The impact of different dietary sources of marine polyunsaturated fatty acids on the fatty acid composition of rat brain, liver and red blood cells.

by

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"The food that is good for the heart is likely to be good for the brain"
- Hippocrates

Cover illustration: Mouth of Truth, Rome, Italy

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Abstract

There is a general consensus that the physiological activity of fish oil can be ascribable to eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). However, the form in which they are introduced in diet might affect the rate of incorporation in tissue. The aim of this study was to examine the effects of n-3 polyunsaturated fatty acids (PUFA) of different marine origin and the incorporation of EPA and DHA in rat brain, liver and red blood cells (RBC). Eight dietary groups received different diets, in which six were based on marine resources (rich in n-3 PUFA), differing in the structural form of the fatty acids (triacylglycerol (TAG), phospholipids (PL) and ethyl ester), and two diets were based on vegetable resources (TAG and PL rich in n-6 fatty acids). The marine diets included; salmon, seal oil or cod liver oil all rich in TAG, cod, marine PL, and ethyl esters from commercially available n-3 capsules

After 3 weeks of feeding, animals were sacrificed and tissue fatty acid composition was determined. The fatty acid profile changed profoundly in the liver and the RBC of animals receiving marine n-3 PUFA compared to n-6 diets, whereas the changes in the brain were only moderate. Animals fed the marine diets showed increased levels of EPA and DHA with a concomitant lower level of n-6 fatty acids compared to animals fed vegetable diets. The brain and liver responded to the structural forms of the fatty acids, whereas the RBC responded in a dose dependent matter. The brain, the liver and the RBC differed in their response to the different diets.

In conclusion, salmon, seal oil, cod liver oil and marine PL are good sources for increasing DHA levels in brain, whereas the incorporation of EPA and DHA from capsules containing the fatty acids as ethyl esters was poor.

Abbreviations

AA Arachidonic acid (20 : 4$n-6$)

ADHD Attention Deficit Hyperactivity Disorder

ALA α linolenic acid (18 : 3n-3)

CNS Central nervous system

DHA Docosahexaenoic acid (22 : 6n-3)

DPA Docosapentaenoic acid (22 : 5n-3)

DGLA Homoγ-linoleic acid (20 : 3n-6)

EFA Essential Fatty Acid

EPA Eicosapentaenoic acid (20 : 5n-3)

FA Fatty Acid

IUPAC International Union of Pure and Applied Chemistry

LA Linoleic acid (18 : 2n-6)

LCPUFA Long-Chain PolyUnsaturated Fatty Acids with >20 carbon atoms

LPO Lipid peroxide

LTP Long-Term Potentiation

MDD Major Depressive Disorder

n-3 omega-3

n-6 omega-6

NMDA *N*-methyl-D-aspartate

PE Phosphatidylethanolamine

PG PhosphatidylgGlycerol

PI Phosphatidylinositol

PL Phospholipid

PO Phosphate group

PS Phosphatidylserine

PUFA Polyunsaturated Fatty Acid

RME Reference Memory Error

TAG Triacylglycerol

VLDL Very Low Density Lipoprotein

WME Working memory error

WHO World Health Organization

1

Introduction

Polyunsaturated fatty acids (PUFA) are long chained fatty acids containing two or more double bonds. PUFA are grouped in two series based on the position of the terminal double bond, being either at the 3 or 6 carbon from the terminal omega (ω) carbon. PUFA are involved in a wide range of biological properties in the cell, as well as precursors for conversion to metabolites regulating biological functions. There is an augmented interest in PUFA because of their potential in therapeutic, food and nutritional applications. PUFA occur naturally throughout animal, plant, algae, fungi and bacteria.

At the Workshop on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids held in 1999 [1], there was consensus of the importance of reducing the n-6 and increase the n-3 PUFA in diet of both adults and newborns for optimal brain and cardiovascular health and function. The workshop claimed that an increase in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) together with a reduction of vegetable oils high in linoleic acid (LA), are necessary to reduce the adverse effects of excess arachidonic acid (AA) and its eicosanoid products, to achieve a healthier diet in Western countries.

PUFA derived from n-6 and n-3 fatty acids (FA) are fundamental structural components of brain membrane lipids [2]. DHA plays an important role in the growth, development and nutrition of the brain [3, 4, 5], however the mechanisms of DHA action in the brain is not clear. Early, perinatal, n-3 status has been shown to profoundly affect adult physiology [6] and more investigation is needed to establish the cellular and molecular mechanisms of DHA in the brain.

1.1 Omega-3 (n-3) Fatty Acids

Long-chain omega-3 (n-3) fatty acids are produced by terrestrial and marine plants. Fish and other seafoods are rich sources of long n-3 FA with 20 and 22 carbons in length, whereas vegetable oils, cereals and all vegetables and fruits only contain 18 carbon n-3 FA acids.

If α-linolenic acid (ALA, 18 : 3n-3) can be fully synthesized to EPA and DHA in the human body, one could expect the same health benefits from 18 : 3n-3 rich vegetable oil as direct intake of marine oil. This has not yet been demonstrated, and there is little evidence that the consumption of vegetable oils rich in ALA will have the same beneficial effect in preventing or treating chronic diseases such as coronary heart disease [7].

Unlike saturated FA, which have been shown to have negative health consequences, the polyunsaturated n-3 FA have been associated with many health benefits [8]. Fatty acids of the n-3 family have been found to have a positive effect in treating hyper-tension, Crohn's Disease, rheumatoid arthritis, and asthma. n-3 FA have also been reported to decrease the risk of primary cardiac arrest, coronary artery disease and decrease serum triglycerides (see references within [8]). New research is also suggesting a potential positive role of n-3 PUFA in a number of psychiatric disorders [8].

1.1.1 Docosahexaenoic acid (DHA)

Docosahexaenoic acid (DHA, 22 : 6n-3), a 22 carbon n-3 fatty acid with six double bonds (figure 1.1) is the most unsaturated membrane FA present in the mammalian biological system [9]. Dietary DHA can only be found in marine foods such as fish and other sea life, where it has been synthesized by the phytoplankton of the waters.

DHA is very tissue specific, compared to arachidonic acid (AA, 20 : 4n-6) that is distributed in relatively large amount in most tissues [10]. Nervous tissue and reproductive organs are unique in their phospholipid composition, their cell membranes contain large amounts of n-3 FA, particularly in synaptic membranes in gray matter [11, 12], especially synaptic membranes [13], and in the rod outer segment in retina [4, 14]. The rod outer segment in retina contains the highest level of DHA in the human body. Up to 50 % of the membrane phospholipids are n-3 PUFA, most of which is DHA. [3, 10].

Adequate supply of long-chain polyunsaturated fatty acids (LCPUFA) in has shown to be important in infant mental development [15]. DHA is especially important during prenatal human brain development. Both the placenta, and breast feeding supply predominantly DHA and AA to growing infants [15]. DHA in infant diet is associated with higher mental development scores [16], better visual

acuity as measurement of infant brain maturation [17], and better problem-solving scores [18].

Upon deficiency of n-3 FA, a decrease in the levels of DHA are followed by an increase in the end products of the n-6 metabolism, DPA n-6 [19]. DPA n-6 will accumulate to a certain degree instead of DHA [20, 21], in order to keep the total content of 22 carbon FA constant. How well the content of LCPUFA is kept stable depends on the tissue [5]. The LCPUFA are kept at a more stable content in the brain and retina than other tissues [22], and the DHA concentration in these areas are little influenced by diet. The brain and retina have efficient conservation mechanisms to preserve a constant high level of DHA in their membranes [3, 23]. Neurons and astrocytes are reported [21] to be kept most constant with respect to n-3 / n-6 ratio, whereas the long-chain PUFA content seems to be tightly regulated in synaptosomal membranes and oligocytes.

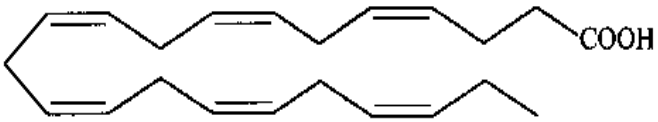

Figure 1.1: Structure of the n-3 fatty acid docosahexaenoic acid (DHA), the most unsaturated fatty acid present in the mammalian biological system, containing 22 carbon and 6 double bonds.

1.2 Polyunsaturated Fatty Acids and Central Nervous System

PUFA are known to constitute an important environmental factor in the central nervous system (CNS). The CNS contains large amounts of DHA and AA, which mainly accumulate during the brain growth spurt [24]. The CNS requires large amounts of DHA during early postnatal development, when cellular differentiation, active synaptogenesis, and photoreceptor membrane biogenesis takes place [25]. DHA is a ubiquitous and essential component of the CNS, and the high content, specific incorporation and strong retention within nervous tissue strongly suggest that DHA play a specific role, and is an important compound for optimal brain function [26]. Studies on n-3 FA have shown that long-chain PUFA deficiency in rats pertubates the learning behavior and performance in several learning tasks involving learning abilities, and sensory, motor or motivational processes [27, 28]

1.2.1 Polyunsaturated Fatty Acids and the Brain

Lipids account for about 10 % of the fresh weight and 50 – 60 % of the dry matter of the brain [29]. 35 % of the lipids are found as PUFA, mostly long-chain PUFA. DHA constitutes almost 17 % by weight of the total FA in the brain of adult rats, and 33 % of total FA in the retina, but only a small percentage in other tissues of the body. The PUFA in the nervous system participate in the structure of phospholipids, but not that of sphingolipids [29]. Brain lipids are mainly structural, and they are not related to energy metabolism; they participate directly in the functioning of cerebral membranes [30]. The lipids are mainly found in nerve cell membranes as complex lipids. The cerebral essential FA can either be derived from the dietary precursors, linoleic acid (n-6) and α-linolenic acid (n-3) by intermediary metabolism in the body, or originate from diet rich in long-chain PUFA, e.g. seafood. The most prominent FA in the brain are of 20- and 22-carbon atoms, and the most common FA in phospholipids are AA (20 : 4n-6) and DHA (22 : 6n-3). These long-chain PUFA are important for the structure and function of many membrane proteins, including receptors, enzymes and active transport molecules. The biochemical effects induced by dietary LCPUFA on the cellular membranes may result in functional changes including alteration in membrane architecture and fluidity [31], enzymatic activities [11] and ion transport [32], membrane electrophysiology [32] and gene expression [33, 34].

During embryonic and postnatal development of the nervous system, long-chain PUFA, especially DHA and AA, accumulate in retina and synaptic membranes in the brain [4]. The largest accretion of PUFA takes place during the last trimester and the first 6 – 10 months after birth in humans, when the brain growth spurt is taking place [24]. The fetus recieves PUFA through the placenta and the newborn mammal from the maternal milk. Human infants requires an adequate supply of n-6 and n-3 fatty acids in order to sustain development of neurological structures of the brain and retina, and to support normal growth and development of other tissues [10]. Preterm infants have shown the ability to synthesize long-chain PUFA from 18-carbon precursors [35], but more research is needed to establish whether these metabolic systems capacity proceeds at a sufficient rate to meet tissue needs for DHA.

The identification of important developmental periods in the ontogeny of brain membrane lipids, especially regarding the supply of essential FA influenced by extrinsic factors to the brain, such as nutrition and maternal -placental circulation, suggests vulnerable time points at which events that disrupt the normal ontogenetic pattern of accumulation could produce long-lasting effects on normal development [36]. The developing brain appears to be more susceptible to dietary changes than the adult brain [27, 37, 38, 39]. The total fatty acid content in the brain augments 4 – 5 times during brain maturation due to rapid accretion of

lipids. After maturation the fatty acid composition remains fairly constant [29].

There are many reports on the effect of n-3 PUFA deficiency, but few on the supply of DHA and learning abilities. Chronic administration of DHA has shown to improve reference memory*-related learning ability in young [40] and aged rats [41]. Diets containing long-chain n-3 PUFA affect the fatty acid profile of the brain [42] and cognitive functions [43, 44, 45].

The fatty acid profile in the brain can be recovered from n-3 FA deficiency as shown in rats [46], and monkey [47], but the rate is very slow compared to other tissues [46, 48, 49]. The slow recuperation rate may be that regulation occurs at the level of either synthesis in liver, transport across the blood-brain barrier, or the enzymatic activities of desaturation and elongation [50]. It is interesting that the recuperation rate of cerebral microvessels and capillaries also were slow [51], even though they are in contact with plasma lipoproteins of normal composition, since the liver recuperates very rapidly (2 weeks).

Hippocampus

The hippocampus is a bilateral, cortical structure located in the temporal lobe (figure 1.2). The hippocampus is an important structure in learning and consolidation of explicit memories†, from short-term memory to long term memory storage in other cortical regions. Damage to the hippocampal complex can lead to irreversible anterograd amnesia‡ [52]. Although the mechanisms of DHA in memory formation are unclear, results strongly suggest that the ratio of DHA/AA in different cerebral structures, like the hippocampus, may be considered a novel indicator of learning ability [40, 41].

DHA may be involved in protection against neuronal degenerative stress. Lipid peroxide (LPO) from peroxidation of PUFA are know to damage genes, membrane lipids and enzyme proteins. The accumulation of LPO induces disorders of cellular function in the aging. Chronic administration of DHA decreases LPO levels in the hippocampus [41], as well as reduces neural damage after forebrain ischemia, probably due to an increase in the DHA/AA ratio in the hippocampus [9].

DHA and Long-term potentiation DHA does not only have neuronally protective effects on the hippocampus, it also exerts direct effects. DHA has been suggested to plays an important role in long-term potentiation (LTP). LTP is a

*Reference memory involves utilizing information that remains constant over time

†Explicit memory involves memories based on facts and events

‡Anterograd amnesia refers to the inability to form new memories following brain trauma. In severe cases a person may be completely incapable of learning anything new

long-lasting, activity-dependent change in the efficacy of synaptic communication. Since its discovery by Bliss and Lømo [53] in 1972, LTP has been a well studied example of synaptic plasticity[§]. This phenomena is regarded as a neuronal base of learning and memory, and is believed to provide information regarding the understanding of the cellular and molecular mechanisms involved in forming and storing of memories in the brain. LTP has been well documented in the hippocampus, where it is induced by activation of the *N*-methyl-*D*-aspartate (NMDA) receptor.

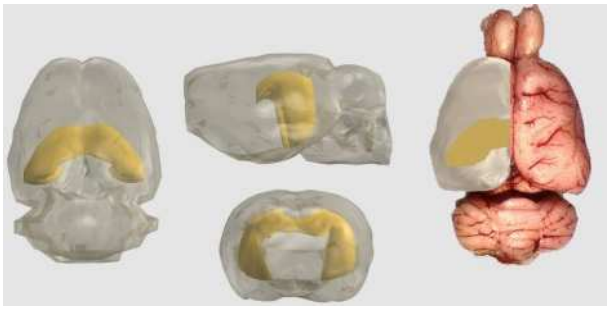

Figure 1.2: 3D positioning of the hippocampus (yellow) in rat brain. Adapted from Synapse Web, Medical College of Georgia, http://synapses.mcg.edu/

1.3 Mental Health

Major depressive disorder is a recurrent illness leading to severe impairment of the persons mental health [54]. Despite advances in pharmacotherapy, a significant proportion of depressed patients are resistant to treatment [55]. There has been little focus on the nutritional influences on depressive symptoms, and new research indicates that dietary influence on mental disorders is currently underestimated. Research is now showing growing evidence for n-3 as a new potential agent in the treatment of depression [56].

According to the World Health Organization (WHO) [54] 25 % of the people, in both developed and developing countries, will experience one or more mental or behavioral disorders at some stage in life. 450 million people worldwide are affected by mental, neurological or behavioral problems at any time. WHO estimates that 121 million people are currently suffering from depression, and 870.000 people die by suicide every year. Even though depression can be reliable diagnosed and treated in primary care, less than 25 % of those affected have access to effective treatments.One of the barriers to an effective treatment of mental

[§]Synaptic plasticity is defined as changes in synaptic strength and modification

illness is the lack of recognition of the seriousness of the illness. There is a discrimination between physical and mental problems at all levels of society; from politicians, insurance companies to the public at large. [54].

Major Depression Disorder (MDD) Depression is a common mental disorder. It is an extremely complex and heterogeneous condition, not to be misconceived with normal mood changes. A major depressive episode is a pathological mood state in which persistent negative emotions and thoughts coexist with disturbances of motivation, sleep, energy, appetite, and libido. MDD is ranked fourth as a cause of disability by the WHO [54], and by 2015 its ranking is expected to rise to second! Today, depression is already the second cause of disability in the age category $15-44$ years for both men and women .

Attention Deficit Hyperactivity Disorder (ADHD) ADHD is the most common mental disorder in children. The symptoms of ADHD appears early in a child's life and can persist into adulthood. The disorder manifests as a persistent pattern of inattention, hyperactivity, and impusitivity that is more frequent and severe than normally observed in individuals at a comparable level of development. These behaviors may severely affect school performance, family relationships, and social interactions. If untreated, ADHD can have long term adverse effects into adolescence and adulthood, and over time children with ADHD may develop depression, poor self-esteem and other emotional problems. There is no specific test for diagnosing ADHD, hence making an accurate diagnosis is difficult, especially in adults. There is no recent cure for ADHD, however, medications can improve the condition, and is an important part of the treatment. In the recent years ADHD has been a subject of great public attention and concern.

In Norway, it is estimated that $5-6$ % of all children have ADHD, whereas $2-3$ % of these have a severe degree of the disorder. 300.000 children between $6-18$ years of age has been diagnosed with ADHD, and about 50 % of these outgrows the disorder. ADHD is $2-3$ times more common in boys than it is in girls [57].

A study [58] found that boys with lower levels of n-3 FA in plasma showed, more problems with behavior, learning and health than boys with higher level of n-3. ADHD is believed to be a multifactorial disorder, with both genetic and environmental etiologies. Among the factors assessed, essential fatty acid status is a factor that may have both genetic and environmental origins [58].

1.3.1 Nutrition and Mental Health

The rates of major depression have increased and the age of onset has decreased in every decade during the last century [59]. The modern US and Western European diet has changed dramatically over the past hundred years. In the 20^{th} century in particular, the consumption of dietary lipids has increased almost exponentially . In addition, the type of fats consumed has changed. In the pre-industrial period, the diet contained a balance of n-6 and n-3 FA. Advances in agriculture and food technology have lead to a diet enriched with n-6 FA, e.g. from commercial and processed vegetable oils, at the expense of the n-3 FA. The n-3 and n-6 FA often have opposing physical effects, and the result of the lipid imbalance in modern diets could be pathophysiological states mediated by these lipids [60].

Epidemiologic data suggest that populations with lower n-3 fatty acid consumption, and hence, lower plasma and presumably, tissue contents of n-3 FA, have higher rates of psychiatric disorders [61]. The identification of nutritional factors that correlate with the prevalence rates of psychiatric disorders might provide new strategies for treatment and prevention. Long-chain n-3 PUFA, like DHA and EPA, are important candidates in the study of nutritional insufficiencies that may augment the risk of psychiatric disorders [62]. Greater rates of seafood consumption are assosiated with lower lifetime prevalence rates of bipolar disorders [62]. A causal relationship between seafood intake and bipolar disorder has not been demonstrated. Nevertheless, there is a hypothesis that an insufficient dietary intake of n-3 PUFA increases the risk of affective disorders [63].

Several authors have reported of altered fatty acid composition in plasma [58, 64, 65, 66] and erythroytes (red blood cells) [67, 68, 69] among subjects with mental disorders compared with healthy subjects. The subjects with psychriatic disorders show lower concentrations of EPA or DHA, together with higher ratio of n-6 to n-3 PUFA. It must be noted, that there is a possibility, that the plasma phospholipid level of DHA is a poor reflector of brain or synaptic DHA content, as reported in piglets [70].

In a study [71] with rats fed fish oil, the results suggested that a lifelong intake of fish oil was able to induce an antidepressant effect, with EPA and DHA concentrations increased in the cerebral cortex and hippocampus. New evidence indicate that treatments affecting phospholipid metabolism and membrane fluidity may have an effect in the treatment of depressive-like symptoms in humans [72]. It has been found that both dietary n-3 and uridine, a stimulant of cytidine 5′-diphosphocholine which is a critical substrate for phospholipid synthesis, have antidepressant-like effects in rat. Moreover, supplement of n-3 during 8 weeks, seems to improve the short-term course of illness in patients with major depressive disorder [73]

The advantages of n-3 FA as mood stabilizers include the lack of toxicity, high

patient acceptance, have no documented adverse drug interactions, and appears to be safe, as well as possibly beneficial, in pregnancy and children. The disadvantage of n-3 in the treatment of psychiatric disorders include their low potency, resulting in relatively large doses needed. High doses have been shown to induce mild gastrointestinal distress and increased bleeding time [60].

Increasing evidence shows that a correct balance between n-3 and n-6 fatty aics in brain cell membranes is important to mental health, however, more data are required to reach conclusive answers in this regard [15].

1.4 Lipids

Lipids occur throughout the living world in microorganisms, higher plants and animals. They occur in all cell types where they contribute to cellular structure, provide and store fuel, and participate in biological processes ranging from transcription of the genetic code to regulation of vital metabolic pathways and physiological responses.

Lipids are a heterogenous group of compounds related more by their physical rather than their chemical properties. They are based on their solubility properties than their chemical structure. Lipids include fatty acids, triacylglycerides (TAG), steroids, prostaglandins, fat-soluble vitamins, oils, waxes and related compounds, all having common properties of being insoluble in water, and soluble in nonpolar solvents. Lipids have four major biological functions; (1) they are the major structural elements of membranes in all cells, (2) the triacylglycerols serve as the main energy storage, (3) many of the vitamines and hormones found in animals are derived from lipids, and (4) the bile acids help to solubilize the other lipid classes during digestion. Most of the fatty acid synthesis occurs in the cytoplasm of the liver. From there they are released into the circulation and taken up by other tissues. Adipose tissue synthesizes triglycerides from glycerol and FA, whereas phospholipids (PL), necessary for membrane biosynthesis, are produced by most tissues [74].

