INTERACTIVE EFFECTS OF FISH OIL AND METHYLMERCURY ON THE

FATTY ACID PROFILE OF ADULT RAT FOREBRAIN PHOSPHOLIPIDS

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INTERACTIVE EFFECTS OF FISH OIL AND METHYLMERCURY ON THE FATTY ACID PROFILE OF ADULT RAT FOREBRAIN PHOSPHOLIPIDS

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VITA

Julie Taylor Baker, daughter of Roger and Dinah Taylor, was born December 10, 1981, in Lebanon, Virginia. She completed her secondary education at Lebanon High School in June, 2000. She entered Southwest Virginia Community College in August, 1999 and then transferred to Radford University in January, 2001. She completed her Bachelor of Science in Foods and Nutrition in May, 2004. In August, 2004, she was accepted as a graduate student in the Department of Nutrition and Food Science at Auburn University. During this time, she married Austin Lafayette Baker in August, 2006.

THESIS ABSTRACT

INTERACTIVE EFFECTS OF FISH OIL AND METHYLMERCURY ON THE FATTY ACID PROFILE OF ADULT RAT FOREBRAIN PHOSPHOLIPIDS

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Docosahexaenoic acid (DHA), a fatty acid found in cold water marine fish is a long chain polyunsaturated fatty acid (LCPUFA). It is important for cognitive and motor function, vision, and infant development. Fish is also the major source of methylmercury (MeHg) exposure, a known environmental pollutant that, when ingested, results in neurological, motor, developmental, and visual deficits. There are similar effects of MeHg toxicity and DHA deficiency, and it is postulated that the detrimental effects of MeHg on neural function could be related to a decrease in DHA in brain phospholipids.

The objective of this research is to determine if chronic exposure to MeHg alters the forebrain fatty acid profile of the adult rat, and if dietary fish oil could assist in overcoming the adverse effects of mercury exposure.

Female Long-Evans rats, approximately five months old, were assigned a dietary treatment which contained a mixture of four oils: palm, safflower, soybean and either

coconut (without n-3 LCPUFA) or fish oil (with n-3 LCPUFA), and three concentrations of mercury as methylmercuric chloride (0.0, 0.5, and 5.0 ppm) dissolved in water. The fish oil diet contained 5% of fatty acids as DHA, whereas the coconut oil diet contained saturated fatty acids in the place of the n-3 LCPUFAs. At 18 months of age the rats were euthanized, tissue samples collected and quick-frozen in liquid nitrogen. Lipids were extracted and phospholipids separated into phosphatidylethanolamine (PE) and phosphatidylcholine (PC) fractions using thin-layer chromatography. These fractions were then analyzed for fatty acid composition using capillary gas chromatography. The differences among groups, main treatment effects, and the interaction between diet and MeHg exposure were assessed using a two way ANOVA procedure with post hoc Tukey's Studentized Range test. The results were reported as adjusted values and were considered significant at $P \leq 0.05$.

In the adult rat forebrain, chronic MeHg exposure had no significant effects on the alteration of the major LCPUFA; DHA and arachidonic acid (ARA), as well as total n-3 and total n-6 fatty acids. However, there was a dietary effect showing a significant increase in DHA in both the PE (23-30% increase) and PC (25-46% increase) fractions in animals consuming dietary fish oil compared with those consuming the coconut oil diet ($P<0.001$). There were significant decreases of ARA in both the PE (18-20% decrease) and PC (12-28% decrease) fractions. Also, there were reciprocal effects of total n-3 (net increase) and total n-6 (net decrease) fatty acids in both PE (n-3, 23-31% increase; n-6, 19-24% decrease) and PC (n-3, 26-47% increase; n-6, 28-29% decrease) fractions in response to dietary fish oil. Overall, there was no evidence showing a relationship between LCPUFAs and MeHg toxicity in the adult rat brain.

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CHAPTER I. INTRODUCTION

Vertebrate animals are incapable of synthesizing long chain unsaturated fatty acids *de novo* due to a lack of desaturase enzymes. Thus, these fatty acids are considered essential and must be provided in the diet. The human diet contains essential fatty acids primarily in the form of α-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6) synthesized by plants. ALA is the 18-carbon precursor for the n-3 fatty acid family which is converted to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). LA is the 18-carbon precursor for the n-6 fatty acid family which is converted to arachidonic acid (ARA; 20:4n-6). The biochemical pathways involve a series of elongation and desaturation steps ending with ß-oxidation.

Among the tissues of the body, nervous tissue is one of the richest in lipid content. About 50-60 percent of the brain dry weight consists of membrane lipids, and 20 percent of these are essential fatty acids. Apart from their quantitative importance, the lipids of the nervous tissue show a great deal of structural diversity. Polyunsaturated fatty acids (PUFA), most importantly DHA, are the main components of cell membranes and contribute to membrane fluidity and may protect against lipid peroxidation and toxicants. Polyunsaturated fatty acids of the n-3 series are important for growth and development, cognitive function, vision, and motor skills.

Methylmercury (MeHg) is a well known environmental toxicant found in the aquatic ecosystem. MeHg accumulates in the aquatic food chain and reaches its highest

concentrations in long-lived predatory fish, such as shark and swordfish, in the oceans, and pike and bass in freshwater. A number of molecular targets and mechanisms have been proposed to be implicated in its neurotoxic effects. Exposure to MeHg has resulted in neurological, motor, developmental, and visual deficits. Central nervous system damage following MeHg exposure in adults is primarily in specific areas, such as the granule layer of the cerebellum and the visual cortex of the cerebrum.

The presence of high levels of the very long-chained n-3 fatty acids, EPA and DHA, are identified as one of the major benefits of ingesting fish-derived lipids and consequently, fish are the most common source of MeHg. Health effects of MeHg chronic exposure may be important in humans, because all the fish we eat contain a trace amount of MeHg. The increasing daily fish consumption typical of modern society as a part of a healthier diet may result in chronic low-level dietary intake of MeHg and thus poses a significant toxicological problem, especially to susceptible individuals, such as developing embryos or fetuses. The functional regions impaired by MeHg overlap those also impaired by n-3 PUFA deficiency.

The working hypothesis of this study is that chronic MeHg exposure will result in the alterations of adult rat brains by decreasing concentrations of long chain (>C-20) polyunsaturated fatty acids in the membrane phospholipids. Another goal of this investigation is to determine if animals supplemented with 5% of DHA through dietary fish oil will have an increased concentration of DHA in brain tissue phospholipids. Most importantly, the experiment is designed to test if the dietary fish oil will counterbalance the detrimental effects brought about by the MeHg toxicity in the adult brain by returning fatty acid concentrations to normal levels.

CHAPTER II. REVIEW OF LITERATURE

Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) are a family of long chain fatty acids (18-24 carbons) with the first double bond situated at the third or sixth carbon atom from the methyl end. Determined by the position of their first double bond, PUFAs are divided into two families: n-3 and n-6 fatty acids.

The first demonstration of the requirement for fat in the diet was in 1929, when Burr & Burr demonstrated that rats reared on a fat-free diet failed to grow, developed pathologies and subsequently died (1). Vertebrate animals are incapable of synthesizing long chain unsaturated fatty acids *de novo* due to a lack of omega-3desaturase and delta-12 desaturase enzymes, and thus are considered essential and must be provided in the diet. However, humans do have the enzymes capable of elongating and desaturating fatty acids to produce long chain polyunsaturated fatty acids (LCPUFA) from their precursors. The human diet contains essential fatty acids primarily in the form of α-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) synthesized by plants. ALA is the 18-carbon precursor for the n-3 fatty acid family which is converted to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). LA is the 18-carbon precursor for the n-6 fatty acid family which is converted to arachidonic acid (ARA; 20:4n-6). The biochemical pathways for the synthesis of LCPUFAs involve a series of elongation and desaturation steps ending with ß-oxidation.

The delta-6 desaturase and the delta-5 desaturase introduce double bonds between the pre-existing double bond and the carboxyl end of the fatty acid and are considered rate limiting steps in the desaturation/elongation pathway. In a recent review, Nakamura et al (2) pointed out that n-3 and n-6 fatty acids are thought to use the same series of desaturase and elongase enzymes (**Figure 1**). The competitive interaction between LA and ALA appears at the level of the delta-6 desaturation, although ALA may also inhibit delta-5 desaturation in n-6 fatty acids. The preferential order for desaturation/elongation is well supported as 18:3 n-3 > 18:2 n-6 > 18:1 n-9 by in vitro and in vivo studies using animal models (3-4). All metabolic conversions occur without altering the methyl end of the molecule that contains the n-3 and n-6 double bonds and once ingested, n-3 and n-6 fatty acids are not interconvertible. It is now generally accepted that n-3 fatty acids are essential, but it is debated whether conversion of ALA to DHA can fulfill the needs for the brain, or whether intake of preformed DHA is the most adequate method for accumulation in the brain (5).

Brain fatty acid composition

Among the tissues of the body, nervous tissue is one of the richest in lipid content. About 50-60 percent of the brain dry weight consists of membrane lipids and 20 percent of these are essential fatty acids. Apart from their quantitative importance, the lipids of the nervous tissue show a great deal of structural diversity. In a comprehensive review of lipids in the nervous tissue, by Sastry, the different types of lipids in nervous tissue are enumerated. The lipids are found mainly in nerve cell membranes as complex lipids participating directly or indirectly in the functions of the membrane-bound

macromolecules (6). In mammalian species, DHA is found in high concentrations only in select tissues, such as synaptosomes (7), sperm (8), retinal rod outer segments (9), and brain (10). Limited tissue distribution of high DHA levels implies a specialized, but as yet undefined, role for DHA in these cells (11).

In neural membranes, phospholipids perform important functions. Certain sets of phospholipids are selected for each membrane to give it unique characteristics suited to its role. These characteristics are not the properties of individual phospholipid classes but properties of an organized neural membrane as a whole. As reviewed by Farooqui et al, (12), among the membranes of the brain, the most abundant phospholipids of mammalian tissues are, phosphatidylcholine (PC), and phosphatidylethanolamine (PE), phosphatidylserine, and phosphatidylinositol. Sastry has pointed out that ethanolamine phosphoglycerides are quantitatively the major phospholipids in the nervous tissues and the predominant form is the ethanolamine plasmalogen. It accounts for 50-60 percent of the ethanolaminephosphoglyceride class of lipids in the whole brain. The alkylacyl analogue content is relatively low in the nervous system and amounts to 3-7 percent of the ethanolamine phosphoglyceride class in the brain. Phosphatidylethanolamine makes up the remaining amount of ethanolamine phosphoglycerides. The predominant form of choline phosphoglycerides occurring in the nervous tissue is PC. The choline plasmalogen and the alkyl analogue account for only about 2 percent of the total choline phosphoglycerides in the brain. About 9-10 percent of dry matter of the brain is PC and it accounts for 30-35 percent of total phospholipids (6).

Phospholipids undergo a rapid deacylation-reacylation process involving phospholipase A_2 and acyltransferases, resulting in continuous shuttling of fatty acids

between different phospholipid subclasses. The deacylation-reacylation cycle is responsible for the introduction of PUFAs into phospholipids. The deacylation-reacylation cycle is an important mechanism for controlling saturated and PUFA phospholipid acyl group composition in neural membranes (13).

A major portion of brain phospholipids contains long chain PUFAs of the two essential fatty acid classes, n-3 and n-6. These fatty acids usually occupy the sn-2 position of brain phospholipid molecules (13). The most prominent essential fatty acids in brain phospholipids are 20- and 22-carbon atoms long and the most common LCPUFA fatty acids in phospholipids are ARA (20:4n-6) and DHA (22:6n-3). Tinoco (14) reported that the general composition in the phospholipid fractions of the brain contained very little LA and very high proportions of 18:1 n-9. Arachidonic acid was found to be an important component in brain phospholipids, but the major PUFA present was usually DHA. Fatty acid composition of PC consists mainly of 16:0, 18:0, and 18:1n-9 acids. PE quantitatively has the highest concentrations of DHA in neural membranes (6).

Comparing results from many studies can be difficult due to the differences in each study. For example, the studies in Tables 1 and 2, did not use the same animal model. The different models represented are as follows: fetal rat (15), F3 ALA deprived rats (16), juvenile Rhesus monkeys (17), adult male mice (18), adult male Wistar rat (19), Wistar-Kyoto rat (20) and developing human (21). Also, in these studies, the results were not reported in the same manner. There were differences in the type of units used for fatty acid concentrations reported and one must be careful in making comparisons. Weight percent (15, 17-20), μmol/g (16), and mol percent (21) were the types of units used in these studies.

In addition, there were differences in the actual fatty acids reported within each study. For example, one study reported only five fatty acids (15) whereas another study reported 15 fatty acids (21).

Brain fatty acid composition is specific to each type of phospholipid. In all the studies summarized in Tables 1 and 2, the PE fraction of the brain as compared with the PC fraction, is much lower in 16:0 (15-21). In the PE fraction of the brain, 16:0 ranged from 4.5 weight percent (18) to 16 weight percent (15) compared with the PC fraction which ranged from 36 weight percent (20) to 50 weight percent (15). As shown in Tables 1 and 2, the 20:4n-6 (15-18, 20-21), 22:5 n-6 (15-17, 21), and 22:6 n-3 (15-18, 21) have greater concentrations in the PE fractions of the brain compared with the PC fractions. For example (15), 20:4 n-6 ranged from 19 weight percent in the PE fraction to 6 weight percent in the PC fraction, 22:5 n-6 ranged from 11 weight percent in the PE fraction to 1 weight percent in the PC fraction, and 22:6 n-3 ranged from 16 weight percent in the PE fraction to 1 percent in the PC fraction.

Diet composition can also affect fatty acid content in both the PE and the PC fractions of the brain. Feeding a diet adequate in n-3 fatty acids can have reciprocal effects on the concentrations of n-3 and n-6 fatty acids. DHA, an n-3 fatty acid, increased in both the PE and PC fractions of the brain in animals fed diets rich in n-3 fatty acids compared with animals receiving an n-3 deficient diet (15, 17-18). In contrast to DHA, ARA an n-6 fatty acid, decreased in the PE fraction of the brain in animals fed n-3 adequate diets compared to those animals receiving n-3 deficient diet. In the PE fraction of the brain, the difference of DHA concentrations in n-3 adequate and n-3 deficient diets ranged from 5.71 to 25.1 percent of total fatty acids and in the PC the difference of DHA

concentrations ranged from 0.45 to 2.6 percent of total fatty acids. Whereas, in the PE fraction of the brain, the difference of ARA concentrations in n-3 adequate and n-3 deficient diets ranged from 0.18 to 4.0 percent of total fatty acids. However, in the PC fraction, ARA did not show a consistent result with any of the dietary treatments (15, 17-18).

The fatty acid profile of brain phospholipids is very specific and is not always subject to dietary manipulation. DHA and EPA are both n-3 fatty acids that are present in fish oil supplemented diets often in approximately equal amounts. As shown in Tables 1 and 2, only one study reported EPA concentrations in the PE and PC fractions of the brain (17). The EPA in the PE fraction of the brain ranged from 3.1 (fish oil diet) to 0.0 (safflower oil diet) weight percent, and the EPA in the PC fraction of the brain ranged from 1.5 (fish oil diet) to 0.0 (safflower oil diet) weight percent (17). These concentrations of EPA are much less than those of DHA in the fish oil supplemented animals.

There are changes in certain fatty acid concentrations during brain development. Martinez et al (21), showed that as age increased from 26 wks to 8 yrs, the DHA in both the PE and PC fractions of human forebrain increased. In the PE, DHA in the 0-6 mo group increased from 9.22 to 10.87 mol% and continued to increase from 10.87 to 14.07 mol% in the 6 mo-8 yr group. In the PC, DHA in the 0-6 mo group increased from 0.55 to 0.73 mol% and continued to increase from 0.73 to 1.00 mol% in the 6 mo-8 yr group.

