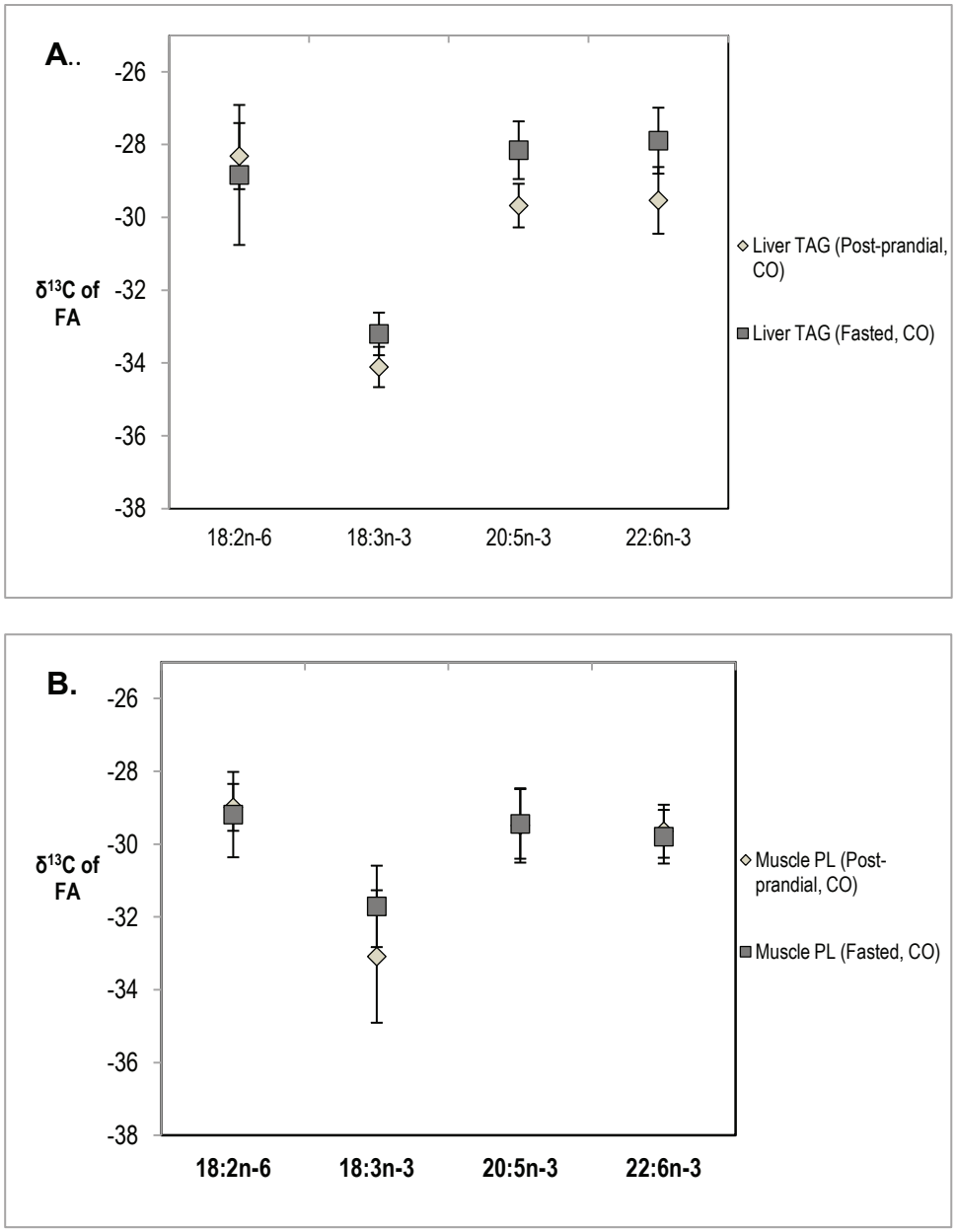

Figure 3.6. Comparison of mean $\delta^{13}C$ values and standard deviation of fatty acids from (A) post-prandial (n=7) and fasted (n=6) liver triacylglycerols of the canola oil-fed fish, and (B) post-prandial (n=8) and fasted (n=6) muscle phospholipids of the canola oil-fed fish.

3.3.4.2. TISSUE-SERUM DISCRIMINATION

The $\delta^{13}C$ values of 18:2n-6 and 18:3n-3 of fasted pollock showed little fractionation between the liver and serum (18:2n-6: Δ_{S-L}= 0.32‰; 18:3n-3: Δ_{S-L}= -0.51‰), while the $\delta^{13}C$ values of 20:5n-3 and 22:6n-3 fractionated such that the liver FA were isotopically enriched relative to the serum (Figure 3.7; 20:5n-3: Δ_{S-L}= -1.61‰; 22:6n-3: Δ_{S-L}= -1.81‰). Significant differences were detected for fasted liver and serum (MANOVA: Wilks' λ= 0.165, $p< 0.004$), and the Tukey's post-hoc test indicated that the $\delta^{13}C$ variance was only derived from 22:6n-3 ($p< 0.002$).

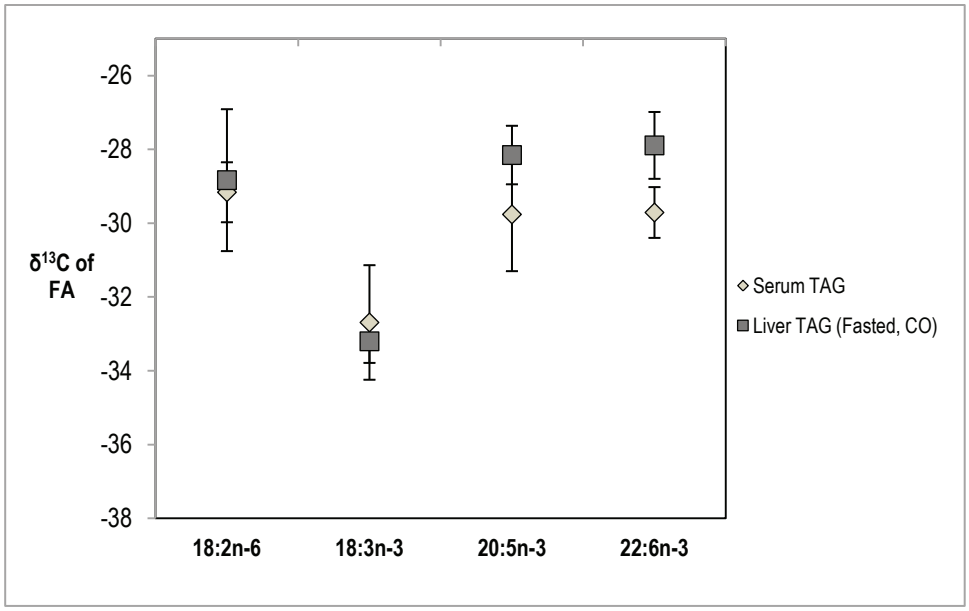

Figure 3.7. Mean $\delta^{13}C$ and standard deviation of liver triacylglycerol fatty acids (n=6) and serum triacylglycerol fatty acids (n=7) of pollock that were fasted for 3 weeks following the canola oil diet.

3.4 ISOTOPE DISCUSSION

3.4.1 FATTY ACID BIOMARKERS

The ultimate goal of my experiment was to examine diet FA biomarkers in pollock tissues to determine the degree to which metabolism changes the $\delta^{13}C$ values of FA in routinely sampled tissues in wild fish. The $\delta^{13}C$ values of the dietary FA 18:2n-6, 18:3n-3, 20:5n-3 and 22:6n-3 were analyzed in post-prandial tissues of each experiment to determine carbon fractionation during FA assimilation. Because of the different origins, there was a difference in $\delta^{13}C$ values in C_{18} FA, reflecting that of a terrestrial source, and the C_{20} and C_{22} FA, reflecting a marine origin, in both the CO and FO diets. Due to the low Δ6-desaturase activity present in animals, the dietary FA biomarkers across the two diet groups provided an opportunity to examine the metabolic pathways of FA that were exclusively derived from diets without any biosynthesis in the tissues or modified after assimilation from the digestive tract.

3.4.2 EXPERIMENTAL DIETS

The CO and FO diets were isotopically distinct with major differences lying in the $\delta^{13}C$ of 18:2n-6 and 18:3n-3, These FA were considerably more enriched in the FO diet relative to the CO diet. The history of the C_{18} FA present in the diets can explain variation in their $\delta^{13}C$ values. The CO diet lipid was solely supplied by the canola oil with trace amounts of residual lipid that originate from fish meal, soy and corn meal. The FO diet FA are derived predominantly from their fish oil supply, with some contribution from poultry fat that is incorporated in the commercial FO feed produced by the Corey Feed Mills. Poultry fat was an ingredient in the FO diet, and produced by a commercial poultry processor; however, it is likely that chickens were fed diets containing high

amount of corn (a C_4 plant) prior to slaughter (Frank 1982). For this reason, the lipid in the FO diet likely contains a higher proportion of lipid from a C_4 source than the CO diet, as canola is a C_3 plant and dominates the lipid composition of the CO diet. Canola is a C_3 plant; therefore the contributions of C_3 and C_4 plants in each diet likely account for differences in the C_{18} FA. The bulk $\delta^{13}C$ of C_3 plants is typically -25 to -29‰ and C_4 plants range from -12 to -16‰ (O'Leary 1988), therefore the $\delta^{13}C$ of terrestrial FA (18:2n-6 and 18:3n-3) in the FO diet correspond to a dietary mixture that was a predominantly C_4 plant source, while the CO diet is more closely related to a C_3 signature.

Despite having a similar fish oil source, 20:5n-3 and 22:6n-3 also had distinct $\delta^{13}C$ values in each diet, though not as substantial as the differences seen in the C_{18} FA. The difference found in 20:5n-3 and 22:6n-3 was likely due to inevitable differences in the fish oil and meal used. It is unknown whether the proportions of herring and anchovy oil are consistently maintained throughout all feed formulations, since their purposes as an oil would be considered equivalent in industrial use.

3.4.3 POST-PRANDIAL TISSUE COMPARISONS TO DIET

The carbon isotopes were fractionated from diet to different degrees across all four FA biomarkers in all the post-prandial tissues examined. The liver and muscle tissue of fish fed the FO diet showed minimal fractionation of C isotopes in 18:2n-6 and 20:5n-3 when compared to the diet FA; however, 22:6n-3 had $\delta^{13}C$ values for liver and muscle that were more depleted than the diet (Figure 3.3). These results indicate that individual FA differ in their degree of fractionation from diet to tissue, for reasons that do not include FA modification. These anomalies in FA isotopes were similarly found for

previous experiments (Pond et al., 2006; Budge et al., in review). As mentioned in the previous chapter, FA appear to differ in their degrees of retention and oxidation in the tissues, and certain FA tend to be used for energy, while other FA are retained for other biological purposes (Osmundsen1981; MacKenzie et al., 1998; Turchini et al., 2003; Menoyo et al., 2004).