1.4.1 Fatty Acids

Fatty acids (FA) are the simplest of the lipids. They have a chain-like structure with an acid or carboxyl group (COOH) at one end, and a methyl group (CH_3) at the other. The rest of the molecule consists of a hydrocarbon chain varying in length. Figure 1.3 shows the essential features of a fatty acid. Most FA are straight-chain compounds containing an even number of carbon atoms. FA are usually found as components of complex lipids esterified through the carboxyl

carbon to various alcohols such as glycerol, ethanolamine, or choline, and are rarely found as free fatty acids.

Saturation

Fatty acids can occur at different levels of saturation. A saturated fatty acid contains no double bonds, whereas an unsaturated fatty acid will contain one or more double bonds. Based on the number of double bonds, unsaturated FA are further subdivided into monounsaturated fatty acids, containing only one double bond, and polyunsaturated, containing two or more double bonds. Unsaturated FA are found in plants and animals, and most concentrated in seeds, nuts, certain fruits and fish.

Chain length

Short-chain FA have less than 8 carbon atoms, they are water soluble and absorbed directly from the intestine into the bloodstream. They are usually metabolized for immediate energy needs, and are most commenly found in dairy products.

Fatty acids with more than 12 carbon atoms are considered long-chain. Long-chain FA are the major components of the complex lipids present in the membranes. They are stored as phospholipids and TAG in membranes where they serve as an efficient source of energy, or are available as precursors for several metabolic pathways.

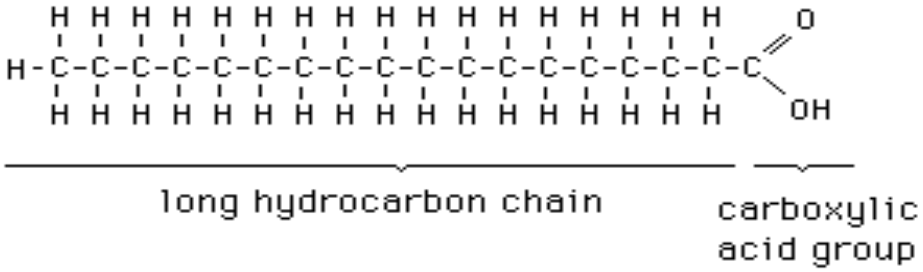

Figure 1.3: Essential features of a saturated fatty acid (stearic acid, 18 : 0). A fatty acid consists of a hydrocarbon chain that may vary in length and saturation, a carboxyl group (COOH) at one end, and a methyl group at the other (CH_3). Modified from http://medlib.med.utah.edu/NetBiochem/FattyAcids/3_1.html

Fatty Acid Nomenclature

Most FA have a trivial name in addition to a name given by International Union of Pure and Applied Chemistry (IUPAC). IUPAC names follow the nomencla-

ture conventions of the Recommendations of 1976, Commission on biochemical Nomenclature [75, 76]. According to this system the names describe the structures in detail; carbon atoms are numbered from the carboxyl carbon (carbon number 1). The carbon atom adjacent to the carboxyl carbon is know as the alpha (α) carbon, and the terminal methyl carbon is know as omega (ω) carbon. There are various conventions used for indicating the number and positions of double bonds; i.e., $\Delta 9$ indicates a double bond between carbon atoms 9 and 10, counting from the carboxyl end of the fatty acid, and $\omega 6$ indicates a double bond on the sixth carbon counting from the ω-carbon atom. The omega nomenclature is the most commen nomenclature used in the field of nutrition. The term n- is synonymous with the use of ω, and in this thesis all FA will be denoted by omega nomenclature, using n- for the indication of the first double bond from the ω carbon. Table 1.1 shows the most common unsaturated FA. They are listed by their numeric name; indicating the number of carbon atoms in the carbon chain and total number of double bonds (if any, separated by a colon), series, their trivial name, and where they most commonly occur.

Table 1.1: Some unsaturated fatty acids of physiologic and nutritional significance

Numeric name	Series	Trivial name	Occurence
16 : 1**n**-7	**n**-7	**Palmetoleic**	**in nearly all fats**
18 : 0	**saturated**	**Stearic**	**common in all animal and plant fats**
18 : 1**n**-9	**n**-9	**Oleic**	**most common fatty acid in natural fats**
18 : 2**n**-6	**n**-6	**Linoleic**	**corn, peanut, soybean and many plant oils**
20 : 4**n**-6	**n**-6	**Arachidonic**	**found in animal fats and in peanut oil**
18 : 3**n**-3	**n**-3	**α linolenic**	**particularly in linseed oil**
20 : 5**n**-3	**n**-3	**Eicosapentaenoic**	**fish oils**
22 : 5**n**-3	**n**-3	**Docosapentaenoic**	**fish oils, phospholipis in brain**
22 : 6**n**-3	**n**-3	**Docosahexenoic**	**fish oils, phospholipids in brain**

Essential Fatty Acids

Linoleic acid (LA, 18 : 2n-6) and α-linolenic acid (ALA, 18 : 3n-3), as shown in figure 1.4, are classified as essential fatty acids (EFA). The body cannot synthesize them *de novo*, and they must therefore be obtained through food sources providing them “ready-made”. Both LA and ALA are needed for optimal growth and good health, and without them deficiency symptoms (dermatis [77], growth retardation [78] and infertility [79]) can occur. Both LA and ALA are precursors of the n-3 and n-6 PUFA. LA is required for the synthesis of arachidonic acid, a key intermediate in the synthesis of eicosanoids, whereas ALA is used partly

as a source of energy, and partly as a precursor for metabolites and longer chain PUFA.

In a normal diet LA and ALA synthesized by plants are the major source of EFA. Within the human body 18 : 2n-6 and 18 : 3n-3 can be elongated and desaturated to more unsaturated FA, principally arachidonic acid (20 : 4n-6) and DHA (22 : 6n-3) [80] as shown in figure 1.5. The liver is the primary site for essential fatty acid metabolism. Since vertebrates cannot insert double bonds more proximal to the methyl end than the seventh carbon, once ingested, the n-3 and n-6 fatty acids are not interconvertible [5]. All metabolic conversions of EFA occur without altering the methyl end of the molecule containing the n-3 and n-6 double bond. However, the degree of conversion of the EFA to long-chain PUFA in the human body appears to be unreliable and restrict [80].

n-3 and n-6 substrates compete for the same enzymes in the metabolic pathway described in figure 1.5 to their respective long-chain PUFA. In 1963 Mohrhauer and Holman [81] proposed a general hypothesis; all FA compete with essential FA, at all steps of the cascade shown in figure 1.5 for metabolism of the essential fatty acids. In their study [81], it was shown that if dietary 18 : 3n-3 is augmented, it acts as a strong suppressor of n-6 fatty acid metabolism, whereas an equal suppression of n-3 metabolism requires more than 10 times as much 18 : 2n-6 . Nevertheless, equality of competition may not be the cirterion for optimal function. It appears that the dietary ratio of 18 : 3n-3 / 18 : 2n-6 is a more important factor than the absolute quantities of these FA for brain bioavailability, and the central nervous system-mediated effects. The optimal ratio of α-linoenic to linoeic acid is ranging from 3.5 : 1 to 5 : 1 [82].

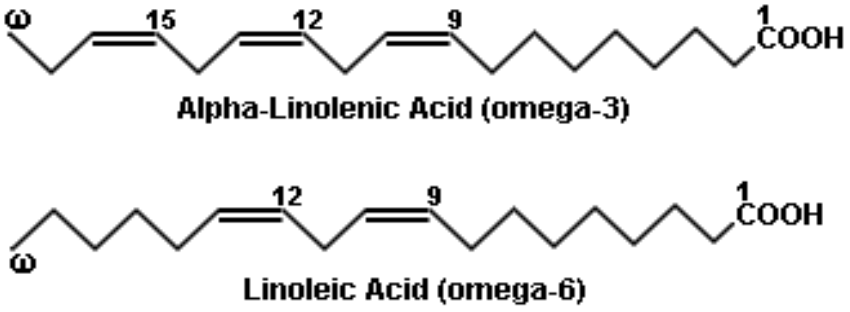

Figure 1.4: The essential fatty acids 18 : 3n-3 and 18 : 2n-6. α-linoleic acid (18 : 3n-3) is a precursor of the n-3 LCPUFA, containing 3 double bond, the first at the third carbon atom from the omega (ω) end. Linoleic acid (18 : 2n-6), is a precursor of the n-6 LCPUFA, containing 2 double bonds, the first at the sixth carbon form the omega (ω) carbon. From http://www.scientificpsychic.com/fitness/fattyacids.html

1.4.2 Phospholipids

Phospholipids are the major constituents of membranes. They consitute about 50 % of the mass of most animal cell membranes, the rest being mostly proteins. In the dry weight of the human brain 22 % of the cerebral cortex and 24 % of the white matter consist of phospholipids [3]. Phospholipids are derivates of triacylglycerols, in which one fatty acid has been replaced by a phosphate group (PO_4^-). A phospholipid molecule consists of a hydrophilic polar head, and a hydrophobic tail. The polar head can contain one or more phosphate groups. The tails are usually made up of two fatty acid chains, varying in length (normally between 14 and 24 carbon atoms) and saturation. One of the tails is usually unsaturated (contain *cis*-double bonds), while the other tail is saturated. The difference in length and saturation of the fatty acid tails are important features because they influence the fluidity of the phospholipid molecule. A higher degree of unsaturation result in higher fluidity.

Most phospholipids in cell membranes belong to phosphoglycerides. Phosphoglycerides are also the most abundant phospholipid group in neural membranes [29]. They are formed from the combination of glycerol, two fatty acids, and phosphoric acid. Phospholipids can be further modified by the covalent binding of additional compounds in the phosphate group. The addition of another small hydroxyl-containing molecule, such as choline, serine, or ethanolamine, creates more complex phospholipids. Some phopholipids contain a long-chain fatty alcohol rather than a fatty acid at carbon number 1 of glycerol, such as the plasmalogens. Plasmalogens are important structural compounds in nervous tissue, and they function as a reservoir for second messengers. They may also act as antioxidants and protect the cells from oxidative stress. Decreased levels of plasmalogens are observed in several neurological disorders, including Alzheimer's disease [84]. Sphingolipids are another group of complex phospholipids, composed of the amino alcohol sphingosine plus carbohydrate. The sphingolipids occur in particularly large concentrations in nervous tissue, and are the major lipid of the myelin membrane, where they play an important role in signal transduction processes [29].

1.5 Dietary Lipids

Triacylglycerols (TAG) are quantitatively the most important lipid component in the human diet, and constitute the major energy contribution from lipids. Dietary TAG is digested and absorbed, under normal circumstances, with more than 95 % efficiency.

The digestion and absorption of dietary lipids takes place in the small intes-

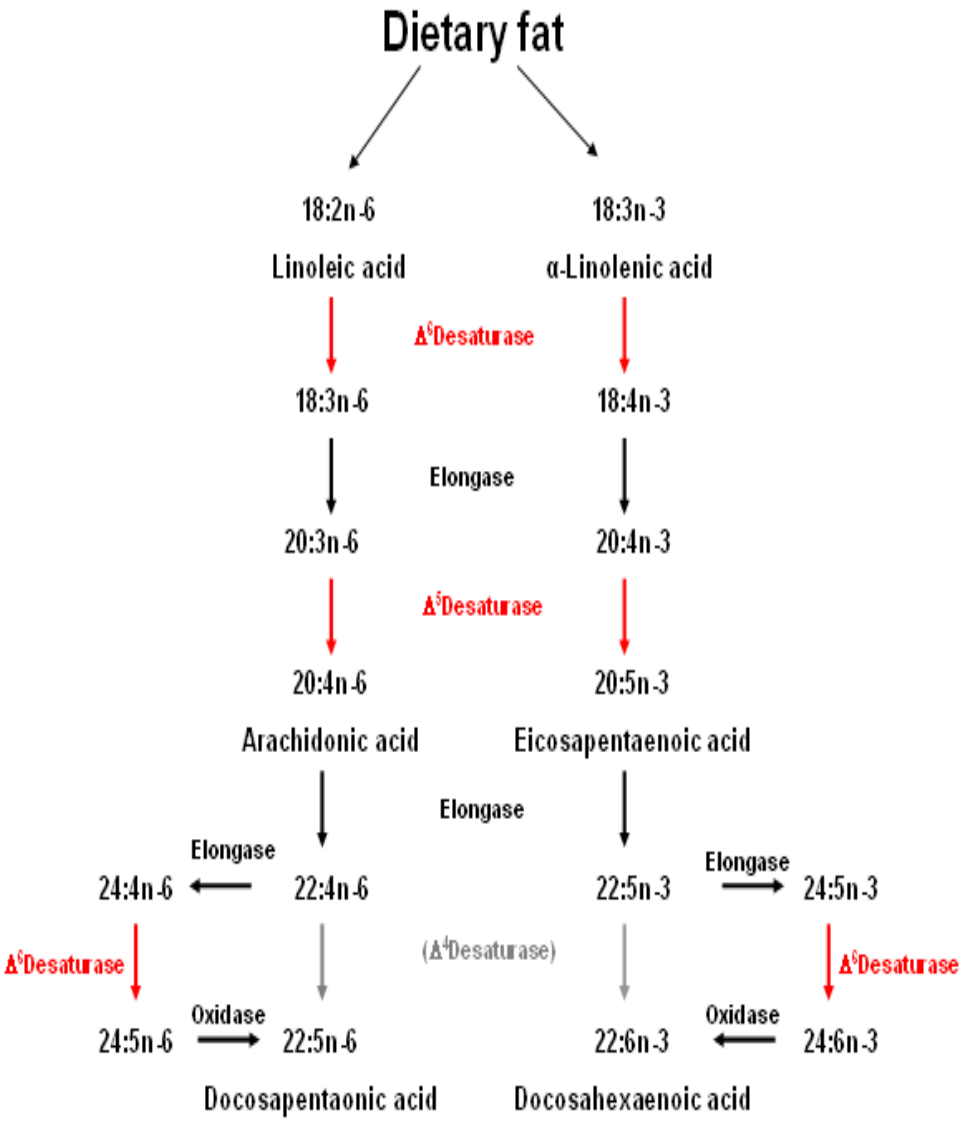

Figure 1.5: Metabolic pathways for n-3 and n-6 fatty acids. The precursor FA, linoleic acid and α-linolenic acid are metabolized to the longer FA through a series of enzymatic desaturation (adding of double bonds) and elongation (adding a 2-carbon unit) steps in the endoplasmatic reticulum. The mechanism of conversion of 22 : 5n-3 og 22 : 6n-3 is not agreed upon. Traditionally, it was believed to occur via a the Δ^4-desaturase, however no proof of the existence of this enzyme has been found. Currently, it is believed that the last step in the conversion of DHA occurs via a step of retroconversion, including a desturation, an elongation step and peroxisomal β-oxidation reaction, as proposed by Sprecher and coworkers [83].

tine. Lipid droplets enters the small intestine from the stomach where they are subjected to the action of pancreatic lipase, phospholipase A_2 and cholesterol esterase. These enzymes hydrolyse triacylglycerol to produce monoacylglycerols and fatty acids, phospholipids to produce lysophospholipids and FA, and cholesterol esters to liberate cholesterol and FA, respectively. These products are emulsified with bile salts (from the gall bladder) to produce a micellar suspension from which components are absorbed across the epithelial cell membranes. Within the epithelial cell, the components are reassembled and packaged into chylomicrons. The chylomicrons do not enter the plasma directly. They are secreted into small lymph vessels, from where they pass via the thoracic duct and enter the circulation in the subclavian vein. They then reach the heart for distribution around the body. Chylomicrons are the main route for the transport of dietary long-chain fatty acids. Short- and medium-chain FA with chain lengths of less than twelve carbon atoms are absorbed in the non-esterified form. They pass directly into the portal blood and are metabolized directly in the liver. Bile salts are re-absorbed along with cholesterol in the lower part of the small intestine [74]. The digestion and absorption of lipids are shown in figure 1.6.

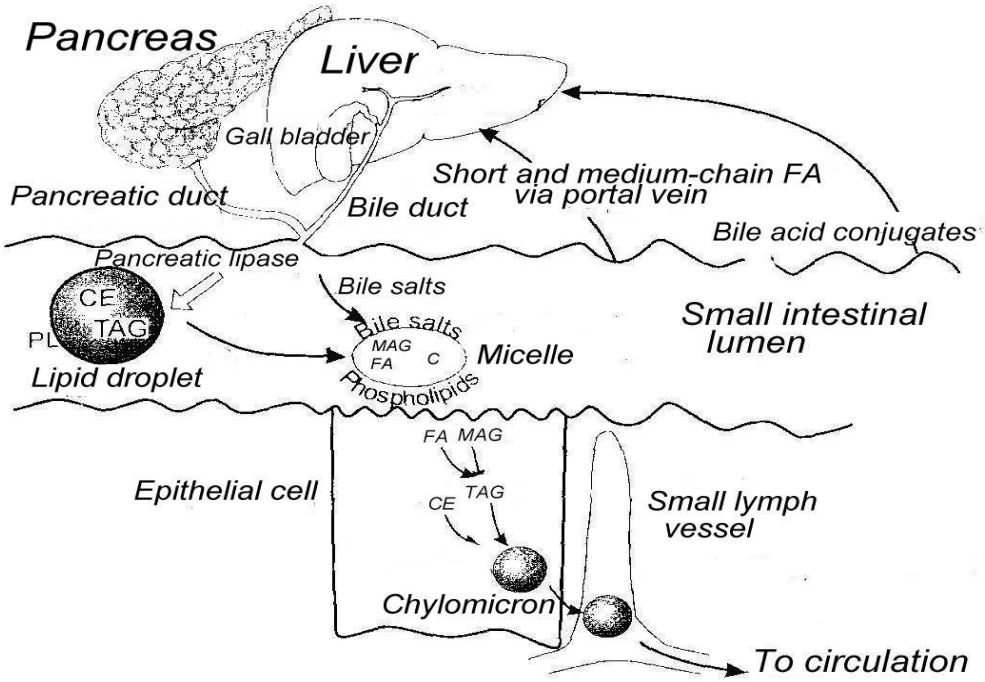

Figure 1.6: The digestion and absorption of dietary fat in the small intestine. To be usable by the body, dietary fats must be digested in the lumen of the small intestine. The digestion products pass through the intestine wall and are re-synthesized in the intestinal epithelial cells from where they are packaged for transport and sent into the bloodstream. Modified from [**?**]

1.5.1 Pancreatic Lipase

Pancreatic lipase is a water-soluble, exocrine enzyme secreted by the pancreas into the duodenum following a hormonal response. The enzyme is responsible for the hydrolization of triacylglycerol to free fatty acids within the small intestine. Pancreatic lipase is position specific, that is, it removes two FA from the *sn*-1 and *sn*-3 positions (first carbon and third carbon respectively) of the TAG molecule, leaving two free fatty acid and a monoacylglycerol (*sn*-2-MAG). After complete degradation of TAG there is a general conservation of approximately 75 % of the FA located in the *sn*-2 position (middle carbon) [85]. DHA in fish or fish oil TAG, is usually located in the *sn*-2 position, while EPA show greater preference for *sn*-2 and *sn*-3 positions, with small amounts in *sn*-1. By contrast, marine mammals distribute EPA and DHA in their TAG mainly in *sn*-1 and *sn*-3 positions. Therefore, the position of the FA within the TAG molecule determines the susceptibility to hydrolysis by pancreatic lipase in the intestine, being mainly *sn*-1 and *sn*-3 specific. FA in the *sn*-2 position are generally not substrates for intestinal lipases [7].

1.6 Statistics

1.6.1 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a multivariate technique for reducing matrices of data to their lowest dimensionality. PCA involves a mathematical procedure for transforming a number of possible related variables into a smaller number of uncorrelated variables (principal components). Principal component 1 (PC1) accounts for as much as possible of the variability in the data set. It is taken to be along the direction with the maximum variance. Principal component 2 (PC2) is constrained to lie in the subspace perpendicular to PC1. Within that sub-space, it points in the direction of the maximum variance. Principal component 3 (PC3), if any, is taken in the maximum variance direction in the subspace perpendicular to the first 2 principal components.

1.6.2 Analysis of Variance (ANOVA)

The purpose of the analysis of variance (ANOVA) is to test differences between means for statistically significance. This is accomplished by analyzing the variance, that is, by partitioning the total variance into the component that is due to true random error, and the components that are due to differences between the means. The comparison between the actual variation of the group averages and that expected is expressed in terms of the *F* ratio. *F*= (found variance of the group

average)/(expected variation of the group average). If the null hypothesis (H_0) is correct one could expect the *F* value to be about 1, whereas a "large" *F* indicate a treatment effect. If the treatments are found to be significant, that is, values laying within 95 % confidence interval, it implies that the means differ more than would be expected by chance alone. When the treatments are significant, the means must then be examined in order to determine the nature of the effect, and a *"post-hoc test"* is used to tell where the differences in the data lies.

1.7 Aims of the study

The molecular mechanisms underlying the positive effects of n-3 polyunsaturated fatty acids are not fully understood, however before these mechanisms can be fully elucidated, it is important to know the extent to which different sources of n-3 fatty acids are incorporated into different body organs. It will be important to know whether incorporation varies with the source and polyunsaturated fatty acid composition of the dietary sources, an issue that has only been studied to a limited extent.

There is a general consensus that the physiological activity of fish oil is ascribable to EPA and DHA. However, the form in which they are introduced and the rate of incorporation following the structural form administered, is less known. This study was based on the lack of data comparing different sources of marine n-3 fatty acids. Based on a literature review, this study is the first research aiming at comparing triacylglycerols, to ethyl ester and phospholipid from different commercially available supplements of marine n-3.

