LIPID COMPOSITION OF SELECTED TISSUES AND MILK OF PHOCID SEALS OF EASTERN CANADA

CENTRE FOR NEWFOUNDLAND STUDIES

TOTAL OF 10 PAGES ONLY MAY BE XEROXED

(Without Author's Permission)

EDWARD A.D. DURNFORD

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

69

This reproduction is the best copy available.

UMI

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

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

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

0-612-54891-0

Canada

LIPID COMPOSITION OF SELECTED TISSUES AND MILK OF PHOCID SEALS OF EASTERN CANADA

By

©Edward A. D. Durnford

A thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for

the degree of the Master of Science

Department of Biochemistry

Memorial University of Newfoundland

April, 1998

St. John's, Newfoundland, Canada

Abstract

Marine oils and seafoods have received much attention since their consumption has been associated with beneficial cardiovascular effects. Much of the research, however, has focused on fish oils and oily fish with some recent reports on harp and grey seal blubber oils. This study investigated the lipid composition of several tissues from all six species of Eastern Canadian Phocid seals. The position of fatty acids of the triacylglycerol (TAG) backbone also has important dietary and biochemical implications. Therefore, the positional distribution of fatty acids of the TAG of harp seal milk was also investigated.

Proximate composition of the samples indicated that blubber, followed by milk, had the highest lipid content and the lowest levels of moisture and protein. Muscle tissues had the highest protein content and milk had the lowest amount of ash.

Thin layer chromatography-flame ionization detection (TLC-FID) studies indicated that lipid class composition was primarily dependent on the tissue as compared to species of seal. Furthermore, some tissues had a very unique composition. Blubber and milk lipids were found to be mainly composed of TAG while brain had undetectable levels of TAG. Brain lipids were high in cholesterol and cerebrosides, the latter being a polar lipid class not found in any other tissue examined.

The fatty acids of all tissue lipids contained relatively high proportions of polyunsaturated fatty acids (PUFA) of the ω3 type (12- 23%) and these were composed of eicosapentaenoic acid (EPA, 0.3-13%), docosapentaenoic acid (DPA, 0-5%), and

docosahexaenoic acid (DHA, 3-16%). Multivariate analysis showed that fatty acid composition varied more from tissue to tissue than species to species. Several unique features in the fatty acid compositions of various tissues were also identified. Blubber was found to be high in monounsaturated fatty acids (MUFA), but low in arachidonic acid and dimethyl acetals. Brain lipids, on the other hand, were high in dimethyl acetals. Brain lipids were also high in DHA. Lung tissue lipids were very high in saturated fatty acids, especially palmitic acid, and heart lipids had a higher content of linoleic acid than lipids of other tissues examined.

Mulitvariate analysis also indicated clear differences in the neutral and polar lipid fractions of corresponding tissues. The polar lipid fraction tended to be higher in dimethyl acetals, saturated fatty acids and PUFA of the ω3 configuration. The neutral lipid fraction of corresponding tissues, however, tended to be richer in MUFA and PUFA of the ω3 configuration.

The positional distribution of the fatty acids of the triacylglycerols of harp seal milk was very similar to that of harp seal blubber. The ω3 PUFA were concentrated in the *sn*-1 and *sn*-3 positions while the saturated fatty acids were preferentially esterified to the *sn*-2 position.

Acknowledgements

I would like to express my sincere appreciation to Dr. F. Shahidi for providing me with the opportunity to complete my degree under his supervision. His guidance, support, and encouragement throughout the course of my studies and the preparation of this thesis are also greatly acknowledged. I would also like to express many thanks to my supervisory committee members, Drs. E. Miller and R. Hoover for their input and guidance throughout this study. The assistance of Dr. E. Miller in multivariate analysis is also greatly appreciated. I would like to thank all of my colleagues in Dr. Shahidi's research group for their support and creating a pleasant environment to study and work. Finally, I would like to thank my loving wife Sonja for her continued support throughout my studies.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	- Arachidonic acid
ACS	- American Chemical Society
BHT	- Butylatedhydroxytoluene
CE	- Cerebroside
CHD	- Coronary heart disease
CL	- Cardiolipin
DAG	- Diacylglycerol
DHA	- Docosahexaenoic acid
DMA	- Dimethyl acetal
DPA	- Docosapentaenoic acid
DPPC	- Dipalmitoylphosphatidylcholine
EPA	- Eicosapentaenoic acid
FAME	- Fatty acid methyl ester
FFA	- Free fatty acid
FID	- Flame ionization detector
FFSC	- Flexible fused silica column
GC	- Gas chromatography
HDL	- High-density lipoproteins
HPLC	- High performance liquid chromatography
LA	- Linoleic acid
LDL	- Low-density lipoproteins
MAG	- Monoacylglycerol

MUFA	- Monounsaturated fatty acids
NMR	- Nuclear magnetic resonance
PA	- Phosphatidic acid
PC	- Phosphatidylcholine
PCA	- Principle component analysis
PE	- Phosphatidylethanolamine
PG	- Phosphatidylglycerol
PI	- Phosphatidylinositol
PS	- Phosphatidylserine
PUFA	- Polyunsaturated fatty acids
SM	- Sphingomyelin
TAG	- Triacylglycerol
TLC	- Thin layer chromatography
TLC-FID	- Thin layer chromatography-flame ionization detection
VLDL	- Very-low-density lipoproteins

CHAPTER 1

INTRODUCTION

There has been continued interest in marine oils since the 1970s when epidemiological studies proposed that low incidences of heart disease in Greenland Eskimos and some Japanese populations were due to their unique diets that were rich in oily fish, whale blubber, and seal blubber (Bang and Dyerberg, 1972; Stansby, 1990b). It is believed that humans originally consumed a diet with a ratio of ω6 to ω3 fatty acids of about 1:1, whereas today the ratio is estimated to range from 10:1 to 20-25:1 in the western diet. Therefore, western diets are deficient in ω3 fatty acids compared with the diet on which humans evolved and from which their genetic profiles established (Simopoulos, 1991). More recently, interest in seal blubber, specifically as a source of ω3 fatty acids has intensified.

Early research on ω3 polyunsaturated fatty acids (PUFA) focused on fish. As a result, much of the literature has provided evidence on the beneficial effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are the predominant ω3 PUFAs in fish oils (Newton, 1996). However, seal blubber oil is rich in docosapentaenoic acid (DPA) as well as EPA and DHA (Shahidi *et al.*, 1996). Existing literature contains little information on the effects of DPA.

Much of the research suggest that the reduction in coronary heart disease (CHD) by marine oils is through lowering of serum triacylglycerols, reduction of the occurrence of arrhythmia, and their ability to act as antiatherogenic and antithrombotic agents

(Abbey *et al.*, 1990). Marine oils have also been studied for their effects on inflammatory and autoimmune disorders, stroke, skin disorders, various cancers, and the development of retina and brain (Simopoulos, 1997a).

Besides fatty acid composition, the specific classes of lipids present also have important biochemical and nutritional implications. For example, cholesterol is an essential component of cellular membranes (Gibbons *et al.*, 1982), however, high levels of cholesterol in the diet may have negative health implications (Shahidi and Synowiecki, 1991). Besides cholesterol the lipid classes commonly found in animal tissues include triacylglycerols, free fatty acids, monoacylglycerols, diacylglycerols, phospholipids (phosphatidic acid, phosphatidylglycerol, cardiolipin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol), and sphingolipids (sphingomyelin and cerebroside) (Gunstone and Norris, 1983).

Previous studies have compared the fatty acid composition of the blubber of several species of seals and others have compared the fatty acid composition of several tissues from the same species of seal. However, there has been no previous study to compare the fatty acid composition of different tissues from all six species of Eastern Canadian Phocid seals. Consequently, the first objective was to compare the fatty acid composition of different organs of all six Eastern Canadian Phocid seal species simultaneously. The hypothesis is that when comparing the fatty acid composition across both species and tissues, the tissue under consideration will have a larger impact than the species under consideration.

Since most studies have focused on the blubber of seals, which is predominantly triacylglycerols (TAGs), there is little information in the literature on the lipid classes of various tissues of different species of seals. There is one study on the lipid class composition of a Mediterranean Monk seal using high performance liquid chromatography (HPLC) (Henderson *et al.*, 1994). Therefore, the objective of this aspect of the study was to determine the lipid class composition of selected tissues of the Eastern Canadian Phocid seals. It is hypothesized that the lipid classes will depend more on the tissue under consideration rather than species and as a result will also be similar to the general trends observed in the analysis of the corresponding tissues in the Mediterranean Monk seal.

Besides the different tissues of seal, milk of these mammals is often of interest to scientists because it is also very unique. The most extraordinary feature of seal milk is its very high fat content (30 to 60%) (Oftedal *et al.*, 1988; Ackman and Lamonthe, 1989; Iverson *et al.*, 1992). Many studies indicate that the fatty acid composition of seal milk is similar to blubber except that the levels of palmitic acid (16:0) are higher in milk. Since the fatty acid composition of seal milk has been well established and it is composed of predominately TAGs, further studies in these areas would be unproductive or uninteresting. However, there have only been a limited number of studies on the positional distribution of fatty acids in the TAGs of seal milk (Iverson *et al.*, 1992; Puppione *et al.*, 1992) and neither of these provided the positional distribution of fatty acids in all three positions of the TAG backbone. Therefore, the final objective of this study was to determine the positional distribution of the fatty acids in all three positions

of TAG backbone of harp seal milk. The hypothesis is that the distribution will be similar to that of harp seal blubber. That is, the ω3 PUFA will be preferentially esterified to the *sn*-1 and *sn*-3 positions while the saturated fatty acids will be preferentially esterified to the *sn*-2 position.

CHAPTER 2

LITERATURE REVIEW

2.1. Seals

Seals belong to the Order Pinnipedia which is divided into two main divisions: the Otarioidea, which includes the fur seals, sea lions and walruses, and the Phocoidea, which includes the "true" seals (King, 1983). The true seals, also known as the "hair" seals, are characterized by negligible external ears, hind flippers that do not turn forward, and the absence of an underfur. The Otariidae, however, have all these features with the walruses, Odolaenidae, being an offshoot of Otariidae (Sergeant, 1991).

2.1.1. Phocid Seals of Eastern Canada

Six species of phocid seals are found in Eastern Canada. They include the bearded, grey, harbour, harp, hooded, and ringed seals. Grey and harbour seals whelp on land while the remaining four species whelp on ice (Malouf, 1986).

2.1.1.1. The Bearded Seal, *Erignathus barbatus*

The bearded seal has a circumpolar distribution (Figure 2.1) and is essentially an Arctic and subarctic seal of relatively shallow waters (Bonner, 1990). Bearded seals tend to be sedentary, but will undertake regular long distance migrations in response

Figure 2.1. Distribution of the bearded seal (*Erignathus barbatus*). Adapted from King (1983).

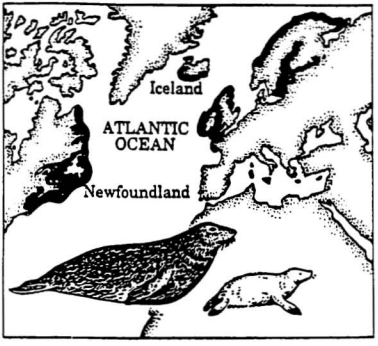


Figure 2.2. Distribution of the grey seal (*Halichoerus grypus*). Adapted from King (1983)

to movements of ice fields (Davis *et al.*, 1980). Since they generally do not maintain breathing holes in the ice they are dependent upon open water. Bearded seals are large with both sexes reaching a weight of approximately 250 kg. They are greyish in colour with a scattering of small dark spots and are darker on the back. Bearded seals have an abundance of long conspicuous whiskers from which it derives its name (Bonner, 1990). The world population of bearded seals is estimated to be between 500,000 and 1,000,000 (Bonner, 1990; King, 1983) and the size of the Canadian stock is unknown, but is much lower than the ringed seal stock (Malouf, 1986).

The bearded seal is hunted by the Inuit for its pelt which is prized for being tough and flexible. The meat is eaten by both humans and dogs (Shahidi, 1998).

2.1.1.2 The Grey Seal, *Halichoerus grypus*

There are three distinct populations of grey seals: the northeast Atlantic, the Baltic and the northwest Atlantic as shown in Figure 2.2 (Bonner,1990). Grey seals are also known as Atlantic seal, horse head, Phoque gris, or Cowmore seal (Beck, 1983a). Grey seals normally inhabit rocky shores and in the northeast Atlantic they are concentrated in the Gulf of St. Lawrence (King, 1983). Grey seals are fairly large with the male being considerably larger than the female. Adult males reach approximately 450 kg and females 270 kg. The male's coat is dark grey in colour with silver, grey spots. The coat of the female is silver grey with small scattered dark spots (Mansfield, 1988).

Prior to 1990, the world population of grey seals was estimated to be between 120,000 and 135,000 (Bonner, 1990). However, in 1993, there were an estimated 144,000

grey seals in Atlantic Canada alone. The Atlantic Canadian population is increasing at about 9 to 13% per year (Shahidi, 1998).

In Canada, grey seals are of concern because of their impact on Canadian commercial fisheries. For example, they damage fishing gear, compete with fishermen for fish, and are the primary host for a parasite that must be removed from the flesh of cod (cod worm) and other groundfish (Malouf, 1986).

2.1.1.3 The Harbour Seal, *Phoca vitulina*

The Habour seal, also known as the common or spotted seal (Godwin, 1990), has a circumpolar distribution similar to the Bearded seal but at a lower latitude, in cold-temperate and temperate waters (Figure 2.3). These are small to medium sized seals with males reaching 120 kg and females 90 kg. The colour and pattern of the coat varies greatly, but is basically a mottle of dark spots on a light background (Bonner, 1990).

The Harbour seal is a shore living animal found principally, but not exclusively, in estuaries and in areas where sand banks are uncovered at low tide (King, 1983). Several subspecies of the Harbour seal have been named including *P. vitulina vitulina*, *P. vitulina concolor*, *P. vitulina mellonae*, *P. vitulina richardsi*, *P. vitulina geronimensis*, and *P. vitulina stejnegeri*. The total world population of Harbour seals is between 300,000 and 400,000 (Bonner, 1990).

Harbour seals are not hunted commercially in Atlantic Canada, except on the Quebec North Shore. They are, however, regarded as a nuisance by inshore fishermen

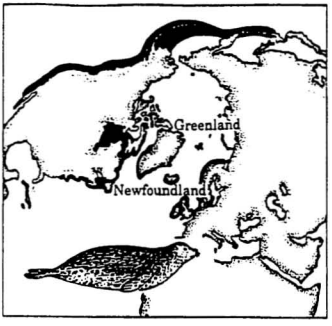


Figure 2.3. Distribution of the harbour seal (*Phoca vitulina*). Adapted from King (1983).

Figure 2.4. Distribution of the harp seal (*Phoca groenlandica*). Adapted from King (1983).

due to damage to fixed fishing gear, competition for fish, and as a non- preferred host for the codworm.

2.1.1.4 The Harp Seal, *Phoca groenlandica*

The harp seal, also known as the Greenland or saddleback seal, is found in the open sea of the Arctic Atlantic (King, 1983). As shown in Figure 2.4 there are three populations of harp seals breeding in the Western Atlantic, off Jan Mayen, and in the White Sea (Bonner, 1990).

Harp seals are medium sized seals ranging from 100 to 130 kg, depending on the time of the year (Seargent, 1991). The coat of the harp changes with maturation. The adult male is light silvery grey with an irregular horseshoe shaped band of black straddling the back. The black band or 'harp' is paler and less well defined in the adult female. Harp seals are born yellowish, quickly turn white, and pass through a number of 'coats' during maturation. Due to varying commercial value for the different coats, each one has a common name as given in Table 2.1.

Harp seals are the third most abundant species of seal in the world and have been hunted for their skin and oil since historical times (Shahidi, 1998). Harp seals are still the focus of the Canadian commercial seal hunt and much effort has been placed into accurately determining their population. Various approaches have been used to estimate the Northwest Atlantic harp seal population based principally on pup production, catch-at-age, and pregnancy rate data (Shelton *et al.*, 1992; 1995). Several models

Table 2.1. Common names used for harp seals of different ages (Commeau, 1989)

Common Name	Description
WhiteCoat	A harp seal pup from 3 to 10 days old, named for its distinctive long, white hair.
Overgang	A weaned harp seal pup during the preparatory stage of its first moult: long, white hair is still firmly attached, but silver- grey beater coat hairs have begun to appear at approximately 12 days of age.
Tanner	A weaned harp seal pup during mid- stage of its first moult, white hair is loose and cane be easily pulled out at approximately 16 days of age.
Ragged jacket	A weaned harp seal pup during the last stages of its first moult, from 2.5 to 4 weeks old.
Beater	A fully moulted harp seal pup from 3.5 weeks to 1 year old. Beaters have short- haired, dark spotted, silver- grey coats.
Bedlamer	An immature harp seal from 1 to 5 years old with a spotted coat. The distinctive saddle or harp- shaped markings of the adult harp seal develop gradually.
Old Harp	A mature seal of over 5 years old with distinctive saddle or harp- shaped markings.

use this data to estimate the population; using estimated populations and estimated replacement yields, the commercial seal harvest can be managed effectively. According to Stenson (1995), the population of harp seals in the Northwest Atlantic ranges from 3.6 to 4.3 million.

2.1.1.5 The Hooded Seal, *Cystophora cristata*

The Hooded seal has a distribution similar to that of the harp seal, but is less numerous [(Figure 2.5);(Bonner, 1990)]. Hooded seals are also migratory and breed on heavy ice flows, but are not as gregarious as the harp seal. They tend to be scattered into individual family groups of a male, female, and pup (Malouf, 1986).

Male hooded seals are large, weighing 300- 400 kg, while females are smaller at about 180 kg. Both sexes are pale grey with large black blotches and darker heads and hind flippers. The world population of hooded seals is estimated at 250,000 to 400, 000 (Bonner, 1990).

2.1.1.6 The Ringed Seal, *Phoca hispida*

The ringed seal is found around the whole Arctic and subarctic regions and in the Baltic Sea, as shown in Figure 2.6 (Bonner, 1990). The ringed seal is the most abundant seal in the Arctic and is found wherever there is open water in the fast ice, even as far as the North Pole, and in fjords and bays. They are rarely found in the open sea or on floating pack ice (King, 1983).

Ringed seals are small with males ranging from 65 to 95 kg and females from 45

Figure 2.5. Distribution of the hooded seal (*Cystophora cristata*). Adapted from King (1983).

Figure 2.6. Distribution of the ringed seal (*Phoca hispida*). Adapted from King (1983).

to 80 kg. The pelt is light grey with black spots. The spots are often surrounded with lighter ring markings (hence the name), and on the back the spots become confluent, giving the appearance of a dark stripe down the back. The belly is a lighter silvery grey (Bonner, 1990).

Ringed seals are the most abundant species of seals in the world with the total population estimated at 7 million (King, 1983; Malouf, 1986; Bonner, 1990; Shahidi, 1998). The size of the Canadian stock is unknown, but probably number over a million individuals (Malouf, 1986).

Although ringed seals are an important source of food and clothing for the Inuit (Malouf, 1986), they are not hunted commercially in Canada.

2.2 The Canadian Sealing Industry

2.2.1 History of the 'Seal Hunt'

Seals have been hunted by humans for approximately 400,000 years in Europe and 40,000 years in North America (Bonner, 1990). However, most pre-industrial sealing in the world was for subsistence and not for commercial exploitation. Early in the sixteenth century Basque fishermen came to Newfoundland to catch cod and discovered that they could catch harp seals by setting nets in the Strait of Belle Isle. By the early 18th century a traditional sealing industry, using nets, was well established in Newfoundland (Bowen, 1985; Bonner, 1990).

The first step towards the development of the offshore seal hunt was the participation in 1794 of the first wooded sailing ships to hunt seals (Bowen, 1985). By 1800 the Newfoundland schooner fleet was under construction. Sealing increased rapidly

to become second only to cod fishing in the Newfoundland economy. By the second quarter of the eighteenth century up to 300 schooners with 12,000 men were taking more than half a million seals annually (Bonner, 1990). Harvests of 680,000, 740,000, and 686,000 seals were reported in 1831, 1832, and 1844, respectively (Colman, 1937).

In 1863 another important development in the sealing industry occurred when steam powered ships were used for the first time. In 1906 the first steel- hulled ship was used in the seal hunt (Bowen, 1985).

In 1938 the large Norwegian sealing ships began to hunt the Northwest Atlantic seals (Bowen, 1985) resulting in the demise of the Newfoundland offshore sealing vessels. However, the Norwegians established companies in Canada to operate the sealing enterprises (Bonner, 1990).

Seal landings between 1863 and 1894 averaged 341,000 and declined to an average of 249,000 between 1895 and 1911, and 159,000 between 1912 and 1940. There was little sealing conducted during the war, but from 1949 to 1961 an average of 310,000 seals were harvested. A quota management system was introduced in 1971 and the harp seal harvest from 1971 to 1981 averaged 172,000 (Bowen, 1985).

Throughout the history of sealing small vessels continued to hunt seals. Fishermen who hunted seals from small boats (<65 feet) were known as 'landsmen'. From 1949 to 1982 the 'landsmen' hunt represented 15- 45% of the total landings (Bowen, 1985).

By the 1980s, the efforts of anti- sealing groups began to have their effects on the sealing industry. In 1983 the importation of pelts of harp and hood seal pups into

countries of the European Economic Community (EEC) was banned. This destroyed markets for seal skins resulting in an immediate reduction in the seal hunt. In 1987, the Canadian government officially prohibited the harvesting of seal pups, defined as those seals that had not started their first moult (i.e. whitecoats and bluebacks)(Bonner, 1990). The use of large vessels (65 feet or larger) for harvesting seals was also banned.

The basic technology of sealing was simple. Adult seals were killed by shooting with high-powered rifles and pups were killed by clubbing. Canadian sealers used a wooden club, shaped like a baseball bat, but Norwegian sealers used an instrument with a heavy iron hook on a long handle, called "*hakapik*".

Initially the products of the sealing industry were oil and some leather. However, in the late 1940s the Norwegians developed preservation and tanning methods that allowed pelts to be used in the fur industry. Pelts for fur soon became the main product of the seal hunt. Carcasses were left on the ice for the most part. Landsmen did take some flippers for personal consumption and they were marketed just outside the immediate sealing areas (Bonner, 1990).

2.2.2 The Modern Seal Hunt

After the collapse of the markets for seal pelts in 1982 and subsequent banning of large vessels and hunting seal pups, a small seal industry survived. It has become and remains an 'inshore' fishery pursued by 'landsmen' or by those using small (<65 feet) fishing vessels (Shahidi, 1998).

From 1982 to1995 the quota or total allowable catch (TAC) remained at 186,000, but only an average of about 50,000 seals per year were taken during this period

(Stenson, 1994), as a result of limited markets. In 1996, the quota was raised to 250,000 and for the first time since 1982 a substantial portion of the quota was harvested. A further increase to 275,000 occurred in 1997 which was nearly completely harvested (DFO, 1997).

The technology of the industry is relatively unchanged, however, the final products are more varied. The policy of the modern seal industry is full utilization. Pelts are still one of the most important components and are primarily used for the furrier trade. The blubber is left attached to the pelt and it used for industrial oil products, but greater percentages are being converted to pharmaceutical/nutraceutical products each year. The meat of the seal is also used. Flippers are sold as a delicacy in local markets and the remainder of the carcass used as human food or as animal feed. Some organs are currently marketed and efforts are being placed into finding opportunities for some of the internal organs.

2.3 Marine lipids and their significance

2.3.1 Chemistry and composition of marine lipids

The term lipid is used to describe a wide range of natural products. However, a specific definition used frequently is one that restricts it to fatty acids and their naturally-occurring derivatives and to compounds closely related biosynthetically to fatty acids (Christie, 1982; Gunstone and Norris, 1983).

2.3.1.1 Fatty acids - Background

Fatty acids are generally aliphatic monocarboxylic acids. Trivial names have been given to many of the more common fatty acids, but systematic names, based on the International Union of Pure and Applied Chemists (IUPAC) system of nomenclature, indicate the chain length of the acid, the position, nature, and configuration of any unsaturated bonds, and the position and nature of substituents. The IUPAC system numbers double bonds with relation to their position from the carboxyl end group. However, another common system, accounting for biological activity of molecules, numbers the double bonds from their methyl end group. Since most natural fatty acids containing multiple bonds are methylene- interrupted, it is only necessary to designate the first double bond (Gunstone and Norris, 1983).

One group of common fatty acids are fatty acids which are straight-chained, even-numbered acids containing 12-22 carbon atoms (Gunstone, 1994). Another common group of fatty acids are the monoenoic acids of which more than one hundred have been identified, although most are very rare. The 9-*cis* isomer (palmitoleic) is the most widely occurring hexadecenoic acid. The 9-*cis* isomer (oleic) octadecenoic acid is the most widely distributed and among the most extensively produced of all fatty acids (Gunstone, 1994).

The third common group of fatty acids are polyenoic acids having two to six *cis* double bonds arranged in a methylene- interrupted pattern. They are usually divided into families depending on the distance of the nearest double bond to the methyl end group. The three main families are ω9, ω6, and ω3. Some of the more common important

methylene-interrupted polyenic acids are linoleic (18:2 ω6), α-linolenic (18:3 ω3), arachidonic (20:4 ω6), eicosapentaenoic (20:5 ω3), docosapentaenoic (22:5 ω3), and docosahexaenoic (22:6 ω3) acids (Gunstone and Norris, 1983).

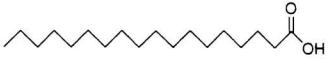
There are also some less common groups of fatty acids. Branched fatty acids with a methyl group on the penultimate (*iso* acids) or antepenultimate (*antesio* acids) are sometimes found naturally in small quantities. Acids containing a cyclopropane or cyclopropene group and several oxygenated acids such as ricinoleic acid also exist (Gunstone and Norris, 1983).

Structures of some of the common saturated, monounsaturated, and polyunsaturated fatty acids are provided in Figure 2.7.

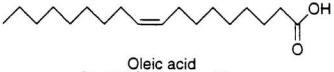
2.3.1.2 Fatty Acids of Marine Mammals

The composition of virtually all marine oils can be described by 8 to 10 major fatty acids, ignoring isomers. Numerous minor acids are also present. A wide variation is observed within identical tissues of the same species. This can be due to both seasonal variations and geographical location, but the most important factor is dietary intake (Padley *et al.*, 1994).

The fatty acid composition of marine mammalian muscle tissue, blubber, and milk has been the subject of many investigations (Ackman and Burgher, 1963; Jangaard and Ke, 1968; Ackman *et al.*, 1971; 1972; Ackman and Hooper, 1974; Engelhart and Walker, 1974; West *et al.*, 1979; Ackman and Lamonthe, 1989; Grompone *et al.*, 1990;

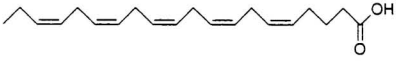


Stearic acid
Octadecanoic acid

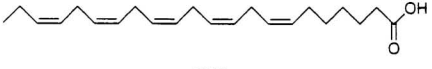


Oleic acid
9c-octadecenoic acid

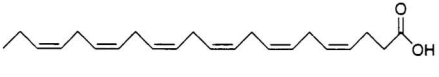
Arachidonic acid
5c,8c,11c,14c-eicosatetraenoic acid



EPA
5c,8c,11c,14c,17c-eicosapentaenoic acid



DPA
7c,10c,13c,16c,19c-docosapentaenoic acid



DHA
4c,7c,10c,13c,16c,19c-docosahexaenoic acid

Figure 2.7. Structures of some common fatty acids.