The post-prandial tissues of fish that were fed the CO diet showed different patterns of fractionation than those of the FO experiment, with enrichment of 18:2n-6 and depletion of 20:5n-3 relative to the diet, whereas 22:6n-3 had no tissue fractionation. However, the $\delta^{13}C$ of FA in the post-prandial liver from fish fed CO strongly resembled the $\delta^{13}C$ of the same FA in post-prandial liver of fish fed FO, which suggests that the FO lipid did not fully turnover in the tissues of fish fed CO. The apparent fractionation patterns of FA in the post-prandial tissues of the CO-fed fish likely still expressed a FO $\delta^{13}C$ signal in the tissues due to remaining stored FO lipid. It is therefore difficult to establish whether variance in FA in the CO-fed fish tissues was due to isotopic fractionation, or failure of the tissues to turnover their FA. For this reason, less emphasis was given to the isotopic data of 18:2n-6, 20:5n-3 and 22:6n-3 in the CO post-prandial tissues; however, the $\delta^{13}C$ 18:3n-3 was likely still reliable due to a consistent input. This FA was only present in trace amounts in the FO diet, and consequently the 18:3n-3 signal was too weak to be properly identified from post-prandial tissues of FO-fed fish. For this reason, any 18:3n-3 present in the tissues of fish fed the CO diet was likely derived from the CO diet, and problems with lipid turnover should not be a complicating factor for this FA.

Carbon isotope fractionation must rely on a mechanism that changes the overall ratio of heavy to light C in the tissues, under the assumption that EFA are conserved in tissues, the only metabolic processes that EFA are likely to undergo are hydrolysis and re-esterification associated with assimilation and mobilization in the tissues. In general, chylomicrons are transported in the blood and supply lipid to the liver for storage, and to the muscle and other peripheral tissues for oxidation. Prior to tissue deposition, chylomicron TAG must be hydrolyzed to FFA and MAG to cross the cell membrane of endothelial cells, where they are re-esterified to TAG. Hydrolysis is the process of cleaving the bond between the oxygen on the fatty acyl structure and the C on the glycerol backbone; thus, the C within the FA chain are not involved in the cleavage or formation of bonds (Figure 3.8). Likewise, the process of re-esterification to TAG molecules involves the formation of a bond between the O of the FA structure and the C of the glycerol, similarly preserving the integrity of the FA structure. Since kinetic fractionation of carbon is associated with the formation or breaking of a C bond, these processes cannot be responsible for the isotopic fractionation of C in FA; thus assimilation and mobilization of FA can be eliminated as potential points of carbon isotope fractionation during FA metabolism.

Since the FA that were examined here are not readily synthesized *de novo*, there are a limited number of possible biochemical explanations for the diet-tissue variability. One possibility for tissue fractionation is the preferential utilization of specific FA for β-oxidation and energy production. The published research on selective oxidation of FA is uncertain, and previous studies with tuna and Antarctic fishes also showed obvious patterns of FA retention in the tissues that appeared species-specific (Sidell et al., 2005;

Saito et al., 1996). Studies on β-oxidation across individual FA show that 22:6n-3 is limited to peroxisomal β-oxidation while 20:5n-3 is readily oxidized in the mitochondria of both mammals and fish, and may provide support for the hypothesis that oxidation is FA-specific (Henderson et al., 1985; Crockett et al., 1993; Jump, 2002). The rate of peroxisomal β-oxidation was found to be substantially slower than the rate of mitochondrial β-oxidation of rats (Mannaerts et al., 1979); therefore, the isotopic fractionation may be a function of FA-specific rates of oxidation, depending on the organelles in which the reactions occur. Likewise, a slower rate of oxidation of 22:6n-3 in the peroxisomes may result in a smaller degree of fractionation in 22:6n-3 relative to other FA that are oxidized in the mitochondria. However, the opposite was found for 22:6n-3 in the tissues of fish fed the FO diet as its fractionation was more substantial than any other FA, which suggests that another physical mechanism or biological phenomena must also be occurring in the metabolism of the fish.

The factors that govern the β-oxidation rates for individual FA in fish and other vertebrates show some contradiction in the literature, and the complex nature of this activity is presumably responsible for the different degrees of fractionation found in this study. The β-oxidation rates of FA in Antarctic fishes and Atlantic salmon were shown to vary across individual FA as a result of the energy requirements of the muscle tissue, resulting in a preferential retention of certain FA that was independent of dietary intake (MacKenzie et al., 1998; Menoyo et al., 2004). However, fractionation from diet to tissue may also be linked to increased rates of oxidation for the most abundant FA, such that diet input could affect the rates of oxidation (Torstensen et al., 2004; Budge et al., in review). β-oxidation results in the production of Acetyl-CoA, which in turn generates

ATP, and intuitively one would expect that abundant, non-essential FA be oxidized to a greater extent for energy, while essential FA retained to serve other biological purposes when an animal was not fasting (Turchini et al., 2009). Essential fatty acids are classified based on their requirements for cellular metabolism and their ability to synthesize these FA, and although there is no evidence that 18:2n-6 and 18:3n-3 satisfy essential fatty acid deficiencies, they do provide the necessary substrates for the generation of 20:5n-3 and 22:6n-3 via Δ6-desaturase and elongase enzymes (Tocher, 2003).

If proportions influence selective oxidation, one would expect that 20:5n-3, for instance, to also be substantially fractionated due to its propotion in the FO diet and liver (12% diet FA, compared to 22:6n-3 at 5% in the diet); however, this is not the case in these results, as fractionation of 22:6n-3 in the liver relative to the diet suggests greater oxidation. If oxidation were a matter of FA proportions in the diet and resulted in fractionation, one would expect 18:2n-6 to have substantial fractionation relative to the other EFA due to its large proportion (9% diet FA, 11% liver FA), however this theory also does not match my results, as 18:2n-6 had non-significant fractionation with tissue assimilation. These results also do not agree with the suggestion that fractionation associated with oxidation is the result of FA essentiality and proportion in the diet, due to the inconsistencies found across the FA analyzed. Significant fractionation was only identified in 22:6n-3 of the post-prandial tissues of fish fed the FO diet and may be a result of the particular importance 22:6n-3 has in animal metabolism (Spector, 1999; Cunnane, 2003). However one would expect β-oxidation to cause 22:6n-3 in the tissue to be enriched relative to its dietary input, as isotopically-lighter FA are the preferred substrate; the opposite was found. The observed depletion of 22:6n-3 alongside the

absence of fractionation in other FA of the FO tissues is not supported by any one theory of metabolic activity that could lead to fractionation. These results indicate that mechanisms for the fractionation of individual FA may be the result of independent biochemical pathways of FA which are not fully understood.

3.4.4 CHYLOMICRONS

The uncertainty that was associated with lipid turnover in the CO post-prandial tissues of fish fed the CO diet is eliminated in the examination of chylomicron TAG-FA, as there is no influence from prior consumption of FO diet. The chylomicron FA showed no significant fractionation from the diet when examining the $\delta^{13}C$ of 18:2n-6, 20:5n-3 and 22:6n-3. Chylomicron assembly only involves hydrolysis and re-esterification of TAG, which is not expected to result in fractionation, for reasons described in Section 3.4.3 (Budge et al., Fry, 2006). Oxidation of FA does not occur during digestion and transport of chylomicrons, which eliminates this mechanism as a source of $\delta^{13}C$ variance between chylomicron FA and diet. The $\delta^{13}C$ of 18:2n-6, 20:5n-3, and 22:6n-3 in chylomicrons reflected diet more accurately than the FA of storage tissues, which confirms that the majority of fractionation must occur during the deposition, mobilization and oxidation steps. Contrarily, 18:3n-3 in chylomicrons did not maintain the same $\delta^{13}C$ as diet, and showed a substantial amount of enrichment relative to diet ($\Delta_{C\text{-}D}$=2.21). Reasons for this discrepancy could be related to digestion and absorption of individual FA; the levels of 18:3n-3 are naturally quite low in the diets of marine fish (Kirsch et al., 1998), and it may be that 18:3n-3 in chylomicrons are not absorbed to the same degree as the FA that are typically more plentiful in dietary components (Figure 2.5). These results

add to the conclusion that individual FA vary in their degree of carbon fractionation, and patterns within the same diet regime do not appear to follow a simple trend.

A recent study demonstrated that diet composition can influence the isotopic fractionation of C in the bulk tissues of goats as a result of varying degrees of fiber digestibility in mixed diets that oversupply some carbon sources (Codron et al., 2011). Similarly, the digestibility of individual FA was thought to be a source of underrepresentation of 22:1 FA proportion in the tissues of rats and pinnipeds (Thomassen et al., 1979; Cooper, 2004), which could presumably also lead to differences in C input. If such is the case in this experiment, differences in digestibility that initially arise in the proportions of chylomicron FA could influence the composition of C in the post-prandial tissues. If the dietary input consistently provides an abundance of particular FA while only providing the minimum requirements of other FA, the FA available for oxidation may cause some variability in the $\delta^{13}C$ composition across FAs that are stored in the tissues.

3.4.5 COMPARISON OF TISSUES IN POST-PRANDIAL AND FASTED STATES: CANOLA OIL DIET

No significant difference was apparent in the FA proportions of post-prandial and fasted tissues; however clear differences were present when $\delta^{13}C$ of the FA were examined. Patterns of enrichment were found for all the FA in the post-prandial liver of fish fed the CO diet with the exception of 18:2n-6, which supports the general assumption that kinetic reactions, such as β-oxidation, lead to isotopic enrichment of the substrate. These findings clearly demonstrate that CSIA can reveal information that would otherwise go unnoticed when relying only on FA analysis.

In addition to a long-term site for TAG storage, the liver can oxidize FA for energy, or transport FA out of the hepatocytes to supply energy to other tissues via VLDL particles. Therefore, the enrichment found in fasted liver in comparison to the post-prandial liver was likely due to a combination of oxidation and mobilization of FA as a result of fasting. This $\delta^{13}C$ enrichment in FA of fasted tissues was expected, as the lighter FA would be preferentially selected over isotopically heavier FA. This effect would be augmented in the liver when the fish are relying on the stored energy. The liver FA were derived from TAG molecules, whereas the muscle FA were from PL, which may explain the similarity found between the $\delta^{13}C$ of the fasted muscle tissue FA relative to the post-prandial muscle. Muscle PL is not a major contribution to β-oxidation for energy, but rather an important component of cell structure (Henderson et al., 1996); therefore, it was expected that the change in FA proportions would be minimal in comparison to TAG. The proportions of FA in the muscle would remain relatively consistent for a small period of fasting such as the time interval experienced by the pollock, as muscle FA loss generally occurs as a result of dramatic fasting periods (Kjaer, 2009). It is therefore reasonable for the $\delta^{13}C$ of the fasted and post-prandial muscle PL to be similar; however, the differences in $\delta^{13}C$ of 18:3n-3 in muscle tissue was unexpected. These large differences in $\delta^{13}C$ of 18:3n-3 were also seen in the chylomicron-diet comparison, and suggests that 18:3n-3 is an anomalous FA that may not accurately follow patterns seen in other dietary FA, perhaps due to its high proportion in the diet.