Diau et al, examined the influence of long chain PUFA supplementation on DHA and ARA in baboon neonate central nervous system (CNS). Baboons were randomized into a breastfed and four formula fed groups: term, no DHA/ARA supplementation;

term, DHA/ARA supplemented; preterm, no DHA/ARA supplementation; preterm and DHA/ARA supplemented. Pregnant and lactating females (in the breastfed group) consumed a conventional commercial primate meal which contains fish meal as a source of protein, which also adds n-3 long chain PUFAs. The breastmilk of these baboons contained about 0.68 ± 0.22 percent DHA and 0.62 ± 0.12 percent ARA (DHA and ARA concentrations are expressed as weight percentages of total fatty acids from 14-24 carbons). The formula supplemented groups contained 0.30 and 0.61 percent DHA, and 0.55 and 1.21 percent ARA for the term and preterm groups respectively. For all lobes of the cerebral cortex, and for the cerebellum, supplementation with DHA did not support tissue DHA concentrations similar to those of the breastfed group. All other areas of gray matter investigated, including the basal ganglia, limbic regions, thalamus and midbrain, did show that supplemental dietary DHA restored DHA concentrations to breastfed levels. Another dominant trend in the gray matter regions is that they contain significantly greater DHA concentrations in the DHA supplemented groups compared to unsupplemented groups. Trends in white matter were not as consistent as the two classes of gray matter. Two of the six white matter regions investigated did not respond to treatments (internal capsule and spinal cord). In the breastfed group DHA was significantly greater than in the others in three regions but, less than a supplemented group in the corpus callosum. The trends in ARA are very different from those for DHA. In the brains of the breastfed group, lower ARA concentrations were observed than in the preterm unsupplemented group in every region. The investigators concluded that DHA and ARA concentrations in the CNS are highly region-specific and are unexpectedly high

in the deep CNS regions embedded in white matter of much lower DHA and ARA concentrations (10).

Craig-Schmidt et al, conducted a study on neonatal piglets receiving one of four dietary treatments: i. STD-similar to infant formula, ii. STD+DHA, 0.7 percent DHA, iii. STD+ARA, 0.9 percent ARA, iv. STD+Both, 0.8 percent DHA plus 1 percent ARA. In the retina, DHA supplementation resulted in a 2-fold increase in PC-DHA while ARA supplementation exhibited a 1.3 to 1.4-fold increase in PC-ARA. However, in PE there were no significant increases except for a 1.2-fold increase in DHA when consuming the STD+Both dietary treatment compared to STD. PE was less responsive with only one significant dietary effect altering DHA concentrations. In the PE fraction only STD + Both resulted in increased DHA. In conclusion, consumption of both DHA+ARA supported significant accretion of DHA in the retina of neonatal pigs while simultaneously supplying ARA necessary for membrane PC (22).

Many studies have shown either diet or supplement manipulation can have an effect on the fatty acid profiles of PE and PC. The following studies show different methods of achieving this.

When n-3 fatty acids are deficient in experimental diets of pregnant rats, significant effects on essential fatty acids of the brain are observed. In this study, Green et al, investigated pregnant Wistar rats that were fed fat free diets supplemented with either sunflower oil (SFO; n-3 deficient) or soybean oil (SBO; n-3 adequate) (**Tables 1-2**). Intraamniotic administration of pure ethyl-docosahexaenoate (Et-DHA) was performed through the uterine wall and amniotic sac of some of the SFO-fed dams either 24 or 48 hrs prior to delivery. When n-3 fatty acids are deficient, docosapentaenoic acid

(DPA; 22:5n6) was shown to be at increased levels while DHA levels were significantly decreased. Feeding pregnant rats an n-3 deficient diet for only two weeks resulted in more than a 30 percent decrease in fetal brain DHA and an almost 120 percent increase in DPA compared to the SBO treated diet. Following injection of Et-DHA, there was a gradual increase at 24 and 48 hours prior to delivery in total brain DHA and a decline in DPA. The changes evident in the individual phospholipids (PL) in the SFO-treated dams were quite characteristic: pronounced decreases of DHA accompanied by major increases in DPA were observed in PE, both in fetal brains and livers (**Table 1**), but only minor changes ($P>0.05$) in any of the fatty acids were seen in PC (**Table 2**). In fetal brains of SFO-treated dams, DHA content in PE lipids was lower by 36 percent of the respective value of the SBO-treated controls, while the increase in DPA was approximately two fold. These investigators suggest that rapid modification of fetal brain DHA content is possible within the span of pregnancy (15).

Suzuki et al, fed mice either an n-3 PUFA deficient diet (palm oil) or an n-3 abundant diet (sardine oil). Brain stem phospholipids were analyzed for fatty acid composition. Both percentages of ARA and docosatetraenoic acids (22:4n-6) of PE in the sardine oil diet group were significantly lower than those in the palm oil diet group (**Table 1**). The ARA level of PC (**Table 2**) also showed a similar result. However, the level of DHA in PE in the sardine oil diet group was significantly higher than that in the palm oil group. A definite increase in DHA level and decrease in n-6 PUFA level of brain stem PE were observed in this study. These changes seem to influence the function of brain membrane in the animals (18).

Contreras et al, analyzed half brains of third generation α-linolenic acid deprived male rats. Two experimental diets were used, an n-3 adequate and n-3 deficient diet. Esterified DHA was significantly decreased in brain phospholipids of n-3 deficient rats, by 89 percent in PC (**Table 2**), and by 87 percent in the PE fraction, compared with the n-3 adequate group (**Table 1**). In contrast, significant compensatory increases in DPA were observed in the PE fraction (16).

The importance of DHA in brain tissue is further supported through a study done by Connor et al, on juvenile rhesus monkeys whom had developed n-3 fatty acid deficiency since intrauterine life and were repleted with a fish oil diet rich in the n-3 fatty acids, DHA and EPA. Safflower oil was used as the sole fat source for the deficient diet because it has a very low content of ALA and a very high ratio of n-6 to n-3 fatty acids. Beginning at 10-24 months of age, the monkeys were given the same diet with fish oil replacing 80 percent of safflower oil as the fat source. Biopsies of frontal cerebral cortex were obtained before and up to 28 weeks after the initiation of fish oil feeding, and also at autopsy. Dramatic changes in the fatty acids of the gray matter of frontal cortex could be detected within 1 week after the fish oil diet was initiated. By 12-28 weeks, the total n-3 fatty acids in PE increased from 4.2 percent to 36.2 percent, with the major increase occurring in DHA (4.2-29.3% **Table 1**). Total n-6 fatty acids reciprocally decreased from 44.1 to 15.8 percent, with major reductions occurring in DPA from 17.5 to 2.1 percent, and 22:4 n-6 from 11.5 to 3.7 percent. There was also a moderate decrease in PE ARA from 12.8 to 8.9 percent of total fatty acids. Although the content of n-3 fatty acids in PC is relatively small even in the normal brain, this fraction also showed an increase in total n-3 fatty acids from 0.4 to 5.1 percent after fish oil feeding (**Table 2)**. In summary, fish

oil feeding resulted in reciprocal changes in the levels of n-3 and n-6 fatty acids in the phospholipids of cerebral cortex. (17).

Experimental diets nutritionally adequate but differing in fat composition can modify the fatty acid composition of brain phospholipids. Kitajka et al, investigated essential fatty acid sufficient rats fed from conception either a diet high in ALA (perilla oil) or a diet high in EPA + DHA (fish oil), or kept on normal rat chow. The expression of genes in brains was tested when the rats reached adulthood. In this study, an attempt was made to correlate the alterations in molecular composition of PE caused by dietary n-3 PUFAs with their effect on gene expression and cognitive performance using microarrays. As a result, fatty acid composition of PE (**Table 1**) is almost identical in the three dietary regimens with the exception of DHA which is slightly higher in the brains receiving fish oil. There was only a minor difference in ALA, which does not accumulate or is even absent in brain phospholipids. DHA, the end product of ALA desaturation, did not accumulate in the PE of perilla oil-fed rats. The reported data correspond with the dietary composition, and reinforce that dietary fatty acid content is reflected in the phospholipid structure of brain tissue. The expression levels of 102 cDNAs, representing 3.4 percent of the total DNA elements on the array, were significantly altered in brains of animals fed either perilla oil or fish oil. The changes ranged from –5-fold to +7-fold. Both dietary treatments exerted almost the same effect on the gene expression profile. Altogether 55 genes increased whereas expression of 47 genes were decreased by fish or perilla oil feeding (19).

López et al, investigated age associated changes in the content and fatty acid composition of brain phospholipids. Wistar-strain rats were fed a standard pellet diet.

The cerebral cortex, subcortical white matter, cerebellum and medulla oblongata/pons were obtained from groups of 4, 21.5, and 28 month old rats. The amount of PUFAs such as 22:4 n-6, and DHA were slightly lower in aged rats than in young adult rats (21.5 months). A similar pattern of changes was observed in the fatty acid composition of total lipids from the subcortical white matter of aged rats with respect to adult rats, these changes being more marked in 28 month old rats than in 21 month old rats. The content of total PUFAs in the subcortical white matter of aged rats diminished by 14 percent with respect to adult rats, DHA being the most affected with a 30 percent decrease. The most dramatic changes in the cerebellum and medulla oblongata/pons regions took place in ARA and DHA, which decreased 39 and 35 percent with respect to adult rats, respectively. In the PC fraction, the amount of PUFA (DHA) in subcortical white matter and medulla oblongata/pons and ARA and DHA in the cerebellum is lower in 28 month old rats with respect to adult rats. In PE the fatty acid composition was modified in 28 month old rats through a marked increase in MUFA (18:1 n-9 & 20:1 n-9) in all brain areas. These results show that changes in biochemical properties may be associated with age related changes in the fatty acid composition of myelin-rich areas and neuron enriched areas. It was observed that aging produced an increase in the proportion of MUFAs, and a decrease in PUFAs (23).

The fatty acid composition of tissues is known to change throughout development. Martinez et al, examined the fatty acid composition of phospholipids in 22 human forebrains, ranging in age from 26 prenatal weeks to 8 postnatal years. Postmortem brain tissue was collected from 22 children who died at different ages from causes unrelated to the CNS. There is a general tendency toward an increase in the

proportion of n-3 PUFAs at the expense of a relative decrease in n-6 PUFAs in both PE and PC during development. In PC, both n-3 and n-6 percentages increases with age (21).

The beneficial effect of dietary n-3 PUFAs on developing hypertension has been repeatedly demonstrated. De Wilde et al, performed a comprehensive analysis of cerebral fatty acid concentration in hypertension after long term PUFA-rich dietary treatment compared to a control diet. Both diets reduced blood pressure, increased PUFA and MUFA concentration and reduced SFA content in the brain. The level of PUFAs and MUFAs were lower in hypertensive than in normotensive rats (20).

Docosahexaenoic acid functions

PUFAs are the main components of cell membranes and contribute to membrane fluidity. Many investigators report increases in membrane fluidity from animals fed DHA-rich fish oil diets (24-26), while a DHA-deficient diet resulted in a brush border membrane with decreased fluidity (27). The addition of acyl chain double bonds is generally assumed to increase fluidity, on the basis of which it may be postulated that a membrane rich in DHA should be exceptionally fluid (11). By increasing membrane fluidity, high DHA concentrations enhance serotonin receptor sensitivity which may be a factor in many neurological health implications involving DHA (28). DHA may also increase permeability of membranes (29). The n-3 long chain polyunsaturated fatty acids are derived from the diet, being found mainly in fish and other seafood products.

Health implications of n-3 fatty acids

Domains of behavior may be broadly categorized as sensory, motor, motivational and arousal, cognitive, and social. Because of the high concentrations of n-3 and n-6 long chain PUFAs in neural tissue, all domains of neural and psychologic function are potentially influenced by long chain PUFA status. Studies have shown that DHA is a major structural component of the brain that is negatively affected when treated with diets deficient in essential fatty acids (15-19).

There is an increasing interest in the relationship of n-3 PUFAs and the association with neurological function. Polyunsaturated fatty acids of the n-3 family have beneficial effects on various disorders in the human body. One of the major functions of DHA can be related to its involvement in cognitive processes. Grenier et al, compared male pups who were weaned to the diet of the dam (either n-3 deficient or n-3 adequate) and were tested at 9-12 wks of age. An olfactory-based discrimination and Morris water maze task were used to assess performance. The results indicate that rats with decreased DHA levels in the CNS perform poorer in these tasks compared to rats with higher DHA levels and suggest the presence of learning deficits in these animals (30).

Polyunsaturated fatty acids of the n-3 series are also important for infant growth and development. As discussed in a review by Innis et al (31), long chain PUFAs are known to be necessary nutritional components for infant development. DHA is essential for visual and neurological development of infants, and DHA supplementation in the diet improves learning ability (32). A study conducted by Helland and colleagues investigated pregnant women who were supplemented with either cod liver oil or corn oil starting in

week 18 of their pregnancy until 3 months after delivery. Children who were born to mothers who had taken cod liver oil during pregnancy and lactation scored higher on the Mental Processing Composite of the K-ABC at 4 years of age as compared to children whose mothers had taken corn oil. Adequate maternal intake of long chain PUFAs during pregnancy and lactation may be favorable for later mental development of children (32).

Mothers are the sole source of nutrients for the fetus during development. Without adequate nutritional replenishment, mothers can become depleted of critical nutrients during pregnancy with adverse consequences for both mother and infant. Mothers selectively transfer DHA to their fetuses to support optimal neurological development during pregnancy (33). Maternal DHA status can be reduced by half during pregnancy and not fully restored at 26 weeks postpartum (34). Without sufficient dietary intake, mothers become depleted of DHA and may increase their risk of suffering major depressive symptoms in the postpartum period.

Hibbeln conducted a cross-national, ecological study investigating if higher DHA concentrations in breast milk would predict lower rates of postpartum depression (35). This study found that a 50-fold difference in prevalence rates of major post partum depressive symptoms across countries is substantially associated with n-3 fatty acid nutritional status. Other observational studies of perinatal women indicate that the recovery of maternal DHA status from delivery to 32 weeks postpartum was significantly slower in women who had postpartum depression compared with non-depressed control subjects (36).

There are observations that substance abusers have poor dietary habits and the association between behavior and PUFA status has been examined. Buydens-Branchey

et al, explored the possibility that the fatty acid profiles of cocaine addicts with and without aggressive tendencies would differ. Fatty acids were measured in cocaine addicts shortly after their admission to an inpatient unit. Patients with a history of aggressive behavior were found to have significantly lower levels of DPA, total n-3 PUFAs and DHA and an almost significantly higher n-6/n-3 ratio when compared with patients with no aggression history (37).

Bipolar disorder (manic-depressive illness) is a neuropsychiatric illness. Recent research suggests that all of the current available mood-stabilizing drugs have inhibitory effects on neuronal signal transduction systems. These findings have led to the hypothesis that overactive cell-signaling pathways may be involved in the pathophysiological mechanisms underlying bipolar disorder (38). In a study conducted by Stoll et al, it was hypothesized that orally administered n-3 fatty acids may exhibit inhibitory effects on signal transduction mechanisms in human neuronal membranes, and that high dose n-3 fatty acids would be an effective mood stabilizer in bipolar disorder. N-3 fatty acids used as an adjunctive treatment in bipolar disorder resulted in significant symptom reduction and a better outcome when compared with placebo in this pilot study over 4 months of treatment. Improvement was significantly greater in the n-3 fatty acid group than the placebo group on almost every assessment measure. The striking difference in relapse rates and response appeared to be highly clinically significant (38).

On the basis of the previous findings, Noaghiul and Hibbeln designed a study postulating that lower lifetime prevalence rates of bipolar disorders would occur in countries with greater rates of seafood consumption. The results show a correlational relationship between greater seafood consumption and lower prevalence rates of bipolar I

(r=-0.52) disorder, bipolar II disorder (r=-0.70), and bipolar spectrum disorder (r=-0.67) (39).

Morris et al, examined whether fish consumption and intake of DHA and other n-3 fatty acids were associated with protection against Alzheimer's disease in a biracial community. This was a prospective study conducted from 1993 through 2000, of a stratified random sample from a geographically defined community. Participants were followed up for an average of 3.9 years for the development of Alzheimer's disease. A total of 131 out of 815 sample participants developed Alzheimer's disease. Participants who consumed fish once per week or more had a 60 percent decreased risk of Alzheimer's disease compared with those who rarely or never ate fish. Total intake of n-3 PUFAs, as was intake of DHA, was associated with reduced risk of Alzheimer's disease (40).