The general aim of this study was to investigate 8 different dietary supplements originating from various sources, differing in the structure of the fatty acids, to changes in fatty acid profile in brain, liver and RBC. Finally it must be pointed out that the emphasis of this work was on brain tissue, for further interest and investigation concerning marine n-3 and mental health.

This study addressed the following specific questions:

- Can the fatty acid profile in brain liver and RBC be changed by dietary means during 3 weeks of feeding.
- Can the level of EPA and DHA in brain, liver and RBC be increased by dietary means by 3 weeks of feeding.
- Do different structural forms, i.e., TAG, ethyl ester and phospholipid, of n-3 affect the incorporation of EPA and DHA differently, in brain, liver and RBC.
- Do different positioning of the fatty acids in the TAG molecule affect the rate of incorporation of EPA and DHA, in brain, liver and RBC.

2

Materials & Methods

2.1 Animals

Male Wistar rats (initially weighing 200 g, Tactonic, USA) were housed five per cage (Makrolon IV, Tecniplast Gazzada, Italy) for the first days of the habituation period. After three days of habituation, the rats were housed two per cage (Makrolon III) and maintained at this arrangement during the experiment. During the habituation period, the rats were fed on normal laboratory chow (Special Diet Service, Witham Essex, England). The animals were kept in a 12 h light/dark cycle at 22°C and 50 % humidity, for the entire period. Cage and bedding (Beekay Aspen chips, Hull England) were changed once a week.

The protocol was in compliance with the Norwegian Animal Research Authority.

2.1.1 Study Design

The animals were randomly assigned to eight dietary groups of 6 rats per treatment, recieving either n-3 or n-6 enriched diets. The selection of 6 rats per treatment was based on the consideration given by the Analytical Methods Committee (1994) [86], and a recent published report by NIFES [87]. Each group was fed one of the diets listed in table 2.1. Each cage recieved 50 g chow per day, and the

animals had free access to their respective diets during the 21-days experimental period. Water was available *ad libitum*. The rats were weighed and daily food intake was measured every day during the experimental period. The animals were deprived of food 24 h before being sacrificed.

Table 2.1: Animal groups, diets and structural form of n-3 FA

Group	Diets	Form of n-3
A	Soy oil	TAG
B	Salmon	TAG
C	Cod	PL
D	Ethyl ester	EE
E	Marine phospholipid	PL
F	Soya lecitin	PL
G	Seal oil	TAG
H	Cod liver oil	TAG

TAG: triacylglycerol, PL: phospholipid, EE: ethyl ester

2.2 Preparation of the Diets

All diets were prepared with approximately 20 % protein (casein, Sigma), 10 % fat (different sources), 20.05 % basal mix (NIFES, Norway) and 50 % carbohydrates (dextrin, Sagogryn, Asko Hannevik AS). The amount of each ingredient added to the respective diets is given in table 2.2. The diets were equivalent in overall protein, fat, ash, dry matter, carbohydrate and caloric content, and the exact composition of the experimental diets is listed in table 2.3.

Table 2.2: Amount of ingredients added to the diets

	(g/kg diet)							
	Diet							
Ingredient	**Soy**	**Salmon**	**Cod**	**EE**	**MPL**	**Soy lec**	**Seal oil**	**Cod liver oil**
Casein	224	66	66	224	224	224	224	224
Basal mix[1]	234	234	234	234	234	234	234	234
Fat (n-3 source)[2]	100	246[3]	148[3]	33	57	100	100	100
Maiz Oil	-	-	98	75	43	-	-	-
Dextrin	475	487	488	467	475	475	475	475

[1]see appendix table 5.1 for basal mix composition, [2]n-3 source; fish filet, oils, ethyl ester, marine phospholipid, soy lecitin.[3]fish filet added. EE: ethyl ester, MPL: marine phospholipid, Soy lec: soy lecitin.

Each diet contained a different source of fat. The n-3 was given as either triacylglycerol (TAG, diet B, G and H), phospholipids (PL, diet C, E and F) or

ethyl esters (EE, diet D). In diets where the *n*-3 source did not count for 10 % of required fat, maiz oil was used as a neutral lipid source, due to no content of long-chain n-3 fatty acids.

The two fish diets (diet B and C) were balanced on protein, due to the different amount of fat in salmon and cod, (13.4 g / 100 g fish, and 0.3 g / 100 g fish respectively). Diet D and E were balanced on the amount of n-3 fatty acids EPA and DHA (2.5 g/kg/day), calculated from the amount of these fatty acids in 10 % seal and cod liver oil (diet G and H). Diets A, G and H contained equal amount of oil (10 % fat of total diet). All the diets were prepared before starting the feeding and stored at -10°C during the experiment. The fatty acid composition of the diets is shown in table 2.4.

Table 2.3: The overall composition of the different diets, regarding protein, energy, ash, dry matter and fat.

Diet	Protein (%)	Energy (J/g)	Ash (%)	Dry matter (%)	Fat (%)
A	21	19145	3.2	93	9.9
B	21	18910	3.4	94	9.7
C	20	18932	3.6	94	10.4
D	21	19011	3.2	93	10.7
E	22	18375	4.2	93	7.5
F	22	18204	3.9	93	4.0
G	21	19221	3.2	94	10.3
H	21	19081	3.2	93	10.4

Diet A In diet A, commercially available soy oil (Mills Soya Olje), purchased at the local super market (Rema 1000) was used. The oil was added to the dry mixture of casein, dextrin, and basal mix and blended using a Crypto Peerless EF 20 blender.

Diet B and C Diet B and C contained salmon and cod respectively, bought at Strandkaien Fisk A/S fish store in Bergen. The fish were filleted, and grinded by the use of ELECTROLUX assistent food processor. The crushed fish was freeze dried (Hetosic, Heto Birkerød, Denmark), and the dried material was grinded again by ELECTROLUX food processor to powder consistency. The fish powder was mixed with casein, dextrin and basal mix and blended in the Crypto Peerless EF 20 blender.

Diet D The ethyl esters (Fri Flyt, Vesterålens Naturprodukter, Sortland, Norway) used for diet D was kindly donated by Vesterålen Naturprodukter. The ethyl esters were taken out of the capsules by syringe (Sabre 2.5 ml), and stored at -10°C until use. Ethyl esters were added at concentration 2.5 g/kg/day, and maiz oil was added to reach a total fat content of 10 %. The oil was added as described for diet A.

Diet E The fat source of diet E consisted of marine phospholipids (MPL-50Ca, donated from EXIMO AS, Tromsø, Norway) added at a concentration of 2.5 g/kg/day, and maiz oil was added as a neutral fat source. The marine PL was heated slowly in maiz oil to liquid consistency before it was added to the dry material and blended as described for diet A.

Diet F Diet F consisted of soya lecitin (L-α-phosphatidylcholine, Sigma) as sole lipid source. The soy lecitin was crushed in a mortar with dextrin. The soy lecitin and dextrin mixture added to the other ingredients and blended in the Crypto Peerless EF 20 blender.

Diet G Seal oil (Arctic Omega-3 Selolje) was used as sole lipid source in this diet. The oil was added as described above in diet A.

Diet H The last diet contained cod-liver oil (Møller's Tran, Oslo, Norway) as sole lipid source. The oil is commercially available and was purchased at the local supermarket (Rema 1000). The oil was added as soya oil in diet A.

2.3 Tissue Collection

Upon termination of the experiment, the animals were anesthetized intraperitoneal with pentobarbital natrium (0.3 ml / 100 g). Loss of cornea and hind paw withdrawal reflexes were used as indicators of deep anesthesia. When fully anesthetized, the rats were killed by cardiac puncture; the sternum was cut open and a syringe was placed in the heart to collect the blood. Heart, liver, spleen, intestine, brain, hippocampus and cerebellum taken out and snap frozen in liquid nitrogen. The tissues were stored at -80°C until further processing.

The blood was collected into centrifuge tubes containing EDTA as anticoagulant, and immediately centrifuged at 3000 rpm and 4°C for 10 min. The centrifuged blood separated into 3 fractions; plasma on top, buffy coat(white blood cells) in the middle, and red blood cells (RBC) at the bottom of the tube. Plasma and RBC were separated for analysis, and stored at -80°C until use.

Table 2.4: Fatty acid composition of diets

Fatty acid	Soy	Salmon	Cod	EE	MPL	Soy lecitin	Seal oil	Cod liver oil
SFA								
14 : 0	0.1	3.9	0.1	0.2	0.8	0.1	3.8	3.8
15 : 0	n.d	0.3	n.d	n.d	0.1	n.d	0.2	0.3
16 : 0	10.6	13.2	10.6	9.1	8.3	8.3	6.0	9.0
17 : 0	n.d	0.3	n.d	0.3	0.2	0.1	0.1	0.7
18 : 0	3.1	2.5	2.0	2.2	1.2	1.7	0.8	1.9
20 : 0	0.5	0.2	0.4	0.4	0.2	0.1	n.d	n.d
22 : 0	0.4	n.d	0.1	0.1	n.d	0.2	n.d	n.d
24 : 0	0.2	n.d	0.2	0.1	n.d	0.1	n.d	0.1
MUFA								
14 : 1n-9	n.d	n.d	n.d	n.d	n.d	n.d	0.6	n.d
16 : 1n-9	n.d	0.3	n.d	n.d	0.2	n.d	0.4	0.5
16 : 1n-7	n.d	5.4	0.2	0.4	1.3	0.1	13.9	6.3
18 : 1n-11	n.d	n.d	n.d	n.d	n.d	n.d	3.6	1.5
18 : 1n-9	20.1	14.8	27.9	22.5	14.1	3.8	14.1	13.7
18 : 1n-7	1.4	2.5	0.7	0.8	0.8	0.6	3.7	3.3
20 : 1n-11	n.d	0.5	n.d	n.d	n.d	n.d	2.0	1.3
20 : 1n-9	0.2	3.7	0.3	0.5	0.7	0.2	8.4	9.4
20 : 1n-7	n.d	0.2	n.d	n.d	n.d	n.d	0.5	0.2
22 : 1n-11	n.d	3.9	n.d	n.d	0.3	n.d	1.8	6.5
22 : 1n-9	n.d	0.4	n.d	n.d	n.d	n.d	0.4	0.5
24 : 1n-9	n.d	0.5	n.d	0.2	0.1	n.d	n.d	0.3
n-6								
18 : 2n-6	51.0	4.6	49.9	37.8	22.1	25.1	1.7	1.6
20 : 2n-6	n.d	0.4	n.d	n.d	n.d	n.d	0.2	0.2
20 : 3n-6	n.d	0.2	n.d	n.d	n.d	n.d	n.d	n.d
20 : 4n-6	n.d	0.7	0.2	0.5	0.2	n.d	0.4	n.d
n-3								
16 : 3n-3	n.d	0.3	n.d	0.1	n.d	n.d	0.2	0.1
16 : 4n-3	0.2	0.4	n.d	0.1	n.d	n.d	0.4	0.2
18 : 3n-3	5.4	1.1	1.1	1.1	0.6	3.2	0.5	0.9
18 : 4n-3	n.d	1.4	0.0	1.3	0.4	n.d	1.3	2.4
20 : 4n-3	n.d	1.6	n.d	0.2	n.d	n.d	0.4	0.7
20 : 5n-3	n.d	6.9	0.4	9.6	4.4	0.1	6.4	7.9
22 : 5n-3	n.d	3.3	n.d	0.6	0.4	n.d	3.6	1.1
22 : 6n-3	n.d	11.0	1.2	6.6	5.4	n.d	7.5	12.2
∑ **n**-3	5.5	26.0	2.7	19.7	11.3	3.3	20.3	25.5
∑ **n**-6	51.0	6.0	50.1	38.4	22.3	25.1	2.2	1.8
n-3 / **n**-6	0.1	4.4	0.1	0.5	0.5	0.1	9.0	13.8

Results expressed as mg fatty acids/g sample. n.d: not detected. SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, EE: ethyl ester, MPL: marine phospholipid

2.4 Lipid Analysis

Lipids occur in tissues in a variety of forms, and different extraction methods may be necessary to extract lipids from different tissues. In this thesis the lipid extraction of all tissues and diets was based on the procedure of Folch [88] using chloroform/methanol (2 : 1 v/v) to a final volume 20 times the volume of the tissue sample. Some adjustments were made to the extraction procedure for brain tissue due to a problem with emulsion between the water and hexan phase when extracting the lipids after transmethylation.

Brain Tissue Brain tissue was homogenized using a Ultra- Turrak T8 (IKA Labortechnik). The homogenized tissue was kept at -80°C until fatty acid analysis. Total brain lipids were extracted by a modification of the Folch methode [88]. The tissues were first extracted in methanol and agitated at room temperature for 1 hour. Chloroform was added to a final volume of chloroform/methanol (2 : 1), and the mixture was agitated for another hour at room temperature. The samples were left at -20°C overnight.

Red Blood Cells and Liver Lipids from RBC and the liver were extracted in chloroforom/methanol (2 : 1), and stored at -20°C overnight firstly to achieve a maximum extraction, and secondly to avoid oxidation of the lipids.

2.4.1 Preparation of Fatty Acid Methyl Esters

The homogenate of brain, liver and RBC was filtered to remove tissue and protein remnants. The filtered chloroform/methanol solution was evaporated using a Rotavapor with nitrogen supply (BUCHI B480), and ether was added to extract the lipids. The lipid solution was evaporated to dryness under nitrogen, in a block heater (Reacti-Therm) at 60°C. The total lipid extracts were saponified with 0.5M NaOH at 100°C for 15 min, and transmethylated with 20 % BF_3 at 100°C for 20 min for brain tissue, and 5 min for RBC and liver [89]. The esters were extracted from the solution by adding 2 ml hexan and 2 ml NaCl (0.9%) for brain tissue, whereas RBC and liver were added 2 ml water instead of NaCl. The solution was shaken for 15 sec, and centrifuged for 1 min at 3000 rpm, room temperatur. The hexan phase was collected, and subsequently 2 ml of this solvent were added to the lipid extract solution. The solution was shaken for 30 sec and centrifuged for 1 min at 3000 rpm, room temperatur. The hexan phase was collected and added the previous 2 ml collected. The 4 ml hexan solution containing the lipids was stored at -20°C until gas chromatographic analysis.

2.4.2 Gas Chromatographic Analysis

Methyl esters were analyzed using either Carlo Erva gas chromatograph (GC) or Trace GC 2000 ("cold on column" injection, $60^{25^{\circ}\mathrm{C}/min}$ $160^{25^{\circ}\mathrm{C}/min}$ $190^{25^{\circ}\mathrm{C}/min}$ 220°C), equipped with a 50m CP sil 88 (Chrompack) fused silica capillary column (id: 0.32mm) [90]. The fatty acid composition was calculated using an integrator (Turbochrom Navigator, Version 6.1), connected to the gas liquid chromatograph (GLC) and a standard mixtures of methyl esters (Nu-Chek, Elyian, USA) The absolute fatty acid composition was calculated quantitatively using 19 : 0 methyl ester as internal standard.

2.5 Statistics

The software packages used for the statistical analysis were Statsoft. Inc (2004). STATISTICA (data analysis software system). Version 6. www.statsoft.com, and Statgraphics plus 5.1 (Statistical Graphics Corp). All results are expressed as means $\pm$ standard error of the mean (SEM). Calculation of mean, standard error, and standard error of the mean, were performed in Microsoft ®Excel 2002. An Q-test was used to determine, and eliminate outliers, also by using Microsoft®Excel 2002.

Principal Component Analysis Principal component analysis (PCA) was applied to discriminate between the different dietary groups, using the full fatty acid profile. When comparing the organs (brain, liver and RBC), PCA was perfomed on the marine n-3 groups (salmon, ethyl ester, marine PL, seal oil and cod liver oil).

Analysis of Variance Differences in food intake were tested using a one-way analysis of variance (ANOVA). Significant differences between the dietary groups for selected fatty acids were tested using one-way ANOVA. When the *F*-test was significant, multiple comparisons among groups were measured using Tukey's HSD test (for groups with equal n), and unequal *N*-test (for groups with unequal n). Significance level was set at $p < 0.05$.

Figure 2.1 shows the order in which the data were analyzed.

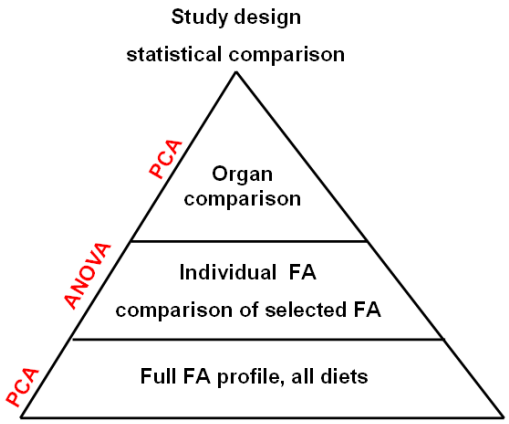

Figure 2.1: Study design, statistical comparison of data. FA: Fatty acid, PCA: Principal component analysis, ANOVA: Analysis of Variance

3

Results

3.1 Diet

Different diets were prepared containing different sources of n-3 and n-6 fatty acids. The diets were analyzed by gas chromatography, and the full fatty acid profil of all the diets is given in table 2.4.

3.1.1 Absolute Amounts of n-3 and n-6 in Diets

Table 3.1 shows the dietary amounts of marine n-3 FA, and two n-6 fatty acid. The salmon and cod liver oil diets contained comparable amounts of marine n-3, the ethyl ester and seal oil diets contained similar amounts of marine n-3, whereas the marine PL together with the diets rich in n-6 FA contained less or no n-3 PUFA.

3.2 Food Intake and Weight Gain

There was no significant difference in food intake between the different dietary groups. The average daily food intake and total weight gain during the experimental period for the dietary groups are listed in table 3.2. The weight gain for marine phospholipid (PL), soy lecitin and seal oil groups were significantly lower than the salmon and cod groups, whereas the ethyl ester and cod liver oil groups weight significantly less at the end of feeding than animals given the cod diet.

Table 3.1: Amount of n-6 and marine n-3 fatty acids in diet.

Diet	18 : 2n-6	20 : 4n-6	18 : 3n-3	20 : 5n-3	22 : 5n-3	22 : 6n-3	∑ marine n-3
Soy Oil	51	n.d	5.4	n.d	n.d	n.d	n.d
Salmon	4.6	0.7	1.1	6.9	3.3	11.0	21.1
Cod	49.9	0.2	1.1	0.4	n.d	1.2	1.6
Ethyl ester	37.8	0.5	1.1	9.6	0.6	6.6	16.2
Marine PL	22.1	0.2	0.6	4.4	0.4	5.4	9.8
Soy lecitin	25.1	n.d	3.2	0.1	n.d	n.d	0.1
Seal oil	1.7	0.4	0.5	6.4	3.6	7.5	17.5
Cod liver oil	1.6	n.d	0.9	7.9	1.1	12.5	21.5

Results expressed as mg. n.d: not detected. Marine n-3: 20 : 5n-3 + 22 : 5n-3 + 22 : 6n-3

Table 3.2: Daily food intake and weight gain

Diet	Daily food intake	Weight gain after 3 weeks (g)
Soy Oil	42 ± 2.0	88 ± 5.1
Salmon	45 ± 2.5	112 ± 8.4
Cod	45 ± 2.9	116 ± 16.1
Ethyl ester	39 ± 1.8	85 ± 8.3^{a}
Marine PL	42 ± 1.4	$80 \pm 8.1^{a,b}$
Soy lecitin	42 ± 2.5	$70 \pm 8.0^{a,b}$
Seal oil	37 ± 1.9	$71 \pm 8.4^{a,b}$
Cod liver oil	39 ± 2.5	86 ± 4.7^{a}

Values represent means ± SEM for 6 rats/group.

[a]Significantly different from cod group, P<0.03

[b]Significantly different from salmon group, P<0.01

3.2.1 Organs

Comparison of relative organ weight indicated that only the liver responded to the diets. No differences were found in the weight of the brain between the different dietary groups. The only significant difference in liver weight was found between the marine PL group and soy lecitin group, where livers from animals fed marine PL weight significantly more than those fed soy lecitin ($p < 0.04$). Fatty liver was observed in animals in 5 of the dietary groups. In the cod group, 1 animal had fatty liver, in ethyl ester group, 3 animals were found with fatty liver, in marine PL group 2 animals had fatty liver, whereas the other 4 showed tendency towards fatty liver. In the 2 groups recieving seal oil and cod liver oil, all the animals had developed fatty livers.

3.3 Full Fatty Acid Profil

The fatty acid profil of brain and liver tissues, together with red blood cells (RBC) was analyzed by gas chromatography, and the resulting fatty acid compostitons are given in table 3.3, 3.4 and 3.5, respectively. From these tables, it can be found that diets rich in marine n-3 PUFA (salmon, ethyl ester, marine PL, seal oil and cod liver oil) resulted in a considerably accumulation of the marine fatty acids; EPA, DPA and DHA, in brain, in liver, and in RBC. The animals fed diets rich in n-6 resulted in remarkably higher levels of LA and AA.