3.4.6 FASTED TISSUE COMPARISONS TO SERUM

Because FA in PL of muscle are unlikely to be mobilized during fasting, their FA $\delta^{13}C$ values were not compared to those of serum. Although VLDL secretion of TAG is

the only process by which lipid is mobilized from the hepatocytes, in most animals lipid mobilization can also occur by mobilization of FFA in adipocytes of the muscle tissue (Ballantyne et al., 1993). FFA release during fasting is typical of mammals and birds that store their fat in extrahepatic tissues (Gruffat et al., 1996; Chapman et al., 1985). Although there is some possibility for FFA release from the muscle of lean fish (Pliesetskaya, 1980; Tocher, 2003; Kjaer et al., 2009), it was assumed to be very low for fish that store major proportion of lipid in their liver. I attempted to extract FFA samples from pollock serum; however their proportions were either low or absent, which confirmed their limited importance as a source of energy in the pollock. Small concentrations of FFA can occur as a result of TAG breakdown during the laboratory analysis (Christie, 2003), and FFA extracted from the fish samples may not be associated with fasting metabolism when present in trace amounts. For these reasons, fasted tissue comparisons in this study focused on the dynamics between liver and serum to examine fractionation that occurs during VLDL secretion from the liver into the blood.

The $\delta^{13}C$ of 20:5n-3 and 22:6n-3 in the liver was enriched relative to the serum, despite the similarities in FA proportions of these tissues. Selective mobilization of the isotopically lighter FA could explain these fractionation patterns observed from fasting fish. During FA mobilization from the liver, apoB proteins attach to TAG molecules in the cytosol and are transported to the smooth endoplasmic reticulum (SER) where TAG are aggregrated into VLDL particles, after which they are transferred to the Golgi complex (Figure 3.9; Swift, 1995; Gruffat et al., 1996). Inside the Golgi, particles are either assigned to their destinations in other organelles, or to the plasma membrane for secretion (Gruffat et al., 1996; Neushwander-Tetri, 2006). This regulation is

accomplished by vesicular trafficking proteins, also known as SNARE proteins, and their attachment to VLDL in the Golgi leads to VLDL secretion outside the cell by exocytosis (Van Meer, 1989; Munro, 1998). Because this mechanism does not involve an enzymatic reaction, VLDL secretion from the liver is not a likely cause of isotope fractionation during FA mobilization. Taking this into account, initial VLDL formation in the cytosol from TAG molecules seems to be a more likely cause of C fractionation. TAG that have an overall lighter mass could be selected for apoB attachment, thus resulting in VLDL particles that are isotopically lighter than the TAG that remains as storage lipid. Alternatively, the VLDL that is formed in the ER could be preferentially assigned to exit the cell by SNARE protein selection, although this idea has not been explored. This selection must also be dependent on the FA structure, as FA were previously found to be selectively mobilized from adipocytes depending on their degree of unsaturation (Raclot, 2003) in rats and humans in the form of FFA from TAG (Raclot, 2003).

3.4.7 IMPLICATIONS

The varying degrees of fractionation found in this study are assumed to mainly be the result of selective β-oxidation of FA. The biochemical requirements of the animal for specific FA likely drives the degree of fractionation found in the FA biomarkers. Although 18:2n-6 and 18:3n-3 are considered essential in animals, these FA might be used as a source of energy more frequently than 20:5n-3 and 22:6n-3. Studies on chicks showed that 22:6n-3 is preferred over 20:5n-3 and 18:3n-3 for the development of brain and retina tissue (Anderson et al., 1990), and similar preference was found in brain lipids of juvenile turbot (Mourente et al., 1991).

Similar to the results from the FA analysis, the $\delta^{13}C$ between chylomicron and diet FA showed more similarities than did the comparisons of post-prandial tissues and diet, and I suggest that the C analysis of chylomicron FA may provide a reliable alternative to tissue analysis in the estimation of diets. As with all group comparisons conducted in my isotopic analysis, the results for fractionation across individual FA were not consistent for the chylomicrons. Consequently, some caution must be taken when choosing FA of interest for the interpretation of diet. Very little ^{13}C fractionation occurred between the liver and serum of the fasted pollock, further suggesting that blood lipids may be a convenient method of analyzing diet. The FA analysis of blood chylomicrons and VLDL (Chapter 1) also support this idea, and zonal centrifugation can be used for both the extraction of chylomicrons and VLDL (Bachorik, 1982; Babin, 1987). The post-prandial tissue FA showed a greater degree of fractionation, but are still considered useful as they provide preliminary results for the corrections necessary to $\delta^{13}C$ in fish tissues in future estimations of diet using CSIA.

This experiment has provided some insight into understanding the processes involved in the $\delta^{13}C$ fractionation of individual FA in order to apply CSIA to diet estimations. Although some confusion with endogenous C is eliminated by applying CSIA to essential FA biomarkers, questions remain to be addressed on the effects of individual FA metabolism, particularly with post-prandial tissue deposition and oxidation, where tissue fractionation was greatest. Discrimination factors determined from my experiment provide only preliminary results on the degree of fractionation that is associated with consumer metabolism, but the factors that I have calculated can be applied to specific situations where lean marine fish diets are estimated. These

discrimination factors can also be applied to simple mixing models using general sources of prey for wild fish in order to assess the contribution of prey items to their diet. The identification of items that comprise a mixed diet is feasible when isotopically distinct sources exist, such as pelagic and benthic diet items that are known to differ in ^{13}C composition (Hobson et al., 1995; Hobson et al., 2002; Søreide et al., 2006). However, the high-fat diets that were fed to these captive fish should be noted when applying the discrimination factors of individual FA to lean marine fish in the wild. I speculate that FA proportions may play a role in the degree to which FA are oxidized, and the proportions of FA used in experimental diets were different from the natural diet of wild fish, which are likely to have a much lower level of lipid in their diet. If the diet composition does indeed influence the fractionation of ^{13}C (Codron et al., 2011), difficulties would arise when applying the discrimination factors from a captive feeding experiment to another study. Additionally, the degree to which individual FA are oxidized might play an independent role in fractionation, which further complicates interpretation. The discrimination factors calculated from captive pollock fed a high-fat diet should therefore be used with caution when estimating the diets of wild fish. However, this is the first experiment that examined the discrimination factors for marine fish and this data is should be considered a useful set of preliminary results. Because *de novo* synthesis and FA modification were limited in the FA biomarkers chosen for my analysis, the varying degrees of fractionation found in FA illustrate the activity that dietary compounds can undergo during consumer metabolism. These results are evidence that the $\delta^{13}C$ of diet is not the result of an equally weighted $\delta^{13}C$ contribution of all the dietary items in a

consumer, an assumption that both bulk and compound-specific analyses have relied on to date.

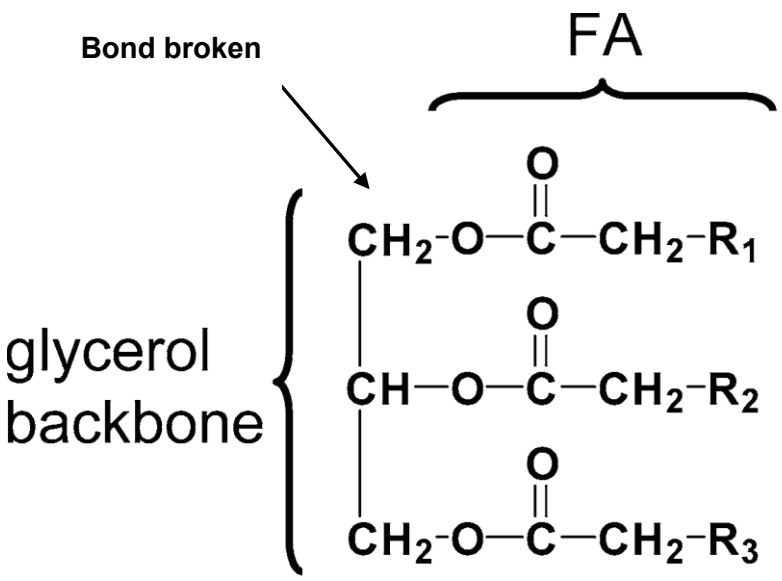

Figure 3.8. A triacylglycerol molecule taken from Budge et al., 2006; which demonstrates the bond between FA and glycerol (indicated by the arrow) that is broken during lipid hydrolysis.

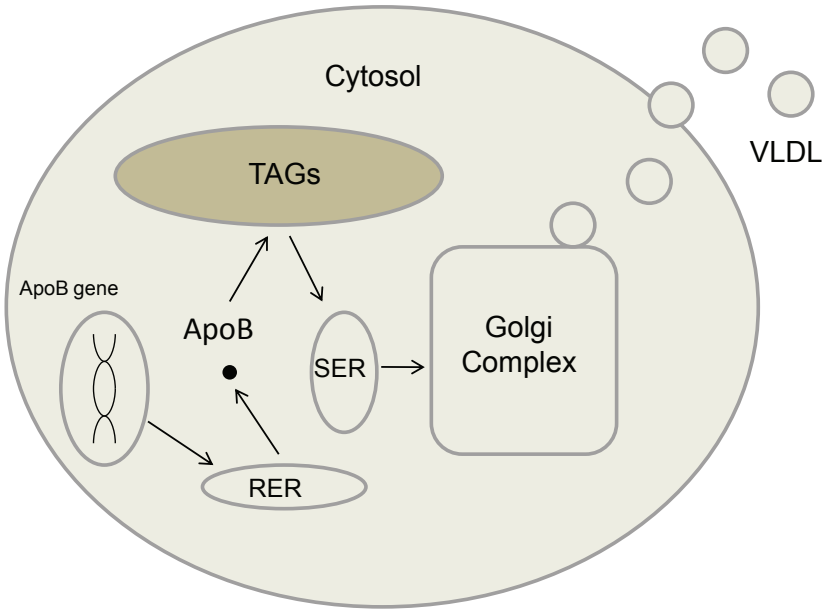

Figure 3.9. Hepatocyte diagram adapted from Gruffat et al. (1996) demonstrating apoB protein production in the rough endoplasmic reticulum (RER), attachement to triacylglycerols, transfer to the smooth endoplasmic reticulum (SER), and transfer to the Golgi complex prior to VLDL secretion.

CHAPTER 4.0 GENERAL CONCLUSION

Rather than establishing a method to estimate wild animal diets, the overall objective of feeding experiments was to provide preliminary data on the variation in dietary FA proportions and $\delta^{13}C$ fractionation upon assimilation in a consumer. These experiments aim to improve the current models that are used for diet estimation of wild animals. The results indicate that the oxidation rates of FA are likely influenced by the animal's diet, resulting in variable $\delta^{13}C$ fractionation. More captive feeding experiments will be required to test my results and to accurately apply discrimination factors to wild animals, especially when the dietary input and feeding state of the animal differs from a laboratory setting. An interesting expansion of my study would involve the use of mixed diets to more precisely determine how diet composition affects oxidation and consequently $\delta^{13}C$ fractionation of diet FA.

There are a number of areas where improvements could be made to the experimental design and future work should take these into account. The experimental fish received a high-fat diet; the impact of that on the overall fractionation of $\delta^{13}C$ from diet to tissue is unknown and may differ from the degree of fractionation that would occur in the wild. It is recommended that future studies examine the fractionation that results from more relevant diets. The effects of fasting following a lower fat diet are also unknown and should be considered to determine the accuracy of tissue to serum fractionation.

Ideally, I would have organized the treatments such that the post-prandial feeding experiments were done concurrently, rather than subjecting the CO-assigned fish to the FO feed prior to their feeding trial; however, this was not possible due to logistics with

animal care facilities. Theoretically, if feeding experiments were conducted concurrently, the discrimination factors for diet and tissue samples could have been compared to determine dietary effects on isotopic fractionation, and the CO tissues would have no residual signature from the FO diet. In addition, lipid samples from the pollock upon arrival into the aquarium facility should have been collected to serve as initial samples; this would have allowed me to confirm dietary lipid turnover in the tissues.