In summary, these studies have shown that consumption of n-3 PUFAs has been inversely linked to impulsivity/aggression/hostility (Buydens-Branchey), as well as a number of neurologically based disorders such as depression (33-36) bipolar disorders (38-39), and forms of dementia such as Alzheimer's Disease (40).

Health implications of methylmercury

Methylmercury (MeHg) is a well known environmental toxicant found in the aquatic ecosystem. Inorganic mercury originates from anthropogenic and natural sources. Methylmercury is produced environmentally by biomethylation of the inorganic mercury present in aquatic sediments (41-42). Piscivorous fish are higher in Hg than are fish that subsist on plants and insects (43). The fish at the top of the food chain that feed on

smaller fish accumulate the highest levels of MeHg and therefore represent the greatest risk to the population (44). MeHg accumulates in the aquatic food chain and reaches its highest concentrations in long-lived predatory fish, such as shark (1.33 μgHg/g fresh wt) and swordfish (0.95 μgHg/g fresh wt) in the oceans, and pike (0.810 μgHg/g fresh wt) and bass (0.752 μgHg/g fresh wt) in freshwater (45). As reviewed by Mahaffey, MeHg is bound to amino acids in fish and shellfish muscle tissue and is not removed from fish by routine soaking or cooking processes, including frying, baking, smoking, and roasting (43).

At the present, human exposure to MeHg is believed to occur almost exclusively via consumption of seafood (42). However, in the past, several large outbreaks of acute organic mercury poisoning have occurred. Examples include the industrial release of methylmercury in Minamata Bay and the Agano River in Japan which resulted in the accumulation of the toxicant in fish, and consumption of bread mistakenly made from grains treated with organomercurial fungicides in Iraq.

Ingested MeHg is almost completely absorbed from the gastrointestinal tract (95%) and accumulates in the brain. MeHg is slowly metabolized to inorganic mercury mainly by microflora in the intestines, at a rate of about 1% of the body burden/day. Most of the MeHg is eliminated from the body by demethylation and excretion of the inorganic form in the feces (46). MeHg can easily cross the barriers at the blood-brain interface and at the placenta (47). MeHg enters the brain where it is demethylated and is contained in the inorganic form.

The high mobility of MeHg in the body is not due to lipid solubility. MeHg is present in the body as water soluble complexes mainly if not exclusively attached to the

sulfur atom of thiol ligands. It enters the endothelial cells of the blood brain barrier as a complex with L-cysteine. Structurally, the MeHg-L-cys complex is similar to the large neutral amino acid L-methionine and is carried across the cell membrane on the large neutral amino acid carrier (48). There are distinct differences in the distribution of pathological changes in young compared to adult brain upon MeHg exposure. A number of molecular targets and mechanisms have been proposed to be implicated in its neurotoxic effects. CNS damage following MeHg exposure in adults is primarily in specific areas, such as the granule layer of cerebellum and the visual cortex of the cerebrum (49).

Apoptosis and necrosis are two modes of cell death characterized by distinct morphological and molecular features and different implications for the surrounding tissue. Neuronal degeneration upon MeHg exposure has been reported to occur by either necrosis (50-51) or apoptosis (52). In a study performed by Nagashima et al, rats received 10 mg/kg per day of MeHg for 10 days, and histopathologic findings revealed that neuronal necrosis occurred in the cerebellar cortex and brain stem nuclei (53).

After adult exposure, MeHg is found throughout the brain, and the localization does not correlate with pathological changes, suggesting distinct vulnerability of various regions to this metal (49). Following exposure, MeHg accumulates in the blood-brain barrier and the blood-cerebrospinal fluid barrier. Autopsy data from a Minamata Bay accident victim showed that total Hg remained high in the brain as long as 26 years after exposure (54). There is usually a latent period of weeks or months between exposure and the onset of symptoms (55-56).

The first symptom to appear in adults is a tingling sensation (paresthesia) (57). This may progress to difficulty in pronouncing words (dysarthria), hearing defects, followed by visual-field constriction resulting from the loss of neurons in the visual cortex, incoordination (ataxia), mental deterioration, and death (57-58). These signs and symptoms are caused by regional destruction of neurons in the visual cortex and cerebellar granule cells. In cases of acute intoxication with organic mercury, there is a correlation between the volume of ingested MeHg containing food and the severity of the clinical symptoms and signs (57). Following the Minamata poisoning, some participants displayed no impairments until well after the peak exposure period, when difficulties in fine motor control during daily activities appeared (59-60).

Acute effects of methylmercury

Levels of exposure seen in some fish-eating populations have been reported to be associated with developmental delays in children whose mothers were exposed during pregnancy. While high-level poisoning episodes in Minimata and Nigata, Japan, and in Iraq demonstrated pronounced MeHg-induced neurological deficits, in the United States, the only reported cases in the past 35 years involved a family that consumed the meat of a pig fed treated grain (61). A university professor was accidentally exposed to dimethylmercury in the laboratory and subsequently died (62). There is also concern that MeHg can cause more subtle developmental delays or other neurological effects at lower levels of exposure more consistent with the usual patterns of fish consumption seen in the United States (63).

Chronic effects of methylmercury

Recently the chronic effects of organic mercury have drawn increased attention around the world (59). Health effects of MeHg chronic exposure may be important in humans, because all the fish we eat contain a trace amount of MeHg. The increasing daily fish consumption typical of modern society as a part of a healthier diet may result in chronic low-level dietary intake of MeHg and thus poses a significant toxicological problem, especially to susceptible individuals, such as developing embryos or fetuses (46).

Animals have been used as models of chronic toxicity due to their physiological similarities with humans and their short lifespan. Mitsumori et al. found that when rats were given a MeHg contaminated diet at 10 ppm level, the animals showed typical neurological signs and a significant increase in mortality before the end of the second year of treatment (64). Dietrich et al. researched the effects of oral exposure to MeHg on locomotor control and activity in adult mice. The results show significant dose- and duration-dependent effects of MeHg exposure on behavioral/functional parameters related to motor performance. Marked decreases in locomotor activity, increases in beam walking latency and clasping score, and progressive gait impairment were observed following chronic MeHg exposure (65).

Interactive effects of n-3 and mercury

The presence of high levels of the very long-chained n-3 fatty acids, EPA and DHA, are identified as one of the major benefits of ingesting fish-derived lipids and ironically, fish are the most common source of MeHg. Effects of MeHg exposure

overlap the effects exhibited by n-3 PUFA deficiency. Thus, visual, motor, and reinforcement systems are all affected by MeHg exposure and n-3 PUFA deficiency (30, 66, 67). Therefore, n-3 PUFAs in fish have been hypothesized to ameliorate MeHg's neurotoxic effects and decrease the risks associated with eating MeHg containing foods.

Newland and colleagues investigated whether the presence or absence of n-3 PUFAs in fish influences blood or brain concentrations of mercury. Daily exposure to an estimated 400 μg/kg of MeHg resulted in neurological disorders including hind limb crossing, gait disturbances, and decreased forelimb grip strength. However, no influence of n-3 fatty acids was seen on any measure of blood or brain mercury levels (68).

In the Faroe Islands a large cohort of 7-year-old children (*n*=917) has been studied with regard to neurologic development effects induced by prenatal exposure to MeHg and polychlorinated biphenyls. Such exposure was primarily determined by maternal consumption of pilot whale containing up to 3 ppm of mercury and resulted in a median maternal hair concentration of 4.5 ppm. Pronounced mercury related neuropsychological deficits were found in the domains of language, attention, and memory (69). Conversely, a previous investigation by Davidson et al studied a MeHg exposed population in the Seychelles, in which fish was their major dietary component, containing mercury concentrations of about 0.05 to 0.25 ppm (70). Such mercury levels are comparable with those found in fish species in the United States. Thus, the higher dietary intake of MeHg in the Seychelles, as compared to the U.S. population, results from the larger consumption of fish, and not to abnormally high metal levels in seafood (71). The increased MeHg exposure was not associated with any behavioral or cognitive deficits (70).

Another region of the world affected by high consumption of seafood and MeHg toxicity are the gold mining communities in the Brazilian Amazon Basin. The gold mining has resulted in the contamination of freshwater fish in downstream areas with concentrations of MeHg often exceeding 0.5 ppm (72).

Methylmercury and DHA also affect antioxidant function in opposing manners. Glutathione is the major intracellular antioxidant. After exposure to MeHg, glutathione concentrations decline and then increase. Cells that are made resistant to MeHg toxicity had an increase in the rate of efflux of MeHg and had 4-fold higher glutathione concentrations than normal cells (73). However, Wang et al, found that DHA increased the activities of antioxidant enzymes of glutathione reductase and glutathione peroxidase, suggesting that DHA may be a potent neuroprotector against toxicants (74).

Public health implications of fish consumption

There has been significant promotion of fish intake in recent years because of its high quality protein content, low fat content, and because it is the only food source of long-chain PUFAs. Good sources of DHA include salmon (0.89 g/100g), bluefin (0.88 g/100g) and albacore tuna (0.62 g/100g), mackerel (2.16 g/100g), and herring (0.71 g/100g) (75). At the same time, high MeHg content has been discovered in some fish, leading numerous government agencies to publish advisories for the consumption of fish, particularly in at-risk subpopulations.

In 2004, the Environmental Protection Agency (EPA) and the US Food and Drug Administration (FDA) jointly released an updated consumer advisory on MeHg in fish. Shark, swordfish, king mackerel, and tilefish were placed on the "do not eat" list due to

the high levels of MeHg contained in these types of fish. However, Albacore tuna, shrimp, canned light tuna, salmon, pollock, catfish and various others were listed as being consumable up to 6 ounces-12 ounces/week in the at-risk populations (76).

The consumption of fish is highly variable across the United States. In a study report to Congress issued by the Environmental Protection Agency (1997) they found that overall 88 percent of adults in the U.S. consume fish and shellfish at least once a month with 58% of adults consuming fish at least once a week. Shrimp, which has little or no mercury, has the highest reported frequency of consumption and is consumed by the majority of adults eating fish (85%). Tuna is the second most frequently consumed fish and approximately half of all fish-eating adults reported its consumption (77). Only 1% of all respondents indicated they ate fish and shellfish daily.

Due to the health hazards of excess mercury exposure, the EPA created a reference dose (R_fD) for mercury of 0.1 µg/kg body weight/d (78). The R_fD is generally interpreted to be a concentration of a chemical which can be consumed on a daily basis over a lifetime without expectation of adverse effects (79). The R_fD is derived from a critical effect level: either a no-observed-adverse-effect level or lowest-observed-adverse-effect level identified from a subchronic or chronic study. The R_fD is calculated by dividing the critical effect level by the product of one or more uncertainty factors and a modifying factor. In contrast to the EPA the Joint FAO/WHO Expert Committee on Food Additives calculated an R_fD called the "provisional tolerable weekly intake (PTWI) of 1.6 µg/kg body weight/week (80). However, greater than average fish intakes correspond to greater methylmercury exposure.

Thesis Statement:

The objective of this research is to determine if chronic exposure to MeHg alters the brain fatty acid profile of the adult rat, and if supplementation with dietary fish oil could assist in overcoming the potential adverse effects of mercury exposure. The hypotheses of this study are as follows:

1.Chronic methylmercury exposure will result in the alterations of adult rat brains by decreasing concentrations of polyunsaturated fatty acids in the membrane phospholipids: phosphatidylethanolamine and phosphatidylcholine.

2. Animals consuming a diet containing fish oil will have an increased concentration of DHA in brain tissue in both the PE and PC fractions compared to those consuming a diet in which coconut oil replaced the fish oil.

3. The dietary fish oil will counterbalance the detrimental effects brought about by the methylmercury toxicity by returning fatty acid concentrations to normal levels.

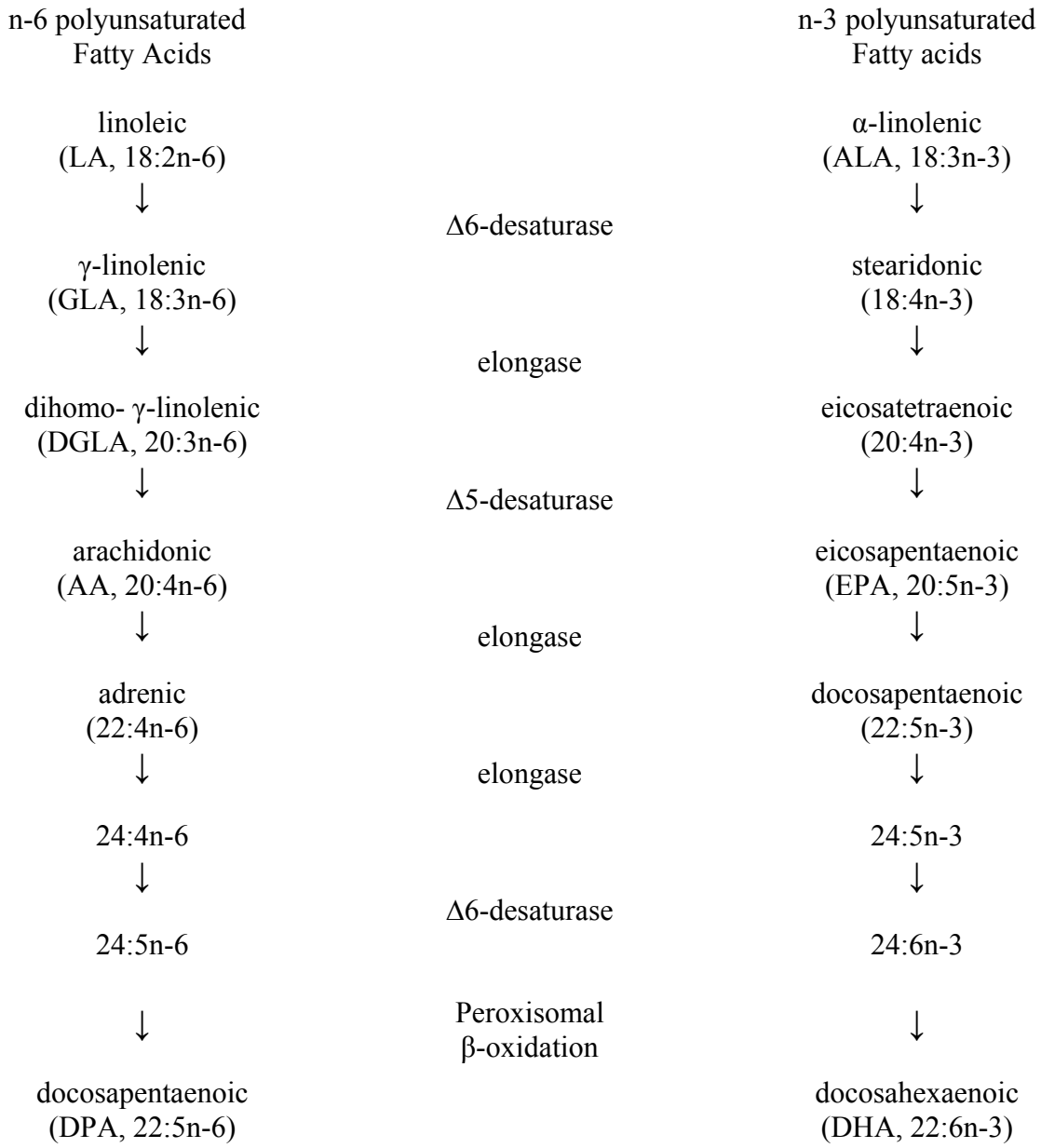

Figure 1. Elongation and desaturation of essential fatty acids.