Table 3.3: Relative fatty acid composition in the brain

Fatty acid	Soy Oil	Salmon	Cod	EE	MPL	Soy lec	Seal oil	Cod liver oil
14 : 0	0.13 ± 0.00	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	0.13 ± 0.00	0.12 ± 0.02	0.15 ± 0.01	0.14 ± 0.01
16 : 0	19.7 ± 0.18	17.8 ± 0.09	20.6 ± 0.32	21.1 ± 0.22	19.1 ± 0.28	18.6 ± 0.40	19.4 ± 0.09	18.7 ± 0.17
17 : 0	0.25 ± 0.01	0.18 ± 0.006	0.28 ± 0.01	0.28 ± 0.00	0.23 ± 0.01	0.21 ± 0.01	0.22 ± 0.0	0.21 ± 0.01
18 : 0	20.8 ± 0.43	18.1 ± 0.11	21.7 ± 0.21	22.6 ± 0.22	20.0 ± 0.28	19.8 ± 0.22	20.4 ± 0.09	19.7 ± 0.06
20 : 0	0.34 ± 0.03	0.35 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	0.31 ± 0.02	0.33 ± 0.02	0.32 ± 0.01	0.31 ± 0.03
22 : 0	0.40 ± 0.04	n.d	0.53 ± 0.00	0.48 ± 0.02	0.46 ± 0.05	0.42 ± 0.04	0.35 ± 0.01	0.38 ± 0.02
24 : 0	0.39 ± 0.02	0.36 ± 0.03	0.47 ± 0.03	0.47 ± 0.01	0.56 ± 0.02	0.63 ± 0.06	0.53 ± 0.03	0.54 ± 0.06
∑ **SFA**	42.0 ± 0.66	36.9 ± 0.19	44.0 ± 0.49	45.5 ± 0.43	40.8 ± 0.57	40.0 ± 0.54	41.4 ± 0.14	39.9 ± 0.14
16 : 1n-7	0.37 ± 0.01	0.44 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.39 ± 0.02	0.38 ± 0.03	0.50 ± 0.02	0.41 ± 0.01
16 : 1n-9	0.13 ± 0.00	0.13 ± 0.003	0.14 ± 0.0	0.14 ± 0.00	0.13 ± 0.01	0.06 ± 0.03	0.13 ± 0.01	0.12 ± 0.00
18 : 1n-7	3.4 ± 0.04	3.3 ± 0.07	3.4 ± 0.04	3.4 ± 0.04	3.8 ± 0.11	3.9 ± 0.11	3.8 ± 0.03	3.7 ± 0.03
18 : 1n-9	17.9 ± 0.23	17.9 ± 0.10	18.1 ± 0.11	18.8 ± 0.05	18.1 ± 0.38	17.5 ± 0.52	19.1 ± 0.11	18.9 ± 0.21
20 : 1n-7	0.45 ± 0.03	0.50 ± 0.02	0.46 ± 0.02	0.43 ± 0.02	0.45 ± 0.03	0.34 ± 0.08	0.47 ± 0.01	0.42 ± 0.03
20 : 1n-9	1.6 ± 0.07	1.5 ± 0.06	1.6 ± 0.07	1.6 ± 0.04	1.5 ± 0.0	1.6 ± 0.07	1.5 ± 0.03	1.6 ± 0.04
22 : 1n-9	0.22 ± 0.01	n.d	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
24 : 1n-9	n.d	n.d	n.d	n.d	0.43 ± 0.05	0.53 ± 0.09	0.39 ± 0.03	0.36 ± 0.04
∑ **MUFA**	24.1 ± 0.279	24.0 ± 0.21	24.4 ± 0.12	24.97 ± 0.08	25.4 ± 0.56	24.8 ± 0.67	26.3 ± 0.15	26.1 ± 0.30
18 : 2n-6	0.88 ± 0.02	0.48 ± 0.02	0.89 ± 0.03	0.89 ± 0.02	0.86 ± 0.01	0.80 ± 0.03	0.32 ± 0.02	0.35 ± 0.02
20 : 3n-6	0.46 ± 0.01	0.43 ± 0.01	0.46 ± 0.01	0.50 ± 0.01	0.53 ± 0.03	0.40 ± 0.02	0.38 ± 0.01	0.40 ± 0.01
20 : 4n-6	9.7 ± 0.12	8.6 ± 0.06	9.3 ± 0.05	8.5 ± 0.08	8.7 ± 0.1	9.3 ± 0.22	8.6 ± 0.07	8.1 ± 0.05
∑ **n**-6	11.2 ± 0.10	9.48 ± 0.07	10.8 ± 0.05	10.0 ± 0.05	10.0 ± 0.18	10.5 ± 0.24	9.4 ± 0.08	8.9 ± 0.06
20 : 5n-3	n.d	0.24 ± 0.01	n.d	tr	0.10 ± 0.04	n.d	0.18 ± 0.01	0.23 ± 0.02
22 : 5n-3	0.16 ± 0.01	0.54 ± 0.03	tr	0.35 ± 0.01	0.38 ± 0.03	n.d	0.43 ± 0.03	0.47 ± 0.01
22 : 6n-3	12.9 ± 0.36	15.0 ± 0.14	12.3 ± 0.12	12.0 ± 0.07	14.11 ± 0.09	13.0 ± 0.14	14.4 ± 0.08	14.7 ± 0.21
∑ **n**-3	13.0 ± 0.36	15.8 ± 0.15	12.3 ± 0.12	12.4 ± 0.06	14.6 ± 0.10	13.0 ± 0.14	15.1 ± 0.10	15.3 ± 0.15
n-3 / **n**-6	1.2 ± 0.02	1.7 ± 0.02	1.1 ± 0.01	1.2 ± 0.01	1.5 ± 0.03	1.2 ± 0.03	1.6 ± 0.02	1.7 ± 0.02

Results expressed as % of total fatty acids, mean ± SEM, n= 5 – 6. EE: ethyl ester, MPL: marine phospholipid, Soy lec: soy lecitin, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n.d: not detected, tr: trace

Table 3.4: Relative fatty acid composition in the liver

Fatty acid	Soy Oil	Salmon	Cod	EE	MPL	Soy lec	Seal oil	Cod liver oil
14 : 0	0.34 ± 0.02	0.44 ± 0.03	0.33 ± 0.04	0.27 ± 0.01	0.43 ± 0.04	0.21 ± 0.01	0.51 ± 0.04	0.50 ± 0.06
15 : 0	0.16 ± 0.01	0.24 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	0.25 ± 0.02
16 : 0	19.3 ± 0.30	20.8 ± 0.30	19.8 ± 0.60	19.7 ± 0.30	19.2 ± 0.40	19.0 ± 0.30	19.6 ± 0.40	20.0 ± 0.10
17 : 0	0.25 ± 0.01	0.38 ± 0.03	0.24 ± 0.02	0.24 ± 0.01	0.31 ± 0.01	0.43 ± 0.02	0.27 ± 0.02	0.33 ± 0.01
18 : 0	11.1 ± 0.40	11.6 ± 0.90	11.9 ± 0.70	10.7 ± 0.60	7.8 ± 0.70	16.9 ± 0.60	10.3 ± 0.80	11.2 ± 0.80
∑ **SFA**	32.1 ± 0.96	33.4 ± 0.98	32.4 ± 0.26	31.1 ± 0.98	28.0 ± 0.53	36.8 ± 0.28	30.9 ± 1.06	32.2 ± 0.79
16 : 1n-7	1.5 ± 0.18	3.3 ± 0.35	1.7 ± 0.30	1.3 ± 0.09	4.2 ± 0.60	1.1 ± 0.11	4.7 ± 0.15	3.7 ± 0.49
16 : 1n-9	0.26 ± 0.03	0.24 ± 0.01	0.25 ± 0.02	0.31 ± 0.03	0.43 ± 0.03	0.06 ± 0.04	0.30 ± 0.01	0.33 ± 0.03
18 : 1n-7	2.8 ± 0.21	2.8 ± 0.14	2.4 ± 0.10	1.7 ± 00.09	3.6 ± 0.21	2.8 ± 0.20	3.7 ± 0.23	3.2 ± 0.20
18 : 1n-9	10.1 ± 0.71	12.1 ± 0.53	11.2 ± 0.85	10.7 ± 0.45	16.2 ± 0.77	5.1 ± 0.47	13.1 ± 0.66	13.7 ± 0.48
20 : 1n-7	0.21 ± 0.02	0.16 ± 0.04	0.19 ± 0.02	n.d	0.32 ± 0.02	0.30 ± 0.02	0.20 ± 0.05	0.06 ± 0.04
∑ **MUFA**	14.9 ± 0.93	19.2 ± 0.84	16.04 ± 1.21	14.9 ± 0.44	25.0 ± 1.50	9.5 ± 0.58	23.8 ± 1.01	22.5 ± 1.22
18 : 2n-6	26.0 ± 1.41	9.4 ± 0.46	24.1 ± 0.66	23.8 ± 0.68	20.9 ± 0.63	20.8 ± 0.74	8.10 ± 0.86	7.47 ± 0.75
20 : 3n-6	0.49 ± 0.03	0.64 ± 0.03	0.46 ± 0.03	0.56 ± 0.02	0.64 ± 0.03	0.46 ± 0.08	0.39 ± 0.04	0.44 ± 0.02
20 : 4n-6	16.7 ± 0.54	8.07 ± 0.37	16.9 ± 1.09	10.2 ± 0.66	6.4 ± 0.68	23.6 ± 0.90	7.3 ± 0.54	6.6 ± 0.53
∑ **n**-6	44.7 ± 0.67	18.6 ± 0.98	41.8 ± 1.0	34.8 ± 0.40	28.2 ± 1.22	45.2 ± 0.41	15.8 ± 1.23	14.2 ± 1.06
20 : 5n-3	0.50 ± 0.05	7.8 ± 0.36	0.76 ± 0.08	5.8 ± 0.45	4.8 ± 0.23	0.45 ± 0.02	8.5 ± 0.49	9.4 ± 0.79
22 : 5n-3	0.82 ± 0.04	3.6 ± 0.28	0.69 ± 0.04	3.0 ± 0.23	2.6 ± 0.17	1.06 ± 0.05	4.56 ± 0.05	3.44 ± 0.14
22 : 6n-3	3.8 ± 0.32	15.3 ± 0.65	6.5 ± 0.38	10.6 ± 0.51	9.7 ± 0.43	4.3 ± 0.08	14.7 ± 0.66	15.8 ± 0.36
∑ **n**-3	6.6 ± 0.29	27.7 ± 1.31	8.5 ± 0.46	20.0 ± 1.04	17.7 ± 0.55	6.6 ± 0.14	28.5 ± 1.30	29.4 ± 1.22
n-3 / **n**-6	0.15 ± 0.01	1.5 ± 0.14	0.20 ± 0.01	0.58 ± 0.04	0.64 ± 0.03	0.15 ± 0.004	1.9 ± 0.22	2.2 ± 0.24

Results expressed as % of total fatty acids, mean ± SEM, n= 5 – 6. EE: ethyl ester, MPL: marine phospholipid, Soy lec: soy lecitin, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n.d: not detected.

3.4 Discrimination Between Diets

At the first level of comparison, the full fatty acid profile of all the diets were analyzed by prinicpal component analysis (PCA) for brain, liver and RBC. The PCA plots in figures 3.1,3.2, and 3.3 show patterns and correlations between the diets and the brain, the liver and the RBC, respectively.

In all 3 organs, the diets rich in n-3 PUFA (salmon, seal oil and cod liver oil) clearly separated from the diets rich in n-6 PUFA (soy oil, soy lecitin and cod), whereas the diets containing high levels of both n-3 and n-6 FA (marine PL and ethyl ester) did not show the same correlation pattern in all organs.

Table 3.5: Relative fatty acid composition in the RBC

Fatty acid	Soy Oil	Salmon	Cod	EE	MPL	Soy lec	Seal oil	Cod liver oil
14 : 0	0.24 ± 0.02	0.43 ± 0.04	0.22 ± 0.01	0.25 ± 0.02	0.25 ± 0.02	0.23 ± 0.02	0.75 ± 0.07	0.64 ± 0.04
15 : 0	0.23 ± 0.01	0.39 ± 0.01	0.21 ± 0.01	0.27 ± 0.01	0.32 ± 0.02	0.31 ± 0.02	0.33 ± 0.01	0.35 ± 0.01
16 : 0	25.9 ± 0.20	27.7 ± 0.26	27.1 ± 0.28	31.3 ± 0.65	28.2 ± 0.30	26.4 ± 0.21	26.4 ± 0.27	26.1 ± 0.12
17 : 0	0.42 ± 0.01	0.56 ± 0.01	0.35 ± 0.01	0.49 ± 0.01	0.51 ± 0.01	0.47 ± 0.01	0.42 ± 0.01	0.37 ± 0.03
18 : 0	12.5 ± 0.46	11.1 ± 0.13	12.0 ± 0.24	14.7 ± 0.14	10.5 ± 0.11	12.9 ± 0.16	11.7 ± 0.14	12.14 ± 0.21
24 : 0	0.12 ± 0.01	0.15 ± 0.01	n.d	n.d	0.17 ± 0.08	0.06 ± 0.04	0.17 ± 0.04	0.15 ± 0.03
∑ **SFA**	39.5 ± 0.59	40.3 ± 0.29	40.0 ± 0.43	47.0 ± 0.59	40.0 ± 0.35	40.4 ± 0.20	39.8 ± 0.38	39.8 ± 0.33
16 : 1n-7	0.45 ± 0.05	1.2 ± 0.10	0.57 ± 0.08	0.48 ± 0.05	0.85 ± 0.10	0.49 ± 0.03	1.7 ± 0.13	1.2 ± 0.13
16 : 1n-9	0.45 ± 0.05	0.20 ± 0.00	0.17 ± 0.00	0.22 ± 0.01	0.25 ± 0.01	0.16 ± 0.01	0.24 ± 0.01	0.28 ± 0.01
18 : 1n-7	2.9 ± 0.08	3.1 ± 0.05	2.8 ± 0.07	3.0 ± 0.06	3.3 ± 0.12	2.6 ± 0.06	3.9 ± 0.07	3.6 ± 0.12
18 : 1n-9	6.7 ± 0.31	8.8 ± 0.14	7.3 ± 0.19	8.3 ± 0.13	7.5 ± 0.20	5.4 ± 0.13	8.1 ± 0.10	8.9 ± 0.12
20 : 1n-9	0.13 ± 0.01	0.45 ± 0.01	0.06 ± 0.04	n.d	0.20 ± 0.01	n.d	0.82 ± 0.04	0.94 ± 0.02
∑ **MUFA**	10.6 ± 0.45	13.9 ± 0.24	11.1 ± 0.30	12.5 ± 0.19	12.3 ± 0.37	8.6 ± 0.17	16.0 ± 0.28	16.3 ± 0.19
18 : 2n-6	12.6 ± 0.37	5.6 ± 0.22	12.1 ± 0.26	13.3 ± 0.35	12.8 ± 0.13	14.0 ± 0.34	4.2 ± 0.25	4.2 ± 0.19
20 : 3n-6	0.49 ± 0.02	0.52 ± 0.01	0.58 ± 0.03	0.55 ± 0.02	0.56 ± 0.02	0.44 ± 0.02	0.38 ± 0.01	0.37 ± 0.01
20 : 4n-6	21.6 ± 0.25	12.7 ± 0.22	18.6 ± 0.36	14.7 ± 0.03	11.8 ± 0.37	18.9 ± 0.45	15.4 ± 0.35	13.1 ± 0.24
∑ **n**-6	35.0 ± 0.40	19.1 ± 0.37	31.7 ± 0.32	26.9 ± 0.28	25.4 ± 0.49	33.7 ± 0.27	20.1 ± 0.57	17.8 ± 0.43
18 : 3n-3	0.26 ± 0.02	0.09 ± 0.02	n.d	n.d	n.d	0.26 ± 0.02	n.d	n.d
20 : 5n-3	0.43 ± 0.02	6.7 ± 0.17	0.52 ± 0.03	4.4 ± 0.41	3.6 ± 0.18	0.42 ± 0.02	7.2 ± 0.19	9.0 ± 0.57
22 : 5n-3	1.9 ± 0.04	3.1 ± 0.07	1.6 ± 0.04	3.3 ± 0.07	2.4 ± 0.2	2.1 ± 0.04	3.2 ± 0.06	2.9 ± 0.11
22 : 6n-3	2.7 ± 0.08	6.3 ± 0.06	3.8 ± 0.06	5.3 ± 0.17	4.2 ± 0.29	2.4 ± 0.05	5.4 ± 0.11	6.9 ± 0.16
∑ **n**-3	5.3 ± 0.09	16.5 ± 0.20	7.7 ± 0.48	11.7 ± 0.52	11.5 ± 0.47	6.3 ± 0.14	15.9 ± 0.27	18.8 ± 0.68
n-3 / **n**-6	0.15 ± 0.00	0.87 ± 0.03	0.24 ± 0.01	0.43 ± 0.01	0.45 ± 0.02	0.19 ± 0.00	0.79 ± 0.03	1.1 ± 0.06

Results expressed as % of total fatty acids, mean ± SEM, n= 5 – 6. EE: ethyl ester, MPL: marine phospholipid, Soy lec: soy lecitin, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, n.d: not detected.

Brain

The PCA plot in figure 3.1 shows the discrimination between all of the diets in the brain, based on the full fatty acid profil given in table 3.3. The animals recieving marine n-3 PUFA was found separated from animals given vegetable diets. The salmon group was found separated from the marine oil groups, whereas the ethyl ester diet correlated with the n-6 rich diets. The soy lecitin diet did not correlate to the n-6 diets and was found closer to the marine PL group in the interphase between the n-3 rich diets and the n-6 rich diets. By the means of 3 components, 68.33% of the variability of the data was described.

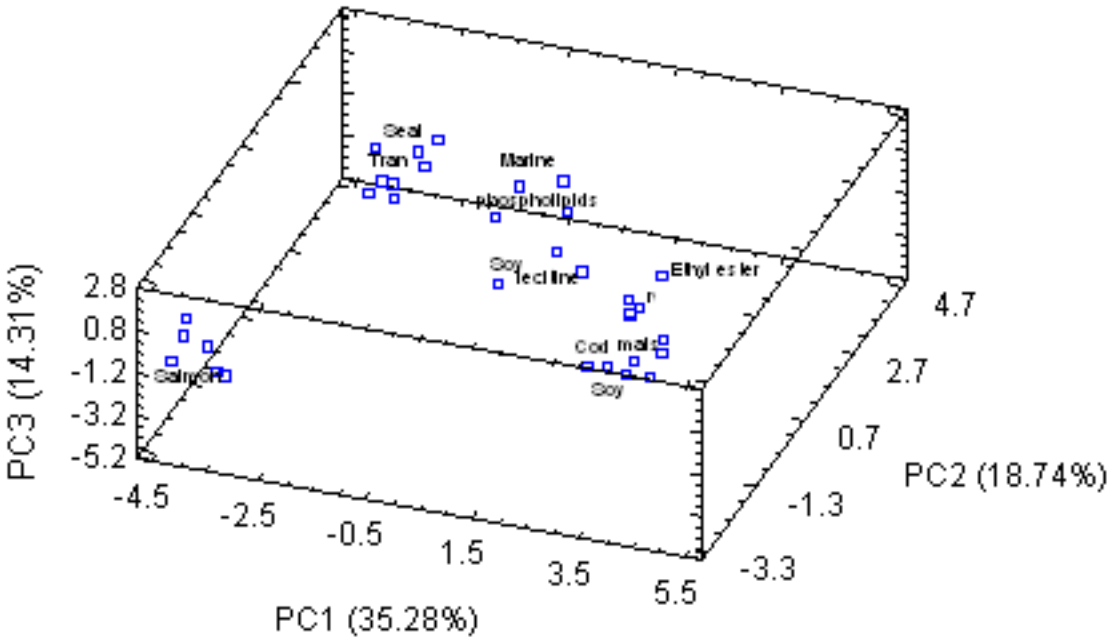

Figure 3.1: PCA plot of the brain, showing the discrimination of the full fatty acid profile in the different dietary groups. The n-3 rich diets were separated from the n-6 rich diets. Marine PL was situated in the interphase between n-3 and n-6 rich diets, whereas ethyl ester was clustered with the n-6 rich diets. PC1, PC2 and PC3 explained 68.33 % of the data in the plot.

Liver

When PCA was applied to the full fatty acid profile (from table 3.4) of all the dietary groups in the liver, the diets separeated into 4 main clusters. Figure 3.2 shows how the salmon, seal oil and cod liver oil diets correlated, and separated from soy oil, soy lecitin and cod. The ethyl ester and marine PL diets were found situated as 2 clusters in the interphase between the n-3 and n-6 clusters. 3 principal components explained nearly 72.45 % of the variability in the data set.

Red Blood Cells

Figure 3.3 shows the discrimination of the full fatty acid profil (from table 3.5) for the dietary groups in the RBC. The diets were arranged in 5 clusters. The groups recieving diets rich in n-3 PUFA were found as 2 clusters, one formed by seal oil and cod liver oil, and one formed by salmon. The groups given dites with high content of n-6 FA highly correlated and were represented as one cluster. The ethyl ester and marine PL diets were observed as 2 clusters in the interphase between the n-3 and n-6 diets. The 3 principal components explained 71.38 % of the data.

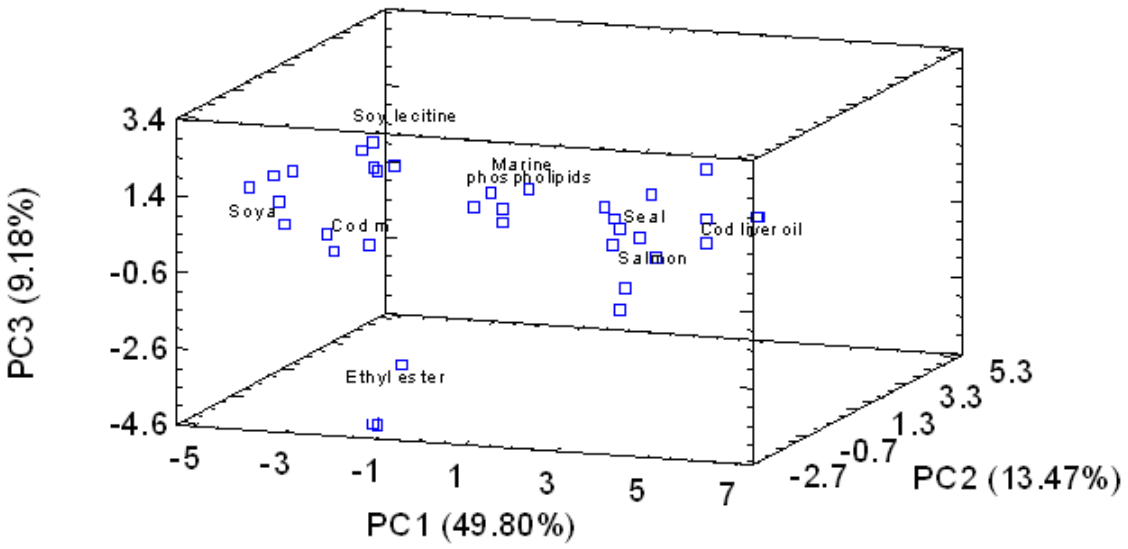

Figure 3.2: PCA plot of the liver showing the discrimination of the full fatty acid profile in the different dietary groups. The groups fed diets rich in n-6 FA were found to the left of the plot, the groups fed diets rich in n-3 PUFA were located to the right, whereas the diets rich in both n-3 and n-6 were situated in the middle. 72.45 % of the variability in the data were explained by 3 components (PC1, PC2 and PC3.)