Kakela and Hyvarinen, 1993; Kakela *et al.*, 1993; Shahidi *et al.*, 1994; Henderson *et al.*, 1994; Grahl- Nielson and Mjaavatten, 1995; Fredheim *et al.*, 1995; Wanasundara and Shahidi, 1997).

There are several unique features of marine oils that differentiate them from most vegetable and land based animal lipids. Most notably, the level of polyunsaturated fatty acids (PUFA) in marine oils is high and is mainly from those with 5 or 6 double bonds. Most of these fatty acids are long-chain (20 to 22 carbon atoms) and of the omega-3 family.

Most of the PUFA in marine oils are formed in unicellular phytoplankton and multicellular marine algae that get passed through the food chain and eventually become incorporated into the bodies of fish and marine mammals (Yongmanitchai and Ward, 1989). The high levels of omega-3 fatty acids in marine lipids are believed to be a result of cold temperature adaptation, because at low temperatures omega-3 PUFAs remain liquid and resist crystallization (Ackman and Lamonthe, 1989).

The fatty acid composition of seals is dependent upon the species and tissue analyzed. In the blubber of harp seal 14:0, 16:0, 16:1, 18:1, 18:2, 20:1, 20:5, 22:1, 22:5, and 22:6 are the principal fatty acids (Jangaard and Ke, 1968; Ackman *et al.*, 1971; Shahidi *et al.*, 1994; Wanasundara and Shahidi, 1997). The predominant fatty acids of the ringed and harbour seal blubbers are the same as those for harp, except 18:2, while the main fatty acids for grey seal blubber include 14:1 (Ackman and Hooper, 1974; West *et al.*, 1979). The principal fatty acids of bearded seal blubber are also similar to harp seal with the addition of 18:0. In hooded seal blubber 22:5 is not one of the predominant fatty

acids (Jangaard and Ke, 1968). The reasons for these interspecies differences in fatty acid composition remain elusive. Fatty acid composition of seal blubber also depends on the age of the animal (Grahl-Nielson and Mjaavatten, 1995).

Unlike the milk of cows and goats, which have 15 to 23% of their fatty acids with chain length of shorter than 14 carbon atoms, milk of marine mammals contains only trace amounts (0.6%) of the shorter chain (<14 C atom) fatty acids (Ashworth *et al.*, 1966). The concentration of fat in the milk of marine mammals is high (30 to 60%) and triacylglycerols are always the main class of lipids (Oftedal *et al.*, 1988; Ackman and Lamonthe, 1989; Iverson *et al.*, 1992). The fatty acid composition of seal milk lipids resembles that of the blubber lipids except that milk lipids have a higher content of 16:0 than the blubber lipids (Jangaard and Ke, 1968).

The main fatty acids in harp seal muscles are 14:0, 16:0, 18:0, 16:1, 18:1, 20:1, 22:1, 18:2, 20:5, 22:5, and 22:6. Muscle lipids are richer in both saturated and polyunsaturated fatty acids than blubber lipids, but blubber is richer in monounstaurated fatty acids (Shahidi and Synoweicki, 1991; Shahidi *et al.*, 1994).

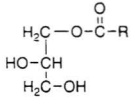
Since marine mammals evolved on land and later became purely aquatic the fatty acid composition of the internal organs is similar to that of terrestrial animals (Ackman and Lamonthe, 1989). The principle fatty acids of seal heart, liver, and lung tissues are 14:0, 16:0, 18:0, 16:1, 18:1, 20:1, 18:2, 20:4, 20:5, 22:5, and 22:6 (Ackman *et al.*, 1972; Ackman and Hooper, 1974; Engelhart and Walker, 1974). Several investigations have reported differences in the fatty acid composition between different internal organs of the same seal. Ackman and Hooper (1974) reported differences between heart, liver, and

blubber fatty acid compositions from both harbour and grey seals. Engelhart and Walker (1974) demonstrated differences in the fatty acid composition between brain, liver, aorta, muscle, and testes of harp seal.

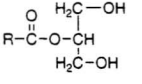
Brain tissue has a very unique lipid composition. Brain lipids contain less than 1% triacylglycerols and more than 70% polar lipids (Henderson *et al.*, 1994). The main fatty acids of brain lipids are 16:0, 18:0, 14:1, 16:1, 18:1, 20:4, and 22:6 (Engelhart and Walker, 1974; Henderson *et al.*, 1994).

2.3.1.3 Acylglycerols

The most common lipids are acyl esters of glycerol known as monoacylglcerols, diacylglycerols, and tricaylglycerols. Monoacylglycerols may be 1- acyl (α-) or 2- (β-) acyl isomers (Gunstone and Norris, 1983).

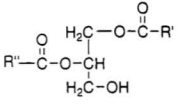


1- acylglycerol

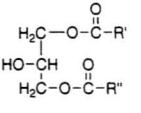


2- acylglycerol

Diacylglycerols may be αβ (1,2- and 2,3- diacylglycerols) or αα′ (1,3- diacylglycerol).



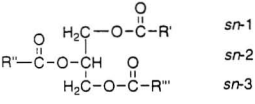
1,2- diacylglycerol



1,3- diacylglycerol

Mono- and diacylglycerols are rarely present in more than trace amounts in fresh animal tissues (Christie, 1982).

Triacylglycerols, the major component of natural oils and fats, are composed of a glycerol molecule esterified with three fatty acids:



1,2,3- triacylglycerol

where R′, R″, and R‴ designate the hydrocarbon chains of fatty acids. Natural triacylglycerols seldom contain three identical acyl groups; more likely they contain two or three different acyl groups selected from all those present in the source material. The number of possible triacylglycerols rises quickly with the number of available acyl groups. Assuming that 30 different acyl groups are present in marine oils, a total of 27,000 isomers could be formed. Even if optical and positional isomerization were omitted, about 5,000 different triacylglycerol molecules could be derived theoretically. Such complexity along with similarity in physical and chemical characteristics makes analyses of marine oils for their triacylglycerol analysis time-consuming, extremely difficult and a complete analysis is virtually impossible (Holmer, 1989).

Hydrolysis of acylglycerols by acid or alkali gives glycerol and a mixture of fatty acids. Enzymatic hydrolysis is more specific; pancreatic lipase, for example, hydrolyzes the acyl groups attached to the two primary hydroxyl groups (*sn*-1 and *sn*-3) and leaves the 2- monoacylglycerols in the intact form (Gunstone and Norris, 1983).

Triacylglycerols may be characterized in a number of ways. The fatty acids in triacylglycerols may be identified and quantified. Triacylglycerols may also be characterized on the basis of the number of double bonds or according to carbon number. Positional distribution of fatty acids on the glycerol backbone is another means of characterizing triacylglycerols.

Brockeroff *et al.* (1968) reported that in harbour seal blubber the two α positions, especially *sn*-3, were occupied by the polyenoic fatty acids 20:5 ω3 and 22:6 ω3. The *sn*-2 position is esterified with high proportions of saturated acids and C_{16}- and C_{18}-monoenoic fatty acids. The longer monoenes are found in the *sn*-1 position. Similar findings have been reported for harp seal (Brockerhoff, 1965; Wanasundara and Shahidi, 1997).

The most common method of stereospecific analysis of triacylglycerols uses a Grignard reaction, phospholipase A_2, and pancreatic lipase to elucidate the positional distribution of the fatty acids (Brockerhoff *et al.*, 1968; Wanasundara and Shahidi, 1997). However, several recent publications have reported similar results using high-resolution ^{13}C nuclear magnetic resonance spectroscopy (e.g. Aursand *et al.*, 1995).

Several studies on positional distribution of fatty acids in seal milk indicate that the PUFA are preferentially esterified at the α position of the triacylglycerol molecules, similar to that of the depot fats (Iverson *et al.*, 1992; Puppione *et al.*, 1992). Existing literature does not indicate fatty acid distribution over all three positions (i.e., *sn*- 1, *sn*- 2, and *sn*- 3) of the milk triacylglycerols.

2.3.1.4 Sterols

Most lipid samples contain some free sterol. In plants, stigmasterol, β-sitosterol, campesterol, and ergosterol are the main sterols while in animals it is predominantly cholesterol. A fraction of the sterol may be esterified with a fatty acid (Gunstone and Norris, 1983). Shahidi and Synowiecki (1991) reported that the cholesterol content of harp seal meat was similar to meats from other animals. However, the cholesterol content is higher in organ tissues as compared to corresponding muscle tissues. Henderson *et al.* (1994) reported the cholesterol content (% total lipid) of Mediterranean monk seal blubber, muscle, liver, heart, and brain to be 1.2, 9.1, 12.3, 12.8, and 23.5, respectively. In fact, cholesterol was reported to be the most abundant neutral lipid in brain tissues.

2.3.1.5 Phospholipids

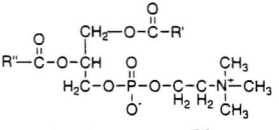
The term phospholipid denotes any lipid containing phosphoric acid as a mono- or diester and includes the glycerophospholipids and the sphingolipid, sphingomyelin (Christie, 1982). They occur widely throughout the animal and plant kingdoms, being particularly associated with biological membranes which provide a physical barrier separating cells and subcellular organelles from their environment and are intimately involved in essential life processes. They facilitate and control the transport of metabolites between the environments they separate, and are involved in many cell functions (Gunstone and Norris, 1983).

Glycerophospholipids are the most widespread and abundant group of membrane lipids. They are similar in that they are a diacylglycerol with a phosphate group esterified

to the primary hydroxyl group of the *sn*-glycerol structure. With the exception of phosphatidic acid, the phosphate forms a phosphodiester bond with the hydroxyl group of a polar headgroup substituent. The most common glycerophospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), diphosphatidylglycerol or cardiolipin (CL), and phosphatidic acid (PA). (Christie, 1982; Mato, 1990a).

Within each class of phospholipid there is a large variation in the fatty acid composition. In the majority of mammalian membrane lipids unsaturated fatty acids are found in position 2 of the glycerol backbone and saturated fatty acids in position 1 (Mato, 1990a).

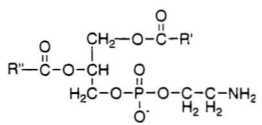
Phosphatidylcholine is the most abundant glycerophospholipid in animals and higher plants (Prasad, 1996, Vaskovsky, 1989). Position *sn*-1 in PC of animals is



phosphatidylcholine (PC)

almost always occupied largely by saturated fatty acids, while position *sn*-2 contains most of the C_{18}, C_{20}, and C_{22} polyunsaturated fatty acids (Christie, 1982). Lysophosphatidylcholine (LPC), where only one of the two available hydroxyl groups is esterified to a fatty acid, is often found when PC is present (Prasad, 1996). It is generally accepted that in most instances position *sn*-1 is esterified to a fatty acid (Christe, 1982).

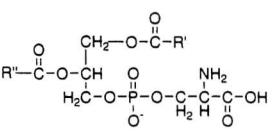
Phosphatidylethanolamine (PE) is generally the second most abundant glycerophospholipid in animals and plants (Prasad, 1996). Its content usually ranges



phosphatidylethanolamine (PE)

from 20 to 25% of the total amount of phospholipids in marine animals (Vaskovsky, 1989). Phosphatidylethanolamines in animals usually contain more PUFAs than the PCs from the same tissue, and the PUFAs are concentrated at the *sn*-2 position (Christie, 1982).

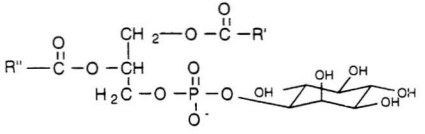
Phosphatidylserine (PS) is a common component of marine animals (Vaskovsky, 1989). It is weakly acidic and is usually isolated as the potassium salt, but may also be associated with calcium, sodium, or magnesium ions (Christie, 1982). Phosphatidyl-



phosphatidylserine (PS)

serine has a similar chromatographic behaviour to PE and some authors present together the data for both of the lipid classes.

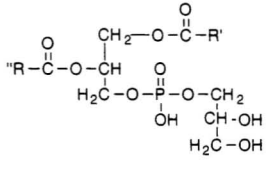
Phosphatidylinositol contains a head group of six carbon cyclic sugar alcohol, inositol, and is a common glycerophospholipid of animal and plant cells (Prasad,



phosphatidyl-1'-myo-inositol (PI)

1996). Phosphatidylinositol is strongly acidic and is usually isolated in association with magnesium or calcium ions (Christie, 1982). Phosphatidylinositol may be further phosphorylated to give polyphosphoinositides such as diphosphatidylinositol and triphosphatidylinositol (Vaskovsky, 1989).

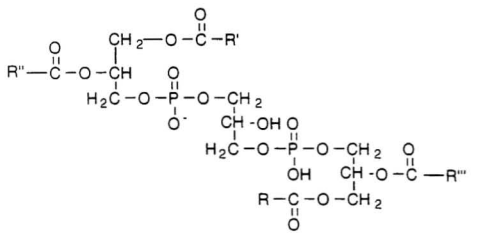
Phosphatidylglycerol (PG) contains a second molecule of glycerol esterified to phosphoric acid. Phosphatidylglycerol is normally present in small quantities in



3-*sn*-phosphatidyl-1'-*sn*-glycerol (PG)

animal tissues and is a minor glycerophospholipid in marine mammals (Vaskovsky, 1989; Prasad, 1996). However, it is possible that PG is more widespread than has been accepted because most of the common solvent systems for thin layer chromatography (TLC) of glycerophospholipids do not resolve the mixture of PG and PE (Vaskovsky and Terekhova, 1979).

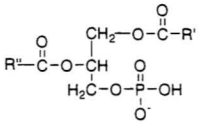
Diphosphatidylglycerol, which is commonly known as cardiolipin (CL), is a major glycerophospholipid of mitochondrial membrane lipids, especially of heart muscle.



1',3'-di-O-(3-*sn*-phosphatidyl)-*sn*-glycerol, or cardiolipin (CL)

It is made up of a phosphatidylglycerol molecule, whose 3'-hydroxyl group of the second glycerol moiety is esterified to the phosphate group of a second phosphatidic acid (Prasad, 1996). The occurrence and properties of cardiolipin have been reviewed (Ioannou and Golding, 1979).

Phosphatidic acid (PA) is a widely distributed minor glycerophospholipid (Vaskovsky, 1989). Although it is a minor glycerophospholipid it is extremely important biosynthetically because it is the precursor to all other glycerophospholipids and of triacylglycerols (Christie, 1982). Phosphatidic acid is strongly acidic and is often isolated as mixed salts. Similar to most glycerophospholipids in animal tissues, position *sn*-1 is occupied predominately by saturated and some monoenoic fatty acids, while position *sn*-2 usually contains polyunsaturated fatty acids.



1,2-diacylglycerol-3-phosphate
phosphatidic acid (PA)

2.3.1.6 Ether Lipids

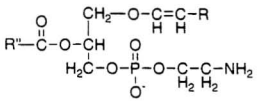
Ether lipids are variations of structures previously discussed. In these compounds the acyl group attached to the *sn*-1 is replaced with an alkyl or alkenyl group. There are four types of these compounds depending on whether the ether link is introduced into a triacylglycerol or a phosphatidyl ester (Figure 2.8). The alkyl and alken-1-yl groups have the same chain lengths as the common fatty acids (Gunstone and Norris, 1983).

The alken-1-yl ether phospholpids are commonly called plasmalogens. They frequently have ethanolamine or choline as their head groups (Mato, 1990b). The plasmalogens are common in many tissues but are found at high levels in various tissues of marine mammals (Henderson *et al.*, 1994).

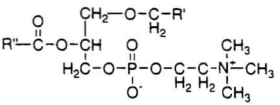
Under acidic transmethylation dimethyl acetals (DMA) are produced from the 1-alk-1′-en-1′-yl linked ether chains.

2.3.1.7 Sphingolipids

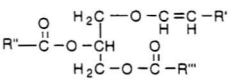
In contrast to all the ester lipid types previously discussed, sphingolipids contain fatty acids combined as amides of long-chain compounds containing an amino and two



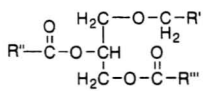
Alk-1-enyl-ether phospholipid or Plasmalogen



Alkyl-ether phospholipid



Alkenyldiacylglycerol (Neutral plasmalogens)



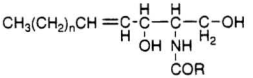
Alkyldiacylglycerol

Figure 2.8. Four types of ether lipids.

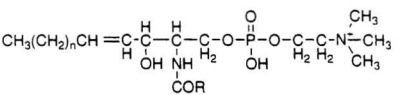
or more hydroxyl groups (Gunstone and Norris, 1983). Sphingolipids include long- chain amines, cerebrosides, gangliosides, and sphingomyelins (Figure 2.9).

Ceramides are amides of fatty acids with long-chain di- or trihydroxy bases, containing twelve to twenty-two carbon atoms in the aliphatic chain (Christie, 1982). In some sphingolipids the ceramide is linked with a sugar moiety through its primary hydroxyl group. The sugar may be simple (glucose or galactose) or complex. Monoglycosylceramides are known as cerebrosides while the more complex ceramides are known as gangliosides. The name cerebroside is derived from brain where it was first isolated. However, they are minor components of most animal tissues and have also been found in plants. The associated fatty acids are mainly alkanoic and 2-D-hydroxyalkanoic acids (Gunstone and Norris, 1983).

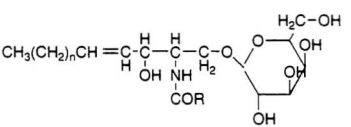
In sphingomyelin, position 1 of the ceramide unit is esterified to phosphorylcholine or phosphorylethanolamine (Gunstone and Norris, 1983). Sphingomyelin is found as a major component of the complex lipids in nearly all animal tissues (Christie, 1982). Sphingomyelins differ in the nature of the sphingosine base and in the acyl group. The most common sphingosine is the 18-carbon aminediol, 1,3-dihydroxy-2-amino-4-octadecene (Mato, 1990a). The most common fatty acids in sphingomyelin are palmitic (16:0) and nervonyl (24:1, 22:0, and 24:0) acids (Svennerholm *et al.*, 1966).



ceramide



ceramide phosphorylcholine (a sphingomyelin)



galactosylceramide (a cerebroside)

Figure 2.9. Structures of some common sphingolipids

2.3.2 Marine lipids in human nutrition and disease prevention

Recently, there has been considerable publicity about the value of marine oils in the diet as a means of minimizing certain diseases, especially those of the heart. However, research in this area has been ongoing for several decades. Probably the first reported use of marine oils in the treatment of disease relates to the use of cod-liver oil in the treatment of arthritis at the Manchester Infirmary in England. This study was later reported in the London Medical Journal (Percival, 1783). In another study, investigators in Norway reported that the rate of heart disease fell drastically during the Second World War, when the availability of meat decreased and the consumption of fish increased (Strøm, 1948; Strøm and Jensen, 1951). Two long-term studies were later conducted to investigate the effects of marine oil consumption on heart disease. A 19 year study by Averly Nelson (Nelson, 1972) in the United States and a 20 year study in Holland by Kromhout-Zutphen (Kromhout *et al.*, 1985) showed that the risk of fatal heart attacks decreased with increased consumption of fish (Stansby, 1990a).

Interest in marine oils intensified in the 1970s when epidemiological studies proposed that the low incidence of coronary heart disease (CHD) in Eskimos might be related to their unique diet. Greenland Eskimos were consuming large amounts of oily fish as well as whale and seal blubber in their diet (>100g/ day), (Pique, 1986). The purpose of the epidemiological studies in the 1970s was to pinpoint the possible adverse effects of a high fat, high protein, low fibre, and low vitamin C diet on the Eskimo's health. Surprisingly, the researchers found that Eskimos were generally healthy and remarkably free from cardiovascular disease (Bang *et al.*, 1976). In a previous study,

these researchers found that the Greenland Eskimos had significantly lower concentrations of serum cholesterol, triacylglycerols, very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) and a higher concentration of high-density lipoproteins (HDL) than their Danish counterparts (Bang and Dyerberg, 1972).

Another situation similar to that of the Greenland Eskimo diet and its effect on heart disease was noted in Japan. Researchers found that families of fishermen living along the coast, who ate much more fish than other Japanese families, demonstrated a decreased incidence of CHD (Stansby, 1990b). A review of some of the Japanese investigations has been compiled by Hirai *et al.* (1987).

The effect of marine oils on CHD has been extensively studied (Burr et al., 1989; Morris *et al.*, 1995; Delorgeril *et al.*, 1996; Davidson *et al.*, 1997; Mizutani *et al.*, 1997; Mori *et al.*, 1997; Pietinen *et al.* 1997), and many reviews published (Bruckner, 1992; Newton, 1996; Nordoy, 1996; Simopoulos, 1997a,b). However, the specific mechanism of the action of marine lipids in prevention of CHD is still unclear. Much of the research suggests that the reduction in CHD by marine oils is through lowering of serum triacylglycerols, reducing the occurrence of arrhythmia and their ability to act as antiatherogenic and antithrombotic agents (Abbey *et al.*, 1990).

Many physiologic and pathophysiologic reactions such as vascular resistance, thrombosis, wound healing, and inflammation are affected by eicosanoids. The eicosanoids include prostaglandins, prostacyclins, thromboxanes, leukotrienes, lipoxins, and hydroxy fatty acids (Weber *et al.*, 1986). The activity of the eicosanoids depends on

minor structural differences and often two different types of prostaglandins can have opposing effects on the body (Pique, 1986).

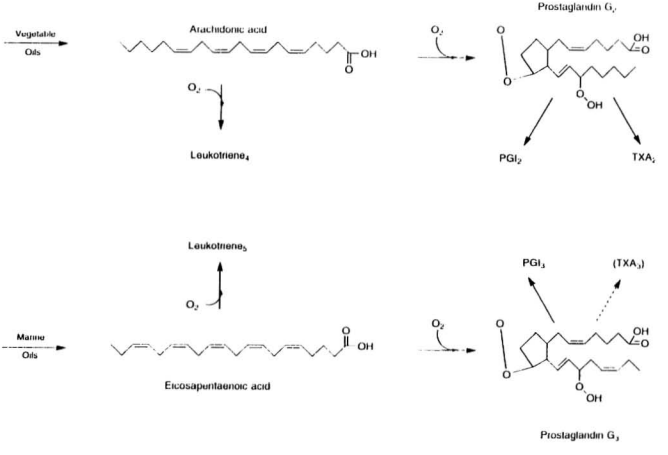
Linoleic acid (LA) is the predominant PUFA in the Western diet and is the precursor of arachidonic acid (AA). Arachidonic acid is in turn the precursor of the prostanoids (prostaglandins and thromboxanes) of the 2 series and of leukotrienes of the 4 series. Eicosapentaenoic acid and DHA, common PUFAs of marine oils, are precursors of the prostanoids of the 3 series and leukotrienes of the 5 series (Needleman *et al.*, 1979; *Weber et al.*, 1986; Simopoulos, 1990; Hwang, 1992). A diagram of the formation of these different eicosanoids is shown in Figure 2.10.

The eicosanoids produced from EPA have a spectrum of biological activity that is more desirable than that of the eicosanoids derived from AA. Thromboxane A_2 derived from AA is proaggregatory and vasoconstrictory whereas thromboxane A_3 derived from EPA is not proaggregatory and is only weakly vasconstrictory. Prostaglandin I_2 derived from AA and prostaglandin I_3 derived from EPA are both antiaggregatory and vasodilatory. Leukotriene B_4 from AA is strongly chemotactic in contrast to leukotriene from EPA which is only weakly chemotactic (Weber *et al.*, 1986).

By far the greatest amount of research has been conducted on the relationship between marine oils and heart disease. However, there has been, and continues to be, a great deal of effort placed on determining the effect of marine oils on other diseases.

Many studies indicate that omega-3 PUFAs may have some therapeutic benefits by reducing inflammatory and autoimmune disorders (Simopoulos, 1991; Boissonneault

Figure 2.10. Biosynthesis of eicosanoids from arachidonic (20:4 ω6) and eicosapentaenoic (20:5 ω3) acids.


Vegetable
Oils
Arachidonic acid
OH
O
O_2
Leukotriene$_4$
O_2
Prostaglandin G$_2$
OH
O
O
O
OH
PGI$_2$
TXA$_2$
Leukotriene$_5$
O_2
Marine
Oils
O
OH
Eicosapentaenoic acid
O_2
PGI$_3$
(TXA$_3$)
OH
O
O
O
OH
Prostaglandin G$_3$

and Hayek, 1992; Endres *et al.*, 1995) including rheumatoid arthritis (Kremer and Jubiz, 1987; Fortin, 1995; Joe and Lokesh, 1997), nephritis (Thais and Stahl, 1987; Robinson *et al.*, 1987), lupus erythematosis (Accinni and Dixon, 1979; Kelly *et al.*, 1985), multiple sclerosis (Bates *et al.*, 1989), and ulcerative colitis (Newton, 1996). The mechanisms of action for the beneficial effects of marine oils on inflammatory and autoimmune diseases are not as well understood as they are for CHD. However, Higgs (1986) studied the role of prostaglandins and leukotrienes in inflammatory diseases, and Boissonneault and Hayek (1992), have concluded that both the ratio of ω3 and ω6 fatty acids, as well as their absolute amounts, may have a profound effects on immune function. These latter investigators suggested that there were three possible mechanisms including alterations in eicosanoid synthesis, changes in lipoprotein levels, and modification in membrane composition that affected transmembrane signal transduction.

There is some debate about the effects of ω3 PUFA consumption and the risk of stroke. A study on Greenland Eskimos suggests that of the populations studied, the one with the higher intake of ω3 PUFAs had a higher incidence of stroke (Kromann and Green, 1980). However, a Japanese study suggests that the incidence of stroke decreased in populations with a higher rate of ω3 PUFA consumption (Hirai *et al.*, 1987). Whether or not consumption of ω3 fatty acids affects the incidence of stroke, a study by Lands (1982), using cats showed that there was reduced damage done to cerebral tissue when the consumption of ω3 fatty acids was high.

There is also evidence that consumption of ω3 fatty acids lowers blood pressure (Lands, 1986; Knapp, 1989; Gerster, 1993; Howe, 1995; Adreassen *et al.*, 1997; Huang *et*

al., 1997). However, a study by Russo *et al.* (1995) indicated that low doses of ω3 PUFAs alone were not effective in lowering blood pressure.

Omega-3 fatty acids may also be important in the relief of some skin disorders. Some relief of psoriasis can be achieved with increased intake of ω3 fatty acids (Ziboh *et al.*,1986; Bittiner *et al.*, 1988; Terano *et al.*, 1989). Furthermore, a study by Kromann and Green (1980) reported that Danes had 20 times the rate of psoriasis compared to Greenland Eskimos.