CSIA is costly and arguably time consuming, and for these reasons many researchers are skeptical about using CSIA as an alternative or even complementary technique to bulk analysis. Bulk analysis is considered convenient and cost effective for the examination of stable isotopes, as the extent of laboratory preparation is limited to lipid extraction, drying and grinding prior to combustion of the tissue sample. However, the laboratory methods and associated time for CSIA are essentially equivalent to those required for FA analysis; therefore, this should not deter ecologists that are interested in maximizing the amount of diet information that can be derived from tissue samples. The examination of individual essential FA can provide a more direct link to their dietary source, for the simple reason that less biochemical alteration has occurred to FA biomarkers. Bulk analysis relies on the assumption that the material chosen for analysis is relatively similar to its original diet composition, while CSIA eliminates the need to make this assumption. Unlike bulk analysis, with CSIA the contribution of biosynthesized compounds to the $\delta^{13}C$ signal can be eliminated; therefore, the $\delta^{13}C$ signal of interest is not masked by other material. Future experiments that are designed to examine the $\delta^{13}C$ fractionation in FA should consider comparisons to the $\delta^{13}C$ of the appropriate bulk material to confirm the accuracy of sample analyses.

Despite the limitations that prevented the direct comparison of feeding experiments, along with the issues with diet turnover, successful comparisons were possible between the tissue groups of fish from the same treatment. I was able to reliably determine the discrimination factors for isotope fractionation of pollock liver from FO diet, along with chylomicron fractionation from CO diet, and serum fractionation from CO fasted liver. The discrimination factors that I have estimated for pollock FA metabolism could eventually be used to delineate food webs when more data is collected on the isotope fractionation associated with the metabolism of zooplankton and higher animals. This is necessary background information in order to begin to use CSIA to estimate diets in wild fish and represents a starting point for other studies investigating discrimination in $\delta^{13}C$ of FA from diet to tissue.

REFERENCES

Ackman, R.G. 1989. *Marine Biogenic Lipids, Fats, and Oils,* Volume 1. CRC Press, Inc. Florida.

Alkanani et al., 2005. Lipid class and nonesterified fatty acid profiles in plasma of North Atlantic cod *(Gadus morhua). Can. J. Fish. Aquat. Sci,* 62(11): 2509-2518.

Anderson, G.J., Connor, W.E., Corliss, J.D. 1990. Docosahexaenoic acid is the preferred dietary n-3 fatty acid for the development of the brain and retina. *Pediatric Research*, 27(1): 89-96.

Arnold-Reed, D.E., Bentley, P.J., Phan, C.T., Redgrave, T.G. 1996. The clearance of lipid from the plasma of a teleost fish, the black bream (*Acanthopagrus butcheri*). *Comp. Biochem. Physiol.,* 116A(2): 167-172.

Austreng, E., Skrede, A., Eldegard, A. 1980. Digestibility of fat and fatty acids in rainbow trout and mink. *Aquaculture,* 19(1): 93-95.

Babin, P.J. 1987. Plasma lipoprotein and apolipoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *Biochem. J.,* 246: 425-429.

Bachorik, P.S., Albers, J.J., Ellefson, R.D., Kane, J.P., Wood, P.D. 1982. Collection of blood samples for lipoprotein analysis. *Clin. Chem.,* 28(6): 1375-1378.

Ballantyne, J.S., Glemet, H.C., Chamerlin, M.E., Singer, T.D. 1993. Plasma nonesterified fatty acids of marine teleost and elasmobranch fishes. *Marine Biology,* 116(1) 47-52.

Bell, M.V., Henderson, R.J., and Sargent, J.R. 1985. Changes in the fatty acid composition of phospholipids from turbot (*Scophthalmus maximus*) in relation to dietary polyunsaturated fatty acid deficiencies. *Comp. Biochem. Physiol.,* 81B(1): 193-198.

Bigeleisen, J. and M. G. Mayer (1947). Calculation of equilibrium constants for isotopic exchange reactions. *Journal of Chemical Physics* 15: 261-267.

Bigeleisen, J. and M. Wolfsberg (1958). Theoretical and experimental aspects of isotope effects in chemical kinetics. *Advances in Chemical Physics*. I. Prigogine. New York, Interscience Publishers, Inc. 1: 15-76.

Black, D. and Love, R.M. 1986(b). The sequential mobilization and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. *J. Comp. Physiol. B*, 156: 469-479.

Black, D., and Skinner, R. 1986 (a). Features of the lipid transport system of fish as demonstrated by studies on starvation in rainbow trout. *J. Comp. Physiol. B* (156): 497-502.

Boschker, H.T.S., Middelburg, J.J. 2002. Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiology Ecology,* 40: 85-95.

Bowling, D.R., Pataki, D.E., Randerson, J.T. 2008. Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. *New Phytologist*, 178(1): 24-36.

Brawn, V.M. 1961. Reproductive behaviour of the cod (*Gadus callarias L.*). *Behaviour,* 18(3): 177-198.

Buckley, L.J., Lough, R.G. 1987. Recent growth, biochemical composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. *Can. J. Fish. Aquat. Sci.,* 44(1): 14-25.

Budge, S.M., Iverson, S.J., Bowen, W.D., Ackman, R.G. 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.,* 59: 886-898.

Budge S.M., Iverson, S.J., Koopman, H.N. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Marine Mammal Science,* 22(4): 759-801.

Budge, S.M., Springer, A.M., Iverson, S.J., Sheffield, G. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. *Marine Ecology Progress Series*, 336: 305-309.

Budge, S.M., Springer, A.M., Iverson, S.J., Sheffield, G., Rosa, C. 2008a. Blubber fatty acid composition of bowhead whales, Balaena mysticetus: Implications for diet assessment and ecosystem monitoring. *Journal of Experimental Marine Biology and Ecology,* 359: 40-46.

Budge, S.M., Wooller, M.J., Springer, A.M., Iverson, S.J., McRoy, C.P., Divoky, G.J. 2008b. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia,* 157:117-129.

Budge, S.M., Wang, S.W., Hollmén, T.E., and Wooller, M.J. 2011. In review. Carbon isotopic fractionation in eider adipose tissue varies with fatty acid structure: Implications for trophic studies.

Campbell, M.K., and Farrell, S.O. 2009. *Biochemistry*. 6th Edition, Thomson Brooks/Cole. Belmont, CA, USA.

Carey, E.M., and Dils, R. 1972. The pattern of fatty acid synthesis in lactating rabbit mammary gland studied *in vivo*. *Biochem. J.,* 126(4): 1005-1007.

Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P. 2004. Lipid content and carbon assimilation in Collembola: implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia,* 139: 325-335.

Chapman, M.J. and Forgez, P. 1985. Lipid transport systems: some recent aspects in swine, cattle and trout during development. *Reprod. Nutr. Dévelop.,* 25(1B): 217-226.

Christie, W.W. 2003. *Lipid Analysis*. Oily Press, Dundee, Scotland.

Clarke, K., Warwick, R. 1994. Change in marine communities: an approach to statistical analysis and interpretation. 2nd edition, Primer-E Ltd, Plymouth.

Codron, D., Codron, J., Sponheimer, M., Bernasconi, S.M., Clauss, M. 2011. When animals are not quite what they eat: diet digestibility influences 13C-incorporation rates and apparent discrimination in a mixed-feeding herbivore. *Can. J. Zool.* 89: 453-465.

Cooper, M.H. 2004. Fatty acid metabolism in marine carnivores: implications for quantitative estimation of predator diets. Ph.D. Thesis. Dalhousie University, Halifax, Nova Scotia.

Cooper et al., 2005. Dynamics of blood chylomicron fatty acids in a marine carnivore: implications for lipid metabolism and quantitative estimation of predator diets. *J. Comp. Physiol.,* 175(B): 133-145.

Cooper M.H., S.M. Budge, A.M. Springer, and G. Sheffield. 2009. Resource partitioning by sympatric pagophilic seals in Alaska: monitoring effects of climate variation with fatty acids. *Polar Biology,* 32: 1137–1145.

Copeman, L.A., Parrish, C.C., Gregory, R.S., Jamieson, R.E., Wells, J., Whiticar, M.J. 2009. Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua. Marine Ecology Progress Series,* 386: 237-251.

Corey Feed Mills, 2010. http://www.coreyaqua.ca/page/AquaBrood. Retrieved on May 15, 2011.

Crawford, K., Mcdonald, RA, Bearhop, S. 2008. Application of stable isotope techniques to the ecology of mammals. *Mammal Review,* 38(1): 87-107.

Crockett, E.L. and Sidell, B.D. 1993. Substrate selectivities differ for hepatic mitochondrial and peroxisomal β-oxidation in an Antarctic fish, *Notothenia gibberifrons*. *Biochem. J.,* 289: 427-433.

Cunnane, S.C. 2003. Problems with essential fatty acids: time for a new paradigm? *Progress in Lipid Research:* 42: 544-568.

Davis, R.A. 1991. Lipoprotein structure and secretion. In: Vance DE, Vance J (eds) Biochemistry of lipids, lipoproteins and membranes. Elsevier, Amsterdam, pp 403-426.

Dahl, T.M., Falk-Petersen, S., Gabrielsen, G.W., Sargent, J.R., Hop, H., Millar, R.M. 2003. Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: a trophic study from an Arctic fjord. *Marine Ecology Progress Series,* 256: 257-269.

Dos Santos, J., Barkow, I.C. and Jobling, M. 1993. Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fish-based feeds. *Aquaculture,* 110: 173-189.

DeNiro, M.J., Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta,* 42: 495-506.

Eaton, S., Bartlett, K., Pourfarzam, M. 1996. Mammalian mitochondrial beta-oxidation. *Biochem. J.,* 320(2): 345-357.

Evershed, R.P.Bull, I.D., Corr, L.T., Crossman, Z.M., Dongen, B.E., Evans, C.J., Jim, S., Mottram, H.R., Mukherjee, A.J., Pancost, R.D. 2007. Compound-specific stable isotope analysis in ecology and paleoecology. In: *Stable Isotopes in Ecology and Environmental Science*, 2nd Ed., Blackwell Publishing Ltd, UK.

Falk-Petersen, S., Hagen, W., Kattner, G., Clarke, A., Sargent, J. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Can. J. Fish. Aquat. Sci.,* 57(3): 178-191.

Fantle, M. S., A. I. Dittel, et al. (1999). A food web analysis of the juvenile blue crab, Callinectes sapidus, using stable isotopes in whole animals and individual amino acids. *Oecologia,* 120(3): 416-426.

Farquhar, G.D., Ehleringer, J.R., Hubik, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 40: 503-537.

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

Frank, F.R. Chicken feed composition. US Patent, 4310522. January 12, 1982

Froyland, L., Madsen, L., Eckhoff, K.M., Oyvind, L., and Berge, R.K. 1998. Carnitine palmitoyltransferase I, carnitine palmitoyltransferase II, and acyl-CoA oxidase activities in Atlantic salmon (*Salmo salar*). *Lipids*, 33(9): 923- 931.

Fry, B. 1981. Natural stable carbon isotope tag traces Texas shrimp migrations. *Fishery Bulletin,* 79(2): 337-345.