3.5 Discrimination of Individual Fatty Acid Composition

EPA, DHA, and AA have distinct biological functions, accordingly, a major objective in this study was to determine whether the different diets containing marine n-3 PUFA resulted in differences in the levels of each of these FA, in brain, liver and RBC.

After finding particular patterns between the diets, one- way analysis of variance (ANOVA) was performed on selected FA, in order to establish differences in the relative level of FA incorporated in the organs studied in this work. Concequently, at the second level of comparison, ANOVA, was perfomed on the n-3 FA ALA, EPA, DPA and DHA, and n-6 FA LA, DGLA and AA, for the salmon, ethyl ester, marine PL, seal oil and cod liver oil groups.

3.5.1 Fatty Acid Comparison in the Brain

The ANOVA analysis of the n-3 and n-6 FA was conducted from the values in table 3.3, for the TAG (salmon, seal oil and cod liver oil), ethyl ester and marine

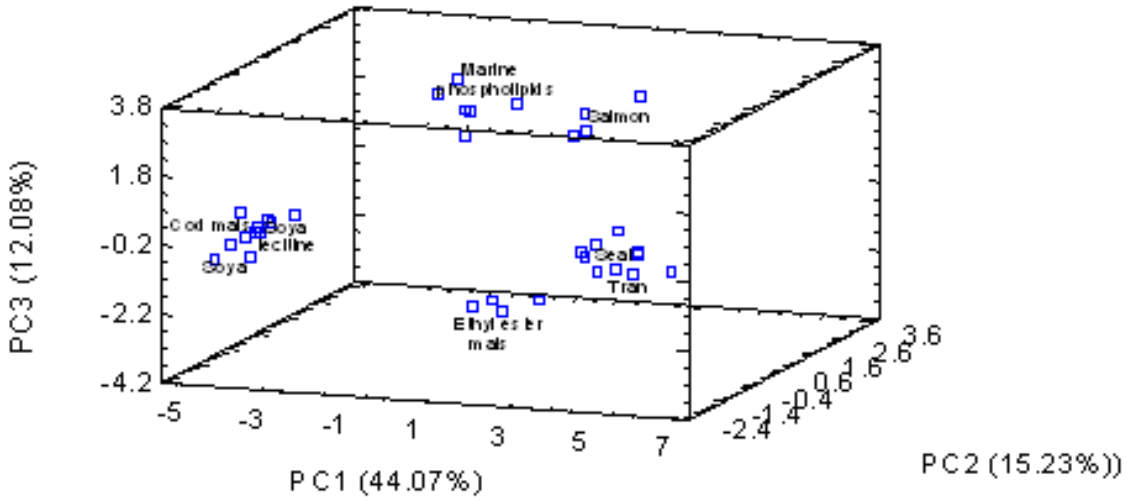

Figure 3.3: PCA plot of the RBC, showing the discrimination of the full fatty acid profile from different dietary sources. The n-6 rich diets appeared as one cluster, separated from the n-3 rich diets, clustered in 2 groups. Marine PL and ethyl ester were found as 2 clusters in the interphase between the n-3 and n-6 diets. The 3 principal components explained 71.38 % of the data.

PL diets. The result from the ANOVA is summarized in table 3.6.

EPA was the only FA exclusively found in animals recieving long-chain n-3 PUFA. DPA was found in small amounts in the soy oil group as the only group not recieving marine n-3 PUFA. DHA was found at high levels in all the groups, however, the highest levels were found in animals who recieved marine n-3 diets.

TAG; Fish versus Oils

When comparing salmon with the marine oils (seal oil and cod liver oil), there was no significant difference in the relative amount of the marine n-3, EPA, DPA and DHA incorporated between these groups. The animals given salmon showed significant higher levels of LA incorporated ($p < 0.0004$), whereas the cod liver oil diet had a significant lower level of AA than the salmon ($p < 0.01$) and seal oil ($p < 0.002$). The cod liver oil diet resulted in a significant higher ratio of n-3 / n-6 than the seal oil diet ($p < 0.02$).

TAG versus Ethyl Ester

Traces of EPA were found in the brain of animals recieving ethyl ester. These animals showed significant lower level of DHA ($p < 0.0001$), and DPA was significant lower than the salmon ($p < 0.001$) and cod liver oil ($p < 0.05$) groups.

Significantly more LA ($p < 0.0001$) and DGLA ($p < 0.05$) were found in the brains of animals given ethyl ester than those given TAG diets, whereas AA levels did not differ between the groups as to establish statistical differences. As for liver and RBC, the n-3 / n-6 ratio in the brain was significantly lower in animals fed ethyl ester as their source of n-3 ($p < 0.0001$).

TAG versus Marine Phospholipids

EPA was found to be significantly lower in animals recieving marine PL than those recieving salmon ($p < 0.002$) and cod liver oil ($p < 0.002$). DPA ($p < 0.002$) and DHA ($p < 0.0005$) were significantly lower than the salmon group. However, DHA was significantly higher in marine PL compared to the ethyl ester ($p < 0.003$). LA was found at significant higher levels in the marine PL fed animals ($p < 0.0004$), whereas the level of AA was similar to the TAG diets. The overall ratio of n-3 / n-6 in the brains of rats fed marine PL differed significantly from rats fed the TAG diets ($p < 0.001$).

Table 3.6: Summary of the relative n-3 and n-6 fatty acid composition in brain

Fatty acid	Salmon	EE	MPL	Seal oil	Cod liver oil
18 : 2n-6	$0.48 \pm 0.02^{a,b}$	$0.89 \pm 0.02^{a,b,c}$	$0.86 \pm 0.01^{a,b,c}$	0.32 ± 0.02	0.35 ± 0.02^{c}
20 : 3n-6	0.43 ± 0.01	$0.50 \pm 0.01^{a,b,c}$	0.53 ± 0.03	0.38 ± 0.01	0.40 ± 0.01
20 : 4n-6	8.6 ± 0.06	8.5 ± 0.08	8.7 ± 0.13	8.6 ± 0.07	8.1 ± 0.05
20 : 5n-3	0.24 ± 0.01	tr	$0.10 \pm 0.04^{a,c}$	0.18 ± 0.01	0.23 ± 0.02
22 : 5n-3	0.54 ± 0.03	$0.35 \pm 0.01^{a,c}$	$0.38 \pm 0.03^{a,c}$	0.43 ± 0.03	0.47 ± 0.01
22 : 6n-3	15.0 ± 0.14	$12.0 \pm 0.07^{a,b,c,e}$	$14.11 \pm 0.09^{c,d}$	14.4 ± 0.08	14.7 ± 0.21
n-3 / **n**-6	1.7 ± 0.02	$1.2 \pm 0.01^{a,b,c,e}$	$1.5 \pm 0.03^{a,b,c,e}$	$1.6 \pm 0.02^{a,c,d}$	1.7 ± 0.02

Results are expressed as % of total fatty acids. EE; ethyl ester, MPL; phospholipid, tr: trace. [a]significantly different from cod liver oil, [b]significantly different from seal oil, [c]significantly different from salmon. [d]significant from EE, [e]significant from MPL. Significance level $p < 0.05$.

3.5.2 Fatty Acid Comparisons in the Liver

The ANOVA analysis of the selected FA in the liver was carried out on the relative amounts of the FA given in table 3.4. Table 3.7 summarizes the differences found between the different structural forms of n-3 (TAG, ethyl ester and marine PL) administered throught the diets.

TAG groups; Fish versus Oils

When comparing the n-3 FA in livers from the salmon, seal oil and cod liver oil groups, no major differences were found between the diets. The only significant difference was at the level of DPA, being significantly the highest in the seal oil group ($p < 0.05$). Concerning the n-6 FA, the sole differences between the TAG diets was the levels of DGLA, found to be significantly higher in the salmon diet ($p < 0.001$). Animals fed cod liver oil showed the highest ratio of n-3 / n-6 within the TAG diets, but the difference was not significant.

TAG versus Ethyl Ester

The animals recieving n-3 PUFA in the form of ethyl ester showed a significant lower level of EPA and DHA in the liver, compared to the TAG diets ($p < 0.01$). The levels of DPA in the ethyl ester group was only significant lower than the seal oil group ($p < 0.004$), whereas the levels of LA and AA were significantly higher than the TAG group ($p < 0.0001$ and $p < 0.02$ respectively). The total ratio of n-3 / n-6 was significantly lower in the ethyl ester diet than the TAG diets ($p < 0.008$).

TAG versus Marine Phospholipids

The marine PL diet resulted in a significant lower level of EPA ($p < 0.003$), DPA ($p < 0.01$), DHA ($p < 0.001$), whereas the levels of LA were significant higher than the TAG diets. The animals fed the marine PL diet had a significant higher level of LA ($p < 0.0001$). However, the phospholipid diet showed the lowest level of AA in the liver, but the difference was not significant. The phospholipid diet resulted in the lowest n-3 / n-6 ratio in the liver, being significant from the TAG diets ($p < 0.006$).

3.5.3 Fatty Acid Comparisons in Red Blood Cells

The ANOVA analysis was performed on the n-3 and n-6 FA for the TAG, ethyl ester and marine PL diets based on the values in table 3.5. Table 3.8 summarizes the differences found between the fatty acids.

TAG; Fish versus Oils

The diet containing cod liver oil had significant higher levels of EPA incorporated in RBC than the salmon ($p < 0.001$) and seal oil diets ($p < 0.01$). The cod liver oil and salmon groups had significant higher levels of DHA than the seal oil group ($p < 0.02$), whereas the seal oil diet lead to a significant higher level of DPA than cod liver oil ($p < 0.02$). When considering the n-6 FA incorporated in the RBC

Table 3.7: Summary of the relative n-3 and n-6 fatty acid composition in liver

Fatty acid	Salmon	EE	MPL	Seal oil	Cod liver oil
18 : 2n-6	9.4 ± 0.46	$23.8 \pm 0.68^{a,b,c}$	$20.9 \pm 0.63^{a,b,c}$	8.1 ± 0.86	7.5 ± 0.75
20 : 3n-6	$0.64 \pm 0.03^{a,b}$	0.56 ± 0.02	0.64 ± 0.03	0.39 ± 0.04	0.44 ± 0.02
20 : 4n-6	8.1 ± 0.37	$10.2 \pm 0.66^{a,b,c}$	6.4 ± 0.68^{e}	7.3 ± 0.54	6.6 ± 0.53
18 : 3n-3	0.62 ± 0.06	0.55 ± 0.04	0.54 ± 0.03	0.52 ± 0.06	0.58 ± 0.05
20 : 5n-3	7.8 ± 0.36	$5.8 \pm 0.45^{a,b,c}$	$4.8 \pm 0.23^{a,b,c}$	8.5 ± 0.49	9.4 ± 0.79
22 : 5n-3	3.6 ± 0.28	3.0 ± 0.23^{b}	$2.6 \pm 0.17^{a,b,c}$	$4.6 \pm 0.05^{a,c}$	3.4 ± 0.14
22 : 6n-3	15.3 ± 0.65	$10.6 \pm 0.51^{a,b,c}$	$9.7 \pm 0.43^{a,b,c}$	14.7 ± 0.66	15.8 ± 0.36
n-3 / **n**-6	1.5 ± 0.14	$0.58 \pm 0.04^{a,b,c}$	$0.64 \pm 0.03^{a,b,c}$	1.9 ± 0.22	2.2 ± 0.24

Results are expressed as % of total fatty acids. EE; ethyl ester, MPL; phospholipid. ^{a}significantly different from cod liver oil,^{b}significantly different from seal oil, ^{c}significantly different from salmon,^{e}significant from MPL. Significance level $p < 0.05$.

from the diets, the salmon group had a significant higher level of LA than the oil groups ($p < 0.002$). The n-3 / n-6 ratio, was significant higher in animals fed cod liver oil than rats recieving salmon ($p < 0.01$) and seal oil ($p < 0.0003$).

TAG versus Ethyl Ester

The ethyl ester group showed significant lower levels of EPA than the TAG groups ($p < 0.003$), whereas the level of DHA was significantly lower than rats fed salmon ($p < 0.02$) and cod liver oil ($p < 0.0003$). Rats fed ethyl ester contained significantly higher levels of LA ($p < 0.0001$) than TAG diets, significant higher levels of DGLA compared to oil groups ($p < 0.0001$), and significant higher levels of AA compared to salmon ($p < 0.004$) and cod liver oil ($p < 0.03$). The n-3 / n-6 ratio was significant lower in the ethyl ester group compared to the TAG groups ($p < 0.0002$).

TAG versus Marine Phospholipids

In RBC, the marine PL diet resulted in a significant lower level of EPA ($p < 0.0001$) and DHA ($p < 0.0005$), compared to TAG diets. DPA was significantly lower in the marine PL group than salmon ($p < 0.002$) and seal oil ($p < 0.0003$). The animals fed marine PL had a significantly higher level of LA incorporated in their RBC ($p < 0.0001$), whereas the relative amount of DGLA was significant higher than the oil groups ($p < 0.0001$). The level of AA was significant lower in the marine PL diet than the oil groups ($p < 0.04$), and the n-3 / n-6 ratio was significantly lower than the TAG diets ($p < 0.0001$).

Table 3.8: Summary of the relative n-3 and n-6 fatty acid composition in RBC

Fatty acid	Salmon	EE	MPL	Seal oil	Cod liver oil
18 : 2n-6	$5.6 \pm 0.22^{a,b}$	$13.3 \pm 0.35^{a,b,c}$	$12.8 \pm 0.13^{a,b,c}$	4.2 ± 0.25	4.19 ± 0.19
20 : 3n-6	0.52 ± 0.01	$0.55 \pm 0.02^{a,b}$	$0.56 \pm 0.02^{a,b}$	0.38 ± 0.01	0.37 ± 0.01
20 : 4n-6	12.7 ± 0.22	$14.7 \pm 0.03^{a,c}$	$11.8 \pm 0.37^{a,b,e}$	15.4 ± 0.35	13.1 ± 0.24
18 : 3n-3	0.09 ± 0.02	n.d	n.d	n.d	n.d
20 : 5n-3	6.7 ± 0.17	$4.4 \pm 0.41^{a,b,c}$	$3.6 \pm 0.18^{a,b,c,d}$	7.2 ± 0.19	$9.0 \pm 0.57^{b,c}$
22 : 5n-3	3.1 ± 0.07	3.3 ± 0.07^{a}	$2.4 \pm 0.21^{b,c,d}$	3.2 ± 0.06^{a}	2.9 ± 0.11
22 : 6n-3	6.3 ± 0.06	$5.3 \pm 0.17^{a,c}$	$4.2 \pm 0.29^{a,b,c,d}$	$5.4 \pm 0.11^{a,c}$	6.9 ± 0.16
n-3 / **n**-6	0.87 ± 0.03	$0.43 \pm 0.01^{a,b,c}$	0.45 ± 0.02	0.79 ± 0.03	$1.07 \pm 0.06^{b,c}$

Results are expressed as % of total fatty acids. EE; ethyl ester, MPL; phospholipid. ^{a}significantly different from cod liver oil,^{b}significantly different from seal oil, ^{c}significantly different from salmon. ^{d}significant from EE, ^{e}significant from MPL. Significance level $p < 0.05$.

3.5.4 Absolute Compositions of n-3 PUFA in Brain, Liver and Red Blood Cells

In addition to the relative level of EPA, DPA, DHA, LA, DGLA and AA, the absolute amounts of these FA were compared by one-way ANOVA, for all the dietary groups.

Internal standard, used for quantitative measurements of FA, was mistakenly not added to the salmon group in brain, and the cod liver group in liver and RBC, hence, no absolute amounts are available for these groups.

Brain Due to very different amounts of EPA, DPA and DHA in the brain, the graphic illustration of these FA was divided in two figures. Figure 3.4 shows EPA and DPA, whereas figure 3.5 shows the amount of DHA incorporated in the brain. EPA was only found at very low amounts in animals fed marine PL, seal oil and cod liver oil. The amount of EPA was significantly lower in the marine PL group than cod liver oil ($p < 0.0002$). DPA was found in significant lower amounts in the soy oil and ethyl ester groups, whereas DPA in the ethyl ester group was significantly lower than the oil groups ($p < 0.004$). Animals fed ethyl ester, cod and soy oil had significant less amount of DHA in brains than animals fed marine PL ($p < 0.0001$), seal ($p < 0.0001$) and cod liver oil ($p < 0.0001$), and also soy lecitin ($p < 0.001$). The animals fed cod liver oil had the highest significant amount of DHA incorporated in the brain ($p < 0.03$).

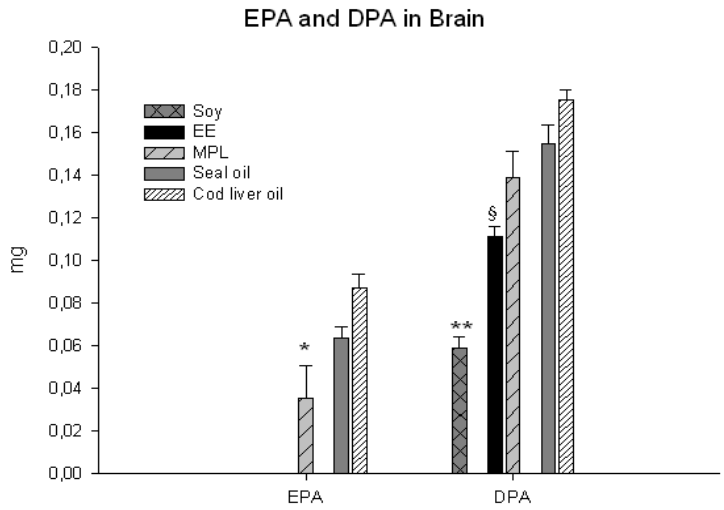

Figure 3.4: Absolute fatty acid composition (mg) of EPA and DPA in brain. Values are presented as mean (n= $5-6$) $\pm$ SEM. The concentration of EPA and DPA in the brain was very low. EPA was only found in animals fed marine PL, seal oil and cod liver oil, however trace amounts were detected in animals fed ethyl ester. DPA was found at slightly higher amounts, and only in animals fed the diets rich in marine n-3, plus the soy oil group. The salmon group is not included in the figure.* statistical significance from cod liver oil, ** statistical significance from all the groups, and § statistical significance from seal oil and cod liver oil. EE: ethyl ester, MPL: marine phospholipid.

Liver Figure 3.6 shows the actual amount of the marine n-3 FA incorporated in the liver of the animals in the different dietary groups. The figure clearly shows how groups recieving the marine n-3 rich diets (salmon, ethyl ester, marine PL, and seal oil), contained significantly higher amounts of EPA ($p < 0.0001$), DPA ($p < 0.0006$) and DHA ($p < 0.002$) than those who were fed diets low (cod) or depleted of marine n-3 (soy oil, soy lecitin). There was no significant difference within TAG groups, or between TAG and ethyl ester regarding the amount of EPA, DPA and DHA incorporated in liver. The amount of EPA in the marine phospholipid group was significantly lower than seal oil ($p < 0.02$).