The impact of increased dietary levels of ω3 PUFAs has been studied is cancer. It has been known for some time that prostaglandins may be involved in the development of cancer (Williams *et al.*, 1968). Much of the effort has been on determining the effects of ω3 fatty acids on breast cancer (Karmeli, 1987; Abou-El-Ela *et al.*, 1989; Abdidezfuli *et al.*, 1997; Bagga *et al.*, 1997; Connolly *et al.*, 1997; Rose, 1997). Other types of cancer where ω3 fatty acids may have beneficial effects are cancer of the colon (Reddy, 1987), prostate (Rose, 1997), and pancreas (Glauert, 1992). Much of the research conducted on ω6 and ω3 fatty acids indicate that polyunsaturated ω6 fatty acids stimulate mammary carcinogenesis, tumor growth, and metastasis, whereas long-chain ω3 fatty acids have inhibitory effects. The precise mechanism is unknown, but Rose (1997) suggests that for breast and prostate cancers a multiplicity of biological actions of eicosanoids derived from tumor cell arachidonate metabolism appear to elicit responses in the tumor and surrounding host cells.

For some time, it has been well established that DHA is involved in the development of the retina. More recently, much evidence has appeared to indicate that the ω3 PUFA in general are essential to certain brain and retina functions and these needs

cannot be met by ω6 PUFAs (Neuringer *et al.*, 1988; Stansby, 1990b). In many neurological disorders including Zellweger syndrome and Adrenoleukodystrophy, tissues, especially brain and retina, are deficient in DHA. As a result, subsequent research has focused on the use of fish oil concentrates to improve both visual symptoms and neuronal dysfunction (Martinez, 1996). There is also a growing body of evidence that ω3 fatty acids, especially DHA, are depleted during depression and that depression is a major predictor of coronary heart disease and hypertension (Hibbeln and Salem, 1995; Peet and Edwards 1997). The effect of DHA on aggression in young adults has also been studied (Hamazaki *et al.*, 1996). Findings indicated that DHA may prevent extraggression from increasing during times of mental stress. The effects or relationships of ω3 PUFAs intake on other diseases including cystic fibrosis (Thies, 1997), bronchial asthma (Masuev, 1997), and diabetes (Bhathena, 1992) have been investigated. Beneficial effects of ω3 PUFAs in these diseases, however, were not conclusive.

Besides in the treatment or prevention of diseases, ω3 fatty acids, particularly DHA, are important to infant development. Prior to birth and for two years afterwards DHA and AA accumulate in brain and retina tissues (Hornsta *et al.*, 1995). Therefore, an adequate supply of DHA to the fetus is essential if its brain and retina are to develop fully and properly. There is no DHA in artificial milk or conventional infant formulas and it has been shown that bottle-fed babies given DHA supplements develop their sight as satisfactorily as breast-fed babies (Carlson, 1995). A study by Bendich and Brock (1997) investigated the rationale for including long chain PUFA that occur naturally in human breast milk to infant formula. They also examined the necessity of increasing the level of

vitamin E along with such a long chain PUFA increase in order to address the increased body demand for natural antioxidants.

2.4 Analysis of lipid composition

Natural lipids are usually mixtures of several classes of compounds with each having a wide range of fatty acids. Therefore, the study of a natural lipid sample can be conducted at several analytical levels. Analyses can be conducted to determine, qualitatively and quantitatively, the nature of the acids, alcohols, or aldehydes associated with the total sample. Alternatively, the sample can be separated into its various classes before analyzing the component acids, alcohols, and aldehydes. Using enzymatic analyses, the fatty acids attached to each of the three carbon atoms in a triacylglycerol or the two carbon atoms in glycerol phospholipids can also be determined. Finally, individual molecular species can sometimes be quantified by combining chromatography and enzymic deacylation techniques (Gunstone and Norris, 1983).

2.4.1 The analysis of fatty acids

If fatty acids are appropriately derivatized so that they absorb strongly in the ultraviolet range they may be detected and quantified by high performance liquid chromatography (HPLC) (Ratnayake and Ackman, 1989). However, about 99% of the analyses of fatty acid composition in foods are determined by gas chromatography (GC) of their methyl esters (Ackman, 1992).

The analysis of fatty acids by GC involves two main steps: the preparation of fatty acid methyl esters (FAMEs) and then the analysis of the FAMEs by GC (Shantha and Napolitano, 1992). The preparation of FAMEs or other derivatives is necessary for two

reasons. First, free acids may associate in pairs in the gas phase and they will absorb to any convenient surface resulting in tailing (Cochrane, 1975). However, this is not a significant problem with modern analyses using flexible fused silica columns (FFSC) (Horjik *et al.*, 1990). The second advantage of FAMEs is that they are more volatile than their corresponding acids.

The reagents that are used to prepare FAMEs are usually considered either "acid-catalyzed" or "base-catalyzed". Acid-catalyzed reagents include boron trifluoride in methanol, methanolic hydrochloric acid, and sulphuric acid in methanol. Base-catalyzed reagents include sodium methoxide in methanol and potassium hydroxide in methanol. Diazomethane and several other reagents are also useful for preparing FAMEs. For samples containing short-chain fatty acids, esters other than methyl esters (i.e., butyl esters) may be prepared (Shantha and Napolitano, 1992).

Probably one of the most important recent developments in GC analysis has been the widespread use of flexible fused silica columns (FFSC) which has made the various packed columns obsolete (Ackman, 1992). However, the modern GC units remain the assembly of three main components: the injection port, the column, and the detector. For most food applications split type injection ports are preferred. As discussed previously, analytical columns are now all FFSC type with various adsorbents, and the flame ionization detector is nearly exclusively used universally (Ackman, 1992).

The tentative identification of components appearing as peaks in a chromatogram is achieved by comparison of their chromatographic behaviour with those of authentic standards. This is suitable for major and more common esters but not for minor and less common constituents. In some cases, it may be necessary to isolate and identify a

particular component using a combination of gas chromatography and mass spectrometry (Gunstone and Norris, 1983).

2.4.2 The analysis of lipid classes

The types and amounts of various lipid classes present in a natural lipid mixture is achieved mainly by chromatographic separation. The most widely used procedures are based on adsorption chromatography with silica or acid-washed florisil. Column or thin layer chromatographic methods may be used with quantitative results obtained through weighing, densitometry, or fluorimetry (thin layer only). (Gunstone and Norris, 1983).

Using column chromatography, neutral lipids are eluted from the column with chloroform, glycolipids with acetone, and phospholipids with methanol. These fractions can then be separated further using a second column and different solvent systems. Similar results can be obtained using thin layer chromatography (TLC). High performance liquid chromatography (HPLC) can also be used in the separation of lipid classes. Columns, and to a lesser extent TLC, are useful for preparative separations. Thin layer chromatography is also very effective in qualitative analyses. However, there are limitations in the application of these methods for quantitative analysis of lipid classes. The application of HPLC to lipid class analysis is restricted by the limitations of the ultraviolet and refractive index detectors for these kinds of compounds.

A relatively recent development has seen the combination of the resolution efficacy of TLC with the quantification capabilities of the flame ionization detector (FID). This system is known as the thin layer chromatography-flame ionization detection (TLC-FID) Iatroscan system. Several reviews (Ackman, 1981; Parish, 1987; Shantha, 1992) and a book (Ranny, 1987) have been published on the Iatroscan system.

The system consists of two independent units, the Chromarods which represents the TLC component and the Iatroscan unit which contains the FID unit. The Chromarod is a quartz rod coated with a thin layer (75 μm) of a mixture of soft glass powder and the adsorbent, either silica gel or alumina. The Iatroscan FID consists of a hydrogen flame jet and an ion collector. The sample is burnt, the ions are collected by the collector electrode, and the signal is amplified in a similar way as in the gas chromatography FID (Shantha, 1992).

Many factors may affect the response of the FID and hence the accuracy of the results obtained. The specific Chromarods being used may affect the response. A study by Indrasena *et al.* (1991) compared alumina and silica Chromarods for separating lipid classes. The nature of the sample may also impact the FID response. Hydrocarbons give higher response than compounds containing oxygen. Natural samples of fish oil triacylglycerols give much lower response than the same amount of pure standard triolein. Studies have shown that fatty acid chain length and degree of unsaturation affect the behaviour of triacylglycerols on Chromarods (Kramer *et al.*, 1985; Ohshima *et al.*, 1987). Other factors affecting response is the amount of sample spotted, sample preparation, spotting technique, Chromarod conditioning, relative position of sample on the Chromarod, and the FID unit set up (Shantha, 1992).

Several techniques have been recommended in order to improve the analysis of lipids using TLC-FID. Response problems occur with the TLC-FID system when degree of unsaturation in a sample varies widely. Hydrogenation of unsaturated samples prior to TLC- FID analysis has been shown to produce an increase in the FID response and is

recommended for many natural samples (Ackman and Ratnayake,1989; Shantha and Ackman, 1990).

Impregnation of the Chromarods may also improve resolution of the samples. Impregnation with copper (II) sulphate results in a more uniform ionization of the sample and as a result a higher response (Kaimal and Shantha, 1984; Kramer *et al.*, 1986a; 1986b). Boric acid impregnation may be used to ensure complete separation of triacylglycerols, 1,3- and 1,2-diacylglycerols, 1-monoacylglycerols, and free fatty acids (Tatara *et al.*, 1983). Silver nitrate impregnation has been reported to be useful in separating positional isomers of fatty acids (Sebedio *et al.*, 1985) and oxalic acid impregnation can be used to give improved separation of phospholipids (De Schrijver and Vermeulen, 1991).

With improvements in instrumentation and the techniques discussed previously to improve response, resolution, and accuracy, the TLC- FID Iatroscan system is now used widely in lipid analysis. It serves as a very useful tool in the identification and quantification of lipid classes.

2.4.3 The structural analysis of lipids

If the triacylglycerol has two different fatty acids esterified to the primary hydroxyl groups of glycerol then the molecule is optically active. In order to specify unambiguously the position of the fatty acid on the glycerol backbone, stereospecific numbering (*sn*) is used. In an optically active molecule drawn in the Fischer projection with the secondary hydroxyl group to left of the middle carbon atom (*sn*-2), the carbon atom above this is *sn*-1 and the one below it is *sn*-3 (Laakso, 1996).

The structure of the triacylglycerol molecule has an impact on its nutritional and biochemical properties. During digestion, lipases hydrolyze triacylglycerols into free fatty acids, predominantly from the *sn*-1/3 positions and 2-monacyl-*sn*-glycerols (Laakso, 1996). Although most dietary fats are in the triacylglycerol form, relatively little is known about the importance of the stereospecific composition of triacylglycerols on the biological activity of dietary fatty acids (Kubow, 1996). With regard to triacylglycerol, the biosynthesis of all glycerol positions are known to be important. The main pathways for triacylglycerol synthesis include *sn*-glycerol-3-phosphate, dihydroxyacetone phosphate, and monoacylglycerols. Generally, the sn-3 position is the last to be esterified (Laakso, 1996). Differences in the positional distribution of fatty acids have also been shown to have specific effects on the profile, structure, and composition of lipoproteins (Kubow, 1996). Studies also indicate that the triacylglycerol structure, in addition to fatty acid composition, affects the cholesterolemic and atherogenic potency of a fat (Kritchevsky *et al.*, 1982; Hayes *et al.*, 1995; Kritchevsky, 1995).

The determination of fatty acyl residues esterified to the primary and secondary hydroxyl groups of triacyl-*sn*-glycerols is most often accomplished with pancreatic lipase (Laakso, 1996). However, other methods are suitable for regiospecific analysis of triacyl-*sn*-glycerols including mass spectrometry (Currie and Kallio, 1993; Kallio and Currie, 1993), carbon 13 nuclear magnetic resonance (^{13}C NMR) spectroscopy (Aursand *et al.*, 1995), and by hydrolysis with Grignard reagent and separation of fractions by TLC (Iverson *et al.*, 1992).

As previously discussed, the differentiation of fatty acids between primary and secondary positions might be essential from a nutritional viewpoint. The methods for the

complete stereospecific analysis of triacylglycerols are almost always based on the formation and separation of diacyl-sn-glycerols (Laakso, 1996). The traditional methods include the phosphorylation of diacylglycerols and their hydrolysis with phospholipases (Brockerhoff, 1971; Litchfield, 1972; Christie, 1982; Gunstone and Norris, 1983; Laakso, 1996). More recently, however, methods for the separation of enantiomeric acylglycerols have been developed. There are two main ways to separate enantiomers. First, by reaction with an achiral reagent, enantiomers form enantiomeric derivatives that may be separated by the chiral stationary phases. Reaction with a chiral reagent may also be carried out, upon which enantiomers form diastereomeric derivatives that are separated by achiral stationary phases (Takagi, 1990; Christie, 1994).

The traditional methods of stereospecifc analysis were pioneered by Brockerhoff (1965). The first step was the partial deacylation of triacylglycerols with pancreatic lipase or Grignard reagent. Currently, Grignard is preferred because it has no fatty acid specificity (Franzke *et al.*, 1973) and causes less acyl migration than other available methods (Yurkowski and Brockerhoff, 1966). Since the Grignard reagent reacts in a random manner with the ester linkages, *sn*-1,2-diacylglycerols, *sn*-1,3-diacylglycerols, monoacylglycerols, and tertiary alcohols are produced (Gunstone and Norris, 1983).

The *sn*-1,2-diacylglycerols can be separated from the *sn*-1,3-diacylglycerols and other fractions by TLC or HPLC using silica gel as the stationary phase. The purified αβ-diacylglycerols are then phosphorylated to their phosphatidylphenols by reacting with phenyldichlorophosphate. The fatty acid in the *sn*-2 position is hydrolyzed using phospholipase A_2 leaving a lysophosphatidylglycerol with the fatty acid in position *sn*-1 and unreacted *sn*-2,3-diacylglycerol phosphatidylglycerol. The fatty acid composition of

position *sn*-2 can therefore be determined from either the free fatty acids or from 2-monoacylglycerols. The fatty acid composition of *sn*-1 is determined by analysis of lysophosphatidylphenols and *sn*-3 is determined indirectly by calculation (Laakso, 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Samples

Tissue samples of bearded (*Erignathus barbatus*), hooded (*Cystophora cristata*), harp (*Phoca groenlandica*), and ringed (*Phoca hispida*) seals were obtained from areas Northeast of Newfoundland by a Department of Fisheries and Oceans research vessel. Harbour seal (*Phoca vitulina*) tissues were obtained from the Southcoast of Newfoundland by a commercial fisherman and Grey seal (*Halichoerus grypus*) tissues were obtained from Nova Scotia by commercial fishermen. All samples were from adult specimens and were collected between April and June of 1995, 1996, and 1997. Upon retrieval and cleaning, samples were vacuum bagged, frozen, and stored at -26°C until analyzed.

3.1.2 Chemicals

All chemicals used in this study were American Chemical Society (ACS) grade or better. Spectroscopic and high performance liquid chromatographic (HPLC) grade chemicals were used for analyses and preparation of reagents as required. Glass distilled water was used for preparation of reagents. Water was demineralized and its organic matter removed using an Ultrapure Barnstead Reverse Osmosis system (Barnstead, Boston, MA) coupled with organic removal, demineralization and submicron filtration.

3.2 Methods

3.2.1 Proximate Composition and Lipid Extraction

3.2.1.1 Moisture Content

Approximately 5.0g of homogenized seal tissue were dried in a pre-weighed aluminum pan (57mm, Fisher Scientific, Unionville, ON) in a forced- air oven (Fisher Isotemp 300, Fair Lawn, NJ) at 104 ± 1°C until a constant weight was obtained. Moisture content was calculated as percentage weight loss (moisture) of the sample during drying (AOAC, 1990).

3.2.1.2 Crude Protein Content

Crude protein content was determined by Kjeldahl analysis (AOAC, 1990). Homogenized samples (200 to 300 mg) were placed into pre-labeled digestion tubes with 20 mL of concentrated sulphuric acid and two catalyst tablets (Kjeltabs, Profamo Analytical Service Inc., Dorval, PQ) containing 5g potassium sulphate and 0.25g HgO. The tubes were placed in a preheated (410°C) digestor (Buchi 430, Buchi Laboratorium-Technik AG, Schweiz, Switzerland) for 50 to 60 min until the solution was clear or pale yellow. To the digested samples, 100 mL of distilled water and 150 mL of 25% (w/v) sodium hydroxide were added. The samples were then steam distilled (Buchi 321, Buchi Laboratorium- Technik AG, Schweiz, Switzerland) and the condensate was collected into 50 mL 4% (w/v) boric acid solution containing 12 drops of methyl red/ methylene blue indicator. The sample was distilled until 150 mL of condensate were collected. After distillation, the collected condensate was titrated with a standardized 0.1N H_2SO_4 to

reach the end point. A sample blank determination was also carried out. Nitrogen and crude protein content were calculated as follows.

$$\% \text{ Nitrogen} = \frac{\text{Volume } H_2SO_4 \text{ (sample- blank) mLs x Normality } H_2SO_4 \text{ x } 14.007}{\text{Weight of sample in mg}} \times 100$$

% Crude protein = % Nitrogen x 6.25

3.2.1.3 Lipid extraction and quantification

Total lipids were extracted and quantified by the procedure of Bligh and Dyer (1959). A 25g sample of tissue was homogenized in a Waring Blender for two min with a mixture of 25 mL chloroform and 50 mL methanol. This was then followed by the addition of another 25 mL chloroform and further blending for 30 s. 25 mL distilled water was then added and blending continued for another 30 s. The homogenate was filtered through a Whatman No. 1 filter paper in a Büchner funnel with slight suction. The residue and filter paper were blended with an additional 25 mL chloroform. The mixture was filtered on a new Whatman No. 1 filter paper through the original Büchner funnel. The combined filtrates were then transferred to a 250 mL separatory funnel and allowed to separate at 4°C overnight. The chloroform layer was recovered and the solvent evaporated in a rotary evaporator at 40°C. The lipid content was then determined gravimetrically.

3.2.1.4 Ash Content

Homogenized seal tissue samples were weighed into pre-cleaned porcelain crucibles and charred using a Bunsen burner flame. The charred samples were transferred to a muffle furnace (Blue M Electric Co., Blue Island, IL) at 550°C and held there until the entire sample turned greyish white, usually within 12 h. The weight of the residue remaining after ashing was used to calculate the percent ash content (AOAC, 1990).

3.2.2 Separation of neutral and polar lipid fractions by column chromatography

Crude lipids extracted in section 3.2.1.3 were separated into neutral and polar fractions by column chromatography according to Christie (1982). Crude lipids (1.5g) were applied to a silicic acid column (1.25 cm internal diameter, 20 cm height, 100 mesh silicic acid powder, Mallinchrodt Canada Inc., Point Claire, PQ). The neutral lipid fraction was eluted first with chloroform (48 times of the column volume). The polar fraction was eluted with methanol (48 times the column volume). The solvents were removed from each fraction under vacuum using a rotary evaporator at 40°C.

3.2.3 Lipid class analysis by thin layer chromatography- flame ionization detection (TLC-FID) Iatroscan

3.2.3.1 Instrumentation

Crude lipid samples extracted in section 3.2.1.3 were chromatographed on silica gel coated Chromarods- SII and then analyzed on an Iatroscan MK V TLC/FID analyzer

(Iatron Laboratories Inc., Tokyo, Japan) connected to a computer loaded with TSCAN software (S.P.E. Limited, Concord, ON) for data handling. A hydrogen flow rate of 160 mL/ min and an air flow rate of 2,000 mL/ min was used. The rods were scanned at a speed of 30s/ rod.

3.2.3.2 Preparation of Chromarods

The Chromarods were cleaned by soaking them in concentrated nitric acid overnight and they were then thoroughly washed with distilled water and acetone. To improve separation, the Chromarods were impregnated with boric acid by dipping in a 3% (w/v) boric acid solution for 5 min and then dried at 120° C for 5 min. The Chromarods were scanned twice to burn off any remaining impurities and to obtain a standard silica gel.

3.2.3.3 Standards and calibration

A stock solution of individual neutral lipids, including free fatty acids (oleic acid), monoacylglycerol (monoolein), diacylglycerol (diolein), triacylglycerol (triolein), and cholesterol as well as individual polar lipids namely phosphatidylcholine, lysophosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidyl-serine, cardiolipin, sphingomyelin, sulphatide, and cerebroside were prepared by dissolving them in chloroform/ methanol (2:1, v/v) and stored at -20°C. A range of dilutions from 1 to 10 μg/μL were prepared and run to obtain the R_f values and standard curves for each standard.

3.2.3.4 Iatroscan (TLC-FID) analysis of seal tissues

A 1 μL aliquot of sample in chloroform/methanol (2:1, v/v) was spotted on silica gel coated Chromarods- SII and developed in two solvent systems. Prior to development, the Chromarods were conditioned in a humidity chamber containing saturated $CaCl_2$ for 20 min. The first development was carried out using hexane/diethyl ether/formic acid (85:15:1, v/v/v) for the separation of nonpolar lipids. Following development, the Chromarods were dried at 100°C to remove solvents and partially scanned to the positions after the monoacylgylcerols peak. The rods were again conditioned in a humidity chamber for 20 min followed by a second development in chloroform/ methanol/ water (80:35:3, v/v/v) for the separation of the polar lipid classes (Innis and Clandinin, 1981). After drying in the oven, the rods were completely scanned. The area of each peak in each chromatogram was calculated with TSCAN Data software. The identity of each peak was determined by comparison with a chromatogram of standards acquired concurrently with the samples. The determination of the weight of the individual classes was achieved by construction of standard curves using a known amount of standards on the Chromarods and developed under identical conditions mentioned earlier.

3.2.4 Stereospecific analysis

3.2.4.1 Purification of triacylglycerols

To obtain pure triacylglycerols from crude lipids, column chromatography (1.25 cm internal diameter and 10 cm height) on silicic acid (100- 200 mesh size, Mallinckrodt Canada Inc., Point Claire, PQ) was employed. First, the column was washed with hexane

and then 1.00 g oil was placed on the top of the column. Hexane (50 mL) was added to the column; elution was then achieved using 250 mL 10% (v/v) diethyl ether in hexane. The solvent was removed under vacuum at 40°C in a rotary evaporator. The recovered oil was then dried over anhydrous sodium sulphate. A few crystals of butylated hydroxytoluene (BHT) were added to prevent oxidation.

3.2.4.2 Grignard reaction on purified triacylglycerols

Grignard reaction was performed on purified triacylglycerols (Section 3.2.4.1) according to the method described by Brockeroff *et al.* (1968) and Brockerhoff (1971) with some modifications. One gram of purified triacylglycerols was dissolved in 50 mL anhydrous diethyl ether followed by the addition of Grignard reagent (3.5 mL, 3.0 M CH_3MgBr, Sigma, St. Louis, MO). The reaction was allowed to proceed with vigorous stirring until the solution became clear. Glacial acetic acid (1.0 mL) was added to the mixture to stop the reaction. This was followed by the addition of a 10% (w/v) boric acid solution (10 mL) to minimize acyl migration. Stirring was continued for another 2-3 min. The mixture was then transferred to a separatory funnel. After separation into two layers, the top ether layer was removed. The aqueous layer was then washed twice with diethyl ether. The ether layers were combined and washed successively with 10 mL of water, 10 mL of 2% (w/v) aqueous sodium bicarbonate, and 10 mL of water. The washed ether layer was then dried over anhydrous sodium sulphate and the solvent was then removed under vacuum at 40°C.

3.2.4.3 Separation of individual lipid classes from Grignard reaction

The products from the Grignard reaction in section 3.2.4.2 were dissolved in a minimum amount of chloroform and applied to several TLC plates (20 X 20 cm; Silica gel, 2- 25 μm mean particle size, 60 Å mean pore diameter, 1000 μm thickness, with dichlorofluorescein, Sigma) impregnated with 5% (w/v) boric acid. The plates were developed in two solvent systems: first, diethyl ether/ petroleum ether (boiling point 30°C- 60°C)(8:92 v/v); and second, diethyl ether/ petroleum ether (40:60 v/v) with a 5 min drying period in air between solvent systems. The bands were located by viewing under short (254 nm) and long (365 nm) ultraviolet light (Spectraline, Model ENF-240C, Spectronics Co., Westbury, NY). From the separated bands of monoacylglycerols (MAG; R_f = 0.02), 1,2- (1,2- DAG) and 2,3-diacylglycerols (2,3- DAG; R_f = 0.4), 1,3-diacylglycerols (1,3- DAG; R_f = 0.5), tertiary alcohol (R_f = 0.8), and triacylglycerols (TAG; R_f = 1.0), the 1,2- and 2,3-diacylglycerol bands were scraped off and then extracted with diethyl ether. The ether was removed under a stream of nitrogen; a small portion of sample was withdrawn for fatty acid analysis and the remaining diacylglycerol fractions were used to prepare synthetic phospholipids.

3.2.4.4 Preparation of synthetic phospholipids from diacylglycerol fraction

The 1,2- and 2,3-diacylglycerols, obtained in section 3.2.4.3, were dissolved in 1 mL diethyl ether and added to a mixture of 1 mL pyridine, 1 mL diethyl ether, and 0.5 mL phenyl dichlorophosphate. The reaction mixture was allowed to stand at room temperature for 1 h, after which 5 mL pyridine, 3 mL diethyl ether, and several drops of

water were added, with cooling. The contents of the flask were then mixed with 86 mL of methanol/ water/ chloroform/ triethylamine (30:25:30:1, v/v/v/v). After thorough mixing the mixture was allowed to stand, the lower chloroform layer containing synthetic phospholipids (1,2-diacyl-3-phosphatide and 2,3-diacyl-1-phosphatide) was recovered and the solvent removed at 40°C using a rotary evaporator.

3.2.4.5 Stereospecific hydrolysis of synthetic phospholipids by phospholipase A_2

The synthetic phospholipids were dissolved in 2 mL diethyl ether and added to a solution containing 15 mL 0.1 M triethylammonium bicarbonate (pH 7.5), 100 µL 0.1 M calcium chloride and 2.0 mg phospholipase A_2 (E.C. 3.1.1.4; Sigma) obtained from snake venom (*Crotalus adamantus*). The mixture was then shaken gently overnight in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ). The water was then evaporated at 40°C in a rotary evaporator with the addition of 20 mL isobutanol to prevent foaming.

The products of hydrolysis were dissolved in 1 mL chloroform/methanol (1:1, v/v) containing one drop of glacial acetic acid. The solution was then applied to TLC plates (20 x 20 cm, Silica gel, 60 Å mean pore diameter, 2- 25 µm mean particle size, with dichloroflourescein, Sigma) impregnated with a 5% (w/v) boric acid solution. The plates were developed in diethyl ether/petroleum ether (40:60, v/v), dried for 5 min in air, then kept over concentrated aqueous ammonia for 10 min, and subsequently redeveloped in concentrated aqueous ammonia/methanol/diethyl ether (2:15:83, v/v/v). After air drying, the bands were located by viewing under short (254 nm) and long (365 nm)

ultraviolet light (Spectroline, Model ENF- 240C, Spectronics Co., Westbury, NY). The separated bands: free fatty acids (hydrolyzed from *sn*-2 position of 1,2-diacyl-3-phosphatide: R_f = 0.70), unhydrolyzed 2,3-diacyl-1-phosphatide (R_f = 0.46), lysophosphatide (R_f = 0.04) and 1,2- and 2,3-diacylglycerols (R_f = 0.94) (Figure 2.2) were scraped off and extracted into chloroform/methanol (1:1, v/v). A small sample of the lipids was removed for fatty acid analysis, as described in section 3.5. The remaining unhydrolyzed 2,3-diacyl-1-phosphatide fraction was hydrolyzed by porcine pancreatic lipase as described in section 3.2.4.6.