Table 1. Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylethanolamine

	Green et al (15)				Suzuki et al(18)	
	Fetal Rat				Adult Male Mice	
	n-3 Adequate[1]	n-3 Deficient-SFO[2]				
	SBO	Control[3]	24 h[4]	48 h[5]	Palm Oil[6]	Sardine Oil[7]
	weight %				weight %	
14:0						
14:1						
16:0	15.47±0.70	15.98±0.77	12.22±0.07	12.24±0.28	5.7±1.3	4.5±0.6
16:1n7						
18:0	22.8 ±0.23	22.58±0.35	21.16±0.61	20.70±0.86	17.5±2.0	15.2±0.9
18:1n9	13.11±1.04	12.51±0.36	10.71±0.37	10.89±0.35	21.1±1.0	21.7±0.9
18:1n7						
18:2n6						
18:3n6						
18:3n3						
20:0						
20:1n9						
20:2n6						
20:3n6						
20:4n6	19.34±0.85	19.52±0.46	19.77±0.51	20.97±0.36	9.0±0.2	5.0±0.3
20:5n3						
22:0						
22:1n9						
22:2n6						
22:4n6					5.1±0.2	2.0±0.1
22:5n6	5.08 ±0.16	11.55±0.32	11.46±0.55	8.97±0.58		
24:0						
22:6n3	15.92±0.70	10.21±0.25	15.61±1.02	16.79±0.77	11.4±0.8	18.4±1.6
24:1n9						

[1] n-3 adequate diet (consumed by dams) = Semipurified fat free diet supplemented with 5% soybean oil
[2] n-3 deficient diet (consumed by dams) = Semipurified fat free diet supplemented with 5% sunflower oil.
[3] Brains from the SFO group that did not receive intraamniotic injection of pure ethyl-docosahexaenoate (Et-DHA).
[4] At days 18, 19, and 20 of gestation dams received intraamniotic administration of Et-DHA to fetus, injected 24 hr prior to delivery
[5] Injected 48 hr prior to delivery
[6] Palm oil=n-3 deficient diet; received dietary treatment for 12 months
[7] Sardine oil (13.8% EPA, 9.3% DHA) = n-3 adequate diet; received dietary treatment for 12 months

Table 1-Continued: Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylethanolamine

	Contreras et al (16)		Connor et al (17)	
	F3 ALA deprived rats		Juvenile Rhesus Monkeys	
	n-3 Adequate[8]	n-3 Deficient[9]	Safflower Oil[10]	Fish Oil[11]
	μmol/g		weight %	
14:0				
14:1				
16:0	5.9±1.0	6.7±1.3	5.7 ±0.9	6.2±1.1
16:1n7			2.5±2.0	1.5±1.2
18:0	19.9±3.4	22.0±3.5	26.2±3.5	25.1±6.4
18:1n9	21.4±4.0	20.6±3.6	6.5±1.0	7.2±0.9
18:1n7				
18:2n6	0.4±0.1	0.30±0.04	0.8±0.4	0.3±0.1
18:3n6				
18:3n3			0	0
20:0				
20:1n9				
20:2n6				
20:3n6				
20:4n6	14.2±2.5	16.8±2.8	12.8±1.5	8.9±1.9
20:5n3			0	3.1±1.4
22:0				
22:1n9				
22:2n6				
22:4n6			11.5±1.7	3.7±1.1
22:5n6	0.9±0.2	19.6±3.6	17.5±4.0	2.1±1.3
24:0				
22:6n3	22.2±4.4	2.8±0.7	4.2±1.2	29.3±2.6
24:1n9				
Total n-3			4.2±1.2	36.2±3.0

[8] n-3 adequate group was fed a diet containing 1.77% (w/w) safflower oil, 0.48% (w/w) flaxseed oil and 7.75% coconut oil

[9] n-3 deficient group was fed a diet containing 1.9% (w/w) safflower oil and 8.1% (w/w) coconut oil

[10] Safflower oil was used as the primary fat source for the n-3 deficient diet for the first 10-24 months

[11] Beginning at 10-24 months of age the same diet was provided with fish oil replacing 80% of the safflower oil as the fat source

Table 1-Continued: Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylethanolamine

	Kitajka et al (19)			Martinez et al (21)		
	Adult male Wistar rat			Developing Human Forebrain		
	Control[12]	Perilla Oil[13]	Fish Oil[14]	26-42 wk	0-6 mo	6 mo-8 yr
	weight %			Mol %		
14:0				0.56±0.13	0.37±0.07	0.44±0.11
14:1						
16:0	8.31±1.53	8.28±2.6	6.34±2.20	10.97±1.0	9.16±0.49	8.25±0.40
16:1n7	0.68±0.21		1.08±0.36	0.30±0.08	0.32±0.10	0.19±0.09
18:0	17.05±2.00	17.13±1.45	19.00±2.90	37.63±2.43	38.35±1.84	36.93±3.02
18:1n9	12.18±1.49	12.23±1.19	12.75±2.56	6.54±0.98	6.53±0.34	8.78±2.35
18:1n7	1.60±0.27	1.57±0.49	1.44±0.30	2.27±0.53	2.30±0.21	3.01±0.81
18:2n6	0.80±0.02	0.88±0.34	0.71±0.32	0.21±0.06	0.29±0.09	0.42±0.16
18:3n6						
18:3n3	1.90±0.17	ND	0.42±0.22			
20:0				0.08±0.02	0.10±0.03	0.13±0.05
20:1n9				0.33±0.08	0.29±0.03	0.66±0.26
20:2n6				0.24±0.11	0.38±0.09	0.45±0.09
20:3n6				0.91±0.17	1.77±0.21	1.60±0.28
20:4n6	9.64±1.57	10.10±1.48	9.07±3.46	15.33±0.77	15.26±1.40	12.72±1.47
20:5n3	ND	ND	0.1			
22:0						
22:1n9						
22:2n6						
22:4n6	4.22±1.29	4.32±0.86	2.68±0.39	9.15±1.06	8.68±1.20	8.44±0.46
22:5n6				4.31±0.57	3.55±0.55	2.74±1.30
24:0						
22:6n3	19.73±2.00	18.39±2.92	22.16±2.86	9.22±1.39	10.87±1.29	14.07±1.78
24:1n9						

[12] Control=standard rat chow; on diet from conception to adulthood
[13] Perilla Oil=standard rat chow supplemented with 8% perilla oil; on diet from conception to adulthood
[14] Fish Oil=standard rat chow supplemented with 8% fish oil; on diet from conception to adulthood

Table 1-Continued: Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylethanolamine

	de Wilde et al (20)		
	Wistar-Kyoto Rat		
	Control[15]	Diet 1[16]	Diet 2[16]
	weight %		
14:0	1.14±0.47	1.43±0.23	1.43±0.23
14:1	0.76±0.28	1.20±0.23	1.20±0.23
16:0			
16:1n7	2.11±0.70	0.95±0.74	0.95±0.74
18:0	13.47±1.59	14.31±3.07	14.31±3.07
18:1n9	17.05±0.48	17.26±1.19	17.26±1.19
18:1n7	4.26±0.22	3.85±0.40	3.85±0.40
18:2n6			
18:3n6			
18:3n3			
20:0			
20:1n9	4.86±0.39	4.96±0.73	4.96±0.73
20:2n6			
20:3n6	0.29±0.02		
20:4n6	7.43±0.31	6.91±1.17	6.91±1.17
20:5n3			
22:0			
22:1n9			
22:2n6			
22:4n6	3.49±0.20	3.25±0.50	3.25±0.50
22:5n6			
24:0			
22:6n3	13.81±0.54	11.76±1.72	11.76±1.72
24:1n9			

[15] Control=standard rat chow; on diet from 4-80 weeks of age

[16] Diet 1 and Diet 2 were almost identical in fatty acid composition; both were PUFA enriched diets containing soybean oil, fish oil, and ARA additives; on diet from 4-80 weeks of age

Table 2. Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylcholine

	Green et al (15)				Suzuki et al (18)	
	Fetal Rat				Adult Male Mice	
	n-3 Adequate[17] SBO	n-3 Deficient-SFO[18] Control[19]	24 h[20]	48 h[21]	Palm Oil[22]	Sardine Oil[23]
	weight %				weight %	
14:0						
14:1						
16:0	49.20±0.43	50.09±1.27	48.60±0.41	46.46±1.34	38.6±4.2	40.3±3.6
16:1n7						
18:0	7.18±0.09	6.35±0.22	6.70±0.13	6.35±0.10	17.5±1.4	16.2±0.4
18:1n9	22.70±0.39	22.09±0.78	21.46±0.22	22.97±0.69	31.1±2.3	32.6±2.1
18:1n7						
18:2n6						
18:3n6						
18:3n3						
20:0						
20:1n9						
20:2n6						
20:3n6						
20:4n6	6.24±0.12	5.68±0.35	6.44±0.27	7.05±0.20	3.7±0.5	2.1±0.5
20:5n3						
22:0						
22:1n9						
22:2n6						
22:4n6						
22:5n6	0.75±0.04	1.38±0.07	1.19±0.08	1.06±0.12		
24:0						
22:6n3	2.06±0.08	1.10±0.06	1.49±0.16	1.89±0.12	4.4±1.5	5.8±1.1
24:1n9						

[17] n-3 adequate diet (consumed by dams)=Semipurified fat free diet supplemented with 5% soybean oil
[18] n-3 deficient diet (consumed by dams)=Semipurified fat free diet supplemented with 5% sunflower oil.
[19] Control are the brains from the SFO group that did not receive intraamniotic injection of pure ethyl-docosahexaenoate (Et-DHA).
[20] At days 18, 19, and 20 of gestation dams received intraamniotic administration of Et-DHA to fetus, injected 24 hr prior to delivery
[21] Injected 48 hr prior to delivery
[22] Palm oil=n-3 deficient diet; received dietary treatment for 12 months
[23] Sardine oil (13.8% EPA, 9.3% DHA)= n-3 adequate diet; received dietary treatment for 12 months

Table 2-Continued: Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylcholine

	Contreras et al (16)		Connor et al (17)	
	F3 ALA deprived rats		Juvenile Rhesus Monkeys	
	n-3 Adequate[24]	n-3 Deficient[25]	Safflower Oil[26]	Fish Oil[27]
	µmol/g		weight %	
14:0				
14:1				
16:0	13.9±1.6	13.9±2.0	48.2±4.8	38.8±5.6
16:1n7			1.3±0.8	2.7±1.0
18:0	4.9±0.6	4.7±0.7	11.9±1.4	11.7±1.2
18:1n9	9.7±1.1	9.5±1.4	20.1±1.6	27.1±3.9
18:1n7				
18:2n6	0.31±0.02	0.23±0.03	2.2±0.5	0.6±0.1
18:3n6				
18:3n3			0	0
20:0				
20:1n9				
20:2n6				
20:3n6				
20:4n6	2.2±0.3	2.3±0.4	4.9±1.9	4.2±0.8
20:5n3			0	1.5±0.5
22:0				
22:1n9				
22:2n6				
22:4n6			0.8±0.3	0.2±0.1
22:5n6	0.05±0.06	1.2±0.3	1.2±0.5	0
24:0				
22:6n3	1.5±0.3	0.16±0.04	0.3±0.2	2.9±0.3
24:1n9				
Total n-3			0.4±0.2	5.1±1.0

[24] n-3 adequate group was fed a diet containing 1.77% (w/w) safflower oil, 0.48% (w/w) flaxseed oil and 7.75% coconut oil

[25] n-3 deficient group was fed a diet containing 1.9% (w/w) safflower oil and 8.1% (w/w) coconut oil

[26] Safflower oil was used as the primary fat source for the n-3 deficient diet for the first 10-24 months

[27] Beginning at 10-24 months of age the same diet was provided with fish oil replacing 80% of the safflower oil as the fat source

Table 2-Continued: Literature Review of Brain Composition-Fatty Acid Composition of Phosphatidylcholine

	Martinez et al (21)			de Wilde et al (20)		
	Developing Human Forebrain			Wistar-Kyoto Rat		
	26-42 wk	0-6 mo	6 mo-8 yr	Control[28]	Diet 1[29]	Diet 2[29]
		mol%			weight%	
14:0	4.19±0.84	2.19±0.62	2.64±3.36			
14:1						
16:0	57.09±3.24	52.12±2.39	45.06±3.59	35.88±2.56	37.42±1.61	36.27±2.11
16:1n7	1.38±0.33	0.95±0.22	0.95±0.27	1.93±0.31	1.46±0.26	1.10±0.50
18:0	8.42±1.13	11.09±1.59	13.16±0.84	17.67±2.22	13.99±1.18	15.33±2.15
18:1n9	14.35±1.56	17.70±1.45	23.48±3.37	22.94±1.76	24.36±1.25	24.04±1.50
18:1n7	4.30±0.38	5.14±0.45	4.86±0.31	6.89±0.94	7.89±0.70	7.75±0.62
18:2n6	0.23±0.06	0.46±0.16	0.77±0.24	1.06±0.71	1.04±0.27	1.08±0.57
18:3n6						
18:3n3						
20:0	0.04±0.02	0.06±0.03	0.09±0.02			
20:1n9	0.31±0.05	0.31±0.02	0.36±0.07	1.82±0.30	1.60±0.08	1.75±0.16
20:2n6	0.13±0.06	0.19±0.03	0.13±0.02			
20:3n6	0.34±0.08	0.79±0.17	0.81±0.16			
20:4n6	3.57±1.46	4.60±0.70	4.23±0.54	3.90±0.52	4.29±0.38	4.31±0.34
20:5n3						
22:0						
22:1n9						
22:2n6						
22:4n6	0.43±0.11	0.49±0.10	0.57±0.11			
22:5n6	0.19±0.07	0.18±0.04	0.18±0.06			
24:0						
22:6n3	0.55±0.19	0.73±0.19	1.00±0.23	3.90±0.60	4.47±0.16	4.35±0.36
24:1n9						

[28] Control=standard rat chow; on diet from 4-80 weeks of age

[29] Diet 1 and Diet 2 were almost identical in fatty acid composition; both were PUFA enriched diets containing soybean oil, fish oil, and ARA additives; on diet from 4-80 weeks of age

CHAPTER III. MATERIALS AND METHODS

Animals

Female adult Long Evans rats were purchased from Harlan Sprague Dawley, Inc., (Indianapolis, IN) and were received at approximately 5 months of age. The rats were housed two per plastic cage but were not allowed to interact due to a plexiglass divider placed diagonally in each cage containing aspen bedding in a room maintained on a 12-h light/dark cycle (lights on 7:00 A.M-7:00 P.M.). The Auburn University Office of Animal Resources Institutional Animal Care and Use Committee approved the protocol.

Experimental Design

Using a staggered start, the rats were assigned to three concentrations of mercury as methyl mercuric chloride (MeHg) and two diets (Coconut oil or Fish oil) in a 3x2 factorial arrangement resulting in 6 treatment groups (Table 3). The two experimental diets were based on the AIN-93 formulation and were obtained from Research Diets, Inc., (New Brunswick, NJ) (81-83). The diets were similar in nutrient composition, but differed in the composition of the dietary fat (Table 4).

Diet

The fat mixture for the coconut oil diet (CO) consisted of 42.8% palm oil, 9.2% safflower oil, 15.0% soybean oil and 33% coconut oil (Table 5). The fish oil diet (FO)

was formulated with EPAX (Pronovo Biocare, Lysaker, Norway) fish oil mixture. The fat mixture for the fish oil diet contained fish oil instead of coconut oil at 33% by weight. The fish oil was added in an attempt to replace saturated fat (8:0, 10:0, 12:0, 14:0) with the n-3 polyunsaturated fatty acids, docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA) which were provided in the fish oil diet at approximately 5% of total fatty acids (Table 5). The fat mixtures contained 1.0-1.6% alpha-linolenic acid (18:3 n-3, ALA), a biosynthetic precursor to EPA and DHA. The n-6 fatty acid content as well as the monounsaturated fatty acids were almost equal in the two diets (Table 5). Composition of the vitamin and mineral content is shown (Tables 6-7). During pregnancy and lactation, the rats were placed on a growth diet consisting of 7% fat from the mixture of oils described above (Table 5). During all other periods, the maintenance diet for mature animals, which contained 4% of the diet as the fat mixture, was used (NRC). Additional vitamin E as a mixture of the tocopherols, Coviox T-70 (10g/25 kg oil) and Copheral F (14g/25 kg oil) was added to the diets in order to minimize oxidation. The diets contained tertiary-butylhydroquinone (TBHQ) to prevent the oxidation of the polyunsaturated fatty acids.