Red blood cells Figure 3.7 represent the quantititative amount of EPA, DPA and DHA in RBC. The RBC contained very low amounts of these fatty acids. The groups fed marine n-3 incorporated significant higher amounts of EPA, DPA and DHA, than animals fed n-6 rich diets. Concerning EPA, the ethyl ester and marine PL groups had significantly lower amount than the salmon ($p < 0.0001$) and seal oil ($p < 0.0001$). DPA was significantly lower in the marine PL group than salmon ($p < 0.001$), seal oil ($p < 0.005$) and ethyl ester ($p < 0.03$). The salmon diet lead to the highest level of DHA in RBC, significantly higher than the other diets ($p <$

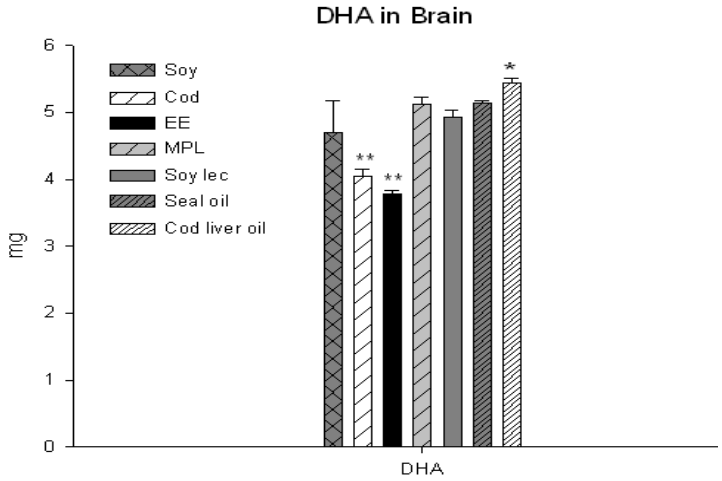

Figure 3.5: Absolute fatty acid composition (mg) of DHA in brain. Values are presented as mean (n= $5-6$) $\pm$ SEM. The level of DHA in brain was fairly constant. Cod liver oil was found to give the highest amount of DHA in the brain, closely followed by seal oil and marine PL. Animals recieving cod and ethyl ester showed a reduction in the amount of DHA compared to soy oil and soy lecitin.* and ** statistical significance from all the groups. The salmon group is not included in the figure. EE: ethyl ester, MPL: marine phospholipid

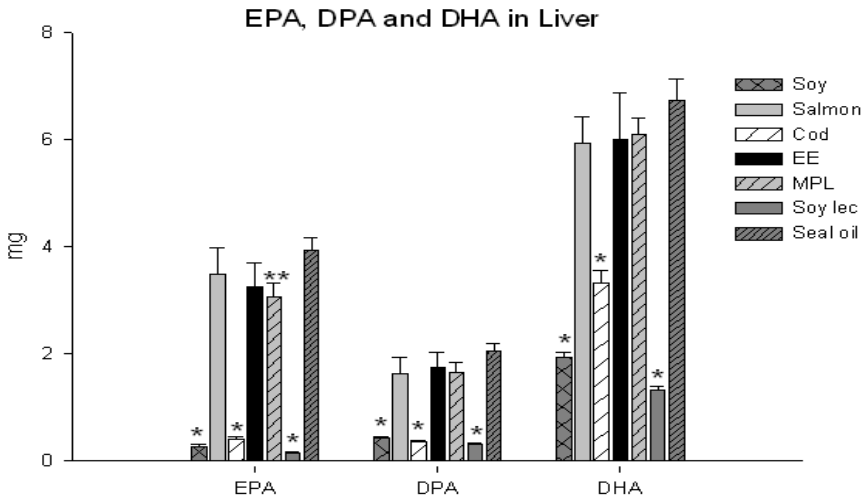

Figure 3.6: Absolute fatty acid composition (mg) of EPA, DPA and DHA in liver. The values are presented as mean (n= $5-6$) $\pm$ SEM. Animals fed diets rich in marine n-3 (salmon, ethyl ester, MPL and seal oil) showed an significant enrichment of EPA, DPA and DHA in the liver. The cod liver oil group is not included in the figure. * statistical significance from the marine diets, ** statistical significance from seal oil. EE: ethyl ester, MPL: marine phospholipid

0.003).

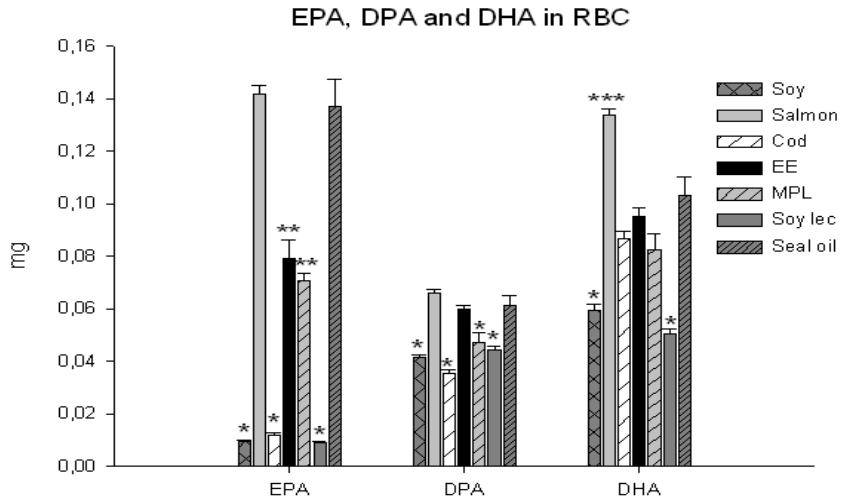

Figure 3.7: Absolute fatty acid compositio (mg) of marine n-3 PUFA in red blood cells. Values are presented as mean (n= 5 – 6) ± SEM. The groups fed marine n-3 showed a higher incorporation of EPA in RBC. The values for DPA were low, and animals fed marine n-3 show higher amounts of DPA then animals fed n-6 rich diets. DHA levels were highest in animals recieving the marine diets, whereas the soy oil and soy lecitin contained the lowest amounts. The cod liver oil group is not included in the figure. * statistical significance from the marine groups, ** statistical significance from salmon and seal oil groups, and *** statistical significance from all the groups. EE: ethyl ester, MPL: marine phospholipid

3.5.5 Absolute Composition of n-6 PUFA in Brain, Liver and Red Blood Cells

The n-6 fatty acid arachidonic acid (AA) is present in most tissue, and is found in high amounts in the brain. DGLA is the fatty acid prior to arachidonic acid in the metabolic pathway of the n-6 fatty acids (see figure 1.5 for the metabolic pathway of n-6 FA), and was therefore included.

Arachidonic acid Figure 3.8 presents the absolute amount of AA in the brain, the liver and the RBC. In the liver, the animals fed marine n-3 showed reduced amounts of AA. The greatest decrease was found for TAG and marine PL, whereas ethyl ester did not reduce the amount to the same extent. In RBC, the diets rich in n-6 contained the highest amount of AA. The brain contained the highest amount of AA, and the soy lecitin group showed the highest amount of AA, whereas the ethyl ester group had the lowest amount of AA.

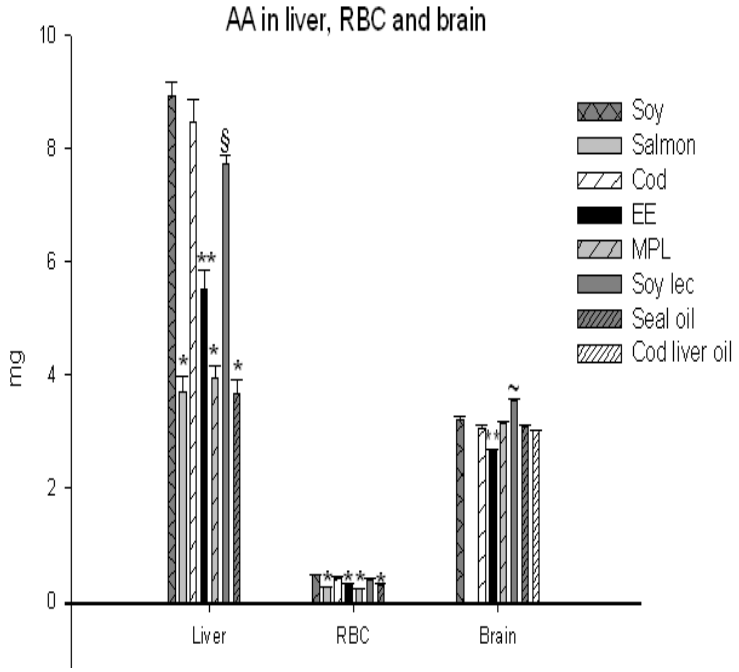

Figure 3.8: Absolute fatty acid composition (mg) of the n-6 fatty acid arachidonic acid (AA) in the liver, the RBC and the brain. Values are presented as mean (n= $5-6$) $\pm$ SEM. In the liver, the marine n-3 diets reduced the amount of AA compared to the n-6 rich diets. In RBC, the changes in AA concentration were minor, however the n-6 rich diets resulted higher amounts than marine n-3 diets. In brain, the highest level of AA was found in animals fed soy lecitin and the lowest in animals fed ethyl ester. In this figure, the cod liver oil group is not included for liver and RBC, and the salmon group is not included for brain. * statistical significance from soy oil, cod and soy lecitin, ** statistical significance from marine and vegetal groups, § statistical significance from soy oil, $\backsim$ statistical significance from all the groups. EE: ethyl ester, MPL: marine phospholipid

DGLA Figure 3.9 shows the amount of DGLA (20 : 3n-6) in the liver, the RBC and the brain. In liver, the greatest concentration of DGLA was found in the animals fed marine PL, whereas the lowest amount of this fatty acid was found in livers of animals fed soy lecitin and seal oil. RBC contained extremely low amounts of DGLA, and no major differences were observed between the groups. In the brain, the amount of DGLA was found to be fairly constant between the dietary groups. Nevertheless, the brains of animals recieving marine PL contained the highest amount of DGLA.

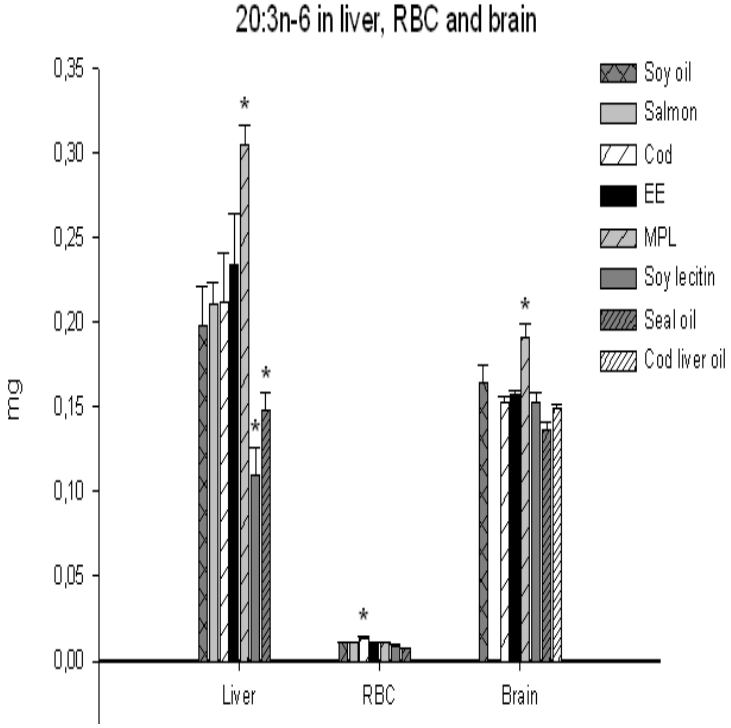

Figure 3.9: Absolute fatty acid composition (mg) of the n-6 fatty acid DGLA (20 : 3n-6) in liver, RBC and brain. In liver and brain, the marine PL group and the seal oil group contained the highest and lowest amount of DGLA respectively. RBC contained extremely low values of DGLA in their cells. In this figure, the cod liver oil group is not included for liver and RBC, and the salmon group is not included for brain. Values are presented as mean (n= $5-6$) $\pm$ SEM. * statistical significance from all the groups. EE: ethyl ester, MPL: marine phospholipid

3.6 Organ Comparison (Brain, Liver and Red Blood Cells)

At the last level of comparison, the dietary influence in the different organs was studied by PCA analysis, regarding the diets containing marine n-3 FA (salmon, ethyl ester, marine PL, seal oil and cod liver oil). The PCA was based on the relative levels of the FA. The PCA plot in figure 3.10 clearly shows how marine PL, ethyl ester, and TAG (seal oil, cod liver oil and salmon) groups, highly correlated in the brain, whereas the same is not true for liver or RBC. The brain as organ was found separated from the liver and RBC. Liver and RBC were found separated in 2 groups, however the 2 clusters were more spread than the brain. The 3 principal components explained 90.18 % of the variability in the data set.

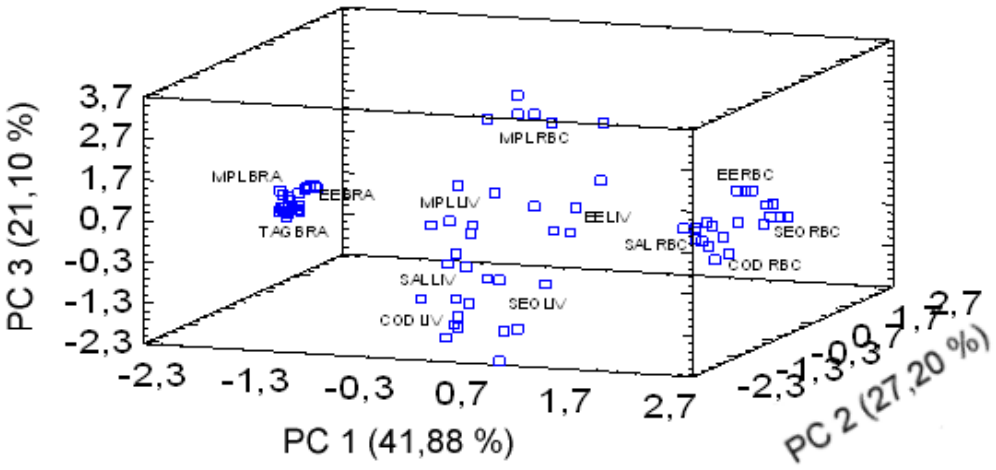

Figure 3.10: PCA plot showing the discrimination of the diets rich in marine n-3 PUFA (marine PL, ethyl ester, salmon, seal oil and cod liver oil) in brain, liver and RBC. The diets influence the organs differently. The brain separated from the liver and RBC, whereas the dietary groups did not correlate to the same extent in liver and RBC. The 3 principal components explained 90.18 % of the variability of data. MPL; marine phospholipid, EE: ethyl ester, TAG: seal oil, cod liver oil and salmon, COD: cod liver oil, SEO: seal oil, SAL: salmon, BRA: brain, LIV: liver, RBC: red blood cells.

3.7 Summary of Results

3.7.1 PCA

Brain The diets rich in marine n-3 PUFA separated in 2 clusters, ethyl ester correlated with cod and soy oil, whereas marine PL and soy lecitin were grouped in the inter-phase between the n-3 rich and n-6 rich diets.

Liver and red blood cells Soy oil, soy lecitin and cod separated in one cluster. Seal oil, cod liver oil and salmon also separated in one cluster, whereas ethyl ester along with marine PL separated in 2 clusters in the inter-phase between the n-3 rich and n-6 rich diets

Organ comparison In brain, all the marine diets highly correlated. The marine dietary groups were found more scattered in the liver, nevertheless as a cluster. The marine diets showed higher correlation in the RBC than liver, however not to the same extent as brain.

3.7.2 ANOVA

Fatty Acid Composition

Brain; relative amounts No differences were found within the TAG groups concerning EPA, DPA and DHA. Ethyl ester diet resulted only in trace amounts of EPA. DHA was considerably lower than in TAG groups, whereas n-6 FA were higher. The marine PL group showed lower levels of EPA, however DPA and DHA was only significant lower than salmon.

Brain; absolute amounts EPA was found in extremely low amounts only in animals fed marine PL, seal oil and cod liver oil. DHA amounts were high in all groups, however, cod and ethyl ester showed the lowest amounts, whereas marine PL, seal oil and cod liver oil resulted in an enrichment of DHA.

Liver; relative amounts No differences within the TAG groups. The ethyl ester and marine phospholipid diets resulted in lower levels of EPA, DPA and DHA, and higher levels of LA.

Liver; absolute amounts Increased amounts of EPA, DPA and DHA were found in animals fed marine diets. No difference was found between the various structural forms of n-3 PUFA (TAG, ethyl ester and marine PL). Marine diets greatly reduced the amount of AA in the liver.

Red blood cells; relative amounts Within the TAG groups, cod liver oil resulted in the highest level of EPA, seal oil resulted in lowest DHA, and highest DPA. Ethyl ester resulted in lower levels of EPA and DHA, and higher levels of n-6 FA compared to TAG. Marine PL resulted in lower levels of EPA, DPA and DHA, higher DGLA and lower AA levels than TAG diets.

Red blood cells; absolute amounts Animals fed n-3 PUFA showed the highest amount of EPA, DPA and DHA. The TAG diets resulted in the highest amount of EPA and DHA. The ethyl ester group showed to the highest amount of DPA. The marine n-3 diets reduced the amount of AA in RBC.

4

Discussion

Polyunsaturated fatty acids (PUFA), especially DHA (22 : 6n-3), is present in high amounts in the central nervous system (CNS) [29]. The n-3 FA are available in the diet as 18 : 3n-3 (ALA) from vegetable origin, or as 20 : 5n-3 (EPA), 22 : 5n-3 (DPA) and 22 : 6n-3 (DHA) originating from marine sources.

Previous studies have established the possibility to affect the fatty acid profile in brain, liver and red blood cells (RBC) through dietary manipulation [30, 37, 91] and it has been shown that fish oil dramatically alters the PUFA composition of brain and liver [92]. Ackman [93] pointed out that the molecular structure of n-3 fatty acid ingested could have a profound effect on lipid production, and its rate and total quantity absorbed. Hence, the physiological and pharmacological properties of n-3 FA could be influenced by the molecular form in which they are ingested.

The effects of dietary n-3 PUFA deficiency on nervous system function are well documented, whereas those of a diet high in marine n-3 PUFA are less known [45]. In this work, normal, healthy animals were fed diets rich, low or depleted of marine n-3, in order to investigate the effect of dietary manipulation, and the accretion of EPA and DHA in brain, liver and RBC. A number of studies have investigated the effect of n-3 PUFA by using pure EPA and DHA fractions [40, 41, 94, 95, 96]. In this thesis, EPA and DHA from naturally available sources were used in order to investigate the accumulation of these FA in rat tissues, following

a dietary intervention. The rate of incorporation of EPA and DHA in brain, liver and RBC was investigated as a function of structure and amount of the dietary lipids.

4.1 Animals

The animals used in this work were initially weighting 200 g. This weight correspond to a rat in young adulthood of $7-8$ weeks of age. When investigating the incorporation of long-chain PUFA in the brain, the age of the animals must be considered. Studies have shown that the adult brain appears to be more resistant to changes in fatty acid composition [38], even when large amounts of dietary n-3 are administered [30, 92]. The developing brain, in contrast, seems to be more plastic and susceptible to changes [27, 38].

Food intake and weight gain The average food intake during the 3 week experimental period did not differ significantly between the groups. Interestingly, as seen in table 3.2, the weight gain show that animals fed the fish diets (salmon and cod) gained significantly more weight than the other animals, apart from the animals in the soy group. According to table 2.3, all diets contained similar amounts of fat, protein and energy, apart from the marine PL and ethyl ester diet, containing less fat and energy, leaving the protein source the only major difference between the diets. The fish diets contained fish protein, from the fish filets, in addition to casein, which might have lead to a increased growth of the animals fed salmon and cod. Others have found a similar increase in weight gain in animals fed fish protein compared to milk protein (unpublished data).

Fatty liver Despite similar amounts of fat in the diet, some of the animals developed fatty liver (hepatic steatosis). It was especially animals recieving diets with high content of marine n-3 (ethyl ester, seal oil and cod liver oil) that developed or showed tendency toward fatty liver. Even animals in the marine PL group recieving less dietary fat showed fatty liver. Fatty liver is an excessive accumulation of fat in hepatocytes (liver cells). Although not a normal condition, fatty liver is usually not harmful or causing damage to the animal. Eating fatty foods does not by itself lead to fatty liver, however dietary components may influence liver in a negative way [97]. Triacylglycerol (TAG) is synthesized in liver, and stored as fat droplets or secreted into the blood as VLDL (very low density lipoprotein). Fatty liver is believed to result from disturbance of the process of TAG synthesis i.e., decreased incoporation of TAG into functional VLDL, or of VLDL synthesis and secretion i.e., impaired export of VLDL from hepatocytes [98].

The fatty liver found in animals fed high amounts of n-3 PUFA may be a result for a surplus of n-3 deposited in the liver, which can have a profound influence on the liver fatty acid profile [91].

4.2 Preparation of Diets

One of the main challenges in this study were to prepare eight comparable diets. This however, was not feasible, since two of the lipid sources (soy oil and soy lecitin) did not contain long-chain n-3 PUFA (EPA, DPA or DHA), and one was extremely poor in marine n-3 fatty acids (cod). The different sources of n-3 did not only differ in the lipid structure, but also in the distribution of the marine n-3 FA within the various sources. Thus, the diets contained different amounts of the marine n-3 FA EPA, DPA and DHA. When preparing the diets, there was another element of consideration; to ensure 10 % fat in all diets, the differences in the amount of n-3 sources added (see table 2.2) was compensated using maiz oil. Maiz oil is a vegetal oil rich in n-6 FA, thereby introducing high amounts of 18 : 2n-6 to some of the marine diets (cod, ethyl ester and marine PL). The extreme variation in the amount of 18 : 2n-6 present in the diets will not be discussed further in this thesis.

Analysis of the diets regarding protein, energy, fat, ash and dry matter, were made after the termination of the feeding trial. The analysis showed that the marine PL diet and the soy lecitin diet contained less fat than the other diets (table 2.3). The analysis of the diets should therefore have been made in advance in order to detect these mistakes, and new diets could have been prepared containing the proper fat content.

4.2.1 Amount of Marine n-3 PUFA in the Diets

The amount of the different n-3 and n-6 FA varied considerably in the different diets (table 3.1). The amount of 18 : 2n-6 was extremely high in soy and soy lecitin diets, as well as for the diets receiving additional maiz oil supplement (cod, ethyl ester and marine phospholipid). The salmon diet contained the highest amounts of 18 : 2n-6 of the diets recieving the marine n-3 PUFA as sole fat source. This can indicate that the salmon used in the experiment was a farm fish, fed a vegetal diet.

As previously mentioned, the various n-3 diets differed in the amounts of EPA, DPA and DHA, together with the total amount of marine n-3. The ethyl ester diet contained the highest amount of EPA, the salmon and cod liver oil diets contained the highest amounts of DHA, and the salmon and seal oil diets contained the highest dietary amount of DPA. Salmon and cod liver oil contained comparable

amounts of EPA, DHA, and total amount of marine n-3 FA (EPA+DPA+DHA). Seal oil and ethyl ester diets contained similar amounts of total marine n-3 FA, however the individual marine n-3 FA differed greatly. Of the diets rich in marine n-3 FA, the marine PL diet containd the lowest amount of the individual marine n-3 FA, and also the lowest amount of total marine n-3, explained by the low fat content in this diet. Although the diets differed greatly in the amount of marine n-3 FA, comparisons will be made regarding the incorporation of EPA and DHA in the brain, the liver and the RBC for the diets rich in n-3 PUFA. Nevertheless, the amount will also be considered.