3.2.4.6 Hydrolysis by pancreatic lipase

The unhydrolyzed 2,3-diacyl-1-phosphatide fraction obtained in section 3.2.4.5 was hydrolyzed with pancreatic lipase according to the method described by Christie (1982) for the determination of the fatty acids at the *sn*-3 position. A 25 mg sample of the lipid was added to a mixture of Tris- hydrochloric acid buffer (5 mL, 1.0 M, pH 8.0), 0.5 mL of calcium chloride (2.2%, w/v) and 1.25 mL sodium taurocholate (0.05%, w/v) in a glass test tube. The mixture was allowed to equilibrate at 40°C in a water bath for 1 min followed by the addition of 5.0 mg of porcine pancreatic lipase (E.C. 3:1:1:3, Sigma). This mixture was then placed in a Gyrotory water bath shaker (Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 200 rpm for 8-10 min at 40°C under nitrogen. Ethanol (5 mL) was added to stop the enzymatic hydrolysis followed by the addition of 5.0 mL of 6.0 N HCl. The products of hydrolysis were extracted three times with 50 mL of diethyl ether; the ether layer was washed twice with distilled water and dried over anhydrous sodium sulphate. The solvent was removed under vacuum

using a rotary evaporator at 30°C. The hydrolytic products were then separated on TLC plates (20 x 20 cm; Silica gel, 60 Å mean pore diameter, 2-25 µm mean particle size, 500 µm thickness, with dichloroflourescein, Sigma) impregnated with 5% (w/v) boric acid. The plates were developed in hexane/ diethyl ether/ acetic acid (70:30:1, v/v/v); air-dried, and the bands located by viewing under short (254 nm) and long (365 nm) ultraviolet light (Spectroline, Model ENF- 240C, Spectronics Co., Westbury, NY). The bands were scraped off the plates and extracted into chloroform/ methanol (1:1, v/v). The fatty acid analysis of the samples was conducted as described in section 3.2.5.

3.2.5 Analysis of fatty acid composition of lipids

3.2.5.1 Preparation of fatty acid methyl esters (FAMEs)

Prior to determination of the fatty acid composition of lipids, fatty acid methyl esters (FAMEs) were prepared. Approximately 10-20 mg of the lipid sample were weighed into a 6 mL Teflon- lined screw- capped conical vial. The internal standard (250 ng/100µL chloroform, methyl tricosanoate, C23:0) was added to the vial and traces of solvent were removed by evaporation under a stream of nitrogen, followed by the addition of 2 mL of freshly prepared transmethylating reagent (3 mL of concentrated H_2SO_4 made up to 50 mL with spectral grade methanol and 8 mg of hydroquinone) to the mixture. The mixture was then thoroughly mixed, by vortexing, and incubated at 60°C for 15 h (Wanasundara and Shahidi, 1997). The mixture was cooled and distilled water (1mL) added. After thorough mixing, the sample was extracted three times with spectral

grade hexane (1.5 mL). Prior to extraction with hexane, a few crystals of hydroquinone were added to each sample. The hexane layers were removed and combined in a clean test tube followed by two washings with distilled water (1.5mL). After the first washing the aqueous layer was removed and discarded and after the second washing the hexane layer was removed and placed in a 2 mL wide mouth crimp GC vial and the hexane evaporated under a stream of nitrogen. Fatty acid methyl esters were then dissolved in carbon disulphide (1 mL) and used for gas chromatographic analysis (Section 3.2.5.2).

3.2.5.2 Analysis of fatty acid methyl esters by gas chromatography

Fatty acid methyl esters (FAMEs) were analyzed using a Hewlett Packard 5890 Series II chromatograph (Hewlett Packard, Toronto, ON) with a Supelcowax- 10 column (0.25 mm diameter, 30 m length, 0.25 µm film thickness; Supelco Canada Ltd., Oakville, ON). A Hewlett Packard 7673 autoinjector (Hewlett Packard, Toronto, ON) was used to inject the samples. The temperature of the oven was set at 220°C for 10.25 min followed by ramping to 240°C at 20°C/min where it was held for 9 min. The injector temperature was 270°C. Helium (15 mL/min) was used as a carrier gas. Hewlett Packard 3365 Series II Chemstation software (Hewlett Packard, Palo Alto, CA) was used for data handling. The FAMEs were identified by comparison of their retention times with those of an authentic standard mixture (GLC-416; Nu-chek) obtained under the same conditions.

3.2.6 Statistical Analysis

All experiments were replicated 3 times with a minimum of three individual seals and mean values ± standard deviation reported for each sample type. However, in the

case of Bearded seal samples only one specimen was available. This sample was not included in the statistical treatment of data, but it was then placed on the graphs to examine its possible relationship with other results. Analysis of variance and Tukey's studentized range test (Snedecor and Cochran, 1980) were used to determine significance of differences between mean values.

A multivariant technique, known as principle component analysis (PCA), first described by Pearson, was used in order to separate information from noise in several data matrices (Manly, 1994). PCA looks for a few linear correlations (principle components) which can be used to summarize the data, losing as little information in the process as possible. As a result some degree of economy is achieved in that the variation in the original number of fatty acids is accounted for by a smaller number of variables, 2 or 3 principle components. In this study the PCA was used to produce two coordinates (principle components) that described the largest and the second- largest variance among the samples. Prior to statistical analysis, data that was reported as percentages were transformed as given below.

$$\text{transformed } x = \arcsin\sqrt{x/100}$$

Chapter 4

Results and Discussion

4.1 Proximate Composition

4.1.1 Moisture Content

The moisture contents of all tissues from the six Canadian species of phocid seals is provided in Table 4.1. As expected, the moisture content of blubber of each species analyzed was much lower than other tissues studied. However, there was no significant ($p>0.05$) difference in the moisture content of the blubber from different species. Harp seal milk had a higher moisture content than blubber, but lower than all other tissues. Muscle tissue was the next lowest with the liver having a similar moisture content in species where both tissues were analyzed. The order of moisture content for heart, kidney, and lung varied from species to species.

There were significant ($p<0.05$) differences in the moisture contents in some of the same tissues between species but no species was consistently higher or lower in moisture content for a majority of tissues examined.

4.1.2. Protein Content

The protein content of samples was generally dependent on the tissue under consideration, but there were also significant ($p<0.05$) differences from species to species for the same tissue (Table 4.2). Similar to the trend in the moisture content, the protein content was lowest in blubber followed by milk. However, in contrast to moisture

Table 4.1. Moisture content (g/100g tissue) of various tissues from six species of seals and milk of harp seal.

Tissue	Harp	Grey	Harbour	Ringed	Hooded	Bearded*
Blubber	5.34 ± 0.10^{b1}	5.88 ± 0.53^{b1}	5.71 ± 0.31^{b1}	3.52 ± 0.82^{a1}	6.22 ± 1.04^{b1}	6.11
Muscle	68.32 ± 0.90^{a3}	72.12 ± 0.45^{c2}	71.50 ± 0.83^{bc2}	72.00 ± 0.47^{c3}	69.79 ± 0.61^{ab23}	73.69
Brain	78.31 ± 0.94^{a4}	77.25 ± 0.28^{a3}	77.09 ± 0.48^{a4}	78.54 ± 0.72^{a5}	77.13 ± 0.92^{a6}	76.08
Kidney	78.91 ± 0.85^{d4}	76.95 ± 0.11^{c3}	NA	74.94 ± 0.04^{b3}	72.15 ± 0.08^{a2}	75.11
Heart	78.15 ± 0.54^{b4}	77.23 ± 1.89^{b3}	78.11 ± 0.55^{b4}	72.60 ± 0.42^{a2}	71.30 ± 0.14^{a2}	72.84
Liver	71.19 ± 0.49^{b3}	71.52 ± 0.16^{b2}	NA	70.01 ± 0.44^{ab1}	68.49 ± 0.25^{a1}	68.25
Lung	79.40 ± 0.34^{d4}	80.63 ± 0.35^{d3}	73.88 ± 0.14^{bc3}	72.08 ± 0.58^{ab2}	74.62 ± 0.87^{c3}	69.86
Milk	38.82 ± 0.69^{2}	NA	NA	NA	NA	NA

NA = Sample was not available for analysis.

The means followed by different alphabetical superscripts in each column are significantly different ($p<0.05$) from each other. Similarly, the means followed by different numerical superscripts in each row are significantly different ($p<0.05$) from each other.

*Bearded seal not included in statistical analysis because only one sample was available for analysis.

Table 4.2. Protein content (g/100g tissue) of various tissues from six species of seals and milk of harp seal

Tissue	Harp	Grey	Harbour	Ringed	Hooded	Bearded*
Blubber	0.31 ± 0.06^{ab1}	0.61 ± 0.08^{b1}	0.45 ± 0.06^{ab1}	0.30 ± 0.12^{a1}	0.58 ± 0.19^{ab1}	0.63
Muscle	28.48 ± 0.31^{c7}	23.04 ± 0.41^{a6}	26.43 ± 0.70^{b4}	25.41 ± 0.64^{b6}	26.59 ± 1.02^{b6}	23.37
Brain	12.49 ± 0.62^{a3}	12.08 ± 0.43^{a2}	12.54 ± 0.77^{a2}	12.33 ± 0.86^{a2}	13.01 ± 0.40^{a2}	11.94
Kidney	16.87 ± 0.19^{a45}	18.07 ± 0.27^{b34}	NA	20.60 ± 0.23^{c3}	23.93 ± 0.26^{d4}	21.04
Heart	19.00 ± 0.44^{a56}	19.98 ± 0.81^{a45}	20.14 ± 0.91^{a3}	23.74 ± 0.11^{b5}	25.15 ± 0.33^{b45}	24.68
Liver	20.93 ± 0.86^{a6}	21.13 ± 0.51^{a56}	NA	22.40 ± 0.04^{a4}	26.18 ± 0.56^{b5}	26.81
Lung	15.83 ± 0.71^{a4}	16.62 ± 0.77^{ab3}	15.71 ± 2.01^{a23}	25.64 ± 0.14^{cd6}	21.97 ± 0.42^{bc3}	29.79
Milk	7.69 ± 0.33^{2}	NA	NA	NA	NA	NA

NA = Sample was not available for analysis.

The means followed by different alphabetical superscripts in each column are significantly different ($p<0.05$) from each other. Similarly, the means followed by different numerical superscripts in each row are significantly different ($p<0.05$) from each other.

*Bearded seal not included in statistical analysis because only one sample was available for analysis.

content, muscle tissue protein content was the highest of all tissues in the species analyzed. Brain protein contents were consistently lower than all other tissues, except for blubber and milk. The protein content of kidney was most often lowest and that of liver was generally the highest among kidney, heart, liver and lung tissues.

Again, trends between species were less apparent. However, of the species analyzed, hooded seal tissues generally had the highest and harp seal tissues the lowest protein content.

4.1.3. Lipid Content

The lipid contents of different tissues of the six species of seals are shown in Table 4.3. Exactly opposite to the trends for moisture and protein contents, the content of lipids was highest in blubber followed by milk. Brain tissue had the highest lipid content of the remaining tissues while muscle had the lowest lipid content. The lipid content of liver was generally the highest while that of lung was generally the lowest among tissues of kidney, heart, liver, and lung. There were significant ($p<0.05$) differences in the same tissues of different seals, but none were consistently higher or lower.

Previous studies on seal lipids have concentrated on blubber (West *et al.*, 1979; Ackman *et al.*, 1971; Shahidi *et al.*, 1996). However, the lipid contents of the tissues of the seals analyzed in this study are consistent with those previously reported for some of the same organs of other seal species (Ackman and Hooper, 1974; Henderson *et al.*,

Table 4.3. Lipid content (g/100g tissue) of various tissues from six species of seals and milk of harp seal

Tissue	Harp	Grey	Harbour	Ringed	Hooded	Bearded*
Blubber	93.88 ± 1.64^{b4}	91.93 ± 1.07^{ab4}	92.72 ± 1.13^{ab3}	93.55 ± 1.98^{ab3}	89.43 ± 1.82^{a3}	90.45
Muscle	1.92 ± 0.03^{a1}	1.82 ± 0.03^{a1}	1.68 ± 0.41^{a1}	1.85 ± 0.53^{a1}	2.36 ± 0.74^{a1}	1.74
Brain	8.10 ± 0.32^{ab2}	10.25 ± 0.10^{c3}	9.86 ± 0.84^{bc2}	6.86 ± 1.01^{a2}	7.40 ± 0.79^{a2}	12.58
Kidney	2.97 ± 0.18^{a1}	3.42 ± 0.04^{bc12}	NA	3.58 ± 0.07^{c1}	3.14 ± 0.05^{ab1}	3.05
Heart	2.19 ± 0.31^{a1}	1.81 ± 0.38^{a1}	1.86 ± 0.14^{a1}	2.32 ± 0.01^{a1}	2.04 ± 0.01^{a1}	1.70
Liver	3.83 ± 0.19^{ab1}	5.60 ± 0.94^{b2}	NA	3.71 ± 0.07^{ab1}	3.66 ± 0.03^{ab1}	2.71
Lung	2.24 ± 0.46^{b1}	2.04 ± 0.03^{b1}	1.88 ± 0.02^{ab1}	2.05 ± 0.02^{b1}	1.76 ± 0.01^{ab1}	1.06
Milk	50.17 ± 0.83^{3}	NA	NA	NA	NA	NA

NA = Sample was not available for analysis.

The means followed by different alphabetical superscripts in each column are significantly different ($p<0.05$) from each other. Similarly, the means followed by different numerical superscripts in each row are significantly different ($p<0.05$) from each other.

*Bearded seal not included in statistical analysis because only one sample was available for analysis.

NOTE TO USERS

Page(s) not included in the original manuscript and are unavailable from the author or university. The manuscript was microfilmed as received.

69

This reproduction is the best copy available.

UMI

1994). The lipid content of harp seal milk (50.17%) was on the high side of the range reported in previous studies (Jangaard and Ke, 1968; Cook and Baker, 1969; Jenness and Sloan, 1970; Lavigne *et al.*, 1982; Stewart *et al.*, 1983; Iverson *et al.*, 1992), but was lower than that reported for hooded seal (61%), apparently the highest fat content for any mammalian milk (Oftedal *et al.*, 1988). The lipid content of the brain tissue of different seals was typical of those of mammals in general (Sastry, 1985).

4.1.4 Ash Content

There were significant ($p<0.05$)differences in the ash content of different seal species and tissues (Table 4.4), but no trends were apparent. Harp seal milk, however, was much lower in ash content than any of the seal tissues.

4.2 Lipid Classes

4.2.1 Neutral and Polar Lipids

The neutral and polar lipid fractions in different tissues of seals and classes constituting each varied. Depending on the tissue under consideration classes that were detectable with the Iatroscan system, with a reasonable level of confidence, were triacylglycerols, free fatty acids, and cholesterol. Similarly, polar lipids were comprised of cardiolipin, phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, and sphingomyelin. Significant levels of cerebroside were found in the brain tissue lipids. Data on the contents of the individual lipid classes in selected tissues of different species of seals are provided in Table 4.5.

Table 4.4. Ash content (g/100g tissue) of various tissues from six species of seals and milk of harp seal

Tissue	Harp	Grey	Harbour	Ringed	Hooded	Bearded*
Blubber	1.174 ± 0.062^{a23}	1.280 ± 0.452^{a1}	1.911 ± 0.087^{b2}	1.633 ± 0.117^{ab4}	1.419 ± 0.096^{ab34}	1.284
Muscle	1.066 ± 0.074^{a2}	1.204 ± 0.069^{a1}	1.178 ± 0.033^{a1}	1.118 ± 0.066^{a23}	1.217 ± 0.049^{a23}	1.095
Brain	1.749 ± 0.010^{cd4}	1.894 ± 0.002^{d1}	1.247 ± 0.066^{a1}	1.554 ± 0.083^{b4}	1.625 ± 0.104^{bc4}	1.763
Kidney	1.240 ± 0.051^{ab23}	1.312 ± 0.005^{b1}	NA	1.100 ± 0.008^{ab123}	0.963 ± 0.134^{a1}	1.134
Heart	0.993 ± 0.027^{a2}	1.181 ± 0.079^{a1}	1.094 ± 0.041^{a1}	1.021 ± 0.011^{a12}	1.043 ± 0.006^{a12}	1.018
Liver	1.408 ± 0.038^{b3}	1.653 ± 0.057^{c1}	NA	1.248 ± 0.025^{b3}	1.067 ± 0.004^{a12}	1.000
Lung	1.114 ± 0.061^{ab2}	1.268 ± 0.020^{b1}	1.004 ± 0.016^{a1}	0.944 ± 0.036^{a1}	1.123 ± 0.056^{ab12}	0.986
Milk	0.412 ± 0.022^{1}	NA	NA	NA	NA	NA

NA = Sample was not available for analysis.
The means followed by different alphabetical superscripts in each column are significantly different ($p<0.05$) from each other.
Similarly, the means followed by different numerical superscripts in each row are significantly different ($p<0.05$) from each other.
*Bearded seal not included in statistical analysis because only one sample was available for analysis.

Table 4.5 (Continued). Lipid class composition (g/100g lipid) of selected tissues of five species of seal

Tissue	TAG	FFA	Chol.	CL	PE/PS	PC	SM	CE
*Bearded kidney	23.5	ND	20.2	3.5	14.3	23.7	11.0	ND
Grey kidney	31.1 ± 0.95^{f}	ND	16.9 ± 0.70^{d}	3.4 ± 1.07^{ab}	18.3 ± 0.84^{e}	22.1 ± 0.76^{bc}	8.2 ± 0.50^{cfg}	ND
Harp kidney	7.0 ± 0.46^{a}	ND	20.3 ± 0.80^{e}	5.4 ± 1.62^{b}	18.9 ± 0.66^{e}	34.0 ± 0.61^{fg}	12.2 ± 0.51^{d}	ND
Hooded kidney	25.9 ± 1.30^{e}	ND	22.7 ± 0.92^{f}	1.8 ± 0.99^{a}	10.4 ± 0.89^{c}	21.6 ± 0.43^{bc}	12.8 ± 0.42^{dfg}	ND
Ringed kidney	32.5 ± 0.46^{f}	ND	17.2 ± 0.38^{d}	4.6 ± 0.25^{ab}	8.5 ± 0.28^{bc}	20.3 ± 0.41^{b}	9.6 ± 0.40^{bcj}	ND
*Bearded liver	55.5	ND	14.4	2.7	5.8	15.6	3.9	ND
Grey liver	47.8 ± 0.68^{h}	2.4 ± 0.34	7.0 ± 0.31^{a}	1.8 ± 0.88^{a}	14.2 ± 1.01^{d}	25.9 ± 0.83^{de}	1.5 ± 0.39^{c}	ND
Harp liver	27.8 ± 0.21^{e}	0.1 ± 0.11	9.7 ± 0.15^{b}	2.1 ± 0.50^{a}	19.8 ± 0.26^{e}	37.1 ± 0.57^{h}	2.3 ± 0.15^{a}	ND
Hooded liver	62.3 ± 0.31^{j}	ND	9.8 ± 0.18^{b}	2.0 ± 0.22^{a}	6.2 ± 0.46^{ab}	13.1 ± 0.46^{a}	3.5 ± 0.06^{ab}	ND
Ringed liver	44.8 ± 0.49^{g}	0.3 ± 0.00	8.6 ± 1.13^{ab}	3.1 ± 0.54^{ab}	10.2 ± 0.84^{c}	27.5 ± 1.38^{e}	2.1 ± 0.46^{bc}	ND
*Bearded lung	14.3	ND	33.5	4.2	6.6	24.8	14.2	ND
Grey lung	11.3 ± 0.94^{b}	ND	28.9 ± 0.68^{g}	3.4 ± 0.24^{ab}	7.5 ± 0.71^{ab}	36.6 ± 1.03^{gh}	10.2 ± 0.28^{ef}	ND
Harp lung	6.9 ± 1.27^{a}	ND	29.9 ± 1.56^{g}	2.7 ± 0.14^{ab}	10.0 ± 0.49^{c}	37.3 ± 1.13^{h}	13.2 ± 1.40^{fj}	ND
Hooded lung	12.2 ± 0.16^{b}	ND	30.0 ± 1.28^{g}	3.0 ± 2.48^{ab}	6.6 ± 1.55^{ab}	32.8 ± 1.29^{f}	11.5 ± 0.42^{fgh}	ND
Ringed lung	10.2 ± 0.13^{b}	ND	29.7 ± 1.01^{g}	3.1 ± 1.72^{ab}	8.3 ± 1.14^{bc}	34.2 ± 1.93^{fg}	9.9 ± 0.43^{e}	ND

Notes: The means followed by different superscripts in each column are significantly different ($p<0.05$) from each other.
*Bearded seal tissues were not included in the statistical analysis because only one sample was avaible.

Only neutral lipids were detected in blubber and milk lipids. Previous studies have reported similar results for blubber, 98.9-100% (Henderson *et al.*, 1994; Shahidi *et al.*,1994), and harp seal milk, >99% (Iverson *et al.*, 1992). Neutral and polar fractions were equally distributed in the kidney lipids of all species, except for harp seal. However, the lipids of liver tissue of all species, except harp, had a higher proportion of neutral than polar lipids, while the lipids of lung tissues of all species contained lower proportions of neutral than polar lipids. The proportion of neutral and polar lipids varied from species to species in muscle and heart tissues. The specific proportion for the tissues of each species varied somewhat, but were generally in the range of that for similar tissues reported in a previous study (Henderson *et al.*, 1994). However, the amount of neutral lipid in harp seal muscle (58%) was much higher than that reported in a previous study (Shahidi *et al.*, 1994), but was closer to the values reported for muscle tissues of a Mediterranean monk seal (Henderson *et al.*, 1994). Of the six species analyzed, harp seal had the lowest proportion of neutral lipids in all tissues except for muscle.

4.2.2 Triacylglycerols

Triacylglycerols (TAG) were the only lipid class detected by Iatroscan in blubber and milk lipids. Previous studies on harp (Iverson *et al.*, 1992) and hooded (Oftedal *et al.*, 1987) seal milk indicated that the TAG contents of the milk lipids were 99.6 and 99.4%, respectively. Henderson *et al.* (1994), however, reported that the TAG content of blubber of a Mediterranean monk seal was 88.3% and that there was a substantial amount of free fatty acids present. The presence of large amounts of free fatty acids in the blubber lipids

of the Mediterranean monk seal may have originated from post mortem decomposition of the lipids.

Brain tissue lipids were the only lipids which showed no detectable TAG as determined by Iatroscan. The low level of TAG in brain tissue lipids is probably due to the role of lipids in brain being primarily structural and functional rather than as an energy reserve. In the tissues of kidney, liver, and lung, the TAG content of the lipids was more dependent on the tissue under consideration than the species. Harp seal tissues, however, were an exception. Generally, lung had a lower TAG content in its lipids than kidney lipids, which had a lower TAG content than liver lipids. Muscle lipids had TAG contents ranging from 27.3% in grey seal to 36.5% in harp seal. Triacylglycerol contents in heart lipids were very low (3.1%) in some species and very high (55.5%) in others.

Harp seal tissue lipids generally had a much lower triacylglycerol content than lipids of corresponding tissues from other species. No other trends in species were apparent.

4.2.3 Free Fatty Acids

It is well known that fatty acids are not usually found to any significant extent in tissues in their free, nonesterified form. Their presence suggests that some post mortem hydrolysis of lipids had occurred. In most tissues of all seal species analyzed in this study the free fatty acid (FFA) content of the samples were very low or undetectable by the Iatroscan method. However, muscle and heart tissue lipids were found to contain significant ($p<0.05$) amounts (3.5 - 26.8%) of FFA. This would indicate that considerable hydrolysis of the natural lipids had occurred post mortem. Since other tissues, namely,

kidney, liver, lung, and blubber from the same animal did not contain substantial amounts of these free fatty acids, the heart and muscle lipids must be more susceptible to hydrolysis or that the hydrolysis is being catalysed in heart and muscle tissues. A similar study on the lipid classes of Mediterranean monk seal, using HPLC, also reported high levels (10.5- 17.0%) of free fatty acids (Henderson *et al.*, 1994). In that study, the free fatty acids were high in all tissues, except blubber. Another study on the lipids of the liver and muscle of the Antarctic sei whale also reported high free fatty acid values of 22.2 and 3.5% for liver and muscle tissues, respectively (Bottino, 1983).

4.2.4 Cholesterol

In animals, cholesterol is present in cell membranes, especially the plasma membranes, and is a major component of myelinated nerves. It is also present in serum lipoproteins and is a predominant lipid of the storage droplets in the cells of steroid-hormone-secreting glands. Consequently, blubber and milk lipids which contain a very high ratio of total lipids to membrane lipids and lipoproteins, had levels of cholesterol that were too low to be detected by the Iatroscan system. Lipids of all other tissues, however, had measurable amounts of cholesterol. In fact, brain lipids, which contain an abundance of myelinated nerves, cholesterol was the most abundant lipid class present (31.4%).

Muscle tissue lipids had a relatively low cholesterol content (4.0- 6.1%) when compared to other tissues. However, these levels are comparable to those reported previously for harp seal muscle and is also similar to that for other animal (beef and chicken) muscle tissues (Shahidi and Synowiecki, 1991). The cholesterol content for

lipids of heart of all species, except ringed seal (12.2%), remained below 10%. For the remaining tissues (kidney, liver, and lung) the lipids of liver generally contained the lowest content of cholesterol followed by those of kidney and lung. Similar trends were reported for the same tissues in adult rats (Gibbons *et al.*, 1982).

The cholesterol content of lipids of grey seal tissues was consistently the lowest among all species examined. From the results summarized above, it is apparent that the cholesterol content of the samples analyzed was influenced more by the tissue rather than species examined. This is consistent with the fact that cholesterol plays important physiological functions in the cell. In cellular membranes cholesterol affects membrane integrity, permeability, shape, fusion, asymmetry, fluidity, and cellular metabolic activity. As well, cholesterol may be metabolized to other steroidal structures in order to fulfill a physiological function (Gibbons *et al.*, 1982). Different tissues and organs play different physiological roles and therefore vary in their cholesterol contents to satisfy those requirements.

The relatively high levels of cholesterol in lung tissue lipids was most likely due to the presence of pulmonary surfactants. The lipids of these surfactants are known to be about 80 to 90% phospholipids and the remaining neutral lipids are predominantly cholesterol (Bourbon, 1991).