Mercury exposure

Methylmercury was administered to the animals 1 week before breeding in three different concentrations, 0, 0.5, and 5.0 ppm, dissolved in their only source of drinking water which they received until 18 months of age. These concentrations provided approximately 0, 40 and 400 μg/kg body weight/day of methylmercury.

Time course of experiment

Treatments were initiated in three week intervals: Dams arrived; dietary treatments began; MeHg exposure began; breeding began with males that were not exposed to MeHg or dietary treatments. The dams were divided in half for breeding with the first group going one week prior to the second group. There was also a third breeding period that was a rebreeding in order to obtain a sufficient number of pups for each experimental group. Mating was confirmed by the presence of a sperm plug. Pups were born after a three week gestation period. Only forebrains of the dams were analyzed and used in this experiment. Dams received MeHg as well as the dietary treatment until 18 months of age.

Fatty acid analysis of forebrain phospholipids

At 18 months of age, the adult dams were anesthetized by sodium pentobarbital (50-100mg/kg/animal), euthanized by decapitation, and the brain removed. The brain was dissected into three sections; the forebrain, medial region, and the hindbrain, and for this experiment only the forebrain was analyzed. The midbrain consisted of a 3 mm section which included the ventral medial hypothalamus (VMH) and the forebrain consisted of primarily cortex and is defined as all of the tissue of the brain in front of the VMH. Upon removal, the forebrain was weighed, blanketed with nitrogen gas, quick frozen in liquid nitrogen, and stored at -80º C for later fatty acid analysis. Approximately 1 mg tissue/ml water was homogenized in UltraPure water. Total lipids of the forebrain were extracted using a modified method of Folch et al (84) (Appendix A). Phospholipids were separated using a one-dimensional thin-layer chromatography system using silica

gel plates (Silica Gel G, 20x20cm, 250 microns; Analtech Inc., Newark, DE) and a chloroform:methanol:acetic acid:water (50/25/6/1.5 by volume) solvent system described by Skipsky et al (85) (Appendix B). The phosphatidylethanolamine (PE) and phosphatidylcholine (PC) fractions were identified using authentic standards (Maytrea Inc., Pleaseant Gap, PA), scraped from the thin-layer plate, and transformed into fatty acid methyl esters using a 10% solution of boron trifluoride methanol (Supelco, Bellefonte, PA) and a 30 minute heating period at 110º C in a dry hot block as described by Morrison and Smith (86) (Appendix C). Methylated fatty acids were extracted by a pentane-water wash (2:1 v/v). Once separated, fatty acid methyl esters (FAME) were dissolved in dicholoromethane and were ready for separation by gas chromatography (Appendix D).

Fatty acid analysis of experimental diets

Two pellets of each experimental diet were homogenized until a fine powder resulted. Approximately 10-15 mg of the powder was then homogenized with 2 ml of UltraPure water. Total lipids of the diet were extracted using a modified method of Folch et al (84) (Appendix A). Samples were then methylated into FAME by the same procedure discussed previously and were ready for separation by gas chromatography (Appendix D).

Gas Chromatography Analysis

The FAME were then separated using a 3900 gas chromatograph (Varian Inc., Sugar Land, TX) equipped with: an Omega-wax fused silica capillary column (0.25mm

internal diameter, 30m; Supelco, Bellefonte, PA); a Varian CP8400 autosampler; a Varian 8410 autoinjector; and a flame ionization detector. The injector and flame ionization detector were held at 250º C and 260º C, respectively. Column temperature was programmed with a continuous gradient (initial temperature of 50º C increasing to 220º C at 4º C/min) followed by a hold time of 35-45 minutes, and helium was used as the carrier gas. Fatty acids were identified by comparison to relative retention times of commercial standards (Maytrea Inc., Pleaseant Gap, PA and NuCheck Prep, Elysian, MN). Fatty acid methyl esters were expressed as percent by weight of total fatty acids by calculating empirical correction factors in the way outlined in Christie (87) (Appendix E).

Statistical analysis

The main treatment effects were diet, either coconut oil or fish oil, and the three mercury treatment groups (0.0 ppm MeHg, 0.5 ppm MeHg, 5.0 ppm MeHg). The main treatment effects and the interaction between exposure groups were tested using a two-way ANOVA (Proc GLM). Tukey's Studentized Range test was used to test for differences in means of fatty acids between exposure groups. The results were reported as post hoc adjusted values and were considered significant when $P < 0.05$. The statistical software used was SAS version 9, (SAS Institute Inc. Cary, NC).

Table 3. Experimental design.

	0 ppm MeHg	0.5 ppm MeHg	5.0 ppm MeHg
Coconut Oil[1]	8	8	8
Fish Oil[1]	8	8	8

[1]Total n in each group represents the number of rats from which forebrain samples were taken and analyzed.

Table 4. Nutrient composition of experimental diets

	CO M[1]	CO G[2]	FO M[3]	FO G[4]
Product #				
		g/100g diet		
Protein	14.3	20.5	14.3	20.5
Carbohydrate	73.1	63.9	73.1	63.9
Fat	4.0	7.0	4.0	7.0
kcal/gm	3.9	4.0	3.9	4.0
Ingredient		g		
Casein, 30 Mesh	140	200	140	200
L-Cystine	1.8	3	1.8	3
L-Methionine	0.8	1.6	0.8	1.6
Corn Starch	496.012	404.006	496.012	404.006
Maltodextrin 10	125	125	125	125
Sucrose	100	100	100	100
Cellulose, BW200	50	50	50	50
Special EPAX Fish Oil[5]	0	0	13.2	23.1
Coconut Oil, Special[6]	13.2	23.1	0	0
Palm oil	17.12	29.96	17.12	29.96
Safflower Oil	3.68	6.44	3.68	6.44
Soybean Oil	6	10.5	6	10.5
t-Butylhydroquinone	0.008	0.014	0.008	0.014
Salts S10022G	0	35	0	35
Salts S10022M	35	0	35	0
Vitamin Mix V10037	10	10	10	10
Choline Chloride	1.38	1.38	1.38	1.38
Total	1000	1000	1000	1000

[1] CO M: Coconut Oil-Maintenance Diet based on the AIN-93 diet for mature rodents; contains a mixture of coconut, palm, safflower, and soybean oils (83).
[2] CO G: Coconut Oil-Growth Diet based on the AIN-93 diet for gestating and growing rodents; contains a mixture of coconut, palm, safflower, and soybean oils (83).
[3] FO M: Fish Oil-Maintenance Diet based on the AIN-93 diet for mature rodents; contains a mixture of fish, palm, safflower, and soybean oils (83).
[4] FO G: Fish Oil-Growth Diet based on the AIN-93 diet for gestating and growing rodents; contains a mixture of fish, palm, safflower, and soybean oils (83).
[5] Special EPAX Fish oil, Mixture 100.01, EPAX 0626TG and EPAX 3000TG. Special EPAX Fish oil has 0.4 g Coviox T-70 and 0.56 g of Copheral F-1300 added/kg of oil.
[6] Add 0.4 g Coviox T-70 and 0.56 g 22of Copheral F-1300 to each kg of Coconut oil.

Table 5. Fatty acid composition of experimental diets[6]

Fatty acid	CO M[1]	CO G[2]	FO M[3]	FO G[4]
		% by weight		
10:0	0.76	0.85	0.07	0.07
12:0	17.88	18.01	1.48	0.49
14:0	8.05	7.63	3.65	3.16
16:0	25.57	25.21	29.17	28.73
16:1 n-7	0.16	0.13	2.52	2.51
18:0	4.12	3.76	4.55	4.38
18:1 n-9	22.48	23.38	25.86	27.06
18:2 n-6	19.97	19.73	21.16	21.05
18:3 n-3	1.02	1.30	1.29	1.61
20:5 n-3	-	-	5.04	5.34
22:6 n-3	-	-	5.23	5.61
∑ SFA[5]	56.38	55.46	38.92	36.83
∑ MUFA[5]	22.64	23.51	28.38	29.57
∑ PUFA[5]	20.99	21.03	32.72	33.61
∑ n-3	1.02	1.30	11.56	12.56
∑ n-6	19.97	19.73	21.16	21.05

[1] CO M: Coconut Oil-Maintenance Diet based on the AIN-93 diet for mature rodents; contains a mixture of coconut, palm, safflower, and soybean oils (83).
[2] CO G: Coconut Oil-Growth Diet based on the AIN-93 diet for gestating and growing rodents; contains a mixture of coconut, palm, safflower, and soybean oils (83).
[3] FO M: Fish Oil-Maintenance Diet based on the AIN-93 diet for mature rodents; contains a mixture of fish, palm, safflower, and soybean oils (83).
[4] FO G: Fish Oil-Growth Diet based on the AIN-93 diet for gestating and growing rodents; contains a mixture of fish, palm, safflower, and soybean oils (83).
[5] Abbreviations are as follows: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids).

Table 6. Composition of AIN-93 vitamin mixture[1]

	AIN 93G	AIN 93M
Ingredient	g/kg mix	
Nicotinic acid	3.000	3.000
Calcium pantothenate	1.600	1.600
Pyridoxine-HCl	0.700	0.700
Thiamin-HCl	0.600	0.600
Riboflavin	0.600	0.600
Folic acid	0.200	0.200
d-Biotin	0.020	0.020
Vitamin B12	2.500	2.500
All-*rac*-α-tocopheryl acetate	15.000	15.000
Retinyl palmitate	0.800	0.800
Vitamin D3	0.075	0.075
Powdered sucrose	974.655	974.655

[1] Research Diets, Inc, New Brunswick, NJ; AIN-93 G Diet for Gestating and Growing Rodents; AIN-93M Diet for Mature Rodents (81-83).

Table 7. Composition of AIN-93 mineral mixture[1]

	AIN 93G	AIN 93M
Ingredient	g/kg mix	
Calcium carbonate	357	357
Potassium phosphate	196	250
Potassium sulfate	46.6	46.6
Potassium citrate	70.78	28
Sodium chloride	74	74
Magnesium oxide	24	24
Ferric citrate	6.06	6.06
Zinc carbonate	1.65	1.65
Manganous carbonate	0.63	0.63
Cupric carbonate	0.30	0.30
Potassium iodate	0.01	0.01
Sodium selenate	0.01025	0.01025
Ammonium paramolybdate	0.00795	0.00795
Sodium meta-silicate	1.45	1.45
Chromium potassium sulfate	0.275	0.275
Lithium chloride	0.0174	0.0174
Boric acid	0.0815	0.0815
Sodium fluoride	0.0635	0.0635
Nickel carbonate	0.0318	0.0318
Ammonium vanadate	0.0066	0.0066
Powdered sucrose	221.026	209.8060

[1] Research Diets, Inc, New Brunswick, NJ; AIN-93 G Diet for Gestating and Growing Rodents; AIN-93M Diet for Mature Rodents (81-83).

CHAPTER IV. RESULTS

Forebrain fatty acid composition

The fatty acid composition in adult rat forebrain phosphatidylethanolamine (PE) (**Tables 8** and **9**) and phosphatidylcholine (PC) (**Tables 10** and **11**) are shown. Ratios of n-3 and n-6 fatty acids are shown also for the PE and PC fractions (**Tables 9** and **11**).

Effects of mercury exposure

None of the n-3 and n-6 fatty acids in the PE fraction were significantly changed by chronic MeHg exposure (**Table 9**). However, in the PE fraction, there was a significant Hg effect in 24:1 n-9 and a mean general effect in 16:1 n-7 (**Table 8**). In the PC fraction, there was a Hg effect on 20:2 n-6 and 22:4 n-6 (**Table 11**). Also, in the PC fraction, 14:0, Sum SFA, 18:1 n-9, and 20:3 n-6 showed a mean general significant Hg effect (**Tables 10** and **11**).

Effects of dietary treatment

n-6 and n-3 fatty acids. Animals receiving the dietary fish oil treatment showed an increase (23-30%) in DHA concentration in the PE fraction ($P<0.0001$) (**Figure 2, Table 9**) and an increase (25-46%) in the PC fractions ($P=0.001$) (**Figure 3, Table 11**) compared to animals on the coconut oil diet. Animals receiving the dietary fish oil treatment showed a decrease (18-20%) in arachidonic acid (ARA) concentration in PE

(P<0.05) (**Figure 4, Table 9**) and a decrease (12-28%) in PC fractions (P<0.05) (**Figure 5**, **Table 11**). In the PE fraction the following PUFAs significantly increased with the addition of dietary fish oil: 18:2 n-6, 20:3 n-6, 20:5 n-3 (**Figure 6, Table 9**) and mutual changes occurred in certain PUFAs in response to dietary fish oil: 22:4 n-6, 22:5 n-6 (**Figure 7**, **Table 9**). In the PC fraction the following PUFAs increased significantly with the addition of dietary fish oil: 18:2 n-6, 20:3 n-6 (**Figure 8**, **Table 11)** and reciprocal changes occurred in certain PUFAs in response to dietary fish oil: 22:4 n-6, 22:5 n-6 (**Figure 9**, **Table 11)**. The following n-3 PUFAs showed a significant overall diet effect in PC, but not any individual effects upon statistical analysis: 18:3 n-3, and 20:5 n-3 (**Table 11)**.

Monounsaturated fatty acids. Animals receiving dietary fish oil demonstrated a significant increase in 24:1 n-9 and no other MUFA in the PE fraction (**Table 8)**. However, in the PC fraction, animals supplemented with FO exhibited a significant decrease in 18:1 n-7, 18:1 n-9, (**Table 10)**. These fatty acids showed an overall diet effect but no individual effects in the PC fraction: 14:1(decreased), 24:1(decreased), 16:1 n-7(increased), 20:1 n-9 (decreased)(**Table 10)**.

Saturated fatty acids. In the PE fraction, 24:0 was significantly increased in the dietary fish oil group demonstrating an overall diet effect (**Table 8)**. In the PC fraction, 22:0 was significantly decreased in the dietary fish oil group demonstrating an overall diet effect (**Table 10)**.

Sums and ratios of fatty acids. In PE there were no significant differences between total SFA, total MUFA, and total PUFA (**Tables 8** and **9)**. However, there were significant increases in total n-3 and total n-6 fatty acids (**Table 9)**. In the PE fraction total n-3 increased 23-31% in the fish oil group compared to the coconut oil group and total n-6 decreased 19-24% in the fish oil group compared to the coconut oil group (**Table 9, Figure 10)**. In the PC fraction total n-3 increased 26-47% in the fish oil group compared to the coconut oil group and total n-6 decreased 28-29% in the fish oil group compared to the coconut oil group (**Table 11, Figure 11)**. In the PC fraction there was an overall significant increase in MUFA in the group receiving dietary fish oil (**Table 10)**.

*Diet*Hg interaction effects*

There were no consistent interactions between dietary fish oil and MeHg in either PE or PC. In the PC fraction 14:0 and the Sum of 18:1 isomers showed a mean general significant effect (**Tables 10)**.