Fry, B. 2006. *Stable Isotope Ecology.* Springer Science + Business Media, LLC, USA.
Gannes, L.Z., O'Brien, D.M., Martinez del Rio, C. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecological Society of America,* 78(4): 1271-1276.

Gelman, A. and Stern, H. 2006. The difference between "significant" and "not significant" is not itself statistically significant. *The American Statistician,* 60(4): 328-331.

Gruffat, D. et al.,1996. Regulation of VLDL synthesis and secretion in the liver. *Reprod. Nutr. Dev.,* 36: 375-389.

Hammer, B.T., Fogel, M.L., Hoering, T.C. 1998. Stable carbon isotope ratios of fatty acids in seagrass and redhead ducks. *Chemical Geology,* 152: 29-41.

Havel, R.J., Kane, J.P., Balasse, E.O., Segel, N., Basso, L.V. 1970. Splanchic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.,* 49: 2017-2035.

Henderson, R.J. and Sargent, J.R.1985. Chain-length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in livers of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* 82B(1): 79-85.

Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Archives of Animal Nutrition,* 49(1): 5-22.

Hobson, K.A. 1995. Reconstructing avian diets using stable carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. *Condor* 97: 752-762.

Hobson, K.A., Ambrose, W.G. Jr., Renaud, P.E. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: insights from delta 13C and delta 15N analysis. MEPS: 128: 1-10.

Hobson, K.A., Schell, D.M., 1998. Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales (*Balaena mysticetus*). *Can. J. Fish. Aquat. Sci.,* 55: 2601-2607.

Hobson, K.A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia,* 120: 314-326.

Hobson, K.A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M. 2002. A stable isotope (δ13C, δ15N) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep Sea Res II:* 49: 5131-5150.

Hooker, S.K., Iverson, S.J., Ostrom, P., Smith, S.C. 2001. Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Canadian Journal of Zoology,* 79(8): 1442-1454.

Hooker, S.K., Whitehead, H., Gowans, S. 2002. Ecosystem consideration in conservation planning: energy demand of forgaing bottlenose whales (*Hyperoodon ampullatus*) in a marine protected area. *Biological Conservation,* 104(1): 51-58.

Howland, M.R., Corr, L.T., Young, S.M.M., Jones, V., Jim, S., Van Der Merwe, N.J., Innis, S.M., and Dyer, R.A. 2003. Brain astrocyte synthesis of docosahexaenoic acid from n-3 fatty acids is limited at the elongation of docosapentaenoic acid. *J. Lipid Research*, 43: 1530- 1536.

Iverson S.J., Arnould, J.P.Y., Boyd, I.L. 1997. Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals. *Can. J. Zool.,* 75:188-197.

Iverson, S.J., Field, C., Bowen, W.D., Blanchard, W. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs*, 74(2): 211-235.

Iverson, S.J., Stirling, I., Lang, S.L.C. 2006. Spatial and temporal variation in the diets of polar bears across the Canadian Arctic: indicators of changes in prey populations and environment. *Top Predators in Marine Ecosystems,* Cambridge University Press, UK.

Iverson, S.J., Springer, A.M., Kitaysky, A.S. 2007. Seabrids as indicators of food web structure and ecosystem variability: qualitative and quantitative diet analyses using fatty acids. *Mar. Ecol. Prog. Ser.,* 352: 235-244.

Jim, S., Ambrose, S.H., Evershed, R.P. 2003. Natural abundance stable carbon isotope evidence for the routing and de novo synthesis of bone FA and cholesterol. *Lipids,* 38(2): 179-186.

Jobling, M., Leknes, O., Saether, B., Bendiksen, E.A. 2008. Lipid and fatty acid dynamics in Atlantic cod, *Gadus morhua*, tissues: influence of dietary lipid concentrations and feed oil sources. *Aquaculture:* 281: 87-94.

Jump, D.B. 2002. The biochemistry of n-3 polyunsaturated fatty acids. *The Journal of Biological Chemistry,* 227(11): 8755-8758.

Käkelä, R., Furness, R.W., Kahle, S., Becker, P.H., Käkelä, A. 2009. Fatty acid signatures in seabird plasma are a complex function of diet composition: a captive feeding trial with herring gulls. *Functional Ecology*, 23: 141-149.

Karmen, A., Whyte, M., Goodman, D.S. 1963. Fatty acids esterification and chylomicron formation during fat absorption: 1. Triglycerides and cholesterol esters. *J. Lipid Research,* 4(3): 312-321.

Karpe et al., 1998. Lipoprotein lipase transport in plasma: role of muscle and adipose tissues in regulation of plasma lipoprotein lipase concentrations. *J. Lipid Research,* 39: 2387-2393.

Kiessling, K.H., Kiessling, A. 1993. Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. *Can. J. Zool.,* 71: 248-251.

Kirsh et al., 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci,* 55: 1378-1386.

Kirsch, P.E. et al., 2000. Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). *Physiological and Biochemical Zoology*, 73(1): 45-59.

Kjer et al., 2009. Characterization of lipid transport in Atlantic cod (Gadus monhua) when fasted and fed high or low fat diets. *Aquaculture,* 288: 325-336.

Kurle, C.M. 2009. Interpreting temporal variation in omnivore foraging ecology via stable isotope modeling. *Functional Ecology,* 23(4): 733-744.

Lall, S.P., Nanton, D.A., Tibbets, S.M., Roy, P.K. and Milley, J.E. 2003. Nutrient requirements and feeding of haddock. Bull. Aquac. Assoc. Canada Special Publ. No. 7, pp. 79-86.

Libes, S.M. 1992. Reading the sedimentary record: the use of stable isotopes in the study of paleoceanography. *Introduction to Marine Biogeochemistry*, Wiley & Sons Inc., NY.

Lichtfouse, E. 2000. Compound-specific isotope analysis (CSIA). Application to archaeology, biomedical sciences, biosynthesis, environmental, extraterrestrial chemistry, food science, forensic science, humic substances, microbiology, organic geochemistry, soil science and sport. *Rapid Communications in Mass Spectrometry.* 14: 1337- 1344.

Lie, O., Lied, E., and Lambertsen, G. 1986. Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. *Aquaculture,* 59(3-4): 187-196.

MacAvoy, S.E., Macko, S.A., Carney, R.S. 2003. Links between chemosynthetic production and mobile predators on the Louisiana continental slope: stable carbon isotopes of specific fatty acids. *Chemical Geology,* 201: 229-237.

MacInnis, M.L., Vavra, M., Krueger, W.C. 1983. A comparison of four methods used to determine the diets of large herbivores. *Journal of Range Management,* 36(3): 302-306.

MacKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G., Randall, D.J. 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (Salmo salar) in seawater. *Fish Physiology and Biochemistry,* 19: 111-122.

Madsen, L., Rustan, A.C., Vaagenes, H., Berge, K., Dyroy, E., Berge, R.K. 1999. Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference. *Lipids*, 34(9): 951-963.

Mannaerts, G.P., Debeer, L.J., Thomas, J., DeSchepper, P.J. 1978. Mitochondrial and peroxisomal fatty acid oxidation in liver homogenates and isolated hepatocytes from control and clofibrate-treated rats. *The Journal of Biological Chemistry*, 254(11): 4585-4595.

Mansbach, C.M. and Parthasarathy, S. 1982. A re-examination of the fat of glyceride-glycerol in neutral lipid absorption and transport. *Journal of Lipid Research,* 23: 1009-1019.

Mansbach, C.M. and Nevin, P. 1998. Intracellular movement of triacylglycerols in the intestine. *Journal of Lipid Research*, 39: 963-968

Mansbach, C.M., Siddiqi, S.A. 2010. The biogenesis of chylomicrons. *Annu. Rev. Physiol.* 72: 315-333.

Marshall, J.D., Brooks, J.R., Lajtha, K. 2007. Sources of variation in the stable isotopic composition of plants. In: *Stable Isotopes in Ecology and Environmental Science,* 2nd ed. Blackwell Publishing Ltd., UK.

Maruyama, A., Yamada, Y., Rusuwa, B. 2001. Stable nitrogren and carbon isotope ratios as migration tracers of a landlocked goby, *Rhinogobius* sp. (the orange form), in the Lake Biwa water system. *Ecological Research,* 16: 697-703.

McMahon, K.W., Fogel, M.L., Elsdon, T.S., Thorrold, S.R. 2010. Carbon isotope fractionation of amino acids in fish muscle reflect biosynthesis and isotopic routing from dietary protein. *Journal of Animal Ecology,* 79: 1132-1141.

Meier-Augenstein, W. 2002. Stable isotope analysis of fatty acids by gas chromatography-isotope ratio mass spectrometry. *Analytica Chimica Acta*, 465: 63-79.

Mellbrand, K., Hamback, P.A. 2010. Coastal niches for terrestrial predators: a stable isotope study. *Can. J. Zool.* 88: 1077-1085.

Menoyo, D., Izquierdo, M.S., Robaina, L., Ginés, R., Lopez-Bote, C.J., Bautista, J.M. 2004. Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. *British Journal of Nutrition,* 92: 41-52.

Michener, R.H. and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. *Stable Isotopes in Ecology and Environmental Science.* Blackwell Scientific Publications Inc., Cambridge, MA: Lajtha and Michener.

Michener, R.H., Kaufman, L. 2007. Stable isotope ratios as tracers in marine food webs: an update. From *Stable Isotopes in Ecology and Environmental Science*, 2nd Edition. Blackwell Publishing Ltd, UK: Lajtha and Michener.

Mills, G.L., Lane, P.A., Weech, P.K. 1984. The isolation and purification of plasma lipoproteins. From *Laboratory Techniques in Biochemistry and Molecular Biology: A guidebook to lipoprotein technique.* Elsevier Science Publishers B.V., Amsterdam, Netherlands.

Mitchell, A.D., Evershed, R.P. 2003. Expression of the dietary isotope signal in the compound-specific $\delta^{13}C$ values of pig bone lipids and amino acids. *Int. J. Osteoarchaeol.,* 13: 54-65.

Munro, S. 1998. Localization of proteins to the Golgi apparatus. *Cell Biology,* 8: 11-15.

Mourente, G., Tocher, D.G., Sargent, J.R. 1991. Specific accumulation of docosahexaenoic acid (22:6n-3) in brain lipids during development of juvenile turbot *Scopthalmus maximus* L. *Lipids*, 26(11): 871-877.

Nanton, D.A., Lall, S.P., McNiven, M.A. 2001. Effects of dietary lipid level on liver and muscle lipid deposition in juvenile haddock, *Melanogrammus aeglefinus* L. *Aquaculture Research*, 32(1): 225-234.

Napolitano, G.E., Pollero, R.J., Gayoso, A.M., Macdonald, B.A., Thompson, R.J. 1997. Fatty acids as trophic markers of phytoplankton blooms in the Bahía Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology*, 25(8) 739-755.

National Marine Fisheries Service, NOAA. FishWatch, U.S. Seafood Facts: http://www.nmfs.noaa.gov/fishwatch/species/atl_pollock.htm. Retrieved on June 9 2011.

Neat, C.E., Thomassen, M.S., and Osmundsen, H. 1981. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. *Biochem. J.* 196: 149-159.