4.2.5 Cardiolipin

Diphosphatidylglycerol or cardiolipin is known to be a major lipid component of mitochondria (Harwood, 1994) and since heart has a large amount of mitochondria to supply the energy required for contraction this lipid class should be relatively abundant in

heart tissue lipids. Table 4.5 shows that the cardiolipin content of heart tissue lipids ranged from 3.3 to 8.6%. This is generally higher than the cardiolipin contents from other seal tissues. Henderson *et al.* (1994) reported a somewhat higher cardiolipin content of 11% in the lipids of Mediterranean monk seal heart.

The analysis of the lipids for cardiolipin by Iatroscan indicated the presence of cardiolipin in all tissues that contained detectable levels of polar lipids except for brain tissue lipids. However, the contents of cardiolipin were relatively low and no statistically significant ($p>0.05$) differences existed among different tissues or species. Similar results were reported previously for Mediterranean monk seal (Henderson *et al.*, 1994). Studies on rat liver and muscle lipids reported cardiolipin contents of 5.1 and 1.4%, respectively (Mato, 1990b).

4.2.6. Phosphatidylethanolamine and Phosphatidylserine

The solvent systems used in this study did not separate phosphatidylethanolamine (PE) and phosphatidylserine (PS), so they were reported as a single group (PE/PS). It is well known that PE is widespread and a major phospholipid while PS, although being widespread it occurs only in minor amounts (Harwood, 1994). Previous studies on lipids of seal and rat tissues, using different analytical techniques, where separation of PE and PS is possible, consistently report PE to be present in greater abundance than PS (Mato, 1990b; Henderson *et al.*, 1994). Therefore, it is most likely that in the tissues analyzed in this study the PE fraction is also the more abundant portion of the PE/PS mixture. However, in brain tissue lipids PS is known to represent approximately 9% of the membrane phospholipids (Debuch *et al.*, 1982).

Phosphatidylethanolamine/phosphatidylserine was detected in all tissues analyzed that contained polar lipids. Brain tissue of grey seal contained 18.3% PE/PS while muscle lipids had a PE/PS content ranging from 4.7% in harp seal to 12.5% in grey seal. In heart tissue, the range varied from 5.1 to 19.3%. Of the tissues kidney, liver, and lung, the PE/PS content of kidney lipids was generally higher that those of liver and lung.

The relatively high level of PE/PS in brain is most likely, at least in part, due to higher levels of PS which is a major acidic phospholipid in the brain. In the brain PS has a role in (Na^+- K^+)- ATPase activity and is responsible for activation of protein kinase C. The use of PS as a therapeutic agent for the treatment of pathological brain aging has been suggested (Nunzi *et al.*, 1990).

4.2.7 Phosphatidylcholine

Phosphatidylcholine (PC) is most often the predominant glycerophospholipid in animal tissues(Harwood, 1994). This held true for different tissues of all species of seals analyzed, except for the brain tissue. PE/PS was the predominant glycerophospholipid in brain tissue. These results lend further support to the findings of Henderson *et al.* (1994) who reported similar results in their studies of Mediterranean monk seal.

PC was the predominant lipid class of the total lipid fraction in lung tissues of all species analyzed, except bearded seal. Since pulmonary surfactants were not removed from the lung tissue prior to analysis, they are most likely responsible for the relatively high content of PC. The PC content of the phospholipid fraction in these lung surfactants is 70 to 80% whereas in other membrane-rich cells it is approximately 50% (Bourbon,

1991). If substantial amounts of surfactants are in the lungs, it is reasonable to expect a significantly higher PC content.

Generally, the PC content was in the range of 20 to 40%. The lowest PC content in tissues that contained detectable levels of polar lipids was in hooded seal liver (13.1%) and the highest amount was in heart lipids of harp seal(48.3%). Since no polar fraction was detectable in blubber and milk lipids no PC was detected in these samples.

4.2.8 Sphingomyelin

Sphingomyelin (SM) is reported to be a major lipid component of certain animal membrane (especially nervous) tissues (Harwood, 1994). Table 4.5 shows that all tissues containing detectable levels of polar lipids contained measurable amounts of SM. Levels ranged from 0.7% in grey seal muscle lipids to 14.2% in bearded seal lung lipids. Of the tissues kidney, liver, and lung, the lipids of liver had the lowest SM content with the lipids of kidney and lung both being significantly ($p<0.05$) higher in all species analyzed.

4.2.9 Cerebrosides

Galactocerebroside is an important lipid in myelin, the multilayered membrane sheath that insulates nerves. Myelin is nothing more than many concentric layers of plasma membrane wound around a nerve fibre by a specialized myelinating cell. A distinguishing feature of these myelinating cells is the large amount of galatocerobroside in their plasma membrane, where it constitutes almost 40% of the outer monolayer. Since it is not present in significant amounts in other membranes, galactocerbroside may play an important role in the membrane wrapping process that is unique to myelination. Consequently, in this study, cerebrosides were only detected in brain tissue lipids by

contributing up to 29.3% of the brain lipids of grey seal. Henderson *et al.* (1994) reported the cerebroside content of the Mediterranean monk seal to be 28.8%.

4.3 Fatty Acid Composition

The fatty acid compositions of the lipids of selected tissues of the six species of Eastern Canadian Phocid seals are provided in Tables 4.6 to 4.13. In comparing similar tissues of different species, one fatty acid at a time, a univariate approach was employed. This approach showed significant ($p<0.05$) differences between similar tissues of different seals. However, when comparing fatty acid profiles of both different tissues and species, using a multivariate approach, it was evident that differences were greater between different tissues than between the same tissues of different species.

4.3.1 Blubber

Most of the previous studies on the composition of seal fatty acids have focused on blubber lipids (Ackman and Jangaard, 1965; Jangaard and Ke, 1968; Ackman *et al.*, 1971; Ackman and Hooper, 1974; West *et al.*, 1979; Grompone *et al.*, 1990; Kakela and Hyvarinen, 1993; Kakela *et al.*, 1993; Shahidi *et al.*, 1996). The data in Table 4.6 compares the fatty acid composition of the blubber of bearded, grey, harbour, harp, hooded, and ringed seals. In most species, 18:1 ω9 was the predominant fatty acid and was found in the highest proportion in grey seal. In harbour and ringed seals 16:1 ω7 was the predominant fatty acid. Palmitoleic acid (16:1 ω7) was the only fatty acid present in significantly ($p<0.05$) different proportions in all species. Significant differences ($p<0.05$) between at least two species were found in twelve fatty acids.

Table 4.6. Fatty acid composition (g/100g lipid) of blubber of various species of seal

Fatty Acid	Bearded*	Grey	Harbour	Harp	Hooded	Ringed
14:0	3.05	3.83 ± 0.03^{a}	4.52 ± 0.13^{b}	4.66 ± 0.49^{b}	4.40 ± 0.38^{ab}	3.36 ± 0.66^{a}
16:0 DMA	ND	ND	ND	ND	ND	ND
16:0	10.14	6.61 ± 0.08^{ab}	8.03 ± 0.38^{bc}	6.24 ± 0.44^{ab}	9.81 ± 1.57^{c}	4.82 ± 2.07^{a}
16:1 ω7	17.77	12.77 ± 0.09^{b}	19.26 ± 0.53^{d}	14.93 ± 0.46^{c}	10.09 ± 0.35^{a}	23.12 ± 0.18^{e}
18:0 DMA	ND	ND	ND	ND	ND	ND
18:1 ω9DMA	ND	ND	ND	ND	ND	ND
18:1 ω7DMA	ND	0.45 ± 0.01	ND	0.46 ± 0.00	ND	ND
18:0	2.15	0.94 ± 0.02^{b}	0.85 ± 0.02^{ab}	0.95 ± 0.03^{b}	1.83 ± 0.31^{c}	0.42 ± 0.19^{a}
18:1 ω9	16.76	24.50 ± 0.44^{c}	18.61 ± 0.55^{a}	18.59 ± 1.01^{a}	22.77 ± 2.66^{bc}	19.72 ± 1.33^{ab}
18:1 ω7	9.49	4.95 ± 0.09^{b}	5.16 ± 0.44^{b}	3.57 ± 0.36^{a}	3.75 ± 0.47^{a}	5.03 ± 0.46^{b}
18:2 ω6	2.30	1.28 ± 0.00^{a}	1.27 ± 0.04^{a}	1.36 ± 0.20^{ab}	1.63 ± 0.20^{b}	2.58 ± 0.02^{c}
20:1 ω9	5.08	12.50 ± 0.43^{b}	9.06 ± 0.33^{ab}	12.56 ± 2.92^{b}	13.00 ± 1.86^{b}	6.71 ± 2.17^{a}
20:4 ω6	0.94	0.51 ± 0.00^{a}	0.44 ± 0.00^{c}	0.36 ± 0.96^{a}	0.31 ± 0.03^{a}	0.30 ± 0.02^{a}
20:5 ω3	8.28	4.85 ± 0.13^{a}	9.31 ± 0.21^{b}	6.82 ± 0.69^{ab}	5.21 ± 1.65^{a}	8.72 ± 1.06^{b}
22:0	0.63	2.26 ± 0.12^{ab}	1.19 ± 0.02^{a}	3.61 ± 1.60^{bc}	5.53 ± 0.79^{c}	0.75 ± 0.67^{a}
22:1 ω11	0.27	0.62 ± 0.03^{a}	0.31 ± 0.01^{a}	0.77 ± 0.61^{a}	0.86 ± 0.33^{a}	0.34 ± 0.01^{a}
22:5 ω3	4.26	5.06 ± 0.05^{cd}	4.22 ± 0.14^{b}	4.78 ± 0.25^{bc}	2.29 ± 0.08^{a}	5.46 ± 0.47^{d}
22:6 ω3	7.22	8.91 ± 0.29^{a}	7.76 ± 0.98^{a}	10.48 ± 1.98^{a}	9.56 ± 2.36^{a}	9.45 ± 1.74^{a}

Notes: ND = not detected and DMA = dimethyl acetal.

Mean ± standard deviation of three samples.

The means followed by different superscripts are significantly ($p<0.05$) different from each other.

*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Generally, blubber lipids have a very unique fatty acid composition when compared to lipids of other tissues. The first notable feature of blubber lipids is a low concentration of 20:4 ω6. Dimethyl acetals, the derivatives of 1-O-alk-1'-enyl linked ether chains, common to plasmalogens, were also noticeably absent or present only in very small concentrations. This was expected as plasmalogens are commonly associated with polar lipids and this fraction accounted for less than 1% of blubber lipids.

Figure 4.1 shows that for blubber lipids of all species of seals, monounsaturated fatty acids were most predominant. Saturated fatty acids were the least abundant group in all species except in hooded seal. Hooded seal blubber lipids had the highest level of saturated fatty acids and the lowest levels of PUFAs. Ring seal blubber lipids, on the other hand, had the lowest level of saturated fatty acids and the highest content of PUFAs. Ring seal blubber lipids were also significantly ($p<0.05$) higher in their content of omega-3 PUFAs than the blubber lipids of several other species while hooded seal blubber lipids tended to have a lower content of omega-3 PUFAs. These results have important implications if the production of seal oil capsules from different species of seals were to be considered. Currently, most seal oil capsules for the nutraceutical market are produced from blubber of harp seals which accounts for the vast majority of seals in the Eastern Canadian annual commercial seal hunt. However, a small proportion of the hunt is comprised of hooded seals. If the blubber of these seals were used in the production of the seal oil capsules, the content of PUFAs, especially ω3 PUFAs, would decrease and the saturated fatty acid content would increase. Consequently, the capsules would become less beneficial in their perceived effects on cardiovascular health.

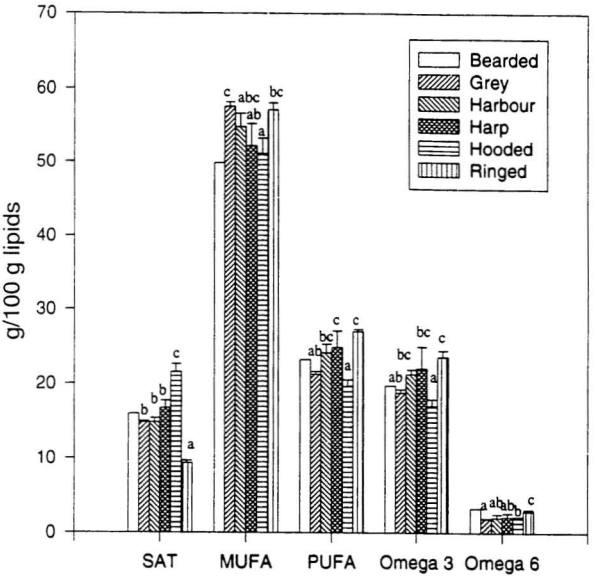


Figure 4.1. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the blubber lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Conversely, ringed seals, which are the most abundant species in the Arctic and are currently unutilized in the commercial harvest, offer potential for inclusion in the production of seal oil capsules. If used, they would decrease the saturated fatty acid content of capsules compared to capsules made from harp seal blubber oil. Obviously, the direct consumption of these seals by a select population would be expected to have similar effects.

Another unique feature of blubber lipids is that the ratio of ω3 to ω6 fatty acids is very high (~10:1). This is a very positive attribute of seal blubber oils because currently there is an imbalance in the ω3 to ω6 ratio in the Western diet with an over abundance of ω6 fatty acids. Seal blubber oil, used as a dietary supplement, may provide a means of reducing this existing imbalance.

4.3.2 Muscle

Table 4.7 provides data on the fatty acid compositions of muscle lipids from bearded, grey, harbour, harp, hooded, and ringed seals. There were twelve fatty acids that were significantly ($p<0.05$) different between at least two species but none that were different in all six species. Harbour and ringed seal muscle lipids were significantly ($p<0.05$) higher in EPA (20:5 ω3) than those of the remaining species.

The predominant fatty acid in lipids of muscle tissues of all species except habour seal was 18:1 ω9. In harbour seal 16:0 was the predominant fatty acid. However, even in harbour seal muscle lipids the total 18:1 (ω9 & ω7) fraction was larger than 16:0. The 20:4 ω6 content of muscle lipids (1.97-7.11%) was much higher than that in blubber lipids (0.30-0.51%). Similar results have been reported in previous studies on muscle

Table 4.7. Fatty acid composition (g/100g lipid) of muscle lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harbour	Harp	Hooded	Ringed
14:0	2.44	2.15 ± 0.05^{a}	1.88 ± 0.50^{a}	2.46 ± 0.71^{a}	3.90 ± 0.35^{a}	2.71 ± 2.05^{a}
16:0 DMA	1.63	1.99 ± 0.08^{b}	1.50 ± 0.16^{ab}	1.86 ± 0.15^{b}	0.35 ± 0.29^{a}	2.08 ± 1.03^{b}
16:0	13.62	13.79 ± 0.73^{a}	14.02 ± 1.10^{a}	12.29 ± 0.91^{a}	12.69 ± 2.38^{a}	16.20 ± 2.49^{a}
16:1 ω7	11.06	3.73 ± 0.40^{a}	5.82 ± 0.34^{a}	7.30 ± 0.79^{a}	5.27 ± 1.21^{a}	5.34 ± 3.01^{a}
18:0 DMA	0.54	0.97 ± 0.07^{b}	0.80 ± 0.56^{ab}	0.78 ± 0.08^{ab}	0.21 ± 0.03^{a}	0.57 ± 0.27^{ab}
18:1 ω9 DMA	0.61	0.84 ± 0.05^{bc}	1.13 ± 0.03^{c}	0.84 ± 0.09^{bc}	0.25 ± 0.28^{a}	0.70 ± 0.06^{b}
18:1 ω7 DMA	1.19	1.14 ± 0.04^{b}	0.75 ± 0.53^{ab}	0.97 ± 0.11^{b}	0.10 ± 0.11^{a}	0.73 ± 0.35^{ab}
18:0	5.65	6.20 ± 0.20^{ab}	12.29 ± 5.57^{b}	5.93 ± 0.19^{ab}	4.84 ± 0.78^{a}	6.22 ± 2.36^{ab}
18:1 ω9	17.08	17.48 ± 0.48^{b}	12.73 ± 1.61^{a}	18.48 ± 0.68^{b}	19.84 ± 0.23^{b}	18.14 ± 1.68^{b}
18:1 ω7	7.70	3.57 ± 0.13^{a}	4.71 ± 0.58^{a}	4.88 ± 1.39^{a}	3.41 ± 0.46^{a}	3.71 ± 0.43^{a}
18:2 ω6	3.01	1.98 ± 0.05^{b}	2.00 ± 0.18^{b}	1.54 ± 0.12^{a}	1.91 ± 0.08^{b}	2.76 ± 0.14^{c}
20:1 ω9	5.03	11.63 ± 0.28^{b}	4.65 ± 1.33^{a}	11.75 ± 1.58^{b}	14.51 ± 2.03^{b}	6.35 ± 2.39^{a}
20:4 ω6	5.10	4.31 ± 0.09^{ab}	7.11 ± 2.29^{b}	3.87 ± 0.63^{ab}	1.97 ± 0.66^{a}	4.26 ± 2.06^{ab}
20:5 ω3	6.10	5.60 ± 0.14^{a}	10.46 ± 1.23^{b}	5.56 ± 0.60^{a}	4.57 ± 0.12^{a}	9.21 ± 1.60^{b}
22:0	1.10	4.46 ± 0.13^{b}	1.44 ± 0.06^{a}	4.46 ± 0.78^{b}	6.82 ± 0.73^{c}	2.02 ± 0.99^{a}
22:1 ω11	ND	0.62 ± 0.02^{b}	0.29 ± 0.01^{a}	0.93 ± 0.23^{c}	ND	0.08 ± 0.00^{a}
22:5 ω3	3.58	2.94 ± 0.25^{b}	2.12 ± 0.06^{a}	2.21 ± 0.32^{a}	1.79 ± 0.16^{a}	2.31 ± 0.08^{a}
22:6 ω3	8.55	9.30 ± 0.82^{a}	10.02 ± 2.12^{a}	6.73 ± 1.30^{a}	8.52 ± 0.91^{a}	9.49 ± 0.45^{a}

Notes: ND = not detected and DMA = dimethyl acetal.

Mean ± standard deviation of three samples.

The means followed by different superscripts are significantly ($p<0.05$) different from each other.

*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

tissues of both harp seal (Shahidi *et al.*, 1994) and Mediterranean monk seal (Henderson *et al.*, 1994). These findings indicate that the majority of the fatty acids present in blubber lipids are those that have been absorbed from the diet and transported directly in chylomicrons to blubber without modification. However, the fatty acids of the lipids of muscles and other tissues are probably modified by the liver before being deposited. As a result, blubber lipid fatty acid composition is similar to the fatty acid composition of the lipids in the animals diet and may also explain some of the variations in fatty acid composition with season, species, geographical location, and age. Similarly, this explains why the fatty acid compositions of the muscle and especially internal organs have a stronger resemblance to the fatty acid composition of similar tissues from terrestrial mammals.

Muscle lipids of all species also contained measurable amounts of dimethyl acetals. These dimethyl acetals are formed when the alkenyl linkage in plasmalogens is cleaved with acidified methanol during transmethylation. Plasmalogens are primarily found in membrane phospholipids and, apart form the general structural function of all membrane phospholipids, no specific function has been attributed to them. The ether glycerophospholipids tend to be rich in PUFAs which does suggest a role as a storage reservoir for these fatty acids. This may be due to the apparent protective nature of the ether bond against hydrolysis of the acyl group at the sn-2 position by phospholipase A_2 (Mato, 1990b).

Figure 4.2 shows that monounsaturated fatty acids were still the dominant group of fatty acids in most species, although to a lesser extent than was observed for blubber. It

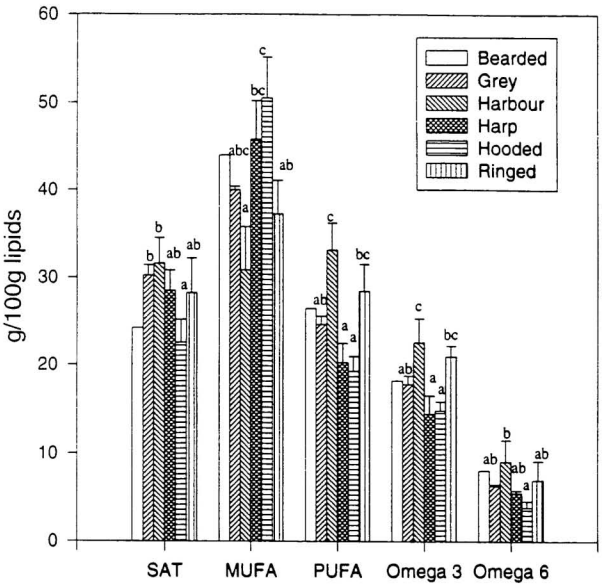


Figure 4.2. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the muscle lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

also shows an increase in the amount of saturated fatty acids. Hooded seal muscle lipids were significantly ($p<0.05$) lower than grey and harbour seal muscle lipids in saturated fatty acids and significantly ($p<0.05$) lower in PUFAs than those of harbour and ringed seal muscle lipids.

4.3.3 Brain

The fatty acid composition of brain lipids for the six species of Eastern Canadian Phocid seals is provided in Table 4.8. The predominant fatty acid was 18:0 for harbour, harp, hooded, and ringed seal brain lipids, while it was 18:1 ω9 in the brain lipids of bearded and grey seals.

The fatty acid composition of brain tissue lipids of all species was very unique when compared to all other tissues. The first notable feature was the high dimethyl acetal content (>7%). This was probably due to the very high polar lipid (~70%) and the very low triacylglycerol contents (<1%). The proportion of EPA (20:5 ω3) was very low while that of DHA (22:6 ω3) was the highest percentage of total lipids of all tissues analyzed. The high DHA content was expected as both brain and retina lipids are known to be rich in DHA. In brain lipids, the content of the saturated fatty acid 14:0 was relatively low while that 18:0 was relatively high.

Figure 4.3 shows that the saturated fatty acids in brain tissue comprised the largest group while the polyunsaturated fatty acids accounted for 20-28g/100g of the total amount. Monounsaturated fatty acids accounted for the remainder (20- 32g/100g lipids).

Table 4.8. Fatty acid composition (g/100g lipid) of brain lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harbour	Harp	Hooded	Ringed
14:0	0.28	0.31 ± 0.00^{a}	0.62 ± 0.20^{b}	0.48 ± 0.07^{ab}	0.48 ± 0.12^{ab}	0.45 ± 0.03^{ab}
16:0 DMA	1.62	2.05 ± 0.12^{c}	1.95 ± 0.01^{bc}	1.66 ± 0.19^{ab}	1.38 ± 0.15^{a}	1.53 ± 0.15^{a}
16:0	11.98	13.96 ± 0.52^{a}	14.74 ± 0.77^{a}	15.45 ± 0.64^{a}	15.48 ± 1.16^{a}	18.18 ± 0.75^{b}
16:1 ω7	0.86	0.94 ± 0.02^{a}	1.15 ± 0.13^{ab}	1.33 ± 0.22^{b}	1.07 ± 0.07^{ab}	1.28 ± 0.16^{ab}
18:0 DMA	2.29	3.00 ± 0.18^{a}	4.01 ± 0.04^{bc}	4.38 ± 0.43^{c}	3.35 ± 0.20^{ab}	2.91 ± 0.26^{a}
18:1 ω9 DMA	1.72	1.84 ± 0.11^{c}	1.96 ± 0.00^{c}	1.82 ± 0.08^{c}	1.18 ± 0.08^{b}	0.84 ± 0.07^{a}
18:1 ω7 DMA	3.51	4.01 ± 0.22^{c}	2.89 ± 0.04^{b}	2.69 ± 0.18^{b}	1.73 ± 0.11^{a}	1.95 ± 0.21^{a}
18:0	14.30	16.33 ± 0.56^{a}	19.22 ± 0.87^{b}	18.08 ± 0.69^{b}	18.06 ± 0.23^{b}	19.08 ± 0.64^{b}
18:1 ω9	15.91	17.25 ± 0.61^{b}	14.86 ± 1.47^{ab}	14.02 ± 0.89^{a}	13.48 ± 1.14^{a}	12.89 ± 0.47^{a}
18:1 ω7	4.25	4.86 ± 0.16^{a}	4.92 ± 0.84^{a}	4.60 ± 0.28^{a}	4.64 ± 0.06^{a}	5.59 ± 0.18^{a}
18:2 ω6	ND	0.12 ± 0.00^{a}	ND	0.15 ± 0.09^{a}	0.28 ± 0.12^{a}	0.32 ± 0.14^{a}
20:1 ω9	3.33	2.09 ± 0.10^{c}	1.72 ± 0.58^{bc}	1.83 ± 0.24^{c}	0.12 ± 0.21^{a}	0.78 ± 0.45^{ab}
20:4 ω6	7.58	5.51 ± 0.04^{a}	5.03 ± 0.57^{a}	5.29 ± 0.22^{a}	6.92 ± 0.99^{b}	5.99 ± 0.30^{ab}
20:5 ω3	0.25	0.34 ± 0.00^{a}	0.57 ± 0.74^{a}	0.70 ± 0.60^{a}	1.02 ± 0.06^{a}	0.94 ± 0.04^{a}
22:0	0.58	ND	ND	0.25 ± 0.09^{a}	0.44 ± 0.02^{b}	0.28 ± 0.00^{a}
22:1 ω11	ND	0.19 ± 0.01^{a}	0.27 ± 0.13^{a}	0.21 ± 0.13^{a}	ND	ND
22:5 ω3	0.57	3.25 ± 0.10^{b}	3.18 ± 0.05^{b}	2.92 ± 0.26^{b}	1.66 ± 0.07^{a}	1.95 ± 0.10^{a}
22:6 ω3	13.51	13.31 ± 0.45^{a}	14.07 ± 1.39^{ab}	15.56 ± 0.64^{bc}	14.49 ± 0.54^{abc}	16.33 ± 0.83^{c}

Notes: ND = not detected and DMA = dimethyl acetal.

Mean ± standard deviation of three samples.

The means followed by different superscripts are significantly ($p<0.05$) different from each other.

*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

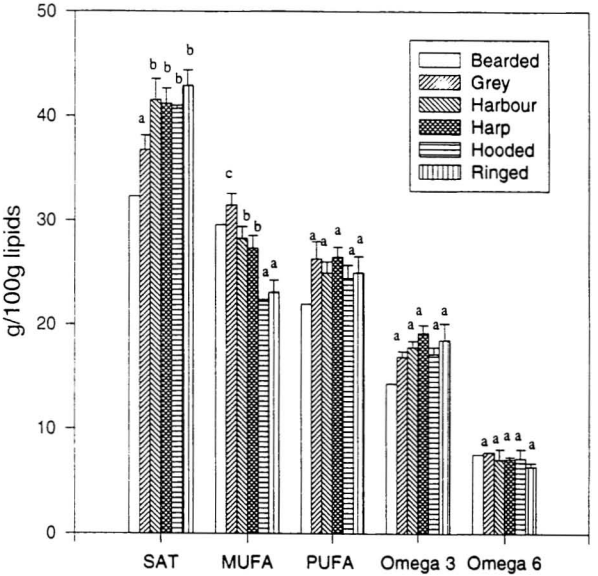


Figure 4.3. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the brain lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

The content of omega-3 fatty acids was generally two to three times greater than that of the omega-6 content.