Table 8.Saturated and monounsaturated fatty acid composition of adult rat forebrain phosphatidylethanolamine after exposure to dietary fish oil and methylmercury

	Coconut Oil 0 ppm Hg n=8	Coconut Oil 0.5 ppm Hg n=8	Coconut Oil 5.0 ppm Hg n=8	Fish Oil 0 ppm Hg n=8	Fish Oil 0.5 ppm Hg n=8	Fish Oil 5.0 ppm Hg n=8	Hg Effect	Diet Effect	Interaction
	% by weight of total fatty acids[1,2,3]							P[4]	
14:0	0.25 ± 0.05	0.32 ± 0.05	0.41 ± 0.0	0.23 ± 0.05	0.35 ± 0.05	0.27 ± 0.05	NS	NS	NS
16:0	6.83 ± 0.85	8.27 ± 0.85	6.46 ± 0.85	6.16 ± 0.85	7.40 ± 0.85	6.69 ± 0.85	NS	NS	NS
18:0	21.52 ± 0.64	22.21 ± 0.64	20.22 ± 0.64	20.09 ± 0.64	20.93 ± 0.64	20.29 ± 0.64	NS	NS	NS
20:0	0.20 ± 0.13	0.18 ± 0.02	0.49 ± 0.14	0.26 ± 0.13	0.23 ± 0.14	0.36 ± 0.13	NS	NS	NS
22:0	0.14 ± 0.12	0.55 ± 0.17	0.09 ± 0.24	0.19 ± 0.14	0.13 ± 0.14	0.27 ± 0.14	NS	NS	NS
24:0[5]	0.23 ± 0.22	0.53 ± 0.19	0.94 ± 0.20	0.97 ± 0.18	0.86 ± 0.18	0.88 ± 0.18	NS	0.0373	NS
∑SFA	29.01 ± 1.48	31.56 ± 1.48	28.30 ± 1.48	27.77 ± 1.48	29.78 ± 1.48	28.59 ± 1.48	NS	NS	NS
14:1	0.23 ± 0.04	0.18 ± 0.04	0.22 ± 0.04	0.14 ± 0.04	0.29 ± 0.04	0.17 ± 0.04	NS	NS	NS
16:1 n-7[5]	1.10 ± 0.14	0.76 ± 0.14	0.88 ± 0.14	1.14 ± 0.14	0.76 ± 0.14	0.96 ± 0.14	0.0392	NS	NS
18:1 n-9	14.19 ±1.2	13.75 ±1.2	15.07 ± 1.2	16.05 ± 1.2	16.82 ± 1.2	15.78 ± 1.2	NS	NS	NS
18:1 n-7	2.77 ± 0.17	2.89 ± 0.17	2.85 ± 0.17	2.79 ± 0.17	2.93 ± 0.17	2.60 ± 0.17	NS	NS	NS
18:1*i*[6]	16.96 ± 1.36	16.64 ± 1.36	17.92 ± 1.36	18.84 ± 1.36	19.75 ± 1.36	18.38 ± 1.36	NS	NS	NS
20:1 n-9	2.38 ± 0.25	2.36 ± 0.25	2.60 ± 0.25	2.59 ± 0.25	2.57 ± 0.25	2.46 ± 0.25	NS	NS	NS
22:1 n-9	0.11 ± 0.03	0.12 ± 0.03	0.10 ± 0.04	0.11 ± 0.02	0.09 ± 0.02	0.14 ± 0.03	NS	NS	NS
24:1 n-9	0.33 ± 0.06[ab]	0.18 ± 0.06[a]	0.61 ± 0.08[b]	0.22 ± 0.04[a]	0.06 ± 0.08[a]	0.24 ± 0.06[ab]	0.0124	0.0072	NS
∑ MUFA	20.79 ± 1.62	20.02 ± 1.62	21.67 ± 1.62	22.87 ± 1.62	23.38 ± 1.62	22.04 ± 1.62	NS	NS	NS

[1]Values reported as LSMEANS±SE; NS (P>0.05)
[2]Means within a row with different superscript letters are significantly different (P<0.05) using two way ANOVA with post hoc Tukey's test.
[3]Abbreviations used are as follows: ∑ (sum of), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids).
[4]P values are reported from two-way ANOVA testing.
[5]No differences among individual groups were observed using post hoc Tukey's test.
[6]Sum of 18:1 isomers (18:1 n-7 + 18:1 n-9)

Table 9.n-3 and n-6 fatty acid composition of adult rat forebrain phosphatidylethanolamine after exposure to dietary fish oil and methylmercury

	Coconut Oil 0 ppm Hg n=8	Coconut Oil 0.5 ppm Hg n=8	Coconut Oil 5.0 ppm Hg n=8	Fish Oil 0 ppm Hg n=8	Fish Oil 0.5 ppm Hg n=8	Fish Oil 5.0 ppm Hg n=8	Hg Effect	Diet Effect	Interaction
	% by weight of total fatty acids[1,2,3]							*P*[4]	
18:2 n-6	0.22 ± 0.02[a]	0.21 ± 0.02[a]	0.18 ± 0.02[a]	0.34 ± 0.02[b]	0.30 ± 0.02[b]	0.30 ± 0.02[b]	NS	<0.0001	NS
18:3 n-6	0.07 ± 0.03	0.16 ± 0.03	0.00 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.3	NS	NS	NS
20:2 n-6	0.18 ± 0.02	0.20 ± 0.03	0.18 ± 0.03	0.20 ± 0.02	0.23 ± 0.02	0.17 ± 0.02	NS	NS	NS
20:3 n-6	0.27 ± 0.05[a]	0.27 ± 0.05[a]	0.31 ± 0.05[a]	0.43 ± 0.05[ab]	0.41 ± 0.05[ab]	0.54 ± 0.05[b]	NS	<0.0001	NS
20:4 n-6	16.33 ± 0.48[b]	15.89 ± .48[b]	16.65 ± 0.48[b]	13.10 ± 0.48[a]	13.07 ± 0.48[a]	13.33 ± 0.48[a]	NS	<0.0001	NS
22:4 n-6	7.30 ± 0.24[b]	6.84 ± 0.24[b]	7.28 ± 0.24[b]	4.87 ± 0.24[a]	4.72 ± 0.24[a]	4.77 ± 0.24[a]	NS	<0.0001	NS
22:5 n-6	2.50 ± 0.12[b]	2.34 ± 0.12[b]	2.61 ± 0.12[b]	0.26 ± 0.12[a]	0.27 ± 0.12[a]	0.23 ± 0.12[a]	NS	<0.0001	NS
∑ n-6	26.90 ± 0.72[b]	25.94 ± 0.72[b]	27.19 ± 0.72[b]	19.28 ± 0.72[a]	19.10 ± 0.72[a]	19.39 ± 0.72[a]	NS	<0.0001	NS
∑ LC n-6	26.31 ± 0.79 [b]	25.22 ± 0.79	26.69 ± 0.79 [b]	18.43 ± 0.79 [a]	18.29 ± 0.79 [a]	18.50± 0.79 [a]	NS	<0.0001	NS
18:3 n3	0.09 ± 0.01	0.12 ± 0.02	0.01 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	NS	NS	NS
20:5 n-3	0.00 ± 0.02[a]	0.00 ± 0.02[a]	0.00 ± 0.02[a]	0.21 ± 0.02[b]	0.24 ± 0.02[b]	0.22 ± 0.02[b]	NS	<0.0001	NS
22:6 n-3	23.26 ± 1.51[ab]	22.36 ± 1.51[a]	22.79 ± 1.51[a]	29.82 ± 1.51[c]	27.42 ± 1.51[abc]	29.53 ± 1.51[bc]	NS	<0.0001	NS
∑ n-3	23.30 ± 1.49[a]	22.47 ± 1.49[a]	22.84 ± 1.49[a]	30.11 ± 1.49[b]	27.74 ± 1.49[ab]	30.00 ± 1.49[b]	NS	<0.0001	NS
∑ LC n-3	23.53 ± 1.52[a]	22.59 ± 1.52[a]	23.09 ± 1.52 [a]	30.49 ± 1.52[b]	28.00 ± 1.52 [ab]	30.29 ± 1.52[b]	NS	<0.0001	NS
∑ PUFA	50.20 ± 2.10	48.41 ± 2.10	50.04 ± 2.10	49.39 ± 2.10	46.84 ± 2.10	49.40 ± 2.10	NS	NS	NS
n-6/n-3	1.16 ± 0.04[b]	1.18 ± 0.04[b]	1.21 ± 0.04[b]	0.66 ± 0.04[a]	0.70 ± 0.04[a]	0.65 ± 0.04[a]	NS	<0.0001	NS
LCn-6/LCn-[5]	1.12 ± 0.04[b]	1.13 ± 0.04[b]	1.17 ± 0.04 [b]	0.62 ± 0.04 [a]	0.66 ± 0.04 [a]	0.62± 0.04 [a]	NS	<0.0001	NS

[1] Values reported as LSMEANS±SE; NS (P>0.05)

[2] Means within a row with different superscript letters are significantly different (P<0.05) using two way ANOVA with post hoc Tukey's test.

[3]Abbreviations used are as follows: ∑ (sum of), PUFA (polyunsaturated fatty acids), LC, (long chain >20 C).

[4]P values are reported from two-way ANOVA testing.

[5] LCn-6/LCn-3=(20:2 n-6 + 20:3 n-6 +20:4 n-6 + 22:4 n-6 + 22:5 n-6)/(20:5 n-3 + 22:6 n-3)

Table 10. Saturated and monounsaturated fatty acid composition of adult rat forebrain phosphatidylcholine after exposure to dietary fish oil and methylmercury

	Coconut Oil 0 ppm Hg n=8	Coconut Oil 0.5 ppm Hg n=8	Coconut Oil 5.0 ppm Hg n=8	Fish Oil 0 ppm Hg n=8	Fish Oil 0.5 ppm Hg n=8	Fish Oil 5.0 ppm Hg n=8	Hg Effect	Diet Effect	Interaction
	% by weight of total fatty acids[1,2,3]							P[4]	
14:0	0.20 ± 0.04[a]	0.38 ± 0.04[b]	0.15 ± 0.04[a]	0.17 ± 0.04[a]	0.20 ± 0.04[a]	0.17 ± 0.04[a]	0.0048	NS	0.0340
16:0	40.83 ± 0.89	40.97 ± 0.89	38.44 ± 0.89	38.97 ± 0.89	41.72 ± 0.89	40.05 ± 0.89	NS	NS	NS
18:0	15.82 ± 0.51	16.50 ± 0.51	16.79 ± 0.51	16.40 ± 0.51	16.10 ± 0.51	15.39 ± 0.51	NS	NS	NS
20:0	0.25 ± 0.11	0.28 ± 0.11	0.24 ± 0.11	0.33 ± 0.11	0.32 ± 0.11	0.14 ± 0.11	NS	NS	NS
22:0[5]	0.18 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	NS	0.0422	NS
24:0	0.18 ± 0.07	0.35 ± 0.07	0.34 ± 0.07	0.18 ± 0.07	0.19 ± 0.08	0.17 ± 0.07	NS	NS	NS
∑SFA[5]	57.45 ± 0.85	58.57 ± 0.85	56.07 ± 0.85	56.21 ± 0.85	58.61 ± 0.85	56.03 ± 0.85	0.0142	NS	NS
14:1[5]	0.18 ± 0.03	0.15 ± 0.03	0.12 ± 0.03	0.06 ± 0.04	0.07 ± 0.04	0.06 ± 0.05	NS	0.0126	NS
16:1 n-7[5]	0.63 ± 0.03	0.60 ± 0.03	0.63 ± 0.03	0.66 ± 0.03	0.70 ± 0.03	0.70 ± 0.03	NS	0.0086	NS
18:1 n-9	21.25 ± 0.39[ab]	20.63 ± 0.39[a]	21.80 ± 0.39[abc]	22.54 ± 0.39[bc]	22.00 ± 0.39[abc]	23.17 ± 0.39[c]	0.0153	0.0001	NS
18:1 n-7	5.80 ± 0.13 [b]	5.54 ± 0.13 [ab]	5.68 ± 0.13 [ab]	5.21 ± 0.13 [a]	5.27 ± 0.13 [a]	5.40 ± 0.13 [ab]	NS	0.0007	NS
18:1*i*[6]	26.17 ± 0.48[a]	28.57 ± 0.48[b]	27.48 ± 0.48[ab]	27.75 ± 0.48[ab]	27.05 ± 0.48 [ab]	27.27 ± 0.48[ab]	NS	NS	0.0093
20:1 n-9[5]	0.96 ± 0.03	0.98 ± 0.03	0.94 ± 0.03	0.96 ± 0.03	0.89 ± 0.03	0.86 ± 0.03	NS	0.0232	NS
22:1 n-9	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	NS	NS	NS
24:1 n-9	0.11 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	0.12 ± 0.03	0.23 ± 0.03	0.10 ± 0.03	NS	NS	NS
∑ MUFA	28.99 ± 0.49[ab]	28.07 ± 0.49[a]	29.37 ± 0.49[ab]	29.59 ± 0.49[ab]	29.19 ± 0.49[ab]	30.34 ± 0.49[b]	NS	0.0293	NS

[1]Values reported as LSMEANS±SE; NS (P>0.05)
[2]Means within a row with different superscript letters are significantly different using two way ANOVA with post hoc Tukey's test.
[3]Abbreviations used are as follows: ∑ (sum of), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids).
[4]P values are reported from two-way ANOVA testing.
[5]No differences among individual groups were observed using post hoc Tukey's test.
[6] Sum of 18:1 isomers (18:1 n-7 + 18:1 n-9)

Table 11.n-3 and n-6 fatty acid composition of adult rat forebrain phosphatidylcholine after exposure to dietary fish oil and methylmercury

	Coconut Oil 0 ppm Hg n=8	Coconut Oil 0.5 ppm Hg n=8	Coconut Oil 5.0 ppm Hg n=8	Fish Oil 0 ppm Hg n=8	Fish Oil 0.5 ppm Hg n=8	Fish Oil 5.0 ppm Hg n=8	Hg Effect	Diet Effect	Interaction
	% by weight of total fatty acids[1,2,3]							*P*[4]	
18:2 n-6	0.36 ± 0.03 [a]	0.37 ± 0.03[a]	0.33 ± 0.03[a]	0.58 ± 0.03 [b]	0.54 ± 0.03[b]	0.54 ± 0.03[b]	NS	<0.0001	NS
18:3 n-6	0.05 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	NS	NS	NS
20:2 n-6	0.06 ± 0.01[ab]	0.12 ± 0.02 [b]	0.07 ± 0.01 [ab]	0.07 ± 0.01 [ab]	0.09 ± 0.01 [ab]	0.06 ± 0.01 [a]	0.0090	NS	NS
20:3 n-6	0.13 ± 0.02[a]	0.12 ± 0.02[a]	0.15 ± 0.02[a]	0.28 ± 0.02[bc]	0.23 ± 0.02[b]	0.31 ± 0.02 [c]	0.0076	<0.0001	NS
20:4 n-6	7.66 ± 0.47 [b]	7.45 ± 0.47[ab]	7.70 ± 0.47 [b]	6.74 ± 0.47[ab]	5.35 ± 0.47[a]	6.01 ± 0.47 [ab]	NS	0.0002	NS
22:4 n-6	0.87 ± 0.05 [b]	0.73 ± 0.05 [ab]	0.92 ± 0.05 [b]	0.61 ± 0.05 [a]	0.55 ± 0.05 [a]	0.61 ± 0.05 [a]	0.0294	<0.0001	NS
22:5 n-6	0.49 ± 0.07 [b]	0.55 ± 0.07 [b]	0.57 ± 0.07 [b]	0.08 ± 0.08 [a]	0.09 ± 0.08 [a]	0.10 ± 0.07 [a]	NS	<0.0001	NS
∑ n-6	9.57 ± 0.50[bc]	9.31 ± 0.50[bc]	9.79 ± 0.50[c]	8.34 ± 0.50[abc]	6.79 ± 0.50[a]	7.66 ± 0.50[ab]	NS	<0.0001	NS
∑ LC n-6	9.06 ± 0.48 [c]	8.79 ± 0.48[bc]	9.24 ± 0.48[c]	7.44 ± 0.48 [abc]	5.99 ± 0.48[a]	6.77 ± 0.48 [ab]	NS	<0.0001	NS
18:3 n-3[5]	0.03 ± 0.04	0.26 ± 0.06	0.08 ± 0.03	0.03 ± 0.00	0.03 ± 0.06	0.03 ± 0.03	NS	0.0312	NS
20:5 n-3[5]	0.00 ± 0.05	0.00 ± 0.05	0.00 ± 0.05	0.13 ± 0.06	0.24 ± 0.06	0.08 ± 0.05	NS	0.0021	NS
22:6 n-3	3.94 ± 0.43[ab]	3.68 ± 0.43[a]	4.73 ± 0.43[abc]	5.74 ± 0.43[bc]	5.26 ± 0.43[abc]	5.89 ± 0.43[c]	NS	0.0001	NS
∑ n-3	3.99 ± 0.45[a]	4.06 ± 0.45[a]	4.76 ± 0.45[ab]	5.85 ± 0.45[ab]	5.47 ± 0.45[ab]	5.98 ± 0.45[b]	NS	0.0002	NS
∑ LC n-3	4.07 ± 0.42[ab]	3.79 ± 0.42[a]	4.88 ± 0.42 [abc]	6.13 ± 0.42[c]	5.70 ± 0.42[bc]	6.27 ± 0.42[c]	NS	<0.0001	NS
∑ PUFA	13.56 ± 0.67	13.36 ± 0.67	14.56 ± 0.67	14.19 ± 0.67	12.26 ± 0.67	13.64 ± 0.67	NS	NS	NS
n-6/n-3	2.41 ± 0.20[c]	2.45 ± 0.20[c]	2.20 ± 0.20[bc]	1.48 ± 0.20 [ab]	1.34 ± 0.20[a]	1.40 ± 0.20[ab]	NS	<0.0001	NS
LCn-/LCn-3	2.23 ± 0.16 [b]	2.39 ± 0.16 [b]	2.02 ± 0.16 [b]	1.25 ± 0.16 [a]	1.13 ± 0.16 [a]	1.16 ± 0.16 [a]	NS	<0.0001	NS

[1]Values reported as LSMEANS±SE; NS (P>0.05)
[2]Means within a row with different superscript letters are significantly different using two-way ANOVA with post hoc Tukey's test.
[3]Abbreviations used are as follows: ∑ (sum of), PUFA (polyunsaturated fatty acids), LC, (long chain >20 C).
[4]P values are reported from two-way ANOVA testing.
[5]No differences among individual groups were observed using post hoc Tukey's test.
[6] LCn-6/LCn-3=(20:2 n-6 + 20:3 n-6 +20:4 n-6 + 22:4 n-6 + 22:5 n-6)/(20:5 n-3 + 22:6 n-3).