4.2.2 Structure of n-3 PUFA

TAG Three groups were given n-3 PUFA in the form of TAG (salmon, seal oil and cod liver oil). Seal oil and fish oil contain large amounts of preformed long-chain n-3 FA located in different positions within the TAG molecule. In seal oil, DHA is located mainly in the *sn*-1/3 position, whereas in salmon and cod liver oil, DHA is mainly found in the *sn*-2 position of TAG. Different positioning of n-3 PUFA within the TAG molecule may affect the intestinal absorption, metabolism and distribution of dietary n-3 PUFA [93, 99].

It has been demonstrated that lymphatic absorption of FA is enhanced when the fatty acid is located in the *sn*-2 position in dietary TAG [100]. Studies have shown that the intramolecular distribution of n-3 FA in dietary TAG affects both the fatty acid distribution of TAG in chylmicrons [101], and the metabolism of chylomicrons [102]. However, Jensen *et al.* [103] found no effects of TAG structure of dietary fat in brain phospholipids when feeding rats either seal oil or fish oil diets. Sommer-Hartvigsen *et al.* [37], on the other hand, showed that brain phosphatidylethanolamine (PE) differed significantly from diet based on specific structured TAG (18 : 3n-3 in *sn*-2 position) compared to randomized TAG, indicating that the metabolism of fatty acid is related to their position within the dietary TAG. Different tissue respond differently to the fatty acid positioning within the TAG molecule, and the bioavailibilty of FA in the *sn*-2 position appears to be higher in brain, whereas DHA equally distributed in TAG lead to in higher levels of DHA in liver [104].

Ethyl ester Ethyl esters are relatively rare in natural foods, and it is therefore natural to assume that the absorption and transport of FA presented as ethyl ester are not identical to that of natural fats [99]. Early studies [105, 106] suggested that n-3 FA as ethyl ester are absorbed differently and hydrolyzed to a lesser degree in humans compared to TAG-EPA or free EPA. The difference is possibly the result of poor hydrolysis of EPA ethyl ester by pancreatic lipase [105]. However, given

sufficient time, EPA as ethyl ester is found to be absorbed equally to TAG-EPA [99]. Hong *et al.* [94] and Frøyland *et al* [107] found in their studies, that EPA and DHA in the form of ethyl ester did not mimic the physiological activity of fish oil, at least in affecting hepatic fatty acid oxidation.

Phospholipid There are evidence indicating that LC PUFA as dietary phospholipids (PL) are more efficiently incoporated than LCPUFA in the form of TAG. The dietary supply of AA in the form phospholipid has shown to have clear effects on brain lipid composition [108, 109]. Wijendran [108], showed that in baboon neonate brain, the accretion of AA provided as phospholipid was 2-fold greater than as TAG. Lemaitre-Delaunay *et al.* [109], provided evidence for higher bioavailability of DHA when provided as phospholipid compared to TAG for incorporation into erythrocytes in human adults. Further, preterm infants fed a formula supplemented with n-3 and n-6 long-chain PUFA in the form of phospholipids, at a significant lower concentration than human milk, developed similar composition of LCPUFA in plasma and RBC as human milk-fed infants, suggesting that the FA from the phospholipid source is more efficiently incorporated [110].

4.2.3 Lipid Sources

The different lipid sources used in this experiment differed in the structures of FA. All sources (apart from marine phospholipid and soy lecitin) were commercially available. The various sources were to be compared concerning the rate of incorporation of EPA and DHA, in rat tissue.

Soy oil The group recieving soy oil was chosen as a “reference” diet, and was meant to reflect Western diets, being extremely rich in n-6 fatty acids, and low or depleted of, long-chain n-3 PUFA. The lipid structure in the soy oil used was in the form of triacylglycerol (TAG), and was a n-6 control to the marine n-3 TAG. Soy oil contains 18 : 3n-3 and 18 : 2n-6, know as the precursors of long-chain PUFA of the n-3 and n-6 families.

Fish oil, ethyl ester and seal oil The fish oil used in this experiment (Møllers Tran) is one of the most common n-3 supplement in Norway. The capsules is together with fish oil traditionally the most common n-3 supplement in Norway. The capsules used in this study (Fri Flyt, Vesterålen Naturprodukt) were chosen based on the chemical structure of the n-3 PUFA, here available as ethyl esters. Seal oil was included in the study for its similarities with fish oil, however differing in the intramolecular structure of the TAG molecule. Seal oil is rapidly increasing as a

n-3 supplement in Norway. The seal oil used in this study (Arctic omega-3 seal oil) is produced from the blubber of harp seal *(Pagophilus groenlandicus)*, and hence does not have the charactheristic adverse taste of fish oil, produced from fish liver.

Marine phospholipids The marine phospholipids (Eximo AS) used in the experiment are extracted from marine sources, and are extremely rich in PUFA of the n-3 family. They are particularly rich in DHA and EPA, which constitute almost 50 % of the total FA in the ratio 2 : 1 (DHA : EPA).

Fish filets Two fish were also included in the experiment. One fatty fish (salmon) and one lean fish (cod). Fat from salmon is available in the form of TAG, whereas in cod, the fat excists in the form of phospolipids. It was not possible to compare the 2 fish diets directly, due to very different amount of fat as previously mentioned in 2.2. However, the most interesting use of the fish diets was to compare the fatty fish with the other n-3 supplements given either as oils, marine PL or ethyl ester. This in order to investigate a "natural" occuring n-3 PUFA source, in comparison to other marine n-3 supplements available, regarding the rate of incorporation of EPA and DHA in rat tissue. The lean fish diet gave clear indications that cod is not a good n-3 source as salmon, or other marine n-3 supplements, for increasing long-chain n-3 PUFA concentrations.

Soy lecitin The soy lecitin diet was added to match the marine PL treatment, hence, it was a n-6 control for the n-3 PL. Soy lecitin from Sigma has high content of FA in the form of phospholipids. Phosphatidylcholine (lecitin) is an emulifier, used mainly as stabilizers of industrially prepared foods [111]. Soy lecitin products has no clear effects on the lipid profile beyond those attributable to its high LA content [112].

4.3 Incorporation of Marine n-3 PUFA

When investigating the incorporation of EPA and DHA in the brain, the liver and the RBC, both relative and absolute amounts were used. Unfortunalty, absolute amounts are missing for the salmon group in brain, and cod liver oil group in liver and RBC. Therefore, the relative amount was mainly used in this thesis, nevertheless, the absolute amounts are also mentioned.

Differences between the relative and absolute amounts of FA were found for FA incorporated in brain, liver and RBC. The relative amount of each FA is influenced by the amount of the other FA present in the tissue, thereby affecting

the relative amount each other. The absolute amount of each fatty acid is the actual amount found in the tissue, and consequently gives a more correct view of the amount incorporated. In this discussion, when referring to the level of a fatty acid, the relative level is implied, whereas the amount indicates the absolute amount of the fatty acid.

There was no control group in this experiments. The animals were considered to be their own controls, and changes in the fatty acid profile in the tissues were assumed to be a response of the dietary intervention. Since the soy oil and soy lecitin diets did not contain long-chain PUFA of the n-3 family, the level of EPA, DPA and DHA in these two groups was undertaken to represent the basal level of these FA in brain, liver and RBC, and can be considered as a control groups [21]. Changes in the levels of EPA, DPA and DHA, must therefore be assumed to arise from dietary manipulation.

Table 4.1 summarize the dietary amount of EPA, DPA and DHA, and the rate of incorporation of these FA in the brain, the liver and the RBC.

Table 4.1: Rate of incorporation of EPA, DPA and DHA in brain, liver and RBC

Fatty acid	Tissue	Rate of incorporation (absolute amounts)
EPA		
	Diet*	**EE > cod liver oil > salmon > seal oil > MPL**
	Brain [1]	**Cod liver oil > seal oil > MPL > EE**
	Liver[2]	**Seal oil > salmon > EE > MPL**
	RBC[2]	**salmon > seal oil > EE > MPL**
DPA		
	Diet	**Seal oil > salmon > cod liver oil > MPL > EE**
	Brain	**cod liver oil > seal oil > MPL > EE**
	Liver	**Seal oil > EE > MPL > salmon**
	RBC	**Salmon > seal oil > EE > MPL**
DHA		
	Diet	**Cod liver oil > salmon > seal oil > EE > MPL**
	Brain	**Cod liver oil > MPL > seal oil » EE**
	Liver	**Seal oil > MPL > EE > salmon**
	RBC	**Salmon > seal oil > EE > MPL**

*Amount in diet, [1] No data for the salmon group, [2] no data for cod liver oil group. EE: ethyl ester, MPL: marine phospholipid.

4.3.1 Brain

The brain is a well-protected organ, regarding polyunsaturated fatty acids [48]. A restiction of n-3 fatty acid for a very short period causes few anomalies in the

PUFA profile of the brain and its organelles [48], however, other organs i.e., liver and plasma, respond rapidly to different amounts of n-3 FA in diets, and the PUFA profile is highly affected by diet [30, 107].

Animals fed marine n-3 FA showed considerably higher relative levels of EPA and DHA in the brain, whereas the differences between the different groups regarding absolute amounts were not as profound. EPA was only found in brains of animals fed diets rich in marine n-3 FA, whereas DHA was found in high amounts in all groups. Nevertheless, animals fed marine n-3 PUFA showed an enrichment of DHA in the brain. There was no major difference in the level of saturated [91] and monounsaturated fatty acids (20, 22 carbon) between the various diets, and the monosaturated fatty acids was not affected by the diet. The level of 18 : 1n-9 was not affected by the diets, which presumably indicates that the myelination of the nerve cells was not affected by the diets [30], since myelin contains high levels of 18 : 1n-9 [113]. 18 : 2n-6 level was highest in the animals fed soy, soy lecitin and cod, as well as the marine diets added maiz oil (ethyl ester and marine PL), indicating that the brain takes up this fatty acid [37], which is not syntesized by mammals. The level of DPA was increased in animals fed marine n-3 diets, suggesting that this fatty acid is either taken up by the brain, or synthesized within the brain [37].

The changes observed in the fatty acid profile of the brain reflects changes in total brain phospholipids. The fatty acid changes occuring in the brain may differ within the different types of phospholipids (PE, PI, PC *), as it is expected that the different phosphoplipid classes exhibit different rates of metabolism [114]. Alsted and Høy [115] found that the fatty acid profiles of the phospholipid subclasses were affected by dietary fats from marine source. The phospholipid subclasses respond differently to dietary manipulation, and PC and PS significantly increased the levels of DHA following fish oil feeding. Sommer-Hartvigsen and coworkers [37] found DHA levels to be similar in the phospholipids in the brain from animals fed different diets, however, the levels of DHA in brain PE differed significantly between the different diets.

EPA EPA was not detected in diets with low (cod) or no (soy and soy lecitin) marine n-3 FA, and was found only at trace amounts in the ethyl ester group. This indicate that unless EPA is administered directly, no significant accumulation occurs in the brain [115]. The content of EPA in brain was extremely low (ranging from 0.04-0.09 mg), even in the groups recieving high amounts of marine n-3 FA. This correspond well with other studies [30, 37], which have found low EPA and DPA levels, although the animals were fed high amounts of fish oil.

The amount of EPA found in brain tissue in the different dietary groups, does

*PE; phosphatidylethanolamine, PI; phosphatidylinositole and PC; phosphatidylserine

not seem to correspond to the amount administered through the diets, as shown in table 4.1. EPA amounts in cod liver oil, seal oil and marine PL groups seemed to be incorporated in a dose dependent matter, and the results suggest that the amount of EPA administered is more important than structural form, ans structural positioning within TAG of fatty acid, regarding the incorporation of EPA in brain. EPA in the form of ethyl ester on the other hand, did not incorporate in a dose-dependent matter, and EPA in the form of ethyl ester resulted in the lowest amount, although the dietary level was the highest. These results indicate that EPA is less incorporated into the brain when administered as ethyl ester.

DHA The amount of DHA in the brain excessivly overcome the amount of EPA and DPA, not surprisingly, since DHA is the most abundant fatty acid in the brain [29], and EPA and DPA are only found at low levels [30]. The levels of DHA remained relatively unaffected by ditary n-3 PUFA, indicating that DHA play an important role in the brain [116, 117], and is not easily manipulated by dietay means [118]. The result of Bourre and coworkers [118] suggests that brain DHA is highly preserved or maintained in the brain, at the expense of other organs.

There was a small, but significant increase in the relative level of DHA in the salmon, cod liver oil, seal oil and marine phospholipid groups compared to soy oil, ethyl ester, cod and soy lecitin. No difference was found at the relative level of DHA within the TAG groups. The absolute amount, however, showed that animals fed cod liver oil had significant higher amounts of DHA incorporated in the brain, than seal oil (no data for salmon). The significant increase of DHA in animals fed cod liver oil was most presumably due to the highest dietary amount of DHA. It is therefore reasonable to suggest that the incoporation of DHA in the brain is independent of the positioning of FA whitin the TAG molecule, when sufficient amounts of n-3 PUFA are available in the diet, as proposed by Christensen and Høy [91]. The results presented indicate that the structural form in which the FA are presented may play a role for the bioavailability of DHA to the brain. If presented as marine phospholipid, even at lower concentrations than TAG, the level of DHA increased to the same amount as seal oil fed animals. This suggests that phospholipids are incorporated at a high rate in the brain, even at lower dietary amounts [108]. Surprisingly, cod and ethyl ester seemed to reduce the level and amount of DHA in the brain compared to soy oil and soy lecitin.

One explanation to the small changes in the level and amount of DHA in the brain, may be the slow renewal of membranes in the brain [11]. 3 weeks of feeding diets high in marine n-3 FA may not be sufficient period of time to change the fatty acid profile in the brain to a great extent. Lim and Suzuki [96] suggested that it takes a certain period of time after the intake of dietary DHA, for it to be incorporated into the brain.

4.3.2 Liver

The liver hepatocyte (liver cell), is the main site for the biosynthesis of 20- and 22-carbon PUFA from 18 carbon precursors, and the formation of lipoproteins that transport fatty acids in the plasma. The liver production of PUFA is both for synthesis of its own membrane PL and for export and uptake by most other cells [10]. It is clear that the liver is able to desaturate ALA to DHA [83] and studies have provided evidence that plasma DHA, which could be derived from synthesis in liver or from diet, is taken up and esterified into brain lipids [25, 119].

Bourre and coworkers [30] showed that increasing amounts of dietary fish oil resulted in a parallel increase in the levels of long-chain n-3 PUFA in the liver. In the same study, it was showen that high dietary content of fish oil affected nearly all fatty acid in the liver, and the liver function remained normal.

The liver is more susceptivle to influences by dietary FA than the brain [30, 91], and the dietary changes regarding EPA, DPA and DHA were more profound than in brain. Animals recieving marine n-3 rich diets showed remarkably higher amounts of EPA, DPA and DHA [94], than animals recieving diets rich in n-6.

EPA The levels of EPA in liver varied considerably between the animals fed diets rich in marine n-3 FA and the animals fed n-6 rich diets, with an average of 7.2 % and 0.57 % respectively. The same was found for absolute amounts, being significantly higher in animals fed marine n-3 compared to vegetal diets ($3-4$ mg versus $0.2-0.4$ mg, respectively).

Surprisingly, no significant difference was found between the amount of EPA in liver of animals fed marine n-3 rich diets. The ethyl ester diet, containing the highest dietary amount of EPA did not result in higher amounts of EPA incorporated. The rate of EPA found incorporated in liver did not correspond to the dietary amount, indicating that the rate of EPA incoporated in the liver is affected by the structure of the FA. The lower rate of incorporation of ethyl ester might be a result of poor hydrolysis by pancreatic lipase compared to TAG, which is highly hydrolysed by this enzyme [105]. The lower EPA amount might be a result of conversion of EPA to DPA, since DPA levels in liver and RBC of animals fed ethyl ester corresponded to the DPA levels in animals fed higher amounts of DPA. The conversion of EPA to DPA is known to occur, whereas further conversion from DPA to DHA is highly restricted [80], due to the limiting step in DHA formation occuring at the level of elongation of DPA to 24 : 5n-3 by a second $\Delta 6$ desaturation [120]. The results might suggest that EPA in the form of ethyl ester is more readily synthesized DPA than TAG-EPA and PL-EPA.

The TAG diets contained higher amounts of DHA than ethyl ester, and the higher amount of EPA can be due to retroconversion of DHA to EPA. High levels of DHA have shown to give retroconversion to EPA [107]. There was no dif-

ference in the rate of incorporation between the structural positioning within the TAG molecule, suggesting that the structural positioning within TAG is not of importance when sufficient amounts of EPA are present in the diet.

These results indicate that the incorportation of EPA in liver is affected by the structural form of EPA administered, and that ethyl ester-EPA is either less effective than TAG and marine PL at increasing EPA levels in liver, corresponding well with other studies [105, 106], or has a higher degree of conversion to DPA.

DHA As for EPA, the levels of DHA was excessivly higher in animals fed diets rich in marine n-3 FA compared to animals fed diets rich in n-6 FA. The animals fed the TAG diets contained the highest level, followed by ethyl ester and marine PL, whereas the amount incorporated did not differ between the groups. The seal oil diet contained considerably lower amount of DHA compared to the salmon and cod liver oil diets, however the absolute amount of DHA incorporated in the liver did not differ significantly between these animals. This might be a result of the differences in the moleculare positioning in the TAG molecule, although, Christensen [91] found no major differences related to TAG structure regarding DHA in liver.

The amount of DHA found in the liver did not differ between the differerent structural forms introduced, even though the dietary amount differed greatly. These results indicate that the liver respond to the structural form of DHA presented in the diets.

4.3.3 Red Blood Cells

The fatty acid composition of the diet has been shown to have a major impact on the fatty acid profile in plasma lipid, platelets and RBC. Vidgren and coworkers [121] showed that fish diet and fish oil supplement increased the proportions of n-3 FA in the membrane of RBC.

The erythrocytes contained very low amounts of EPA, DPA and DHA. EPA levels were most affected by the diets, whereas the dietary changes in the levels of DPA and DHA were only minor. EPA, DPA and DHA was considerably higher in animals fed diets rich in marine n-3 FA compared to animals fed diets rich in n-6 FA.

EPA Animals fed diets diets with low or no marine n-3 (cod, soy and soy lecitin), contained extremely low amounts of EPA. As for the liver, ethyl ester, even at the highest dietary level of EPA, resulted in significantly lower levels incorporated in RBC compared to TAG diets. It does not seems to be any relationship between the dietary dose of EPA administered in the diet and the amount of

EPA found in the RBC. This suggests that the differences in the level of EPA in RBC is caused by the structure of the fatty acid rather than the amount.

DHA The amount of DHA incorporated in RBC appears to be dose dependent. The diets with the highest amount of DHA showed the highest levels of DHA in the RBC. The molecular structure of the TAG molecule does not seem to matter for the incorporation of DHA into RBC. Surprisingly, the amount of DHA in animals fed cod was similar to the marine PL, even though the amount in the diet was considerably lower. It must, however, be noted, that the increase in the cod, ethyl ester and marine PL diets compared the basal level of the soy oil and soy lecitin groups, is not more than about 0.01 mg. It is difficult to draw any conclusions regarding the rate of incorporation of DHA based on the low amounts present in RBC.

4.3.4 n-6 Fatty Acids

The conversion of linoleic acid (LA) to arachidonic acid (AA) appears to be highly regulated. Dietary LA poorly correlate with the AA content in tissues [19], and the level of this fatty acid is not dramatically affected by diets [21].

It has long been know that increasing dietary levels of n-3 PUFA progressively decreases the AA content of tissue phospholipids [96, 115]. Marine n-3 PUFA are more effective than vegetal n-3 FA in altering the FA compostition of tissue PL [19], and the antagonistic effect of EPA to AA and its metabolism are most likely explained by the structural similarities between AA and EPA.

In this study diets containing high levels of marine n-3 LCPUFA affected the levels of AA and DGLA, as found by others [37, 38, 70, 96, 122]. The TAG, marine PL and ethyl ester groups showed decreasing levels of AA in liver and RBC, but not in brain, compared to soy oil, soy lecitin and cod. The amounts of AA in brain did not respond highly to the diets presented. Interestingly, marine PL lowered the levels of AA in liver and RBC, but not in brain, whereas ethyl ester fed animals showed great decreased brain levels of AA. The reduction in AA amounts might result from decreased $\Delta 6$ desaturase activity mediating LA conversion to AA, which has been shown to be reduced in rats fed dietary fish oil [123], or by increasing competition from n-3 FA for the desaturase and elongation enzymes needed for the convertion of LA to AA [81]. The decrease in AA was accompanied by an increase in DGLA in salmon, ethyl ester and marine PL groups. These result might suggest an inhibition of the conversion of 20 : 3n-6 to 20 : 4n-6 by $\Delta 5$ desaturase [37, 104], or an increased synthesis of LA, being the highest in these diets. However, brain levels of AA were unchanged in all diets, although slightly lower in the marine n-3 diets than the n-6 diets.

Excess dietary LA leads to a specific accumulation of DPA n-6 [20]. The levels of DPA n-6 have been shown to be inversely related to the proportion of DHA, and several studies report that increasing levels of DHA are followed by decreasing levels of DPA n-6 [21, 22, 27, 37, 122]. These studies suggest that DHA might be an inhibitior of DPA n-6 synthesis or, alternatively, a better sbustrate for reacylation of the phospholipids. DPA n-6 was not investigated in this thesis, but should be included in further studies.