Ringed seal brain lipids had significantly ($p<0.05$) higher DHA than those of grey and harbour seals. However, for the most part, there were less species to species differences in brain tissue lipids than in the lipids previously discussed. This strong conservation of the fatty acid composition of lipids from brain of different species may be due to the roles of individual fatty acids in the brain lipids. In many tissues, especially blubber, the lipids present are often used as an energy reserve; however, brain tissue does not utilize lipids as an energy source so their presence must be for other purposes. In brain tissues the roles of lipids are most likely structural and functional in nature. Another factor that may result in the strong conservation of fatty acid composition is that there may be requirements for specific lipid classes, i.e., cerebrosides, and these classes may have specific fatty acid compositions, e.g., sphingomyelin generally has a high content of 16:0 (Svennerholm *et al.*, 1966).

4.3.4. Kidney

The fatty acid composition of the lipids of kidney of bearded, grey, harp, hooded, and ringed seals is provided in Table 4.9. The fatty acids 16:1 ω7 and 18:1 ω7 DMA were the compounds most significantly different from species to species. The predominant fatty acid was 18:1 ω9 in bearded, grey, and ringed seal kidney lipids. While in harp seal 16:0 was the predominant fatty acid and 18:0 predominated in hooded seal. The content of 20:4 ω6 was higher in kidney lipids than in the lipids of blubber, muscle, and brain

Table 4.9. Fatty acid composition (g/100g lipid) of kidney lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harp	Hooded	Ringed
14:0	1.86	2.11 ± 0.01^{ab}	1.15 ± 0.88^{a}	2.24 ± 0.16^{ab}	2.39 ± 0.10^{b}
16:0 DMA	2.17	1.82 ± 0.08^{a}	3.09 ± 0.67^{b}	2.09 ± 0.20^{a}	1.87 ± 0.01^{a}
16:0	14.50	14.28 ± 0.09^{ab}	12.71 ± 0.19^{a}	15.24 ± 1.28^{b}	14.64 ± 0.37^{b}
16:1 ω7	6.54	5.00 ± 0.05^{b}	4.05 ± 0.09^{a}	4.60 ± 0.20^{b}	7.05 ± 0.22^{c}
18:0 DMA	1.02	0.56 ± 0.02^{a}	1.65 ± 0.28^{c}	1.07 ± 0.13^{b}	0.83 ± 0.00^{ab}
18:1 ω9 DMA	ND	0.57 ± 0.03^{b}	0.74 ± 0.06^{c}	0.48 ± 0.01^{a}	0.69 ± 0.00^{c}
18:1 ω7 DMA	0.97	0.78 ± 0.03^{c}	0.79 ± 0.07^{c}	0.42 ± 0.02^{a}	0.59 ± 0.01^{b}
18:0	12.29	10.36 ± 0.08^{a}	12.30 ± 0.59^{b}	15.91 ± 1.20^{c}	11.13 ± 0.06^{ab}
18:1 ω9	14.18	15.57 ± 0.31^{b}	12.52 ± 0.45^{a}	15.65 ± 0.81^{b}	15.97 ± 0.25^{b}
18:1 ω7	6.96	6.73 ± 0.09^{b}	5.50 ± 0.35^{a}	5.65 ± 0.33^{a}	6.07 ± 0.08^{a}
18:2 ω6	3.95	3.56 ± 0.06^{c}	3.33 ± 0.03^{b}	3.06 ± 0.01^{a}	3.42 ± 0.09^{bc}
20:1 ω9	2.26	4.33 ± 0.77^{b}	2.20 ± 0.57^{a}	3.80 ± 1.03^{ab}	2.79 ± 0.03^{ab}
20:4 ω6	11.86	10.55 ± 0.21^{b}	10.11 ± 1.08^{b}	6.72 ± 0.71^{a}	7.95 ± 0.18^{a}
20:5 ω3	10.81	5.93 ± 0.47^{a}	9.86 ± 0.53^{b}	11.10 ± 1.75^{b}	10.31 ± 0.33^{b}
22:0	0.94	0.84 ± 0.25^{a}	0.99 ± 0.67^{a}	1.06 ± 0.08^{a}	0.69 ± 0.08^{a}
22:1 ω11	ND	0.83 ± 0.40^{b}	0.21 ± 0.04^{a}	0.72 ± 0.01^{ab}	0.64 ± 0.01^{ab}
22:5 ω3	1.26	2.24 ± 0.03^{b}	2.13 ± 0.05^{b}	0.51 ± 0.11^{a}	2.18 ± 0.04^{b}
22:6 ω3	6.61	4.76 ± 1.22^{ab}	3.29 ± 0.83^{a}	6.51 ± 0.48^{bc}	7.23 ± 0.01^{c}

Note: ND = not detected and DMA = dimethyl acetal.
Mean ± standard deviation of three samples.
The means followed by different superscripts are significantly different ($p<0.05$) from each other.
*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

tissues. In all species, 20:5 ω3 was the dominant ω3 PUFA. The dimethyl acetal content in fatty acids in kidney ranged from 3.73 to 6.27%. Similar trends were reported in a previous study (Grompone *et al.*, 1990), except that dimethyl acetals were not reported and the contents of 20:4 and 20:5 were much lower than those found in this study.

Figure 4.4 shows the proportions of saturated, monounsaturated, and polyunsaturated fatty acids. For kidney lipids of all five species analyzed, fatty acids were fairly evenly distributed in each group. Hooded seal kidney lipids, however, did have significantly ($p<0.05$) higher levels of saturated fatty acids than did those of the other species analyzed. Grey seal kidney lipids had a significantly ($p<0.05$) lower content of ω3 PUFAs than those of hooded and ringed seals and the kidney lipids of both grey and harp seals had significantly ($p<0.05$) higher levels of ω6 PUFAs than did those of ringed and hooded seals. In all species analyzed, the content of ω3 PUFAs was higher than ω6 but less so than in the lipids of blubber, muscle, and brain tissues.

4.3.5 Heart

Table 4.10 provides the fatty acid composition of the lipids of hearts of bearded, grey, harbour, harp, hooded, and ringed seals. The fatty acids 16:1 ω7, 18:0, 20:4 ω6, and 20:5 ω3 were found to be significantly ($p<0.05$) different for most species. In all species, 18:1 ω9 was the dominant fatty acid. Similar to other internal organ lipids, heart lipids had a relatively high 20:4 ω6 content. The fatty acid 20:5 ω3 was the predominant ω3 PUFA in heart lipids of all species. The dimethyl acetal content of heart lipids ranged from 4.82 to 10.75%.

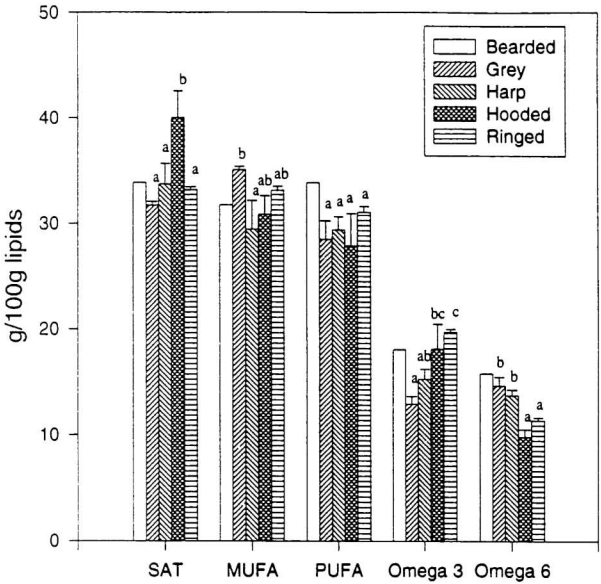


Figure 4.4. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the kidney lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Table 4.10. Fatty acid composition (g/100g lipid) of heart lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harbour	Harp	Hooded	Ringed
14:0	1.86	1.14 ± 0.01^{b}	1.72 ± 0.12^{cd}	0.91 ± 0.03^{a}	1.68 ± 0.04^{c}	1.21 ± 0.01^{b}
16:0 DMA	1.97	2.92 ± 0.06^{b}	2.77 ± 0.09^{b}	3.74 ± 0.10^{c}	2.57 ± 0.03^{a}	3.96 ± 0.07^{d}
16:0	12.85	11.01 ± 0.04^{ab}	10.49 ± 0.43^{a}	10.56 ± 0.81^{a}	12.45 ± 0.13^{c}	12.04 ± 0.06^{bc}
16:1 ω7	12.73	3.50 ± 0.05^{a}	6.21 ± 0.24^{d}	4.32 ± 0.12^{b}	5.18 ± 0.15^{c}	5.18 ± 0.11^{c}
18:0 DMA	0.78	1.32 ± 0.02^{ab}	1.62 ± 0.08^{b}	2.69 ± 0.33^{c}	1.33 ± 0.00^{ab}	1.15 ± 0.10^{a}
18:1 ω9 DMA	0.72	1.66 ± 0.04^{a}	1.79 ± 0.04^{b}	2.58 ± 0.05^{c}	1.62 ± 0.05^{a}	1.57 ± 0.01^{a}
18:1 ω7 DMA	1.35	1.43 ± 0.03^{bc}	1.47 ± 0.05^{c}	1.74 ± 0.03^{d}	0.79 ± 0.02^{a}	1.38 ± 0.01^{b}
18:0	7.63	9.07 ± 0.04^{a}	10.55 ± 0.21^{ab}	11.09 ± 0.34^{b}	10.49 ± 0.01^{ab}	11.61 ± 0.06^{b}
18:1 ω9	16.49	17.46 ± 0.24^{c}	14.62 ± 0.36^{a}	16.69 ± 0.17^{b}	19.81 ± 0.40^{d}	15.31 ± 0.00^{a}
18:1 ω7	9.41	4.01 ± 0.04^{a}	4.14 ± 0.10^{a}	4.56 ± 0.25^{bc}	4.81 ± 0.08^{c}	4.26 ± 0.03^{ab}
18:2 ω6	3.40	3.68 ± 0.08^{c}	2.80 ± 0.07^{a}	3.86 ± 0.08^{c}	3.45 ± 0.08^{b}	5.20 ± 0.01^{d}
20:1 ω9	3.88	3.76 ± 0.38^{a}	3.88 ± 0.09^{a}	4.51 ± 0.05^{b}	9.00 ± 0.10^{c}	4.89 ± 0.12^{b}
20:4 ω6	9.27	11.04 ± 0.20^{d}	5.65 ± 0.24^{a}	9.90 ± 0.13^{c}	5.73 ± 0.17^{a}	7.98 ± 0.05^{b}
20:5 ω3	8.08	6.92 ± 0.06^{a}	11.40 ± 0.31^{c}	9.74 ± 0.13^{b}	11.79 ± 0.40^{c}	12.80 ± 0.13^{d}
22:0	ND	1.72 ± 0.04^{b}	0.82 ± 0.18^{a}	0.58 ± 0.32^{a}	ND	ND
22:1 ω11	0.79	0.37 ± 0.01^{b}	0.18 ± 0.03^{a}	ND	2.44 ± 0.08^{c}	ND
22:5 ω3	2.35	1.62 ± 0.03^{b}	2.42 ± 0.04^{c}	1.17 ± 0.06^{a}	1.16 ± 0.00^{a}	1.57 ± 0.00^{b}
22:6 ω3	5.57	6.08 ± 0.85^{b}	10.40 ± 0.42^{c}	4.11 ± 0.59^{a}	5.87 ± 0.11^{b}	5.29 ± 0.06^{ab}

Note: ND = not detected and DMA = dimethyl acetal.
Mean ± standard deviation of three samples.
The means followed by different superscripts are significantly different ($p<0.05$) from each other.
*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

The content of 18:2 ω6 was higher in heart tissue lipids than in the lipids of others tissues. This was most likely due to a somewhat higher cardiolipin content in heart tissue derived from a high level of mitochondria. In fact, cardiolipin in adult rat is composed of approximately 75% 18:2 ω6. Since cardiolipin is almost exclusively located in the inner mitochondrial membrane, it is most likely that this particular membrane has a very specific composition with respect to both the polar headgroup and the fatty acid composition of its lipids, thus conferring specific properties important to the performance of its functions (Mato, 1990b).

Several studies have reported the fatty acid composition of heart lipids of various seal species. A study on one harbour seal (Ackman *et al.*, 1972) reported the fatty acid composition of the triacylglycerol and phospholipid fractions as did a later study on two harbour seals and a grey seal (Ackman and Hooper, 1974). The general trends outlined above appear to be consistent with these studies. A study of a large number of harp, hooded, and harbour seals, using chemometric methods of fatty acid analysis, has also provided fatty acid compositions of heart lipids (Grahl-Nielsen and Mjaavatten, 1995). Again the general trends were similar, but they did report much higher values for the content of 18:0, 18:1 ω9, 18:2 ω6 and they did not report the presence of any 22:5 ω3. None of these studies reported values for dimethyl acetals. A study on the fatty acid composition of Mediterranean monk seal (Henderson *et al.*, 1994) reported similar values as those in this study; values for dimethyl acetals were also given.

Similar to kidney lipids, the fatty acids of heart lipids were evenly distributed among saturated, monounsaturated, and polyunsaturated components (Figure 4.5). Bearded seal heart lipids had the highest level of MUFAs. Of the remaining species,

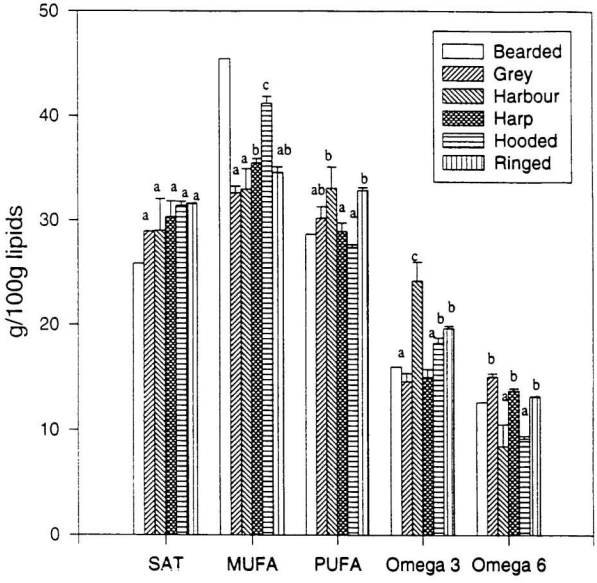


Figure 4.5. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the heart lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Hooded seal heart lipids had a significantly ($p<0.05$) higher proportion of MUFAs. The heart lipids of most species had more omega-3 fatty acids than omega-6. Grey seal, however, had very slightly more omega-6 than omega-3 fatty acids. Harbour seal heart lipids had the highest ratio of ω3 to ω6 fatty acids.

4.3.6. Lung

The fatty acid composition of lung lipids of bearded, grey, harbour, harp, hooded, and ringed seals is provided in Table 4.11. The most apparent difference in the fatty acid composition of lung lipids is the very high content of 16:0 (24.12- 28.37%). This fatty acid was predominant in lung lipids of all seal species analyzed. The content of 20:5 ω3 and 22:6 ω3 was very similar with 20:5 ω3 being the most abundant ω3 PUFA in bearded, hooded, and ringed seals, and 22:6 ω3 being the most abundant ω3 PUFA in grey, harbour, and harp seals. The dimethyl acetal content of lung lipids ranged from 1.38 to 5.54% and 22:5 ω3 was found to vary significantly ($p<0.05$) from species to species.

The very high content of palmitic acid (16:0) in lung tissue lipids is most likely due to a high proportion of dipalmitoylphosphatidylcholine (DPPC) from pulmonary surfactant lipids. Palmitic acid is known to be a major fatty acid present in lung phosphatidylcholine and the saturated phosphatidylcholines are predominant in DPPC, which accounts for approximately half of the total surfactant lipids. It is also generally agreed that DPPC is the major functional agent of lung surfactant, due to its surface-active properties. The presence of rigid acyl chains in the molecule under physiological conditions is linked to these properties (Bourbon, 1991).

Table 4.11. Fatty acid composition (g/100g lipid) of lung lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harbour	Harp	Hooded	Ringed
14:0	1.89	1.75 ± 0.01^{a}	4.02 ± 0.01^{d}	2.40 ± 0.06^{b}	2.52 ± 0.16^{bc}	2.65 ± 0.06^{c}
16:0 DMA	1.38	1.52 ± 0.02^{a}	1.57 ± 0.02^{a}	1.93 ± 0.02^{b}	1.77 ± 0.26^{ab}	1.59 ± 0.12^{ab}
16:0	24.12	25.55 ± 0.13^{a}	24.59 ± 0.22^{a}	24.45 ± 0.67^{a}	28.37 ± 1.70^{b}	26.53 ± 0.57^{ab}
16:1 ω7	6.86	2.41 ± 0.03^{a}	4.54 ± 0.00^{c}	2.54 ± 0.08^{a}	3.64 ± 0.19^{b}	6.17 ± 0.13^{d}
18:0 DMA	ND	1.09 ± 0.13^{b}	1.22 ± 0.05^{b}	1.68 ± 0.08^{c}	0.80 ± 0.13^{a}	0.77 ± 0.04^{a}
18:1 ω9 DMA	ND	0.72 ± 0.02^{b}	0.64 ± 0.04^{a}	0.92 ± 0.02^{c}	0.61 ± 0.00^{a}	ND
18:1 ω7 DMA	ND	0.85 ± 0.01^{b}	0.94 ± 0.03^{c}	1.01 ± 0.00^{d}	0.47 ± 0.00^{a}	0.82 ± 0.03^{b}
18:0	11.74	8.65 ± 0.05^{a}	8.78 ± 0.04^{a}	8.72 ± 0.26^{a}	10.10 ± 0.54^{b}	8.71 ± 0.23^{a}
18:1 ω9	14.05	12.87 ± 0.08^{b}	11.13 ± 0.05^{a}	13.41 ± 0.28^{bc}	15.48 ± 0.87^{d}	14.30 ± 0.19^{c}
18:1 ω7	8.17	4.43 ± 0.02^{b}	3.92 ± 0.01^{a}	3.96 ± 0.27^{a}	3.95 ± 0.26^{a}	9.41 ± 0.03^{c}
18:2 ω6	ND	1.54 ± 0.21^{c}	0.94 ± 0.06^{a}	1.14 ± 0.02^{ab}	1.28 ± 0.02^{bc}	2.03 ± 0.11^{d}
20:1 ω9	ND	3.72 ± 0.78^{bc}	2.74 ± 0.02^{ab}	2.68 ± 0.62^{ab}	4.80 ± 0.25^{c}	2.20 ± 0.18^{a}
20:4 ω6	11.35	6.06 ± 0.07^{cd}	3.78 ± 0.01^{a}	5.81 ± 0.14^{c}	4.23 ± 0.13^{b}	6.22 ± 0.21^{d}
20:5 ω3	9.66	5.56 ± 0.08^{b}	8.12 ± 0.03^{c}	4.86 ± 0.31^{a}	8.28 ± 0.42^{c}	8.75 ± 0.14^{c}
22:0	ND	0.42 ± 0.00^{a}	ND	0.56 ± 0.27^{a}	ND	0.56 ± 0.09^{a}
22:1 ω11	ND	1.08 ± 0.02^{c}	0.62 ± 0.01^{a}	0.81 ± 0.00^{b}	1.32 ± 0.04^{d}	ND
22:5 ω3	ND	3.17 ± 0.05^{c}	3.56 ± 0.02^{d}	2.35 ± 0.04^{b}	1.71 ± 0.00^{a}	4.53 ± 0.06^{e}
22:6 ω3	8.71	6.75 ± 0.04^{ab}	9.05 ± 0.02^{c}	5.80 ± 1.07^{a}	7.96 ± 0.23^{bc}	6.36 ± 0.12^{a}

Note: ND = not detected and DMA = dimethyl acetal.
Mean ± standard deviation of three samples.
The means followed by different superscripts are significantly different ($p<0.05$) from each other.
*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Figure 4.6 clearly shows that the saturated fatty acids make up the largest proportion of the fatty acids in lung lipids of all species analyzed. This was principally due to the high levels of 16:0. The lipids of lung of hooded seal, followed by those of ringed seal, had the highest levels of saturated fatty acids. Harp seal lung lipids were significantly ($p<0.05$) lower in PUFAs than those of grey and harbour seals. In all species, lipids of lung had a higher content of ω3 than ω6 fatty acids. Harbour seal lung lipids had the highest proportion of ω3 to ω6 fatty acids.

4.3.7 Liver

The fatty acid composition of the lipids of liver of bearded, grey, harp, hooded, and ringed seals is provided in Table 4.12. The fatty acid that was present in significantly ($p<0.05$) different proportions in each species analyzed was 16:1 ω7. The predominant fatty acid was 18:0 for bearded, harp, and hooded seals, while 16:0 was the dominant fatty acid in the livers of grey and ringed seals. A notable feature of the liver lipids was the absence or very low concentration of dimethyl acetals. As with other internal organ lipids, the content of 20:4 ω6 was relatively high. The fatty acid 20:5ω3 was the predominant ω3 PUFA present, except for harp seal where 22:6 ω3 was found in greater abundance.

Figure 4.7 shows that the fatty acids are fairly evenly distributed among saturated, mononunsaturated, polyunsaturated for all species tested. The ω3 fatty acid content was higher than that of the ω6 fatty acids for all species analyzed. The MUFA contents of hooded and ringed seal liver lipids were significantly ($p<0.05$) higher than those of grey

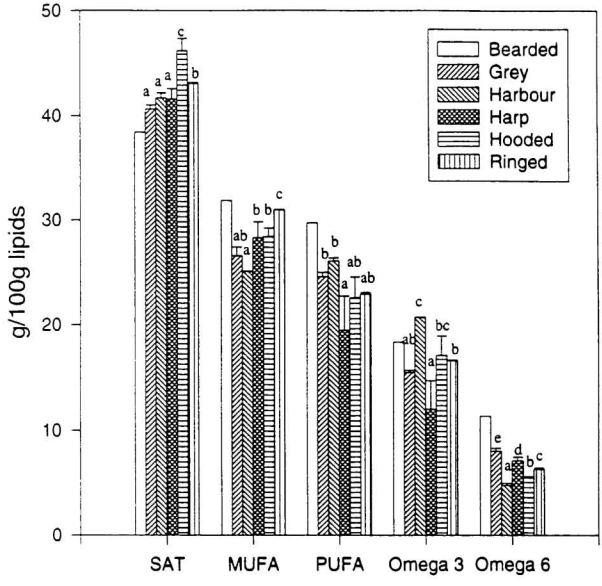


Figure 4.6. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the lung lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

Table 4.12. Fatty acid composition (g/100g lipid) of liver lipids of various species of seal

Fatty Acid	Bearded*	Grey	Harp	Hooded	Ringed
14:0	0.95	1.34 ± 0.04^{a}	1.21 ± 0.20^{a}	1.63 ± 0.00^{b}	1.30 ± 0.00^{a}
16:0 DMA	ND	0.14 ± 0.00^{a}	0.15 ± 0.01^{a}	ND	ND
16:0	13.37	16.08 ± 0.55^{b}	13.63 ± 0.76^{a}	12.64 ± 0.01^{a}	21.02 ± 0.06^{c}
16:1 ω7	6.07	2.94 ± 0.06^{a}	5.34 ± 0.13^{c}	5.09 ± 0.06^{b}	6.28 ± 0.02^{d}
18:0 DMA	ND	ND	0.16 ± 0.04	ND	ND
18:1 ω9 DMA	ND	ND	0.19 ± 0.06	ND	ND
18:1 ω7 DMA	ND	ND	0.13 ± 0.04	ND	ND
18:0	17.27	14.94 ± 0.51^{b}	19.55 ± 0.87^{d}	18.03 ± 0.16^{c}	13.07 ± 0.01^{a}
18:1 ω9	9.91	12.79 ± 0.40^{ab}	11.80 ± 1.45^{a}	15.09 ± 0.04^{c}	14.39 ± 0.07^{bc}
18:1 ω7	10.29	5.39 ± 0.14^{a}	6.10 ± 0.84^{a}	6.05 ± 0.11^{a}	9.41 ± 0.06^{b}
18:2 ω6	2.11	2.61 ± 0.06^{b}	2.04 ± 0.38^{a}	2.29 ± 0.02^{ab}	2.03 ± 0.01^{a}
20:1 ω9	2.80	3.25 ± 0.02^{b}	3.40 ± 0.58^{b}	7.06 ± 0.04^{c}	2.20 ± 0.05^{a}
20:4 ω6	11.47	9.28 ± 0.30^{b}	9.50 ± 0.57^{b}	5.64 ± 0.20^{a}	6.22 ± 0.03^{a}
20:5 ω3	11.66	9.24 ± 0.33^{a}	8.07 ± 1.74^{a}	11.86 ± 0.59^{b}	8.75 ± 0.20^{a}
22:0	ND	0.96 ± 0.11^{b}	0.58 ± 0.04^{a}	ND	0.56 ± 0.01^{a}
22:1 ω11	ND	0.19 ± 0.13^{a}	0.23 ± 0.13^{a}	0.96 ± 0.08^{b}	ND
22:5 ω3	2.82	4.11 ± 0.12^{c}	2.16 ± 0.05^{b}	1.64 ± 0.13^{a}	4.53 ± 0.02^{d}
22:6 ω3	7.67	6.69 ± 0.44^{ab}	8.17 ± 1.83^{ab}	8.89 ± 0.41^{b}	6.36 ± 0.08^{a}

Note: ND = not detected and DMA = dimethyl acetal.
Mean ± standard deviation of three samples.
The means followed by different superscripts are significantly different ($p<0.05$) from each other.
*Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

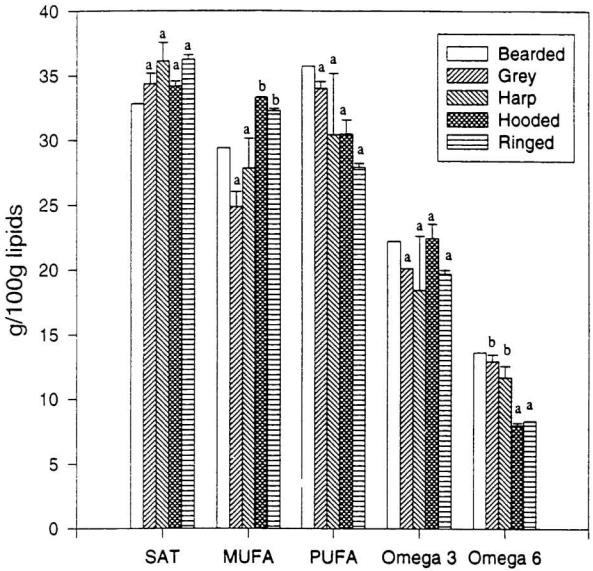


Figure 4.7. Contents of saturated (SAT), monounsaturated (MUFA), polyunsaturated (PUFA), omega 3, and omega 6 fatty acids in the liver lipids of different species of Eastern Canadian Phocid seals.

Notes: The bars within groups having different letters are significantly ($p<0.05$) different from each other.
Bearded seal was not included in the statistical analysis as only one sample was available for analysis.

and harp seals. The situation was reversed for ω6 PUFA contents. There were no other significant differences in groups of fatty acids between liver lipids of different species.