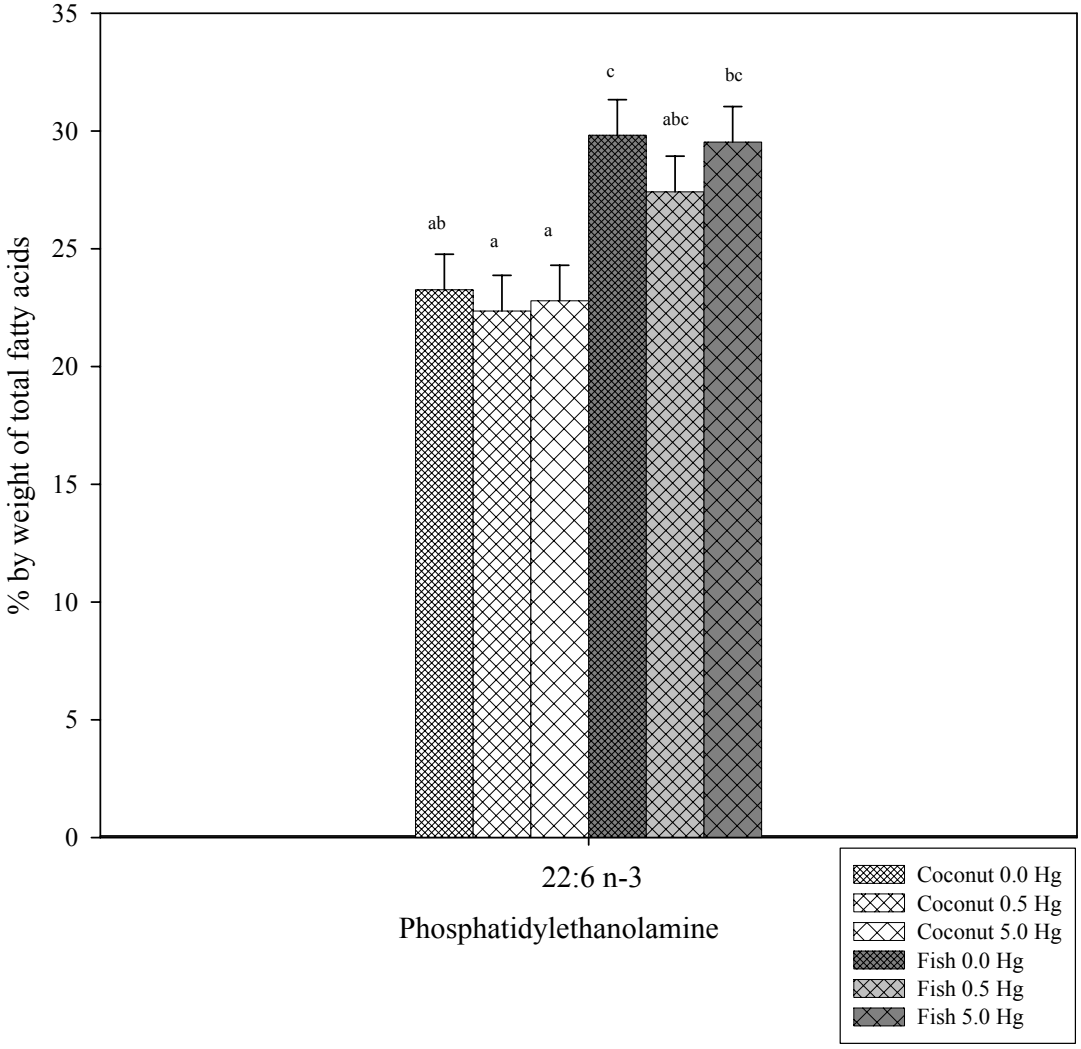

Figure 2. DHA (22:6 n-3) in phosphatidylethanolamine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$) was found. Bars with different letters are significantly different ($P<0.05$).

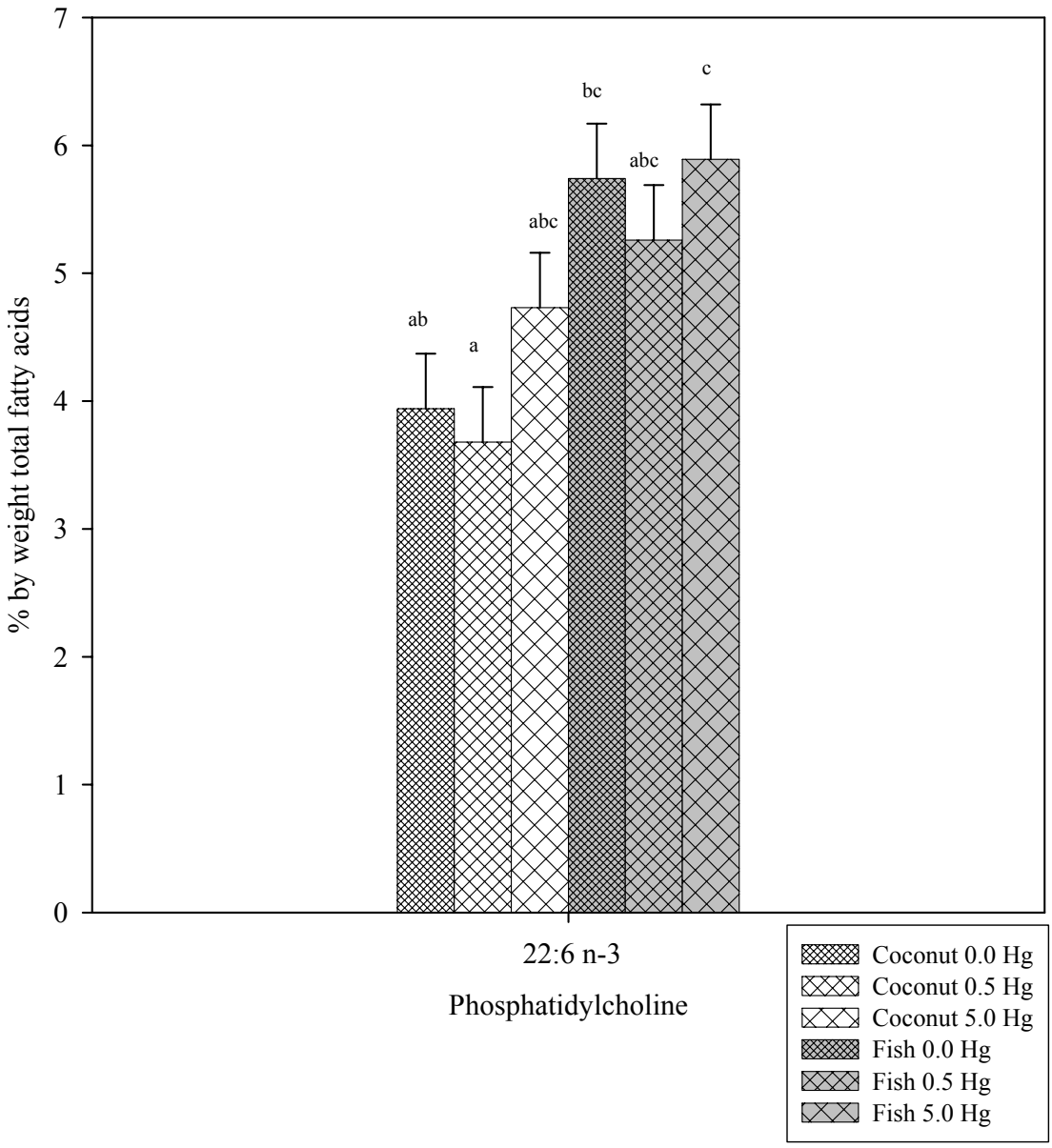

Figure 3. DHA (22:6 n-3) in phosphatidylcholine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect (P=0.0001) was found. Bars with different letters are significantly different (P<0.05).

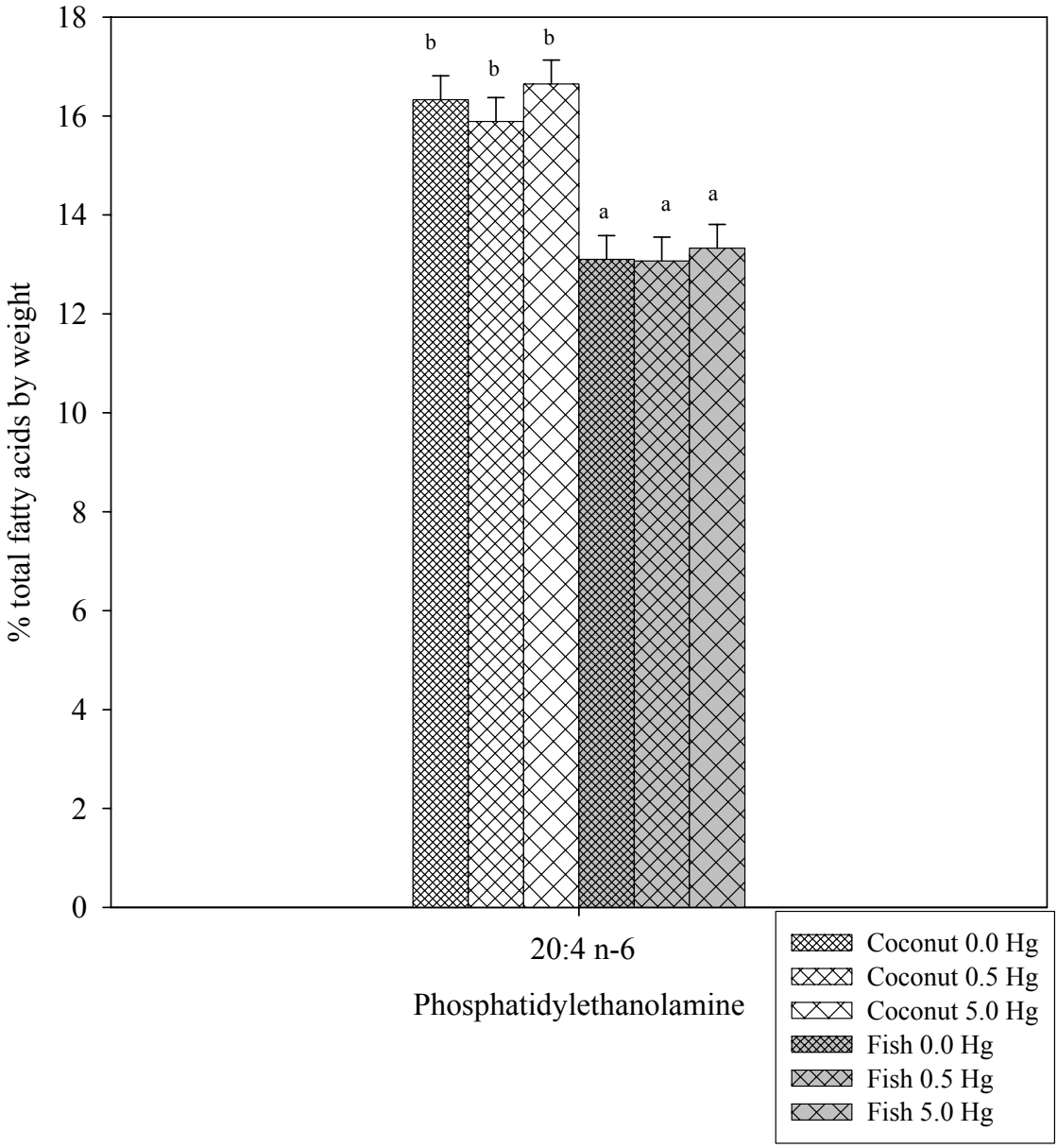

Figure 4. ARA (20:4 n-6) in phosphatidylethanolamine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$) was found. Bars with different letters are significantly different ($P<0.05$).

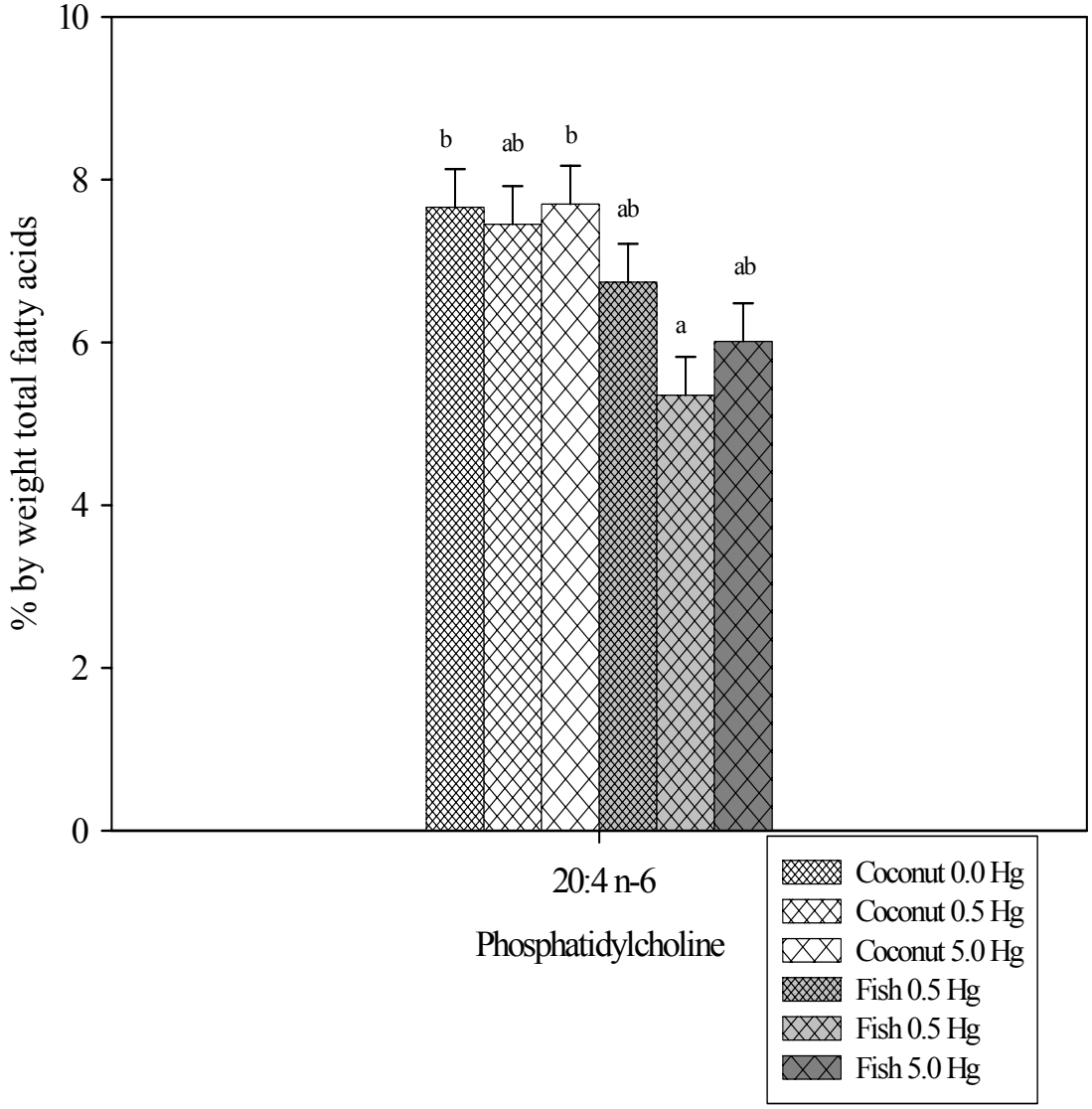

Figure 5. ARA (20:4 n-6) in phosphatidylcholine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect (P=0.0002) was found. Bars with different letters are significantly different (P<0.05).