Neuschwander-Tetri, B.A. 2006. Fatty liver, non-alcoholic steatohepatitis.From *Comprehensive Clinical Hepatology*. Editors: Bacon, B.R. and O'Grady, J.G. Elsevier Ltd, Philadephia, USA.

Nieminen, P., Rouvinen-Watt, K., Collins, D., Grant, J., Mustonen, A.M. 2006. Fatty acid profiles and relative mobilization during fasting in adipose tissue depots of the American marten (*Martes americana*). *Lipids* 41, 231-240.

Nordstrom, C.A. et al., 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbor seals *Phoca vitulina richardsi* in captive feeding studies. *Marine Ecology Progress Series,* 360: 245-263.

Oczkowski, A.J., Pilson, M.E., Nixon, S.W. 2010. A marked gradient in $\delta^{13}C$ values of clams *Mercenaria mercenaria* across a marine embayment may reflect variations in ecosystem metabolism. *Marine Ecology Progress Series,* 414: 145-153.

O'Leary, M.H. 1988. Carbon isotopes in photosynthesis. *BioScience,* 38(5): 328-336.

Olsen, R.E., Henderson, R.J., and Pedersen, T. 1991. The influence of dietary lipid classes on the fatty acid composition of cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. *Journal of Experimental Marine Biology and Ecology,* 148(1): 59-76.

Olsen, R.E., Henderson, R.J. 1997. Muscle fatty acid composition and oxidative stress indices of Arctic charr, Salvelinus alpinus (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition,* 3: 227-238.

Osmundsen, H. 1981. Spectrophotometric procedure for measuring mitochondrial β-oxidation. *Methods in Enzymology*, 72: 306-314.

Penney, S.N. 2010. Estimating diet in Atlantic salmon (*Salmo salar*) with Quantitative Fatty Acid Signature Analysis. M.Sc. Thesis, Dalhousie University, Halifax, NS, Canada.

Peterson, B.J. and Fry, B. 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18: 293-320.

Plisetskaya, E. 1980. Fatty acid levels in blood of cyclostomes and fish. *Env. Biol. Fish.,* 5: 273-290.

Section 1.01 Podoliak, H. 1961. Relation between water temperature and metabolism of dietary phosphorus by fingerling brook trout. *Transactions of the American Fisheries Society,* 90(4): 398-403.

Pond, C.M., Gilmour, I. 1997. Stable isotopes in adipose tissue fatty acids as indicators of diet in arctic foxes *(Alopex lagopus). Proceedings of the Nutrition Society,* 56: 1067-1081.

Pond, D.M., Bell, M.V., Dixon, D.R., Fallick, A.E., Segonzac, M., Sargent, J.R. 1998. Stable-carbon-isotope composition of fatty acids in hydrothermal vent mussels containing methanotrophic and thiotrophic bacterial endosymbionts. *Applied and Environmental Microbiology*, 64(1): 370-375.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* 83(3): 703-718.

Pond, D.W., Leakey, R.L.G., Fallick, A.E. 2006. Monitoring microbial predator-prey interactions: an experimental study using fatty acid biomarker and compound-specific stable isotope techniques. *Journal of Plankton Research*, 28(4): 419-427.

Raclot, T. 2003. Selective mobilization of fatty acids from adipose tissue triacylglycerols. *Progress in lipid research,* 42: 257-288.

Ruess, N., Poulsen, L.K. 2002. Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. *Marine Biology,* 141: 423-434.

Ruess, L., Tiunov, A., Haubert, D., Richnow, H.H., Haggblom, M.M., Scheu, S. 2005. Carbon stable isotope fractionation and trophic transfer of fatty acids in fungal based soil food chains. *Soil Biology & Biochemistry,* 37: 945-953.

Saether, O., Ellingsen, T.E., Mohr, V. 1986. Lipids of North Atlantic krill. *Journal of Lipid Research,* 27: 274-285.

Saito, H., Ishihara, K., Murase, T. 1996. Effect of prey fish lipids on the docosahexaenoic acid content of total fatty acids in the lipid of *Thunnus albacores* yellow fin tuna. *Bioscience, biotechnology, and biochemistry,* 60(6): 962-965.

Sargent, S.R., Bell, J.G., Bell, M.V., Henderson, R.J., and Tocher, D.R. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. From Aquaculture: fundamental and applied research. American Geophysical Union, USA.

Sargent, J.R., Tocher, D.R., Bell, J.G. 1989. The lipids. From *Fish Nutrition*, Third Edition. Elsevier Science, USA.

Schell, D.M., Saupe, S.M., Haubenstock, N. 1989. Bowhead growth and feeding as indicated by $\delta^{13}C$ techniques. *Mar Biol,* 103: 433-443.

Schell, D.M., Barnett, B.A., Vinette, K.A. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. *Marine Ecology Progress Series,* 162: 11-23.

Scott, W.B. and Scott, M.G. 1988. *Atlantic Fishes of Canada.* University of Toronto Press, Toronto, ON.

Shen, H., Howles, P., Tso, P. 2001. From interaction of lipidic vehicles with intestinal epithelial cell membranes to the formation and secretion of chylomicrons. *Advanced Drug Delivery Reviews,* 50: S103-S125.

Sidell, B.D., Crockett, E.L., Driedzic, W.R. 2005. Antarctic fish tissues preferentially catabolize monoenoic fatty acids. *Comparative Physiology and Biochemistry,* 271(2)L 73-81.

Smedbol, R.K., Stephenson, R. 2001. The importance of managing within-species diversity in cod and herring fisheries of the north-western Atlantic. *Journal of Fish Biology,* 59: 109-128.

Smith, R.J., Hobson, K.A., Koopman, H.N., Lavigne, D.M. 1996. Distinguishing between populations of fresh- and salt-water harbor seals (Phoca vitulina) using stable-isotope ratios and fatty acid profiles. *Can. J. Fish. Aquat. Sci,* 53: 272-279.

Søreide, J.E., Hop, H., Carroll, M.L., Falk-Petersen, S., Hegseth, E.N. 2006. Seasonal food web structures and sympagic-pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. *Prog. Oceanogr.* 71: 59-87.

Spector, A.A. 1999. Essentiality of fatty acids. *Lipids,* 34: S1- S3.

Springer, A.M., Byrd, G.V., Iverson, S.J. 2007. Hot oceanography: planktivorous seabirds reveal ecosystem responses to warming of the Bering Sea. *Marine Ecology Progress Series,* 352: 289-297.

St. John, M.A., and Lund, T. 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series,* 131: 75-85.

Sulzmann, E.W. Stable isotope chemistry and measurement. *Stable Isotopes in Ecology and Environmental Science*, 2nd ed. Blackwell Publishing Ltd, UK.

Sweeting, C.J., Polunin, N.V.C., and Jennings, S. 2006. Effects of chemical lipid extractions and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Commun. Mass Spectrom.* 20: 595-601.

Swift, L. 1995. Assembly of very low density lipoproteins in rat liver: a study of nascent particles recovered from the rough endoplasmic reticulum. *Journal of Lipid Research,* 36: 395-406.

Thiemann, G.W., Budge, S.M., Iverson, S.J., Stirling, I. 2007. Unusual fatty acid biomarkers reveal age- and sex-specific foraging in polar bears (*Ursus maritimus*). *Can. J. Zool.* 85: 505-517.

Thomassen, M.S., Strom, E., Christiansen, E.N., Norum, K.R. 1979. Effect of marine oil and rapeseed oil on composition of fatty acids in lipoprotein triacylglycerols from rat blood plasma and liver perfusate. *Lipids,* 14(1): 58-65.

Tocher, D.R., Ghioni, C. 1999. Fatty acid metabolism in marine fish: low activity of fatty acyl Δ5 desaturation in gilthead sea bream (*Sparus aurata*) cells. *Lipids,* 34(5): 433-440.

Tocher, D.R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science,* 11(2): 107-184.

Torstensen, B.E., Lie, O., Froyland, L. 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)- effects of capelin oil, palm oil, and oleic-acid enriched sunflower oil as dietary lipid sources. *Lipids,* 35(6): 653-664.

Trippel, E.A. 1999. Estimation of stick reproductive potential: history and challenges for Canadian Atlantic gadoid stock assessments. *J. Northw. Atl. Fish. Sci.,* 25: 61-81.

Trushenski, J.T., Lewis, H.A., Kohler, C.C. 2008. Fatty acid profile of sunshine bass: II. Profile change differs among fillet lipid classes. *Lipids,* 43: 643-653.

Tso, P., Balint, J.A. 1986. Formation and transport of chylomicrons by enterocytes to the lymphatics. Am. J. Physiol. *Gastrointest. Liver Physiol.* 250: G715-G726.

Turchini, G.M., Mentasti, T., Froyland, L., Orban, E., Caprino, F., Moretti, V.M., Valfre, F. 2003. Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). *Aquaculture,* 225: 251-267.

Van Meer, G. 1989. Lipid traffic in animal cells. *Annu. Rev. Cell Biol.* 5: 247-275.

Voight, C.C., Rex, K., Michener, R.H., Speakman, J.R. 2008. Nutrient routing in omnivorous animals tracked by stable carbon isotopes in tissue and exhaled breath. *Physiological Ecology,* 157(1): 31-40.

Whyte, M., Karmen, A., Goodman, D.S. 1963. Fatty acid esterification and chylomicron formation during fat absorption: 2. Phospholipids. *J.Lipid Research,* 4(3): 322-329.

Yehuda, S. 2003. Omega-6/omega-3 ratio and brain-related functions. Omega-6/Omega-3 Fatty Acid Ratio: The Scientific Evidence. *World Review of Nutrition and Dietetics,* Vol 92. Editor: A.P. Simopoulos. Karger AG, Basel, Switzerland.

Yonezaki S., Kiyota, M., Baba, N., Koido, T., Takemura, T. 2003. Size distribution of the hard remains of prey in the digestive tract of northern fur seal *(Callorhinus ursinus)* and related biases in diet estimation by scat analysis. *Mammal Study,* 28: 97-102.

Zimmerman, R. et al., 2004. Fat mobilization in adipose tissue is promoted by adipose tissue triglyceride lipase. *Science,* 306(5700): 1383-1386.