4.3.8 Comparison of tissues and species

In the previous sections, the individual fatty acids of different species of seals were compared one tissue at a time. This allowed for the identification of any differences between species and some unique features of several tissues. However, much more information may be deduced if the fatty acid composition is compared across species and tissues simultaneously. This was accomplished using a multivariate approach.

The multivariate approach employed was principle component analysis (PCA). Principle component analysis examines a few linear correlations (principle components) which can be used to summarize the data while losing as little information in the process as possible. In this study, the PCA was used to produce two coordinates describing the largest and second- largest variance among the samples from eleven fatty acids. The first two principle components accounted for 74.5% of the variance in the samples. The factor analysis summary and eigenvalues employed for the PCA are given in Tables 4.13 and 4.14, respectively. A plot of the PCA data obtained from the comparison of the fatty acid compositions of the lipids of blubber, brain, heart, lung, and muscle of the six Eastern Canadian Phocid seals is provided in Figure 4.8.

Table 4.13. Factor analysis summary

Number of variables	11
Estimated number of factors	5
Number of factors	3
Number of cases	30
Number missing	0
Degrees of freedom	65
Bartlett's Chi Square	429.347
P- value	< 0.0001

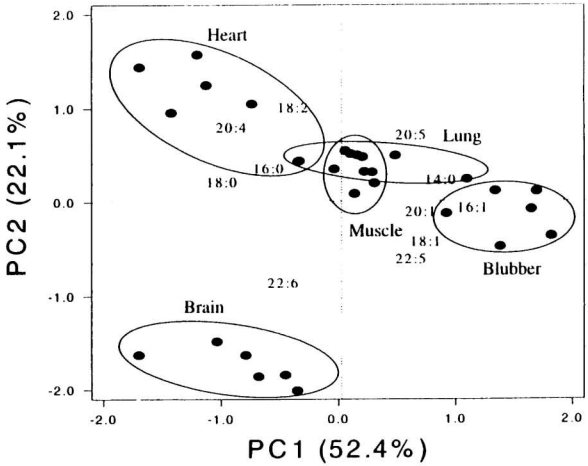
Table 4.14. Eigenvalues

Value	Magnitude	Variance proportion
1	5.768	0.524
2	2.435	0.221
3	1.184	0.108
4	0.663	0.060
5	0.379	0.034

Figure 4.8 clearly demonstrates that the greatest differences are from tissue to tissue and that brain tissue lipids are very unique in their fatty acid composition. This figure also shows how individual fatty acids influence where samples lie in the plot. For example, the fatty acid 22:6 tends to pull samples down along the second principle component (PC2) axis. As a result, any samples high in 22:6 will be pulled in that direction. The fatty acid 20:5 is high up on the PC2 axis and will pull samples high in 20:5 up and allows samples that are low in 20:5, such as brain lipids, to be pulled down even further. The fatty acid 18:2 also causes samples high in it to fall high along PC2, whereas 18:1 and 22:5 have more of a downward impact. Along the PC1 axis the fatty acids 20:4, 18:0, 16:0 and 18:2 pull to the left and 14:0, 22:5, 16:1, and 20:1 pull to the right.

Therefore, using Figure 4.8, we would be able to clearly ascertain that brain

Figure 4.8. A plot of a principle component analysis of the fatty acid compositions of selected tissues of bearded, grey, harbour, harp, hooded, and ringed seals.


Heart
18:2
20:4
16:0
18:0
20:5
Lung
14:0
20:1
16:1
Muscle
18:1
22:5
Blubber
22:6
Brain
2.0
1.0
0.0
-1.0
-2.0
-2.0
-1.0
0.0
1.0
2.0
PC2 (22.1%)
PC1 (52.4%)

tissue lipids tend to be relatively high in 22:6 and 22:5 and low in 20:5 and 18:2. For blubber lipids we would conclude that they tend to be relatively high in 14:0, 22:5, 16:1, 18:1and 20:1 and low in 20:4, 18:2 and 18:0. Heart tissue lipids tend to be high in 20:4 and 18:2 and low in 22:5 and 14:0. Since lung and muscle were lying in the centre of the plot they would tend to have intermediate values for the outlying fatty acids and higher values for the more centralized fatty acids such as 16:0.

The different compositions of lipids found in the individual tissues are not just accidents of evolution, but have evolved, in many cases, because of some specific benefits to the membrane in which they are found. The fatty acid composition is known to be a determining factor of membrane fluidity. Shorter chain lengths and *cis* double bonds provide more fluidity. The degree of unsaturation in the fatty acids of phospholipids has also been shown to affect cell permeability, osmotic fragility, and cholesterol efflux in the outer monolayer of erythrocytes (Keough, 1992).

4.3.9 Comparison of polar and neutral lipid fractions

The fatty acid compositions discussed in previous sections were of the crude lipid extracts of selected tissues from several species of seals. This section compares the fatty acid composition of the neutral and polar lipid fractions of muscle, kidney, heart, and liver of harp seal (Table 4.17). A multivariate approach was employed for the comparison and the factor analysis summary and eigenvalues are provided in Tables 4.15 and 4.16, respectively.

Table 4.15. Factor analysis summary

Number of variables	5
Estimated number of factors	3
Number of factors	3
Number of cases	10
Number missing	0
Degrees of freedom	14
Bartlett's Chi Square	40.97
P- value	< 0.0002

Table 4.16. Eigenvalues

Value	Magnitude	Variance proportion
1	2.701	0.540
2	1.293	0.259
3	0.740	0.148

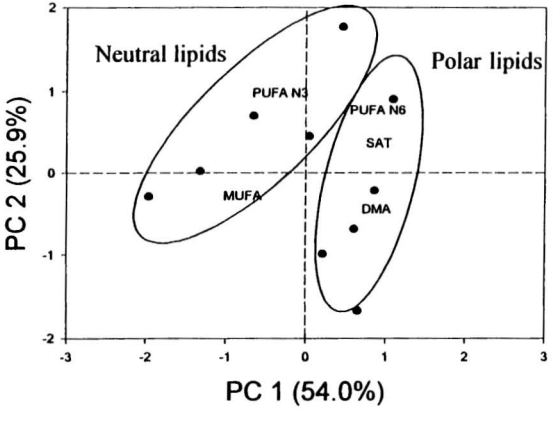
According to Table 4.15, the two principle components used for the two axes in Figure 4.9 account for 79.9% of the variance in the samples. The plot also shows that for all tissues compared there is a similar difference in the fatty acid composition of the polar and neutral lipid fractions. The plot indicates that dimethyl acetals, saturated fatty acids, and polyunsaturated fatty acids of the omega-6 configuration tended to be higher in the polar fraction than in the neutral fraction of corresponding tissue lipids. Monounsaturated fatty acids and polyunsaturated fatty acids of the omega-3 configuration tended to be higher in the neutral fraction than the polar fraction of corresponding tissue lipids.

The trends observed above are reasonable in that the polar lipids are predominantly membrane lipids and would therefore be modified for the specific purpose of the membrane. However, neutral lipids tend to be more for the purpose of lipid storage

Table 4.17. Fatty acid composition (g/100g lipids) of polar and neutral lipid fractions of muscle, kidney, heart, and liver tissues of harp seal.

Fatty acid type	Muscle		Kidney		Heart		Liver	
	Neutral	Polar	Neutral	Polar	Neutral	Polar	Neutral	Polar
Saturated	24.64 ± 0.23	35.42 ± 0.02	35.31 ± 1.39	40.45 ± 1.27	21.15 ± 0.26	36.75 ± 0.97	26.58 ± 0.49	37.70 ± 0.04
Monounsaturated	49.99 ± 0.95	34.88 ± 0.07	29.55 ± 0.65	25.28 ± 2.78	41.73 ± 0.31	29.87 ± 0.59	29.49 ± 0.65	25.16 ± 0.35
Polyunsaturated	19.81 ± 0.08	26.66 ± 0.30	31.05 ± 1.51	30.29 ± 3.16	32.47 ± 0.13	29.49 ± 0.44	37.01 ± 0.18	33.53 ± 0.20
Total ω3	15.93 ± 0.41	15.80 ± 0.18	17.73 ± 1.04	16.96 ± 2.04	19.99 ± 0.01	11.34 ± 0.18	25.38 ± 0.14	20.01 ± 0.15
Total ω6	3.62 ± 0.12	10.86 ± 0.11	13.32 ± 2.53	13.33 ± 1.44	10.94 ± 0.09	17.54 ± 0.55	10.98 ± 0.03	13.12 ± 0.04
DMA	0.33 ± 0.14	12.47 ± 0.00	0.00 ± 0.00	7.38 ± 1.87	0.08 ± 0.02	12.97 ± 1.04	0.37 ± 0.05	1.06 ± 0.08

Figure 4.9. A plot of a principle component analysis of the fatty acid compositions of Neutral and polar fractions of muscle, kidney, heart, and liver of harp seal.


Neutral lipids
Polar lipids
PUFA N3
PUFA N6
SAT
MUFA
DMA
PC 2 (25.9%)
PC 1 (54.0%)
2
1
0
-1
-2
-3
-2
-1
0
1
2
3

and are deposited without modification and therefore resemble more the dietary fatty acids (i.e., low ω6 content, high ω3 content, and high MUFA content).

4.4. Positional distribution of fatty acids in triacylglycerols of harp seal milk

The positional distribution of fatty acids in the triacylglycerols (TAG) of harp seal milk, determined by stereospecific analysis, is provided in Table 4.18. The ω3 PUFAs, EPA, DPA, and DHA were all preferentially esterified to the *sn*-1 and *sn*-3 positions with *sn*-3 being most favoured. The saturated fatty acids 14:0, 16:0, and 18:0 were concentrated in the *sn*-2 position. The mononunsaturated fatty acids 20:1 and 22:1 favoured the *sn*-1 and *sn*-3 positions, while 16:1 preferred the *sn*-2 and 18:1 was most concentrated in *sn*-1 followed by *sn*-2 positions. These results are similar to those reported by Wanasundara and Shahidi (1997) for harp seal blubber lipids.

The positional distribution of fatty acids in milk TAG, which is similar to blubber lipids, is very different from the positional distribution of fish oils. Brokerhoff *et al.* (1968) reported that in fish oils the long-chain PUFAs tend to be concentrated in the *sn*-2 position whereas in marine mammals they favour the *sn*-1 and *sn*-3 positions. More recently, using a similar methodology, Wanasundara and Shahidi (1997) reported similar results for menhaden oil and harp seal blubber oil. Aursand *et al.* (1995) investigated the positional distribution of omega-3 PUFAs in fish oil and marine mammal oil using high resolution ^{13}C nuclear magnetic resonance spectroscopy. Similarly, they found that in fish

Table 4.18. Positional distribution of fatty acids in triacylglycerols of harp seal milk.

Fatty acid	Milk triacylglycerols	*sn*-1	*sn*-2	*sn*-3
14:0	4.23 ± 0.18	3.10 ± 0.14	10.02± 0.21	0.20 ± 0.03
16:0	10.21 ± 0.39	4.73 ± 0.90	23.81± 1.04	3.96 ± 0.16
16:1	11.15 ± 0.32	5.13 ± 0.79	21.88± 0.97	7.69 ± 0.87
18:0	1.94 ± 0.14	2.53 ± 0.11	8.12 ± 0.34	1.80 ± 0.13
18:1	26.40 ± 0.45	40.59± 1.03	24.77± 0.85	18.41± 0.74
18:2	1.35 ± 0.04	0.63 ± 0.04	2.10 ± 0.09	1.76 ± 0.10
20:1	12.69 ± 0.83	15.57± 0.36	2.56 ± 0.41	21.84± 0.44
20:4	0.48 ± 0.00	0.27 ± 0.03	0.17 ± 0.02	1.17 ± 0.09
20:5	8.14 ± 0.81	10.12± 0.43	1.73 ± 0.05	13.45± 0.32
22:1	0.67 ± 0.03	1.07 ± 0.09	0.03 ± 0.01	1.13 ± 0.19
22:5	4.04 ± 0.12	3.96 ± 0.38	0.73 ± 0.04	8.06 ± 0.48
22:6	8.18 ± 0.26	9.66 ± 0.97	1.00 ± 0.07	15.58± 0.97

oils DHA was concentrated in the *sn*-2 position while EPA was more randomly distributed. DHA was predominantly present in the *sn*-1 and *sn*-3 positions in seal oil.

The difference in the positional distributions of ω3 PUFAs has important consequences on their bioavailablity. During digestion, lipases hydrolyze triacylglycerols into free fatty acids, predominantly from the *sn*-1 and *sn*-3 positions, and 2-monoacyl-*sn*-glycerols (Laakso, 1996). Even though most dietary fats are in the TAG form very little is known about the importance of the stereospecific composition of TAG in biological activity of dietary fatty acids (Kubow, 1996). Bracco (1994), however, reported that long-chain saturated fatty acids in the *sn*-1 and *sn*-3 positions may impair fat absorption and consequently the physiological response to the dietary fats.

Chapter 5

Conclusions

Compositional analysis of selected tissues of seals indicated that blubber had the lowest moisture and the highest lipid contents for all six species of Phocid seals of Eastern Canada examined. Milk contained the next lowest moisture and the second highest lipid content. Blubber followed by milk had the lowest protein content and muscle had the highest amount of protein of all tissues examined. Although there were significant differences in the ash content of different seal species and tissues, no apparent trends were discernible. However, harp seal milk had a much lower ash content than any of the other seal tissues.

Analysis of the lipid classes of selected tissues of the same six species of Phocid seals of Eastern Canada was conducted using the Iatroscan TLC/FID system employing a modified two step development process. Results indicated that the lipid class composition was affected more by tissue than the species under consideration. More specifically, blubber and milk lipids were composed mainly of TAG while brain lipids had undetectable levels of TAG. Brain lipids were high in cholesterol and this tissue was the only one containing measurable amounts of cerebrosides. For lipids of most tissues, PC was the predominant polar lipid class.

Comparison of fatty acid compositions of each tissue of the six species identified several unique features in the fatty acid composition of several tissues. Blubber, for example, was high in MUFA, but very low in AA (20:4 ω6) and dimethyl acetals and the ratio of omega-3 to omega-6 in this tissue was much higher than that for other tissues

examined. Brain lipids were very high in dimethyl acetals and DHA (22:6 ω3), but very low in EPA (20:5 ω3). Lung tissue lipids were very high in the content of saturated fatty acids, especially palmitic acid (16:0). Heart tissue lipids had a higher content of linoleic acid (18:2 ω6) than lipids of other tissues. Multivariate analysis showed clearly that the greatest differences in fatty acid composition were from tissue to tissue than from species to species.

In comparing the fatty acid composition of the neutral and polar fractions of lipids of several tissues, multivariate analysis showed that there were clear differences in the two fractions. Differences originated because the polar fraction contained a higher proportion of dimethyl acetals, saturated fatty acids, and PUFA of the ω6 configuration. However, the neutral fraction of corresponding tissue lipids contained a higher proportion of MUFA and PUFA of the ω3 configuration.

Stereospecific analysis of the triacylglycerols of harp seal milk determined that the positional distribution of the fatty acids was very similar to that of harp seal blubber triacylglycerols. The ω3 PUFAs, EPA, DPA, and DHA were all preferentially esterified to the *sn*-1 and *sn*-3 positions with *sn*-3 being favoured. The saturated fatty acids 14:0, 16:0 and 18:0 favoured the *sn*-2 position. The MUFAs 20:1 and 22:1 concentrated in the *sn*-1 and *sn*-3 positions while 16:1 preferred *sn*-2 and 18:1 favoured *sn*-1 followed by *sn*-2.

References

Abbey, M., Clifton, P., Kestin, M., Belling, B. and Nestel, P. 1990. Effect of fish oil on lipoproteins, lecithin, cholesterol acyltransferase, and lipid transfer protein activity in humans. *Arteriosclerosis* 10: 85- 94.

Abdidezfuli, F., Fryland, L., Thorsen, T., Aakvaag, A., and Berge, R.K. 1997. Eicosapentaenoic acid and sulfur substituted fatty acid analogs inhibit the proliferation of human breast cancer cells in culture. *Breast Cancer Res. Treat.* 45: 229- 239.

Abou-El-Ela, S.H., Prasse, K.W., Farrell, R.L., Carroll, R., Wade, A.E., and Bunce, O.R. 1989. Effects of D,L-2-difluoromethylornithine and indomethacine on mammary tumor promotion in rats fed high n-3 and/ or n-6 fat diets. *Cancer Res.* 49: 1434- 1439.

Accini, L., and Dixon, F.J. 1979. Degenerative vascular disease and myocardial infarction in mice with lupus- like syndrome. *Am. J. Pathol.* 96: 477- 492.

Ackman, R.G. 1981. Flame ionization detection applied to thin- layer chromatography on coated quartz rods. *Methods Enzymol.* 72D: 205- 252.

Ackman, R.G. and Burgher, R.D. 1963. Component fatty acids of the milk of grey (Atlantic) seal. *J. Biochem. Physiol.* 41: 2501- 2505.

Ackman, R.G. and Hooper, S.N. 1974. Long- chain monoethylenic and other fatty acids in heart, liver, and blubber lipids of two harbour seals (*Phoca vitulina*) and one grey seal (*Halichoerus grypus*). *J. Fish. Res. Bd. Can.* 31: 333- 341.

Ackman, R.G. and Jangaard, P.M. 1965. The grey (Atlantic) seal; fatty acid composition of the blubber from a lactating female. *Can. J. Biochem.* 43: 251- 255.

Ackman, R.G. and Lamontne, F. 1989. Marine Mammals. In *Marine Biogenic Lipids, Fats, and Oils. Vol. II.* (ed. Ackman, R.G.) pp.179- 381. Boca Raton, FL: CRC Press Inc.

Ackman, R.G. and Ratnayake, W.M.N. 1989. Hydrogenation and improved accuracy of lipid quantitation by Iatroscan TLC/FID. *J. Planar Chromatogr.* 2: 219- 224.

Ackman, R.G. 1992. Application of gas- liquid chromatography to lipid separation and analysis: qualitative and quantitative analysis. In *Fatty Acids in Foods and Their Health Implications* (ed. Chow, C.K.) pp. 47- 64. New York, NY: Marcel Dekker Inc.

Ackman, R. G., Epstein, S., and Eaton, C. A. 1971. Differences in the fatty acid compositions of blubber fats from Northwestern Atlantic finwhales (*Balaenoptera physalus*) and harp seals (*Pagophilus groenlandica*) *Comp. Biochem. Physiol.* 40B: 683- 697.

Ackman, R.G., Hooper, S.N., and Hingley, J. 1972. The harbour seal *Phoca vitulina concolor* Dekay: comparative details of fatty acids in lung and heart phospholipids and triglycerides. *Can. J. Biochem.* 50: 833- 838.

Adreassen, A.K., Hartmann, A., Offstad, J., Geiran, O., Kvernebo, K., and Simonsen, S. 1997. Hypertension prophylaxsis with omega 3 fatty acids in heart transplant recipients. *J. Am. Coll. Cardiol.* 29: 1324- 1331.

AOAC. 1990. *Offical Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed. Arlington, VA: Association of Official Analytical Chemists.

Ashworth, U.S., Ramaiah, G.D., and Keyes, M.C. 1966. Species difference in the composition of milk with special reference to the Northern fur seal. *J. Dairy Sci.* 49: 1206- 1211.

Aursand, M., Jorgensen, L., and Grasdalen, H. 1995. Positional distribution of omega-3 fatty acids in marine lipid triacylglycerols by high resolution 13C nuclear magnetic resonance spectroscopy. *J. Am. Oil Chem. Soc.* 72: 293- 297.

Bagga, D., Capone, S., Wang, H.J., Heber, D., Lill, M., Chap, L., and Glaspy, J.A. 1997. Dietary modulation of omega-3/ omega-6 polyunsaturated fatty acid ratios in patients with breast cancer. *J. National Cancer Instit.* 89: 1123- 1131.

Bang, H.O., and Dyerberg, J. 1972. Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. *Acta Med. Scand.* 192: 85- 94.

Bang, H.O., Dyerberg, J., and Hjrne, N.A. 1976. Composition of the food consumed by Greenland Eskimos. *Acta Med. Scand.* 200: 69- 73.

Bates, D., Cartlidge, N., French, J.M., Jackson, M.J., Nightingale, S., Shaw, D.A., Smith, S., Woo, E., Hawkins, S.A., Millar, J.H.D., Bekin, J., Conroy, D.M., Gill, S.K., Sidey, M., Smith, A.D., Thompson, R.H.S., Zilka, K., Gale, M., and Sinclaire, H.M. 1989. A double blind controlled trial of long chain n3

polunsaturated fatty acids in the treatment of multiple sclerosis. *J. Neurol. Neurosurg. Psychi.* 52: 18- 22.

Beck, B. 1983a. The grey seal in Eastern Canada. *Underwater World.* DFO/1861. UW/26. Department of Fisheries and Oceans, Ottawa, ON.

Beck, B. 1983b. The harbour seal in Canada. *Underwater World.* DFO/846. UW/27E. Department of Fisheries and Oceans, Ottawa, ON.

Bendich, A., and Brock, P. E. 1997. Rationale for the introduction of long chain polyunsaturated fatty acids and for concomitant increase in the level of vitamin E in infant formulas. *Internat. J. Vit. Nutr.* 67: 213- 231.

Bhanthena, S.J. 1992. Fatty acids and diabetes. In *Fatty Acids in Foods and Their Health Implications* (ed. Chow, C.K.) pp.823- 856. New York, NY: Marcel Dekker Inc.

Bittiner, S.B., Tucker, W.F.G., Cartwright, I. and Bleehen, S.S. 1988. A double-blind, randomized placebo-controlled trial of fish oil in psoriasis. *Lancet* i: 378- 380.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911- 917.

Boissonneault, G.A., and Hayek, M.G. 1992. Dietary fat, immunity, and inflammatory disease. In *Fatty Acids in Foods and Their Health Implications.* (ed. Chow, C.K.) pp. 707- 734. New York, NY: Marcel Dekker Inc.

Bonner, W.N. 1990. *The Natural History of Seals.* Facts on File Inc., New York, NY.

Bottino, N.R. 1983. Lipids of the Antarctic sei whale (*Balaenoptera borealis*). *Lipids* 13: 18-22.

Bowen, W.D. 1985. The Harp Seal. *Underwater World.* Ottawa, ON: Department of Fisheries and Oceans.

Bourbon, J.R. 1991. Nature, function, and biosynthesis of surfactant lipids. In *Pulmonary Surfactant: Biochemical, Functional, Regulatory, and Clinical Concepts.* (ed. Bourbon, J.R.) pp. 37- 76. Boca Raton, FL: CRC Press Inc.

Bracco, U. 1994. Effect of triglyceride structure on fat absorption. *Am. J. Clin. Nutr.* 60: 1002S- 1009S.

Brockerhoff, H. 1965. A stereospecific analysis of triglycerides. *J. Lipid Res.* 6: 10-15.

Brockerhoff, H. 1971. Stereospecific analysis of triglycerides. *Lipids* 6: 942- 956.

Brockerhoff, H., Hoyle, R.J., Hwang, P.C., and Litchfield, C. 1968. Positional distribution of fatty acids in depot triglycerides of aquatic animals. *Lipids* 3: 24- 29.

Bruckner, G. 1992. Fatty acids and cardiovascular diseases. In *Fatty Acids in Foods and Their Health Implications*. (ed. Chow, C.K.) pp. 735- 752. New York, NY: Marcel Dekker Inc.

Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C., and Deadman, N.M. 1989. Effect of changes in fat, fish and fibre intakes on death and myocardial function: Diet and reinfarction trial (DART). *Lancet* ii: 757- 762.

Carlson, S. 1995. The role of PUFA in infant nutrition. *INFORM* 6: 940- 946.

Christie, W. W. 1982. *Lipid Analysis* (2nd ed.). Oxford : Pergamon Press

Christie, W. W. 1994. Silver ion and chiral chromatography in the analysis of triacylglycerols. *Prog. Lipid Res.* 33: 9- 18.

Cochrane, G.C. 1975. A review of the analysis of free fatty acids (C_2- C_n). *J. Chromatogr. Sci.* 13: 440-445 .

Colman, J.S. 1937. The present state of the Newfoundland seal fishery. *J. Anim. Ecol.* 6: 145- 159.

Commeau, P.A. 1989. Sealing- A Canadian perspective. *Underwater World.* DFO/4311, UW/60E. Department of Fisheries and Oceans, Ottawa, ON.

Connolly, J.M., Liu, H.H., and Rose, D.P. 1997. Effects of dietary menhaden oil, soy, and cyclooxygenase inhibitor on human breast cancer cell growth and metastasis in nude mice. *Nutr. Cancer Int. J.* 29: 48- 54.

Cook, H. W., and Baker, B.E. 1969. Seal milk. I. Harp seal (*Pagophilis groenlandicus*) milk: composition and pesticide residue contents. *Can. J. Zool.* 47: 1129- 2426.

Currie, G., and Kallio, H. 1993. Triacylglycerols of human milk: rapid analysis by ammonia negative ion tandem mass spectrometry. *Lipids* 28: 217- 222.

Davidson, M.H., Maki, K.C., Kalkowski, J., Schaeffer, E.J., Torri, S.A., and Drennan, K.B. 1997. Effects of docosahexaenoic acid on serum lipoproteins in patients with combined hyperlipidemia: A randomized, double blind, placebo controlled trial. *J. Am. Coll. Nutr.* 16: 236- 243.

Davis, R.A., Finley, K.J., and Richardson, W.J., 1980. The present status and future management of Arctic marine mammals in Canada. Science Advisory Board of the Northwest Territories, Yellowknife, NWT.

Debuch, H., Witter, B., Illig, K., and Gunawan, J. 1982. On the metabolism of ether phospholipids in glial cell cultures. In *Phospholipids in the Central Nervous System. Vol. I, Metabolism.* (eds. Horrocks, L., Ansell, G.B., and Porcellati, G.) pp. 199- 210. New York, NY: Raven Press.

Delorgeril, M., Salen, P., Martin, J.L., Mamelle, N., Monjaud, I., Touboul, P., and Delaye, J. 1996. Effect of a Mediterranean- type diet on the rate of cardiovascular complications in patients with coronary artery disease: Insights into the cardioprotective effect of certain nutrients. *J. Am. Coll. Cardiol.* 28: 1103- 1108.

DeSchrijver, R., and Vermeulen, S. 1991. Separation and quantitation of phospholipids in animal tissues by Iatroscan TLC/FID. *Lipids* 26: 74- 76.

DFO. 1997. The 1996 Seal Quota Report. Statistics Division, Northwest Atlantic Fisheries Centre, DFO, ST. John's, NF.

Dimmitt, S.B. 1995. Recent insights into dietary fats and cardiovascular disease. *Clin. Experiment. Pharmacol. Phys.* 22: 204- 208.

Endres, S., De Caterina, R., Schmidt, E. B., and Kristensen, S.D. 1995. N-3 polyunsaturated fatty acids: update 1995. *Eur. J. Clin. Invest.* 25: 629- 638.