4.4 Organ Comparison

The natural levels of EPA and DHA vary considerably between the brain, the liver and the RBC of rats. EPA was not detected in the brains of animals not recieving marine n-3 FA, and was found in extremely low amount (0.01 mg) in RBC of animals fed diets depleted of marine n-3. The highest basal level of EPA and DHA were found in the liver (0.2 mg) and brain (5 mg) respectively, whereas the RBC contained the lowest basal level of DHA (0.05 mg). The different amount of these FA indicate that EPA and DHA play different roles in the body, and are therefore affected differently by dietary means [22, 42].

The diets rich in marine n-3 PUFA (salmon, ethyl ester, marine PL, seal oil and cod liver oil) affected brain, liver and RBC in different manners. As shown by PCA the marine n-3 diets highly correlated in the brain, indicating that the marine diets not differed considerably regarding the incorporation of EPA and DHA. The dietary influence varied more in the liver, suggesting that the liver respond differently the to diets rich in marine n-3 FA introduced in this experiment than the brain. The RBC showed similar dietary response as the liver, however the diets seemed to correlate more. These results indicate that the various diets result in different response in different tissue, and that individual tissues process PUFA in different ways [10, 22].

The tissues vary in their response to the structural differences of the n-3 PUFA administered. In the brain and liver, the marine PL increased DHA levels to the same extent as the TAG diets, even at considerably lower levels in the diet, suggesting that is is a good structural form for increasing DHA levels in brain. The same is not true for the RBC, where the marine PL diet resulted in significant lower amounts of EPA and DHA. The tissues investigated also differed in their respons to the differences within the TAG molecule. In liver, the seal oil diet resulted in similar amount of DHA incorporated compared to salmon, even at considerably lower dietary level, indicating that the positioning of the FA in the TAG structure affect the bioavailability, whereas the same was not found for the brain and the RBC where the incorporation of TAG appears to be dose dependent. Liver and RBC respond highly to the dietary changes introduces during the 3 week feeding

period, whereas for the brain, 3 weeks did not seem sufficient to introduce major changes in DHA levels.

The different dietary responses by different tissues highlight the importance of examining more than just neural tissues in experiments that undertake dietary manipulation [22]. The physioloical effects induces by dietary marine n-3 in different tissues may influence or affect the response in other tissues.

The diets rich in 18 : 3n-3 (soy and soy lecitin) did not show high levels of n-3 long-chain PUFA in the brain, the liver, or the RBC indicating that the conversion of 18 : 3n-3 to its longer chain metabolites (EPA and DHA) is low in rat, and that 18 : 3n-3 is not as effective as direct intake of EPA and DHA in increasing the level of EPA and DHA in rat [22, 124]. Unfortunatly, there is no real control group to compare the amount of EPA and DHA in the soy and soy lecitin groups. Therefore, it is not possible to say whether or not the levels of EPA and DHA are increased in animals fed soy and soy lecitin compared to a ordinary rat chow diet.

4.5 PUFA and the Brain

Brain EPA and DHA must be obtained either by uptake and further desaturation and elongation of 18 : 3n-3, by other intermediary metabolites, or by uptake of DHA itself from plasma[120]. It has been demonstrated that it is the brain astrocytes that are capable of forming DHA [125], and it has been suggested that astrocytes may be important in supplying DHA to other brain cells.

The brain is able to synthesize DHA from 18 : 3n-3 [126], however, the majority of the accumulating DHA seems to be synthesized in the liver [25] if 18 : 3n-3 is the main source of n-3 PUFA, and transported, possibly by chylomicrons [127], to the brain. If dietary DHA is available, this can probably be transported to the brain either in chylomicrons or in VLDL [91]. Innis and coworkers [120] suggest that the synthesis of DHA from n-3 FA precursors is not the major route through which developing brain accumulates high concentrations of DHA.

It has been shown that a modification in the dietary fatty acid profile affects the fatty acid profile of cellular membranes and thereby the property of the membrane components, receptors and enzymes. Dietary changes may therefore be of consequence for cognitive functions such as learning and memory [37]. These effects on cognitive performance have mostly been demonstarted in animals deficient in n-3 FA, which exhibit impaired learning ability [28].

Studies have shown that DHA or fish oil (rich in both EPA and DHA) enhanced spatial memory or other types of memory in normal and aged animals [45, 103]. However, the durations of the exposure to these diets were over two of four generations, or, alternatively, following high n-3 FA concentrations.

The brain fatty acids are more susceptible to dietary influence at an early stage

of development than when fully developed. [27, 27, 37, 38, 39]. Several studies have shown that an early supply of n-3 PUFA is able to affect brain function and that the reversibility of changes should be dependent on the developmental stage during which the deficiency occurs [39]. Moriguchi and Salem [27] explored the issue of reversibility of losses in spatial task performance associated with n-3 PUFA deficiency in relation to the stage of development. Animals were rendered deficient of brain DHA by deprivation of n-3 dietary fats through 3 generations. Their findings gave indications that the performance in spatial tasks are closely related to the level of brain DHA. When the rats were switched to an n-3 adequate diet at birth, performance on the spatial learning and memory task was very similar to the control group (adequate n-3 diet). When the rats were switched to n-3 adequate diet at weaning, the brain had substantially recovered after 6 or 10 weeks, and performance was significantly different from the n-3 deficient group. The young adult group were switched to the n-3 adequate diet at 7 weeks of age. After 6 weeks of diet repletion, nearly full recovery had occurred in the probe trial and partial recovery for the spatial learning task. These findings are important as they show that brain function can recover from a severe and extended n-3 fatty acid deficiency in the nearly fully developed brain. Thus, juveniles and young adults depleted of adequate n-3 fatty acid sources in early develpment, may be expected to regain brain DHA and improve at least some aspects of brain function once adequate sources of dietary DHA are provided.

However, in most studies, the exact time when changes in the fatty acid composition occured, after supplementation began, is rarely investigated. Another factor to consider in comparing the time course required to change brain fatty acid profil is the concentration of supplement being used, ranging from 5 to 20% of the diet [128]. Improved learning resulting from high intake of DHA may be associated with greater membrane fluidity. Synaptic membranes in mice fed DHA-rich diet have been reported to be more fluid than in control mice [129].

4.5.1 PUFA Deficiency

Effects of fatty acid composition on brain performance have mostly been demonstrated with animals deficient in n-3 PUFA. Animal models of n-3 FA deficiency can be used to help define the nature and extent of the functional deficits in the nervous system that are associated with low or no intake of n-3 PUFA [28]. By inducing n-3 deficiency, the variable to be studied may be magnified and behavioral consequences may be more easily discerned.

A deficiency of dietary n-3 FA will not cause anomalies in the brain unless extremely prolonged, i.e., over several generations. By feeding rats diets depleted of n-3 FA, the brain cells and intracellular organelles will conserve a normal quantity of total quantity of PUFA, however the various cell types and organelles show a

consistent decrease in DHA, that will be compensated for by an increase in DPA n-6 [11, 21, 42, 48].

Nonhuman primates deprived of dietary n-3 FA during gestation and infancy show depletion of n-3 PUFA from plasma lipids, and this depletion is associated with a significnat impairment in the development of visual acuity [26]. Several previous studies have reported that n-3 fatty acid deficiency can impair spatial learning ability [11, 21, 130] and memory, which can be reversed by a diet rich in marine n-3 [27, 41].

Results have shown that nervous system DHA can be restores, although slowly, after dietary depletion of n-3 FA during development [46, 48]. However, these studies used 18 : 3n-3 as the n-3 source, and the synthesis of this fatty acid to long-chain PUFA is know to be slow [80]. Although DHA levels can be restored, there are indications that the functional effects of the DHA decline may not be reversible. Juvenile n-3 deficient rhesus monkeys repleted DHA levels when fed fish oil rich in DHA [47], however the electroretinographic changes observed during n-3 deficiency persisted [4]. Early n-3 PUFA deficiency, regardless of subsequent supply, resulted in hypertension in rats [6]. These studies demonstrate that the duration of the n-3 deficient feeding and therefore the age at which repletion begins, along with the length of repletion can be important variables [27]. The differences in behavioral performance found between n-3 deficient and control animals might reflect irreversible structural damage to the brain induced by the periode of n-3 deficiency in early development [27]. This is supported by observations that adult rat brain hippocampal neurons [131, 132], as well as those in other brain areas [132] are smaller after DHA deficiency.

4.6 Marine n-3 versus Plant n-3

α-linolenic acid (ALA, 18 : 3n-3) is found in vegetable oils, such as flaxseed oil ($45-50$ %), rapseed oil (10 %) and soy oil ($7-10$ %). If ALA can be fully syntesized to EPA and DHA in the human body, one could expect the same health benefits from 18 : 3n-3 rich vegetable oil as direct intake of marine oil [7]. This has not yet been demonstrated, and there is little evidence that the consumption of vegetable oils rich in linolenic acid will have the same beneficial effect in preventing or treating chronic diseases such coronary heart disease. A study by Finnigan and coworkers showed that ALA, and EPA and DHA have different physiological effects [133].

In a study by Sanders and Roshanai [134], long-chain PUFA increased markedly in platelets after supplementation with marine oil, whereas flaxseed oil failed to show the same effect. The flaxseed oil gave only a modest increase in platelet EPA, and DHA was not modified. This confirms that ALA is not readily con-

verted to EPA and still less so to DHA [22, 26], in addition to being less effective in increasing n-3 long-chain PUFA than a direct intake of these FA [133, 134].

Bourre and Dumont [124] showed that dietary porcine brain phospholipids (rich in long-chain PUFA) are much more efficient than soybean phospholipids (rich in 18 : 3n-3) for ensuring a normal level of DHA in tissues and brain subcellular fractions. The study revealed that by giving the same amount of n-3 FA in the diet, lower tissue levels of DHA were observed when soybean PL were used compared to brain PL. The study concluded that dietary porcine brain PL is a better source than soybean PL for attaining normal levels of DHA in tissues and subcellular fractions. This is in accordance with other studies [22, 103, 115] showing that long-chain PUFA are not readily made from 18-carbon precursors.

Numerous studies have compared fatty acid profile in vegans and omnivores [135, 136], and showed that EPA and DHA levels were lower in vegans than in omnivores[†], as well at the ratio of n-3 / n-6 FA. In a study by Agren *et al.* [135], the fatty acid profile of subjects eating a strict uncooked vegan diet for years was compared to ominvore controls. In vegetarians, a prolonged consumption of high levels of ALA resulted in very low levels of n-3 PUFA in RBC, platelets and serum lipids.

Intake of fish oil, or other marine sources rich in EPA and DHA, is accompanied by elevated levels of DHA in all tissue phospholipids, including brain lipids [115], suggesting that preformed long-chain PUFA are more readily incorporated and therefore better to secure optimal brain function than vegetal sources rich in ALA.

In summary, I found that by feeding diets rich in marine n-3 long-chain PUFA, higher levels of these were found in the brain, the liver and the RBC of rats. The liver and RBC responded rapidly to the increased marine n-3 FA, whereas the brain fatty acid profil was less susceptible to dietary changes, also shown by Abedin and coworkes [22].

The tissues investigated in this work responded differently to the different sources of long-chain n-3 PUFA introduced, and caution must be therefore be made when suggesting that RBC levels of n-3 long-chain PUFA reflect brain levels of these FA. In brain and RBC, EPA amounts seem to respond in a dose-dependent matter, apart from the ethyl ester group, whereas the amount of DHA in brain and liver seems to correlate with the structural form of FA introduced in the diets.

EPA in the form of ethyl esters appeared to be less incorporated than TAG-EPA and PL-EPA in all organs studied in this work, and especially in the brain.

[†]Omnivore are animals/humans that consume both plants and animals.

The lower incorporation rate of ethyl esters might result from a lower hydrolyses by pancreatic lipase, hence, their absoption is slower when fed in this form [105, 106]. n-3 PUFA presented as marine PL seems to be a good structural form for increasing levels of EPA and DHA, especially in the brain. Therefore, the industry, when making n-3 supplements containing long-chain n-3 PUFA, should consider the form in which the FA are presented.

4.7 Statistical Considerations

The pyramide in figure 2.1 represents the order in which the data were analyzed. The large data set from tables 3.3, 3.4 and 3.5 made it necessary to apply principal component analysis (PCA) in order to get a general idea of correlation patterns and responses in the organs investigated, before starting the ANOVA analysis. The PCA plots in figure 3.1, 3.2 and 3.3 show how the diets rich in long-chain n-3 PUFA clearly separated from the diets rich in n-6 FA, in all organs investigated. Thus, PCA was a good statistical approach to structure the huge data set generated from the experiments in this work.

Conclusions

The aim of this thesis was to investigate different structural forms of marine n-3 FA introduced in diets, and the incorporation in the brain. Liver and RBC were included, in order to observe the relationship between the rate of incorporation in these organs.

- Liver and RBC fatty acid compostition are more susecptible to dietary FA than the brain, and can be easily manipulated by dietary means during a 3 week feeding period. The rapid increase of n-3 PUFA in liver and RBC levels compared to brain, suggests that it takes a longer period of time after intake of n-3 PUFA for EPA and DHA to be incorporated into the brain. Thus, a longer feeding period is probably needed to markedly change the fatty acid compostition in the brain.

- The structural form of the marine n-3 FA introduced affects the bioavailability for EPA and DHA in the liver and brain. The RBC appears to respond to the dietary FA in a dose dependent matter.

- Dietary DHA introduced in the form of marine phospholipid appears to be an efficient source for increasing the level and amount of DHA in the brain. Further investigations are needed to confirm this result.

- Ethyl ester does not seem to be an efficient form for increasing EPA and DHA levels in the brain, the liver and the RBC compared to TAG and marine PL. However, further investigation is needed in order to establish if the reduction of DHA found in brain is actually caused by the ethyl ester introduced, or an analytical mistake. Feeding animals ethyl ester over a longer time period may aslo be necessary, as it appears that ethyl esters are incoporated at a slower rate than TAG.

- The positioning of the marine n-3 FA within the TAG molecule seems to affect the bioavailability of EPA and DHA in liver and brain. The seal oil diet contained less marine n-3 than salmon and cod liver oil, nevertheless, the level of EPA and DHA incorporated in liver and brain did not differ significantly between the 3 TAG groups.

- There were differences the relative levels versus absolute amount incorporated for some of the FA investigated. Hence, the relative results do not entirely correspond with the absolute amounts.

- Marine n-3 PUFA are more effective in increasing tissue levels of EPA and DHA, than their 18-carbon precursor (ALA).

The overall significance of this study is that different structural forms of n-3 FA seem to incorporate differently in the brain, the liver and the RBC. Marine phospholipid, together with salmon, seal oil and cod liver oil, are good sources for increasing n-3 PUFA levels, whereas the incorporation of EPA and DHA in the form of ethyl esters seem to be poor compared to the other marine sources investigated.

Future Directions

3 weeks do not seem to be sufficient amount of time to change the fatty acid profile in the brain to a great extent. Feeding rats the same experimental diets for a longer period, would give a better general idea of how the brain respond to the dietary n-3 sources introduced in this work.

The different sources used in this trial contained remarkably different amounts of EPA and DHA, and it was not possible to make eight diets with equal amounts of long-chain n-3 PUFA from the sources used. For more acurate comparsions between the different structural forms of the n-3 FA used in this work, more comparable amounts are needed in the diets. In order to achieve equal or comparable amounts of EPA and DHA, or total amount of these two FA (EPA+DHA), fewer diets must be used for comparisons.

In this experiment, the daily food intake did not differ significantly between the different groups, however some of the groups have eaten more, and hence might have gotten higher doses of the marine n-3 FA. To ensure that all animals consume similiar amounts of the marine n-3 FAs, the amount of diet given should be adjusted to the group/cage eating the least.

The brain appears to be more susceptible to dietary changes in the fatty acid profile, at an early stage in development than when fully developed. The animals used in this study were in young adulthood, and the dietary influence might have been different in younger and/or older animals. It is therefore necessary to include all 3 developmental stages (young, adulthood and aged) before making any conclusions on how the brain respond to the dietary marine n-3 FA presented in this study.

Observations that n-3 FA affect gene expression of a number of genes in the brain opens the way towards an understanding of the role these FA play in the function of central nervous tissue. Until recently, it was belived that one of the major functions of long-chain PUFA, particularly DHA, was to maintain proper biophysical property and structural integrity of neural membranes. Kitajka and coworkers [34] assigns an additional function to these FA, namely to control gene expression in nervous tissue. According to the same group, it seems that in different tissues, different genes respond differently to the same fatty acid, although each cell is supplied with the identical genome. It would be of great interest to investigate the diets used in this work at a molecular level. That is, how the dietary sources used in this work affect the level of gene and/or mRNA expression in brain and other tissues.

n-3 FA are known to excert positive health effects in a number of diseases, and there is an increasing interest in n-3 PUFA and mental health. In order to understand cognitive changes presumably induced by n-3 PUFA, a better understanding of the mechanisms in which the PUFA act in the brain is needed.

n-3 PUFA are distributed differently in brain regions in mice, and dietary effects do not affect all regions similarly [137]. Chronic n-3 PUFA deficiency diet alter the PUFA composition differently in brain regions in rats [42]. According to Söderberg *et al.* [12], DHA decrease excessively in the hippocampus of patients with Alzheimer's disease. The hippocampus is an anatomical well defined structure, and easy to separate from the rest of brain tissue. It would be therefore be interesting to invegstigate the effect of the different diets introduced in this work on hippocampus, as well as other brain regions i.e., cerebellum and frontal cortex.

A lot of research has been performed on total brain fatty acid compostition, or different brain regions fatty acid composition, and fewer studies have investigated dietary effects on different brain cell types. Jumpsen *et al.* [138] found that feeding different diets produced larger changes in glial cells compared to neuronal cells in young rats. The majority of DHA incorporated into the brain have been found to be recovered in micosomal, synaptosomal and mitocondrial fractions [139]. It would therefore be of great interest to investigate the accretion of long-chain PUFA from different marine sources in different cell types and brain fractions, i.e., asterocytes, glial cells, neuronal cell bodies, nerve cells, synapses, synaptosomes.

Generally, fats characterized by gas-liquid chromatographt (GLC), as in this thesis, will only provide the fatty acid profile without any information on species compostition or location of FA. A more detailed description, including fatty acid profile in phospholipid subclasses (PE, PC and PI) or regiospecific location of the fatty acids in TAG, would be of interest in order to get a more accurate view of the dietary changes occuring in the fatty acid profile. Especially since it is know that DHA content varies considerably in the different phospholipid species, and DHA is especially abundant in phosphatidylserine and phosphatidylethanolamine of membranes in general [115, 120], in brain gray matter [29], and in synaptosomal membranes [13]. Moreover, it has also been shown [37, 138] that the different phospholipid species resond differently to dietary changes.

Studies report that increasing levels of DHA in the brain result in decreasing levels of DPA n-6 [21, 22, 37, 122]. DPA n-3 was not investigated in this work, but should be included in further studies.

The relative amount and absolute amount found incorporated differed for some FA, and in some tissues. For better understanding the amount of FA, and rate at which they are incorporated, absolute amounts must be used, especially when using diets varying in the amounts of spesific FA, i.e., 18 : 2n-6 in this work.

In terms of animal models, it needs to be recognized that some mammals provide better models of nutritional studies. Differences in retinogenesis, the development of retinal function, has been reported to be more similar in man and guinea pig, than it is in rats. Moreover, the rate of conversion of 18 carbon PUFA to long-chain metabolites is slower in guinea pig than rats and may be more closely related to the rate of conversion seen for humans. According to Abedin *et al.* [22] guinea pig is a better animal model than rat when examining tissue DHA levels and physiological function resulting from dietary manipulation of n-3 PUFA. Rioux *et al.* [70] report of diet induced changes in FA compostition in a studie with piglets, a species that share many similarities in physiology and lipid metabolism with humans.

Diets rich in marine n-3 resulted, in some of the animals, in fatty liver. This needs further investigation, in order to establish the cause of increased fatty liver from diets with high content of n-3 long-chain PUFA.

5

Appendix

5.1 Diet Preparation

5.1.1 Basal mix

The basal mix preparation was kindly prepared by technician Åse Heltveit. Its content is shown in table 5.1.

Table 5.1: Basal mix

Basal mix	Amount
Vitamin mix	**1**%
Mineral mix	**3,5**%
“soupe”	**1**%
L-cysteine	**0,3**%
Choline	**0,25**%
Cellulose	**5**%
Sugar	**9**%
t-butylhydroguinone	**0,001**%

5.1.2 Marine Phospholipids

Certificate of Analysis

The marine phospholipids used in this experiment was extracted from Capelin (*Mallotus villosus*) roe. The specifications of this product is shown in table 5.2 as given by EXIMO AS (Tromsø, Norway)

Table 5.2: Specification of Marine PL

Specification	**Result** [1]
Appearance	**Orange/brown**
Phospholipids [2,3]	**50% of total lipids**
- Lyso Phosphatidylcholine	**2-4%**
- Phosphatidylethanolamine	**10-15%**
- Lyso Phosphatidylethanolamine	**1-3%**
Neutral lipids	**40-50% of total lipids**
- Triacylglycerol	**15-30%**
- Cholesterol	**10%**
- Free Fatty Acids	**10-12%**

[1]Analytical values are based on dry weight material.

[2]Total Phospholipids are caluculated from measurements of total phosphorous

[3]Quantitative analyses of individual lipid classes by HPTLC

The fatty acid composition of the same product is shown in table 5.3. Also as given by EXIMO AS (Tromsø, Norway)

Table 5.3: Fatty Acid Composition of Marine PL

Fatty Acid	**% of total**
Myristic (14 : 0**)**	4.2
Palmitic(16 : 0**)**	23.1
Stearic (18 : 0**)**	2.9
Oleic (18 : 1**n**-9**)**	6.9
Linolei(18 : 2**n**-6**)**	1.0
Linolenic (18 : 3**n**-3**)**	0.6
Aracidonic (20 : 4**n**-6**)**	1.1
Eicosapentaenoic (EPA, 20 : 5**n**-3**)**	9.6
Docosahexaenoic (DHA, 22 : 6**n**-3**)**	25.1

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