APPENDIX A

FATTY ACID PROPORTIONS FROM EXPERIMENTS

FA	FO Diet		Muscle PL (FO, Post-prandial)		Liver TAG (FO, Post-prandial)	
	Mean	SD	Mean	SD	Mean	SD
i-14:0	0.03	0.00	0.06	0.02	0.01	0.01
14:0	5.61	0.06	0.60	0.13	2.27	0.14
14:1n-9	0.09	0.01	0.01	0.01	0.09	0.03
14:1n-7	0.04	0.00	0.05	0.02	0.05	0.01
14:1n-5	0.10	0.00	0.01	0.02	0.08	0.01
i-15:0	0.13	0.00	0.05	0.03	0.09	0.01
15:0	0.34	0.01	0.13	0.01	0.20	0.02
15:1n-8	0.00	0.00	0.05	0.03	0.03	0.03
i-16:0	0.05	0.01	0.16	0.15	0.05	0.01
16:0	18.39	0.16	16.87	0.86	14.18	0.35
16:1n-11	0.27	0.00	0.21	0.01	0.28	0.03
16:1n-9	0.29	0.00	0.26	0.03	0.43	0.03
16:1n-7	7.39	0.05	1.26	0.25	5.91	0.18
16:1n-5	0.16	0.01	0.19	0.02	0.13	0.01
17:1(a)	0.02	0.02	0.06	0.01	0.04	0.01
i-17:0	0.14	0.02	0.08	0.01	0.14	0.03
16:2n-6	0.15	0.00	0.01	0.01	0.07	0.01
ai-17:0	0.05	0.00	0.08	0.01	0.11	0.01
17:1(b)	0.13	0.01	0.16	0.02	0.15	0.03
16:2n-4	0.87	0.00	0.11	0.04	0.34	0.05
17:0	0.29	0.00	0.17	0.01	0.19	0.01
Phytane	0.10	0.01	0.03	0.07	0.04	0.02
16:3n-4	1.12	0.01	0.04	0.02	0.41	0.11
0.709028	0.18	0.00	0.07	0.01	0.18	0.08
16:3n-3	0.04	0.01	0.00	0.00	0.05	0.09
16:4n-3	0.08	0.01	0.11	0.01	0.09	0.03
16:4n-1	1.66	0.01	0.36	0.07	0.39	0.12
18:0	3.86	0.02	5.88	0.50	5.18	0.24
18:1n-11	0.13	0.03	0.61	0.13	0.50	0.10
18:1n-9	16.98	0.09	9.73	1.38	28.17	0.91
18:1n-7	2.70	0.01	2.18	0.24	3.99	0.12

FA	FO Diet		Muscle PL (FO, Post-prandial)		Liver TAG (FO, Post-prandial)	
	Mean	SD	Mean	SD	Mean	SD
18:1n-5	0.14	0.01	0.13	0.02	0.25	0.03
18:2d5,11	0.17	0.01	0.07	0.01	0.20	0.05
18:2n-7	0.06	0.01	0.04	0.02	0.08	0.04
18:2n-6	9.05	0.06	7.10	1.09	11.22	0.91
18:2n-4	0.33	0.00	0.14	0.04	0.25	0.05
18:3n-6	0.22	0.00	0.07	0.01	0.21	0.03
18:3n-4	0.16	0.01	0.16	0.04	0.17	0.03
18:3n-3	0.98	0.02	0.50	0.08	0.98	0.03
18:4n-3	1.46	0.02	0.24	0.04	0.83	0.13
18:4n-1	0.22	0.01	0.09	0.02	0.17	0.03
20:0	0.17	0.00	0.02	0.02	0.07	0.01
20:1n-11	0.19	0.00	0.22	0.12	0.81	0.12
20:1n-9	1.50	0.01	0.73	0.11	3.30	0.49
20:1n-7	0.23	0.04	0.03	0.01	0.42	0.07
20:2n-9	0.13	0.00	0.00	0.00	0.05	0.02
20:2n-6	0.17	0.00	0.36	0.05	0.36	0.03
20:3n-6	0.16	0.00	0.19	0.04	0.13	0.01
20:4n-6	0.80	0.02	2.64	0.30	0.50	0.04
20:3n-3	0.05	0.01	0.07	0.02	0.19	0.29
20:4n-3	0.50	0.01	0.41	0.07	0.37	0.02
20:5n-3	11.82	0.13	15.88	0.84	6.19	1.11
22:0	0.10	0.01	0.03	0.02	0.04	0.01
22:1n-11	1.66	0.02	0.12	0.02	2.74	0.49
22:1n-9	0.26	0.01	0.07	0.02	0.41	0.09
22:1n-7	0.09	0.02	0.03	0.03	0.06	0.01
22:2n-6	0.03	0.03	0.04	0.04	0.05	0.04
21:5n-3	0.47	0.04	0.25	0.06	0.26	0.05
23:0	0.05	0.03	0.09	0.04	0.05	0.05
22:4n-6	0.13	0.03	0.15	0.03	0.10	0.01
22:5n-6	0.20	0.03	0.48	0.04	0.12	0.01
22:5n-3	1.45	0.04	1.85	0.33	0.96	0.07
22:6n-3	5.38	0.09	27.78	3.21	4.45	0.52
24:1	0.27	0.05	0.43	0.14	0.18	0.04

FA	Muscle PL (FO, Fasted)		Liver TAG (FO, Fasted)		Serum TAG (FO)	
	Mean	SD	Mean	SD	Mean	SD
i-14:0	0.20	0.12	0.02	0.02	0.00	0.00
14:0	0.53	0.08	2.51	0.13	2.17	0.09
14:1n-9	0.15	0.11	0.09	0.02	0.24	0.02
14:1n-7	0.02	0.03	0.05	0.01	0.03	0.01
14:1n-5	0.07	0.07	0.08	0.01	0.05	0.00
i-15:0	0.01	0.01	0.09	0.00	0.07	0.01
15:0	0.11	0.05	0.19	0.01	0.19	0.01
15:1n-8	0.07	0.07	0.03	0.02	0.03	0.02
i-16:0	0.32	0.13	0.03	0.03	0.06	0.02
16:0	15.56	1.21	14.41	0.77	13.78	0.44
16:1n-11	0.15	0.05	0.25	0.03	0.29	0.02
16:1n-9	0.30	0.02	0.43	0.03	0.36	0.01
16:1n-7	1.15	0.27	6.33	0.16	5.22	0.13
16:1n-5	0.31	0.11	0.13	0.02	0.11	0.00
17:1(a)	0.09	0.06	0.03	0.02	0.04	0.01
i-17:0	0.06	0.03	0.11	0.01	0.11	0.01
16:2n-6	0.01	0.01	0.09	0.01	0.09	0.00
ai-17:0	0.03	0.02	0.13	0.00	0.10	0.00
17:1(b)	0.16	0.05	0.13	0.01	0.07	0.01
16:2n-4	0.10	0.05	0.43	0.04	0.20	0.02
17:0	0.18	0.02	0.21	0.01	0.35	0.01
Phytane	0.03	0.07	0.03	0.02	0.30	0.05
16:3n-4	0.02	0.01	0.02	0.02	0.47	0.02
0.709028	0.04	0.03	0.55	0.07	0.15	0.04
16:3n-3	0.05	0.03	0.22	0.01	0.01	0.01
16:4n-3	0.06	0.04	0.05	0.01	0.05	0.02
16:4n-1	0.17	0.19	0.54	0.12	0.44	0.04
18:0	6.29	0.57	5.44	0.53	5.21	0.38
18:1n-11	0.64	0.13	0.38	0.15	0.35	0.21
18:1n-9	9.69	1.94	27.18	2.23	24.79	0.83
18:1n-7	2.33	0.33	4.30	0.24	3.53	0.10
18:1n-5	0.12	0.01	0.23	0.02	0.07	0.09
18:2d5,11	0.07	0.03	0.22	0.02	0.18	0.02
18:2n-7	0.04	0.02	0.08	0.01	0.18	0.07
18:2n-6	7.13	1.74	11.72	0.71	9.41	0.18
18:2n-4	0.17	0.05	0.32	0.04	0.26	0.01

FA	Muscle PL (FO, Fasted)		Liver TAG (FO, Fasted)		Serum TAG (FO)	
	Mean	SD	Mean	SD	Mean	SD
18:3n-6	0.07	0.01	0.25	0.02	0.23	0.01
18:3n-4	0.18	0.06	0.15	0.02	0.14	0.02
18:3n-3	0.49	0.11	1.01	0.04	0.77	0.02
18:4n-3	0.21	0.06	0.93	0.09	0.90	0.03
18:4n-1	0.09	0.03	0.19	0.03	0.14	0.01
20:0	0.02	0.01	0.07	0.01	0.10	0.00
20:1n-11	0.26	0.23	0.57	0.17	0.52	0.11
20:1n-9	0.64	0.32	2.65	0.33	2.49	0.09
20:1n-7	0.15	0.11	0.31	0.04	0.24	0.02
20:2n-9	0.02	0.02	0.09	0.01	0.09	0.00
20:2n-6	0.40	0.07	0.34	0.02	0.38	0.02
20:3n-6	0.16	0.10	0.17	0.03	0.15	0.00
20:4n-6	2.83	0.35	0.56	0.05	0.91	0.03
20:3n-3	0.05	0.02	0.05	0.01	0.04	0.02
20:4n-3	0.47	0.08	0.36	0.04	0.38	0.02
20:5n-3	17.63	0.69	7.24	0.88	10.95	0.43
22:0	0.03	0.02	0.02	0.01	0.04	0.01
22:1n-11	0.11	0.01	2.02	0.21	2.31	0.07
22:1n-9	0.06	0.02	0.28	0.08	0.30	0.01
22:1n-7	0.01	0.02	0.05	0.02	0.05	0.01
22:2n-6	0.03	0.02	0.04	0.02	0.06	0.02
21:5n-3	0.30	0.09	0.34	0.03	0.43	0.03
23:0	0.04	0.03	0.01	0.01	0.07	0.06
22:4n-6	0.18	0.05	0.10	0.01	0.16	0.05
22:5n-6	0.52	0.03	0.11	0.01	0.26	0.07
22:5n-3	2.34	0.41	0.96	0.11	1.78	0.18
22:6n-3	25.93	4.11	3.96	0.57	6.77	0.26
24:1	0.38	0.20	0.12	0.02	0.36	0.15

FA	CO Diet		Muscle PL (CO, Post-prandial)		Liver TAG (CO, post-prandial)		Chylomicron TAG (FO)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
i-14:0	0.02	0.01	0.14	0.03	0.00	0.00	0.03	0.04
14:0	1.49	0.03	0.47	0.13	1.71	0.24	1.29	0.09
14:1n-9	0.05	0.01	0.01	0.01	0.06	0.02	0.14	0.01
14:1n-7	0.01	0.01	0.10	0.02	0.02	0.02	0.04	0.03
14:1n-5	0.04	0.02	0.09	0.04	0.04	0.02	0.11	0.06
i-15:0	0.05	0.01	0.01	0.01	0.06	0.01	0.04	0.03
15:0	0.12	0.00	0.12	0.01	0.14	0.02	0.13	0.01
15:1n-8	0.01	0.01	0.00	0.00	0.01	0.02	0.00	0.00
i-16:0	0.03	0.02	0.26	0.06	0.04	0.01	0.02	0.03
16:0	8.99	0.10	14.77	1.39	10.86	0.85	9.00	0.37
16:1n-11	0.13	0.00	0.15	0.07	0.24	0.06	0.21	0.04
16:1n-9	0.09	0.00	0.36	0.11	0.41	0.06	0.18	0.02
16:1n-7	1.75	0.02	0.87	0.12	4.14	0.99	2.01	0.11
16:1n-5	0.10	0.00	0.25	0.03	0.10	0.02	0.13	0.06
17:1(a)	0.03	0.01	0.07	0.01	0.03	0.01	0.04	0.05
i-17:0	0.05	0.00	0.07	0.01	0.10	0.02	0.06	0.06
16:2n-6	0.02	0.01	0.01	0.02	0.06	0.02	0.02	0.03
ai-17:0	0.05	0.03	0.11	0.04	0.07	0.01	0.14	0.05
17:1(b)	0.04	0.00	0.11	0.03	0.12	0.02	0.11	0.01
16:2n-4	0.13	0.00	0.08	0.02	0.26	0.07	0.13	0.01
17:0	0.08	0.00	0.14	0.04	0.15	0.02	0.11	0.01
Phytane	0.05	0.01	0.00	0.01	0.03	0.02	0.24	0.01
16:3n-4	0.04	0.03	0.01	0.01	0.27	0.10	0.11	0.02
0.709028	0.13	0.03	0.07	0.04	0.23	0.11	0.14	0.01
16:3n-3	0.06	0.08	0.00	0.00	0.03	0.07	0.00	0.00
16:4n-3	0.04	0.01	0.06	0.04	0.05	0.01	0.02	0.03
16:4n-1	0.14	0.01	0.31	0.07	0.28	0.09	0.10	0.01
18:0	1.82	0.01	6.03	0.47	4.22	0.48	2.90	0.44
18:1n-11	0.13	0.02	0.49	0.20	0.54	0.16	0.52	0.57
18:1n-9	38.72	0.13	13.32	2.61	35.31	3.40	39.62	0.93
18:1n-7	2.80	0.09	2.51	0.39	4.38	0.26	3.06	0.13
18:1n-5	0.15	0.02	0.17	0.12	0.22	0.02	0.17	0.04
18:2d5,11	0.02	0.01	0.09	0.04	0.17	0.04	0.04	0.04
18:2n-7	0.06	0.01	0.04	0.02	0.06	0.01	0.02	0.03
18:2n-6	19.57	0.13	10.00	1.61	13.72	1.18	16.06	0.48
18:2n-4	0.05	0.01	0.13	0.03	0.23	0.07	0.07	0.02