Engelhart, F.R. and Walker, B.L. 1974. Fatty acid composition of the harp seal *Pagophilus groenlandicus* (*Phoca groenlandica*). *Comp. Biochem. Physiol.* 47 B: 169- 179.

Fortin, P.R. 1995. Validation of a meta-analysis: The effects of fish oil in rheumatoid arthritis. *J. Clin. Epidemiol.* 48: 1379- 1390.

Franzke, C., Hollstein, E., Kroll, J., and Noske, H.J. 1973. Studien zur Glyceridstruktur von Fetten. I: Vergleichende Untersuchungen an Modellsubstanzen uber den Einsatz von Pankreaslipase und

Methylmagnesiumbromid zur structurspezifischen Triglyceridspaltung. *Fette Seifen Anstrichm.* 75: 365- 369.

Fraser, A.J., and Taggart, C.T. 1988. Thin-layer chromatography- flame ionization detection calibration with natural and synthetic standards. *J. Chromatogr.* 439: 404- 407.

Fredheim, B., Holen, S., Ugland, K., and Grahl- Neilson, O. 1995. Fatty acid composition in blubber, heart, and brain from phocid seals. In *Whales, Seals, Fish, and Man.* (eds. Blix, A.S., Walloe, L., and Ultang, O.) pp. 153- 168. Cambridge, UK: Elsevier Science.

Gerster, H. 1993. Fish oil (n-3 long-chain PUFAs) and blood pressure. *Nutr.* 17: 15- 24.

Gibbons, G.F., Mitropoulos, K.A. and Myant, N.B. 1982. *Biochemistry of Cholesterol.* New York, NY: Elsevier Science Pub. Co.

Glauert, H.P. 1992. Dietary fatty acids and cancer. In *Fatty Acids in Foods and Their Health Implications* (ed. Chow, C.K.) pp. 753- 768. New York, NY: Marcel Dekker Inc.

Godwin, S. 1990. *Seals.* New York, NY: Michael Freedman Publishing Group Inc.

Grahl- Nielson, O. and Mjaavatten, O. 1995. Marine mammalian fatty acids: a source of information. In *Whales, Seals, Fish, and Man.* (eds. Blix, A.S., Walloe, L., and Ultang, O.) pp. 141- 152. Cambridge, UK: Elsevier Science.

Grompone, M.A., Sienra, B. and Quilez, J.L. 1990. Fatty acid composition of fats from the Uruguayan fur seal (*Arctocephalus australis* Zimmerman). *Marine Mammal Sci.* 6: 48- 53.

Gunstone, F.D. 1994. Fatty acid structure. In *The Lipid Handbook* (eds. Gunstone, F.D., Harwood, J.L., and Padley, F.B.). pp. 1- 21. London: Chapman and Hall.

Gunstone, F.D. and Norris, F.A. 1983. *Lipids in foods: chemistry, biochemistry, and technology.* New York, NY: Pergamon Press.

Hamazaki, T., Sawazaki, S., Itomura, M., Asaoka, E.M., Nagao, Y., Nisjiuro, N., Yazawa, K., Kuamori, T., and Kobayashi, M. 1996. The effect of docosahexaenoic acid on aggression in young adults. *J. Clin. Invest.* 97: 1129- 1134.

Harwood, J.L., 1994. Lipid structure. In *The Lipid Handbook* (eds. Gunstone, F.D., Harwood, J.L., and Padley, F.B.), pp. 21- 46. London: Chapman and Hall.

Hayes, K.C., Pronczuk, A., and Khosla, P. 1995. A rationale for plasma cholesterol modulation by dietary fatty acids- modeling the human response in animals. *J. Nutr. Biochem.* 6: 188- 194.

Henderson, R.J., Kalogeropoulos, N. and Alexis, M.N. 1994. The lipid composition of selected tissues from a Mediterranean monk seal, *Monachus monachus*. *Lipids* 29: 577- 582.

Hibbeln, J.R., and Salem, J.N. 1995. Dietary polyunsaturated fatty acids and depression: When cholesterol does not satisfy. *Am. J. Clin. Nutr.* 62: 1-9.

Higgs, G.A., 1986. The role of eicosanoids in inflammation. *Prog. Lipid Res.* 25: 555- 561.

Hirai, A., Terano, T., Saito, H., Tamura, Y., and Yoshida, S. 1987. Clinical and epidemiological studies of eicosapentaenoic acid in Japan. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids* (ed. Lands, W.E.M.) pp. 9- 24. Champaign, IL: American Oil Chemists' Society.

Holmer, G. 1989. Triglycerides. In *Marine Biogenic Lipids. Fats and Oils. Vol. I.* (ed. Ackman, R.G.) pp. 139- 174. Boca Raton, FL: CRC Press Inc.

Horjik, C. A., Burges, I., Phylipsen, G. J. M., and Cappenbers, T. E. 1990. Trace determination of volatile fatty acids in sediments by gas chromatography with chemically bonded FFAP columns. *J. Chromatogr.* 511: 317- 323.

Hornsta, G., Al, M.D.M., Van Houwelingen, A.C., and Foreman- Van Drongelen, M.M. 1995. Essential fatty acids in pregnancy and early human development. *Eur. J. Obstet. Gynencol. Reprod. Biol.* 61: 57- 62.

Horrocks, L.A. 1972. Content, composition and metabolism of mammalian and avian lipids that contain ether groups. In *Ether Lipids: Chemistry and Biology*. (ed. Snyder, F.) pp. 177- 272. New York, NY: Academic Press Inc.

Howe, P.R.C. 1995. Can we recommend fish oil for hypertension. *Clin. Experiment. Pharm. Phys.* 22: 199- 203.

Huang, Y.J. Fang, V.S., Juan, C.C., Chou, Y.C., Kwok, C.F., and Ho, L.T. 1997. Amelioration of insulin resistance and hypertension in a fructose fed rat

model with fish oil supplementation. *Metabol. Clin. Experiment* 46: 1252-1258.

Hwang, D. 1992. Dietary fatty acids and eicosanoids. In *Fatty Acids in Foods and Their Health Implications*. (ed. Chow, C.K.) pp. 545- 558. New York, NY: Marcel Dekker Inc.

Indrasena, W.M., Paulson, A.T., Parrish, C.C., and Ackman, R.G. 1991. A comparison of alumina and silica gel Chromarods for the separation and characterization of lipid classes by Iatroscan TLC-FID. *J. Planar Chromatogr.* 4: 182- 188.

Innis, S.M., and Clandinin, M.T. 1981. Separation of phopspholipids on Chromarods. *J. Chromatogr.* 205: 490- 492.

Ioannou, P.V. and Golding, B.T. 1979. *Prog. Lipid Res.* 17: 279

Iverson, S.J., Sampugna, J. and Oftedal, O.T. 1992. Positional specificity of gastric hydrolysis of long- chain n- 3 polyunsaturated fatty acids of seal milk triglycerides. *Lipids* 27: 870- 878.

Jangaard, P.M. and Ke, P.J. 1968. Principal fatty acids of depot fat and milk lipids from harp seal (*Pagophilus groenlandica*) and hooded seal (*Cystophora cristata*). *J. Fish. Res. Bd. Can.* 25: 2419- 2426.

Jenness, R. and Sloan, R.E. 1970. The composition of milks of various species: a review. *Dairy Sci. Abstr.* 32: 599-612.

Joe, B., and Lokesh, B.R. 1997. Prophylactic and therapeutic effects of n-3 polyunsaturated fatty acids, capsaicin, and curcumin on adjuvant induced arthritis in rats. *J. Nutr. Biochem.* 8: 397- 407.

Kaimal, T.N.B., and Shantha, N.C. 1984. Quantitative analysis of lipids on copper II sulphate impregnated Chromarods. *J. Chromatogr.* 288: 177- 186.

Kakela, R. and Hyvarinen, H. 1993. Fatty acid composition of fats around the mystacial and superciliary vibrissae differs from that of blubber in the Saimaa ringed seal (*Phoca hispida saimensis*). *Comp. Biocem. Physiol.* 105B: 547-552.

Kakela, R., Hyvarinen, H. and Vainiotalo, P. 1993. Fatty acid composition in liver and blubber of the Saimaa ringed seal (*Phoca hispida saimensis*) compared with that of the ringed seal (*Phoca hispida botnica*) and grey seal (*Halichoerus grypus*) from the Baltic. *Comp. Biochem. Physiol.* 105B: 553- 565.

Kallio C. and Currie, G. 1993. Analysis of natural fats and oils by ammonia negative ion tandem mass spectrometry- triacylglycerols and positonal distribution of their acyl groups. In *CRC Handbook of Chromatography- Analysis of Lipids*. (eds. Mukhejee, K.D., Weber, N., and Sherma, J.) pp. 435- 458. Boca Raton, FL: CRC Press Inc.

Karmeli, R.A., 1987. Omega 3 fatty acids and cancer: A review. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids*. (ed. Lands, W.E.M.) pp. 222- 231. Champaign, IL: American Oil Chemists' Society.

Kelly, V. E., Ferritti, A., Izni, S., and Strom, T. B. 1985. A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL- lpr mice. *J. Immunol.* 134: 1914- 1919.

Keough, K.M.W. 1992. Unsaturation and the interactions of phospholipids with cholesterol and proteins. In *Structural and Dynamic Properties of Lipids and Membranes* (eds. Quinn, P.J. and Cherry, R.J.) pp. 19-28. Chapel Hill, NC: Portland Press Inc.

King, J.E. 1983. *Seals of the World* (2nd ed.). London: British Museum (Natural History) and Oxford University Press.

Knapp, H.R. 1989. The antihypertensive effects of fish oil: A controlled study of polyunsaturated fatty acid supplements is essential hypertension. *N. Engl. J. Med.* 320: 1037- 1043.

Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R. 1985. The effect of fatty acid chain length and unsaturation on the chromatographic behavior of triglycerides on Iatroscan Chromarods. *Lipids* 20: 617- 619.

Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R. 1986b. Resolution of phospholipids on copper II sulphate impregnated Chromarods. *J. Chromatogr.* 351: 571- 573.

Kramer, J.K.G., Thompson, B.K., and Farnworth, E.R. 1986a. Variation in the relative response factor for triglycerides on Iatroscan Chromarods with fatty acid composition and sequence of analysis. *J. Chromatogr.* 355: 221- 228.

Kremer, J.M., and Jubiz, W. 1987. Fish oil supplementation in active rheumatized arthritis: A double blinded, controlled crossover study. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids* (ed. Lands, W.E.M.). Champaign, IL: American Oil Chemists' Society.

Kritchevsky, D. 1995. Fatty acids, triglyceride structure, and lipid metabolism. *J. Nutr. Biochem.* 6: 172- 178.

Kritchevsky, D., Davidson, L.M., Weight, M. Kriek, L.P.J., and DuPlessis, J.P. 1982. Influence of native and randomized peanut oil on lipid metabolism and aortic sudanophillia in vervet monkeys. *Atherosclerosis* 42: 53- 58.

Kromann, N., and Green, A. 1980. Epidemiological studies in the Upernavik district, Greenland. *Acta Med. Scand.* 208: 401- 406.

Kromhout, D. Bosschieter, E.B., and Coulander, C. de L. 1985. The inverse relation between fish consumption and 20 year mortality from coronary heart disease. *N. Engl. J. Med.* 312: 1205- 1209.

Kubow, S. 1996. The influence of positional distribution of fatty acids in native, interesterified and structure- specific lipids on lipoprotein metabolism and atherogenesis. *J. Nutr. Biochem.* 7: 530- 541.

Laakso, P. 1996. Analysis of triacylglycerols- Approaching the molecular composition of natural mixtures. *Food Rev. Int.* 12: 199- 250.

Lands, W.E.M. 1982. Experiments with strokes produced in cats. In *Nutritional Evaluation of Long-chain Fatty Acids in Fish Oil* (ed. Barlow, S.M., and Stansby, M.E.) pp. 273- 274. London: Academic Press.

Lands, W.E.M. 1986. *Fish and Human Health.* London: Academic Press.

Lavigne, D.M., Stewart, R.E.A., and Fletcher, F. 1982. Change in composition and energy content of harp seal milk during lactation. *Physiol. Zool.* 55: 1-9.

Litchfield, C. 1972. *Analysis of triglycerides.* London: Academic Press.

Malouf, A.H. 1986. *Seals and Sealing in Canada: Report of the Royal Commission. Vol.* 2. Canadian Government Publishing Centre, Ottawa, ON.

Manly, B.F.J. 1994. *Multivariate Statistical Methods: A Primer. 2nd Ed.* London: Chapman and Hall.

Mansfield, A.W. 1988. The grey seal. *Underwater World.* DFO/3884, UW/26, Department of Fisheries and Oceans, Ottawa, ON.

Manzato, E., Bassi, A., Tiozzo, A., Casson, S., Zambon, S., Bariga, A.B., Grossato, O., Veronese, C., Malusa, E., Manoni, F., and Crepaldi, G. 1997. Fish

consumption, phospholipid fatty acids, and plasma lipids in healthy Italian subjects. *Nutr. Metabol. Cardiovascular Diseases* 7: 353- 359.

Martinez, M. 1996. Docosahexaenoic acid therapy in DHA deficient patients with disorders or peroxisomal biogenesis. *Lipids* 31: 5145- 5152.

Masuev, K.A. 1997. The effect of omega- 3 polyunsaturated fatty acids on late phase of allergic reaction in patients with bronchial asthma. *Terapevticheski Arkhiv*. 69: 31- 33.

Mato, J.M. 1990a. Lipid components of cellular membranes. In *Phospholipid metabolism in Cellular Signaling* (ed. Mato, J.M.) pp. 1-8. Boca Raton, FL: CRC Press Inc.

Mato, J.M. 1990b. Phospholipid composition of cellular membranes. In *Phospholipid metabolism in Cellular Signaling* (ed. Mato, J.M.) pp. 1-8. Boca Raton, FL: CRC Press Inc.

Mizutani, M., Asano, M., Roy, S., Nakajima, T., Soma, M., Yomoshita, K., and Okuda, Y. 1997. Omega-3 polyunsaturated fatty acids inhibit migration of human vascular smooth muscle cells in-vitro. *Life Sci.* 61: PL269- PL274.

Mori, T.A., Beilin, L.J., Burke, V., Morris, J., and Ritchie, J. 1997. Interactions between dietary fat, fish, and fish oils, and their effects on platelet function in men at risk of cardiovascular disease. *Arteriosclerosis Thrombosis Vascular Biol.* 17: 279- 286.

Morris, M.C., Manson, J.E., Rosner, B., Buring, J.E., Willett, W.C., and Hennekens, C.H. 1995. Fish consumption and cardiovascular disease in the Physicians Health Study: A prospective study. *Am. J. Epidemiol.* 142: 166- 175.

Needleman, P., Raz, A., Minkes, M.S., Ferrendeli, J.A., and Specher, H. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci. USA*. 76: 944- 948.

Nelson, A. 1972. Diet therapy in coronary disease- Effects on mortality of high protein, high seafood, fat controlled diet. *Geriatrics* 27: 103- 116.

Neuringer, M., Anderson, G.J., and Conner, W.E., 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Ann. Rev. Nutr.* 198: 517- 541.

Newton, I.S. 1996. Long chain fatty acids in health and nutrition. *J. Food Lipids* 3: 233- 249.

Nordoy, A. 1996. Fish consumption and cardiovascular disease: A reappraisal. *Nutr. Metabol. Cardiovascular Diseases* 6: 103- 109.

Nunzi, M.G., Milan, F., Guidolan, A.Z., and Toffano, G. 1990. Therapeutic properties of phosphatidylserine in the aging brain. In *Phospholipids: Biochemical, Pharmaceutical and Analytical Considerations*. (eds. Hanin, I. and Pepeu, G.) pp. 213- 218. New York, NY: Plenum Press.

Oftedal, O.T., Boness, D.J., and Bowen, W.D. 1988. The composition of hooded seal (*Cystophora cristata*) milk: an adaptation for postnatal fattening. *Can. J. Zool.* 66: 318- 322.

Ohshima, T., Ratnayake, W.M.N., and Ackman, R.G. 1987. Cod lipids, solvent systems and the effect of fatty acid chain length and unsaturation on lipid class analysis by Iatroscan TLC- FID. *J. Am. Oil Chem. Soc.* 64: 219- 223.

Padley, F.B., Gunstone, F.D. and Harwood, J.L. 1994. Occurrence and characteristics of oils and fats. In *The Lipid Handbook* (eds. Gunstone, F.D., Harwood, J.L., and Padley, F.B.). pp. 46- 93. London: Chapman and Hall.

Parish, C.C. 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Can. J. Fish. Aquat. Sci.* 44: 722- 731.

Peet, M., and Edwards, R.W. 1997. Lipids, depression and physical diseases. *Current Opinion Psych.* 10: 477- 480.

Percival, T. 1783. Observations on the medicinal uses of the oleum jeconis aselli or cod liver oil in chronic rheumatism and other painful disorders. *London Med. J.* 3: 393- 401.

Pietinen, P. 1997. Intake of fatty acids and risk of coronary heart disease in a cohort of finnish men: The alpha-tocopherol, beta-carotene cancer prevention study. *Am. J. Epidemiol.* 145: 876- 887.

Pique, G.C. 1986. *Omega-3, the Fish Oil Factors: Natural Food Sources and Health Effects*. San Diego, CA: Omega-3 Project Inc.

Prasad, R. 1996. Structure and distribution of membrane lipids. In *Manual on Membrane Lipids* (ed. Prasad, R.) pp. 1- 15. New York, NY: Springer- Verlag Berlin Heidelberg.

Puppione, D.L., Kuehlthau, C.M., Jandacek, R.J., and Costa, D.P. 1992. Positional analyses of triacylglycerol fatty acids in the milk fat of the Antarctic fur seal (*Arctocephalus gazella*). *Lipids* 27: 637- 639.

Ranny, M. 1987. *Thin Layer Chromatography with Flame Ionization Detection*. Boston: D. Reidel

Ratnayake, W.M.N., and Ackman, R.G. 1989. Lipid Analyses. Part II. In *The Role of Fats in Human Nutrition* (2nd ed.)(ed. Vergroesen, A.J., and Crawford, M.) pp. 515- 565. London: Academic Press.

Reddy, B.S., 1987. Dietary fat and colon cancer: Effect of fish oil. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids*. (ed. Lands, W.E.M.) pp. 233- 237. Champaign, IL: American Oil Chemists' Society.

Robinson, D.R., Tateno, S., Patel, B., and Hirai, I. 1987. The effect of dietary marine lipids on autoimmune disease. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids*. (ed. Lands, W.E.M.) pp. 139- 147. Champaign, IL: American Oil Chemists' Society.

Rose, D.P. 1997. Dietary fatty acids and prevention of hormone responsive cancer. *Proceed. Soc. Experment. Biol. Med.* 216: 224- 233.

Russo, C., Oliveri, O., Girelli, D., Azzini, M., Stanzial, A.M., Guarini, P., Friso, S. Defranceschi, L., and Corrocher, R. 1995. Omega- 3 polyunsaturated fatty acid supplements and ambulatory blood pressure monitoring parameters in patients with mild essential hypertension. *J. Hypertension* 13: 1823- 1826.

Sastry, P.S. 1985. Lipids of nervous tissue: Composition and metabolism. *Prog. Lipid Res.* 24: 69- 176.

Sebedio, J.L., Farquharson, T.E., and Ackman, R.G. 1985. Quantitative analyses of methyl esters of fatty acid geometrical isomers, and of triglycerides differing in unsaturation, by the Iatroscan TLC/FID technique using $AgNO_3$ impregnated rods. *Lipids* 20: 555- 560.

Sergeant, D.E. 1991. *Harp Seals, Man, and Ice*. Canadian Special Publication of Fisheries and Aquatic Sciences 114. Department of Fisheries, Ottawa, ON.

Shahidi, F. 1998. *Seal Fishery and Product Development*. St. John's, NF: ScienceTech Publishing Company.

Shahidi, F. and Synowiecki, J. 1991. Cholesterol content and lipid fatty acid composition of processed seal meat. *Can. Inst. Sci. Technol. J.* 24: 269- 272.

Shahidi, F., Synowiecki, J., Amarowicz, R., and Wanasundara, U. 1994. Omega- 3 fatty acid composition and stability of seal lipids. In *Lipids in Food Flavours.* (eds. Ho, C.T. and Hartman, T.G.) pp. 233- 243. Washington, DC: ACS Symposium Series 558, American Chemical Society.

Shahidi, F., Wanasundara, P.K.J.P.D., and Wanasundara, U.N. 1996. Seal blubber oil: A novel source of ω3 fatty acids. *J. Food Lipids* 3: 293-306.

Shantha, N.C. 1992. Thin- layer chromatography- flame ionization detection Iatroscan system. *J. Chromatogr.* 624: 21- 35.

Shantha, N.C. and Ackman, R.G. 1990. Advantages of total lipid hydrogenation prior to lipid class determination on Chromarods-SIII. *Lipids* 25: 570-574.

Shantha, N.C., and Napolitano, G.E. 1992. Gas chromatography of fatty acids. *J. Chromatogr.* 624: 37- 51.

Shelton, P.A., Caddigan, N.G., and Stenson, G.B. 1992. Model estimates of harp seal population trajectories in the Northwest Atlantic. *CAFSAC Research Document*, No. 39.

Shelton, P.A., Stenson, G.B., Sjare, B., and Warren, W.G. 1995. Model estimates of harp seal numbers at age for the Northwest Atlantic. Department of Fisheries and Oceans, *Research Document 95121.*

Simopoulos, A.P. 1990. Omega-3 fatty acids in growth and development. In *Omega-3 Fatty Acids in Health and Disease* (eds. Lees, R.S., and Karel, M.). pp. 115-156. New York, NY: Marcel Dekker, Inc.

Simopoulos, A.P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54: 438- 463.

Simopoulos, A.P. 1997a. Essential fatty acids in health and chronic disease. *Food Rev. Int.* 13: 623- 631.

Simopoulos, A.P. 1997b. Omega- 3 fatty acids in the prevention management of cardiovascular disease. *Can. J. Phys. Pharmacol.* 75: 234- 239.

Snedecor, G.W. and Cochran, W.G. 1980. *Statistical Methods*, 7th ed. Ames, IA: The Iowa State University Press.

Stamler, M.J., Willett, W.C., Colditz, G.A., and Spezer, M.D. 1987. Intake of cholesterol, fish, and specific types of fat in relation to breast cancer. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids*. (ed. Lands, W.E.M.) pp. 248- 252. Champaign, IL: American Oil Chemists' Society.

Stansby, M.E. 1990a. Nutritional properties of fish oil for human consumption- early developments. In *Fish Oils in Nutrition* (ed. Stansby, M.E.) pp. 268- 288. New York, NY: Van Nostrand Reinhold.

Stansby, M.E. 1990b. Nutritional properties of fish oil for human consumption- modern aspects. In *Fish Oils in Nutrition* (ed. Stansby, M.E.) pp. 289- 308. New York, NY: Van Nostrand Reinhold.

Stenson, G.B. 1994. The status of pinnipeds in the Newfoundland region. *NAFO Science Council Studies* 21: 115- 119.

Stenson, G.B., 1995. Report on the status of harp seals in the Northwest Atlantic, Department of Fisheries and Oceans, Ottawa, ON.

Stewart, R.E.A., Webb, B.E., Lavigne, D.M., and Fletcher, F. 1983. Determining lactose content of harp seal milk. *Can. J. Zool.* 61: 1094- 1100.

Strøm, A. 1948. Examination of the diet of Norwegian families during the war years 1942- 45. *Acta Med. Scand. Suppl.* 1- 47.

Strøm, A., and Jensen, R.A. 1951. Mortality from circulation diseases in Norway 1940- 1945. *Lancet* 1: 126- 129.

Svennerholm, E., Stallberg-Stenhagen, S., and Svennerholm, L. 1966. Fatty acid composition of sphingomyelins in blood, spleen, placenta, liver, lung, and kidney. *Biochim. Biophys. Acta* 125: 60- 65

Takagi, T. 1990. Chromatographic resolution of chiral lipid derivatives. *Prog. Lipid Res.* 29: 277- 298.

Tatara, T. Fuji, T., Kawase, T., and Minagawa, M. 1983. Quantitative determination of tri-, di-, monooleins, and free oleic acid by the thin layer chromatography-flame ionization detection system using internal standard and boric acid impregnated rods. *Lipids* 18: 732- 736.

Terano, T., Kojima, T., Seya, A., Tanabe, E., Hirai, A., Makuta, H., Ozawa, A., Fujita, T., Tamura, Y., Okamoto, S., and Yoshida, S. 1989. The effect of highly

purified eicosapentaenoic acid in patients with psoriasis. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 19: 610- 613.

Thais, F. and Stahl, R.A.K. 1987. Effect of dietary fish oil on renal function in immune mediated glomerular injury. In *Proceedings of the AOAC Short Course on Polyunsaturated Fatty Acids and Eicosanoids.* (ed. Lands, W.E.M.) pp. 123- 126. Champaign, IL: American Oil Chemists' Society.

Thies, N.H. 1997. The effect of 12 months treatment with eicosapentaenoic acid in 5 children with cystic fibrosis. *J. Paediatrics Child Health* 33: 349- 351.

Vaskovsky, V.E. 1989. Phospholipids. In *Marine Biogenic Lipids, Fats and Oils. Vol. I.* (ed. Ackman, R.G.) pp. 139- 174. Boca Raton, FL: CRC Press Inc.

Vaskovsky, V.E., and Terekhova, T.A. 1979. HPTLC of phospholipid mixtures containing phosphatidylglycerol. *J. High Resol. Chrom.* 2: 671-677.

Wanasundara, U.N. and Shahidi, F. 1997. Positional distribution of fatty acids in triacylglycerols of seal blubber oil. *J. Food Lipids* 4: 51- 64.

Weber, P.C., Fischer, S., Von Schacky, C., Lorenz, R., Strasser, T. 1986. Dietary omega- 3 polyunsaturated fatty acids and eicosanoid formation in man. In *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (eds. Simopoulos, A.P., Kifer, R.R., and Martin, R.E.). pp. 48- 66. London: Academic Press.

West, G.C., Burns, J.J., and Modafferi, M. 1979. Fatty acid composition of blubber from four species of Bering Sea phocid seals. *Can. J. Zool.* 57: 189- 195.

Williams, E.D., Karim, S.M., and Sandler, M. 1968. Prostaglandin secretion by medullary carcinoma of the thyroid. *Lancet* i: 22- 27.

Yongmanitchai, W. and Ward, O.P. 1989. Omega-3 fatty acids: Alternative sources of production. *Prog. Biochem.* 24: 117- 125.

Yurkowski, M. and Brockerhoff, H. 1966. Fatty acid distribution of triglycerides determined by deacylation with methyl magnesium bromide. *Biochim. Biophys. Acta* 125: 55-59.

Ziboh, V.A., Cohen, K.A., Ellis, C.N., Miller, C., Hamilton, T.A., Kragballe, K., Hydrick, C.R., and Voorhees, J.J. 1986. Effects of dietary supplementation of fish oil on neutrophil and epidermal fatty acids. Modulation of clinical course of psoriatic subjects. *Arch. Dermatol.* 122: 1277- 1282.