FA	CO Diet		Muscle PL (CO, Post-prandial)		Liver TAG (CO, post-prandial)		Chylomicron TAG (FO)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
18:3n-6	0.02	0.01	0.04	0.02	0.17	0.05	0.07	0.04
18:3n-4	0.12	0.03	0.16	0.05	0.14	0.02	0.10	0.04
18:3n-3	5.74	0.04	1.57	0.64	2.29	0.78	4.07	0.22
18:4n-3	0.16	0.14	0.14	0.04	0.56	0.20	0.28	0.01
18:4n-1	0.04	0.02	0.07	0.01	0.13	0.05	0.04	0.03
20:0	0.42	0.01	0.05	0.04	0.09	0.03	0.37	0.03
20:1n-11	0.40	0.02	0.16	0.05	0.67	0.25	0.63	0.07
20:1n-9	3.58	0.01	0.97	0.34	3.18	0.44	4.03	0.32
20:1n-7	0.28	0.07	0.24	0.22	0.33	0.07	0.49	0.24
20:2n-9	0.01	0.01	0.01	0.01	0.06	0.03	0.01	0.01
20:2n-6	0.10	0.01	0.46	0.08	0.37	0.02	0.24	0.03
20:3n-6	0.03	0.02	0.18	0.05	0.08	0.06	0.01	0.02
20:4n-6	0.17	0.01	2.51	0.24	0.41	0.11	0.21	0.03
20:3n-3	0.03	0.01	0.07	0.02	0.06	0.02	0.03	0.03
20:4n-3	0.11	0.01	0.38	0.05	0.29	0.07	0.09	0.05
20:5n-3	2.17	0.04	14.52	1.70	5.10	1.28	2.50	0.30
22:0	0.23	0.02	0.03	0.02	0.03	0.02	0.16	0.06
22:1n-11	4.60	0.05	0.12	0.05	2.16	0.30	5.23	0.25
22:1n-9	0.49	0.02	0.06	0.04	0.31	0.06	0.53	0.07
22:1n-7	0.08	0.01	0.01	0.02	0.02	0.02	0.06	0.05
22:2n-6	0.02	0.01	0.01	0.01	0.03	0.08	0.00	0.01
21:5n-3	0.07	0.02	0.25	0.05	0.23	0.08	0.07	0.04
23:0	0.04	0.02	0.09	0.07	0.04	0.04	0.11	0.05
22:4n-6	0.02	0.01	0.14	0.03	0.07	0.02	0.08	0.06
22:5n-6	0.04	0.01	0.43	0.16	0.09	0.02	0.08	0.05
22:5n-3	0.29	0.02	2.19	0.42	0.82	0.19	0.47	0.12
22:6n-3	3.40	0.09	23.45	2.52	3.76	0.47	2.69	0.36
24:1	0.30	0.03	0.25	0.07	0.12	0.04	0.36	0.08

FA	Muscle PL (CO, Fasted)		Liver TAG (CO, Fasted)		Serum TAG (CO)	
	Mean	SD	Mean	SD	Mean	SD
i-14:0	0.12	0.05	0.00	0.00	0.00	0.00
14:0	0.36	0.04	1.68	0.29	1.28	0.12
14:1n-9	0.01	0.01	0.05	0.03	0.07	0.07
14:1n-7	0.08	0.03	0.04	0.02	0.05	0.05
14:1n-5	0.14	0.15	0.04	0.02	0.03	0.01
i-15:0	0.01	0.02	0.06	0.01	0.06	0.02
15:0	0.18	0.25	0.14	0.02	0.13	0.01
15:1n-8	0.04	0.05	0.01	0.02	0.02	0.05
i-16:0	0.20	0.06	0.05	0.04	0.04	0.01
16:0	14.77	1.09	11.21	0.76	10.05	0.76
16:1n-11	0.18	0.04	0.21	0.06	0.16	0.07
16:1n-9	0.33	0.04	0.42	0.06	0.34	0.03
16:1n-7	0.62	0.06	4.13	1.05	2.65	0.52
16:1n-5	0.23	0.04	0.10	0.02	0.10	0.04
17:1(a)	0.07	0.01	0.03	0.01	0.05	0.03
i-17:0	0.08	0.05	0.11	0.05	0.10	0.02
16:2n-6	0.01	0.02	0.06	0.01	0.03	0.01
ai-17:0	0.18	0.15	0.07	0.01	0.07	0.01
17:1(b)	0.10	0.02	0.11	0.03	0.16	0.04
16:2n-4	0.06	0.01	0.26	0.08	0.15	0.03
17:0	0.16	0.03	0.15	0.03	0.14	0.02
Phytane	0.01	0.01	0.02	0.01	0.21	0.07
16:3n-4	0.14	0.27	0.10	0.13	0.15	0.08
17:1	0.05	0.03	0.30	0.09	0.18	0.01
16:3n-3	0.05	0.03	0.17	0.10	0.04	0.07
16:4n-3	0.08	0.06	0.05	0.04	0.04	0.03
16:4n-1	0.30	0.04	0.26	0.10	0.11	0.03
18:0	5.93	0.49	4.27	0.36	3.91	0.44
18:1n-11	0.41	0.22	0.49	0.17	0.43	0.13
18:1n-9	12.60	2.14	36.05	4.56	37.03	1.43
18:1n-7	2.46	0.16	4.27	0.54	3.71	0.17
18:1n-5	0.17	0.10	0.21	0.07	0.21	0.03
18:2d5,11	0.08	0.05	0.22	0.04	0.14	0.04
18:2n-7	0.05	0.01	0.07	0.06	0.04	0.01

FA	Muscle PL (CO, Fasted)		Liver TAG (CO, Fasted)		Serum TAG (CO)	
	Mean	SD	Mean	SD	Mean	SD
18:2n-6	8.90	1.22	14.03	1.44	13.38	0.69
18:2n-4	0.13	0.04	0.22	0.08	0.14	0.04
18:3n-6	0.06	0.05	0.15	0.06	0.12	0.04
18:3n-4	0.22	0.31	0.13	0.10	0.10	0.04
18:3n-3	1.34	0.46	2.50	0.95	2.64	0.47
18:4n-3	0.12	0.03	0.57	0.17	0.39	0.07
18:4n-1	0.07	0.02	0.14	0.05	0.07	0.02
20:0	0.05	0.03	0.10	0.03	0.21	0.03
20:1n-11	0.13	0.02	0.73	0.05	0.96	0.89
20:1n-9	0.86	0.20	2.97	0.25	3.15	0.88
20:1n-7	0.19	0.13	0.26	0.08	0.33	0.12
20:2n-9	0.02	0.02	0.06	0.02	0.03	0.02
20:2n-6	0.39	0.08	0.35	0.03	0.38	0.04
20:3n-6	0.23	0.13	0.07	0.05	0.06	0.04
20:4n-6	2.37	0.42	0.35	0.11	0.44	0.09
20:3n-3	0.08	0.09	0.04	0.03	0.05	0.03
20:4n-3	0.37	0.07	0.28	0.10	0.21	0.04
20:5n-3	14.39	2.09	4.66	1.60	5.09	1.18
22:0	0.06	0.02	0.06	0.03	0.09	0.05
22:1n-11	0.14	0.07	1.99	0.23	3.14	0.39
22:1n-9	0.19	0.27	0.32	0.06	0.41	0.06
22:1n-7	0.05	0.06	0.05	0.02	0.05	0.03
22:2n-6	0.17	0.16	0.12	0.13	0.09	0.13
21:5n-3	0.27	0.41	0.18	0.16	0.29	0.38
23:0	0.04	0.04	0.03	0.02	0.03	0.01
22:4n-6	0.14	0.05	0.07	0.04	0.07	0.03
22:5n-6	0.48	0.12	0.09	0.04	0.10	0.04
22:5n-3	2.11	0.64	0.70	0.24	0.83	0.16
22:6n-3	25.88	1.86	3.28	0.79	5.06	0.83
24:1	0.30	0.11	0.12	0.07	0.23	0.11

APPENDIX B

ISOTOPE DATA FROM EXPERIMENTS

FO (POST-PRANDIAL)						
	δ^{13}C Diet (n=6) ± SD		δ^{13}C Liver (n=6) ± SD		δ^{13}C Muscle (n=7) ± SD	
18:2n-6	-26.42	± 0.59	-25.86	± 0.92	-25.73	± 1.09
20:5n-3	-29.60	± 0.62	-29.31	± 1.58	-29.66	± 1.13
22:6n-3	-28.11	± 1.03	-30.82	± 1.57	-30.70	± 0.95
CO (POST PRANDIAL)						
	δ^{13}C Diet (n=5) ± SD		δ^{13}C Liver (n=8) ± SD		δ^{13}C Muscle (n=10) ± SD	
18:2n-6	-32.17	± 0.69	-28.32	± 0.91	-28.99	± 0.64
18:3n-3	-36.32	± 0.95	-34.11	± 0.55	-33.08	± 1.82
20:5n-3	-27.52	± 0.63	-29.68	± 0.60	-29.48	± 1.02
22:6n-3	-29.64	± 1.02	-29.53	± 0.92	-29.64	± 0.73
δ^{13}C CHYLOMICRONS (CO) ± SD (n=6)						
18:2n-6	-31.17	± 1.32				
18:3n-3	-33.83	± 0.84				
20:5n-3	-27.75	± 2.30				
22:6n-3	-29.11	± 1.55				
CO (FASTED)						
	δ^{13}C Serum (n=10) ± SD		δ^{13}C Liver (n=11) ± SD		δ^{13}C Muscle (n=11)± SD	
18:2n-6	-29.16	± 0.81	-28.83	± 1.92	-29.19	± 1.17
18:3n-3	-32.69	± 1.55	-33.20	± 0.58	-31.71	± 1.12
20:5n-3	-29.76	± 1.54	-28.15	± 0.79	-29.44	± 0.96
22:6n-3	-29.71	± 0.69	-27.89	± 0.90	-29.79	± 0.74