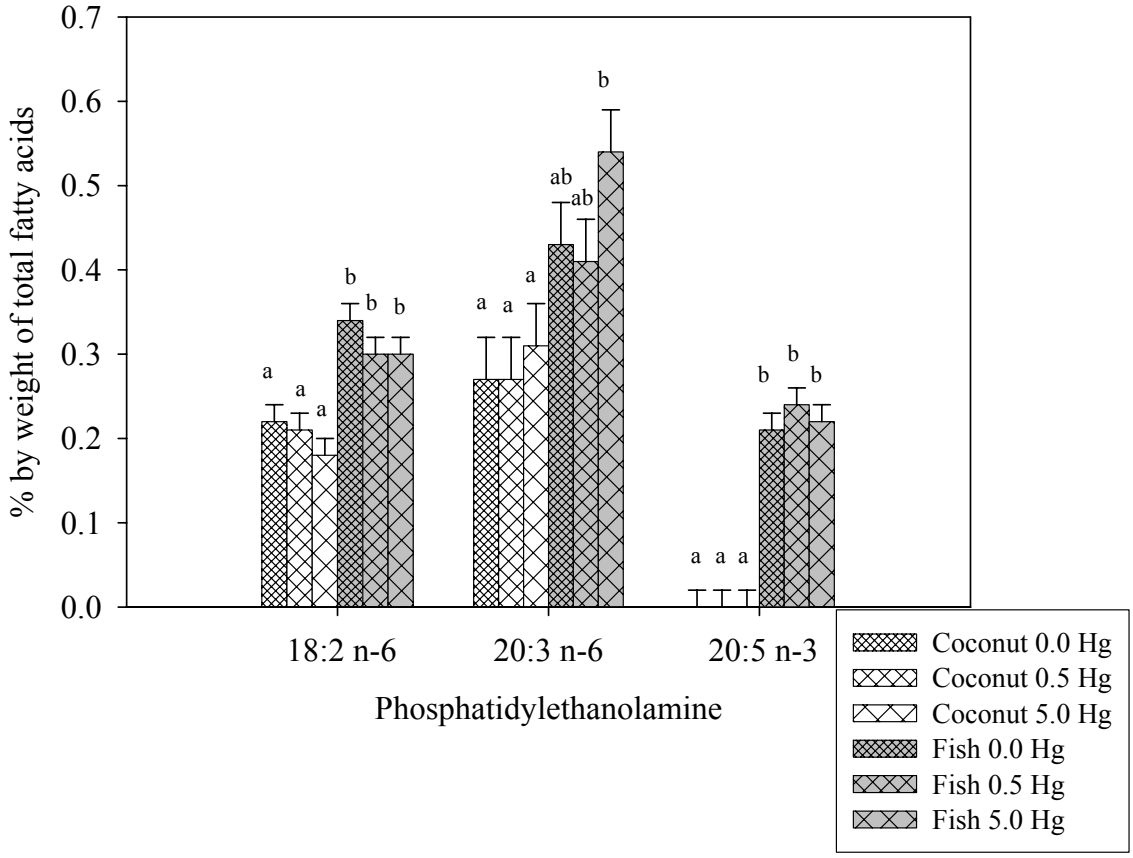

Figure 6. 18:2 n-6, 20:3 n-6, and 20:5 n-3 fatty acids increase in response to fish oil compared with coconut oil in the phosphatidylethanolamine fraction. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$) was found. Bars with different letters are significantly different ($P<0.05$).

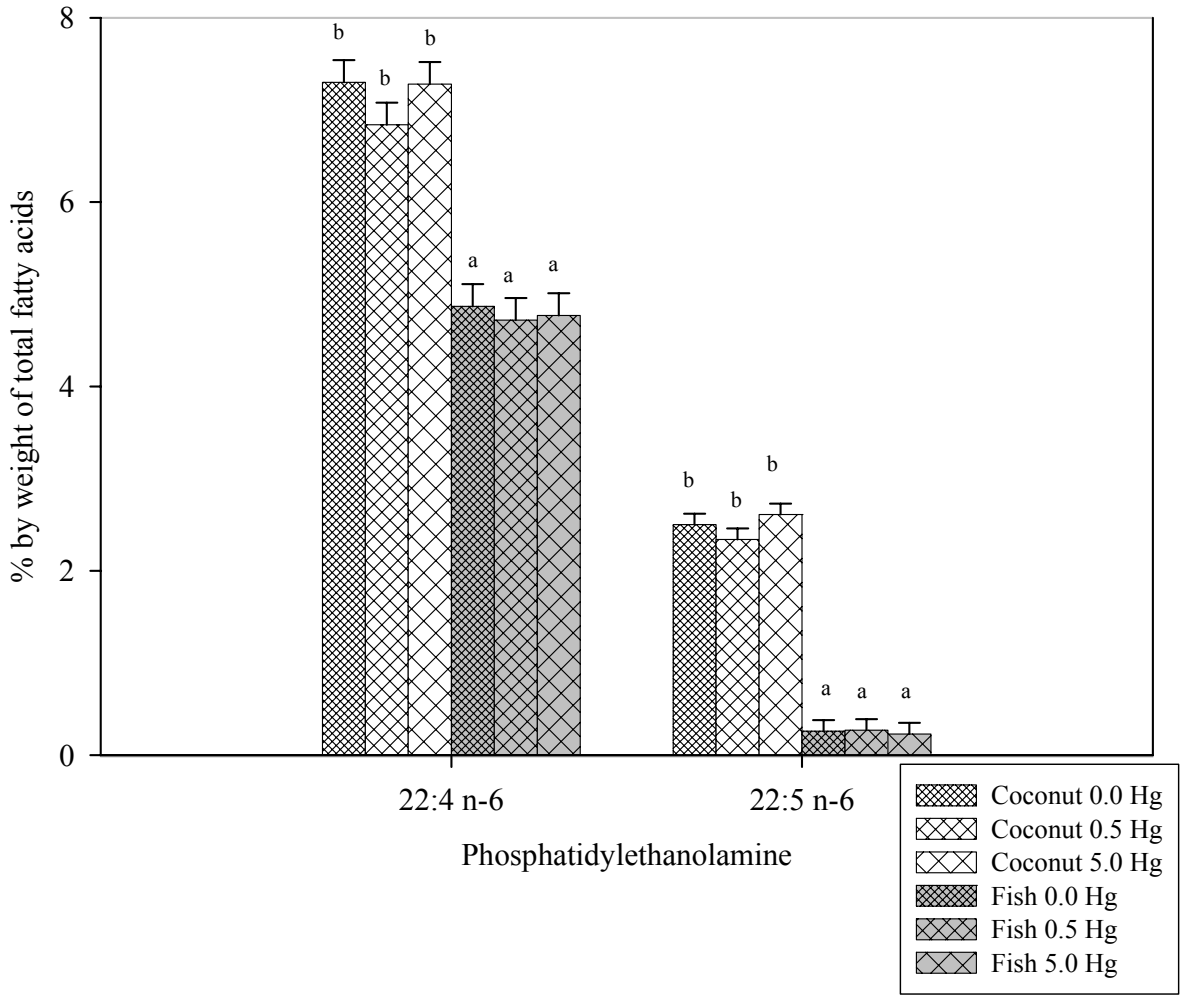

Figure 7. 22:4 n-6 and 22:5 n-6 in phosphatidylethanolamine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$) was found. Bars with different letters are significantly different ($P<0.05$).

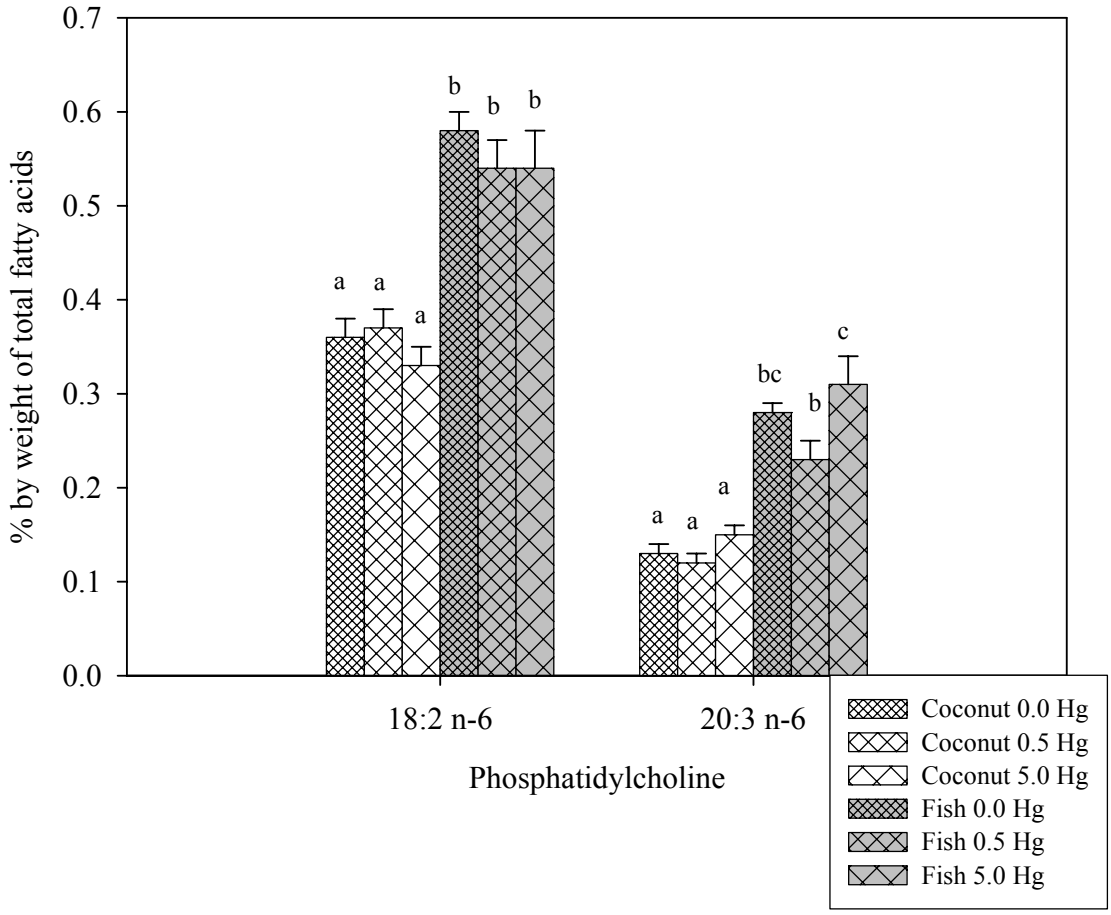

Figure 8. 18:2 n-6 and 20:3 n-6 fatty acids increase in response to fish oil compared with coconut oil in the phosphatidylcholine fraction. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$), and an overall Hg effect for 20:3 n-6 ($P=0.0076$) were found. Bars with different letters are significantly different ($P<0.05$).

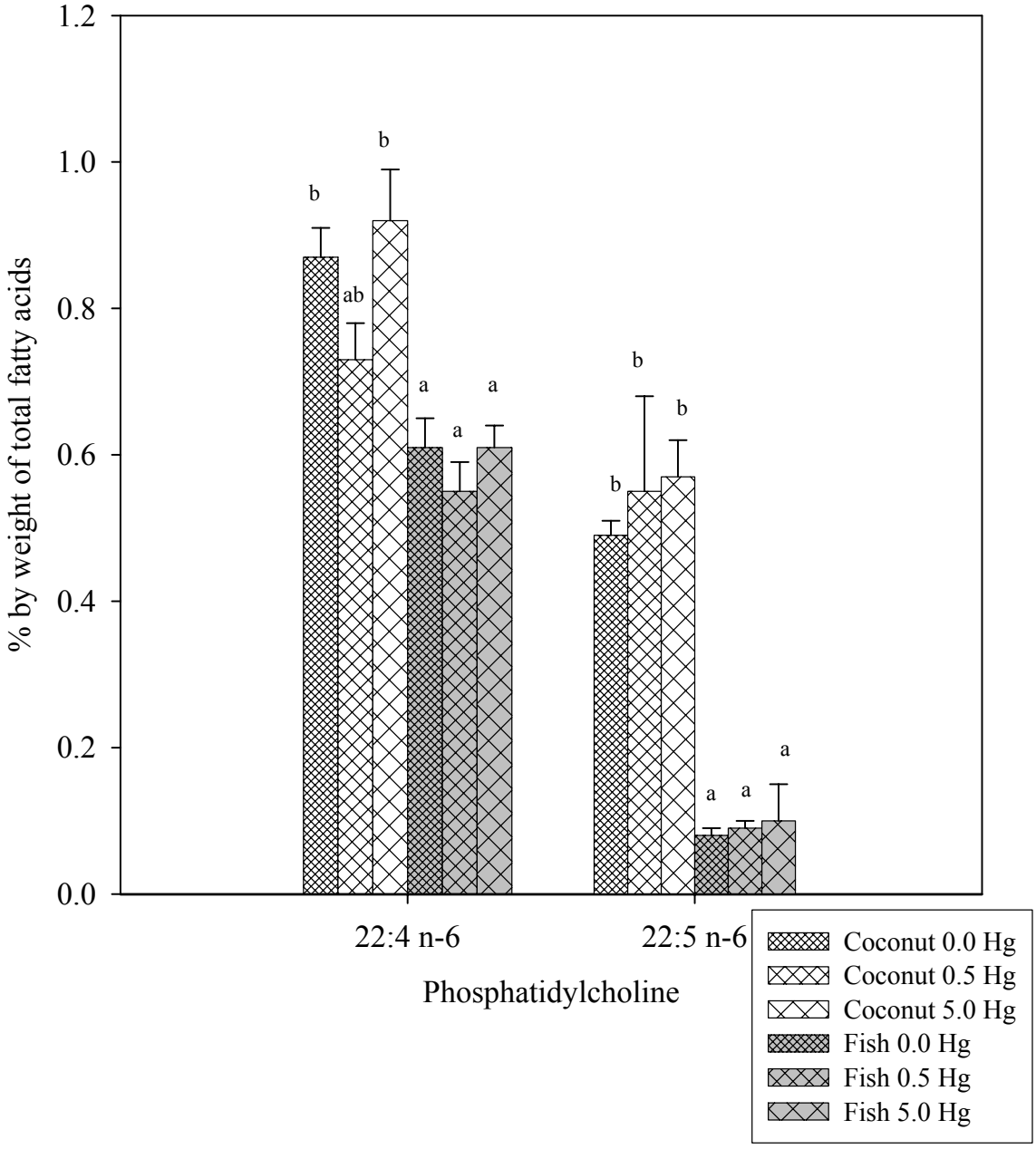

Figure 9. 22:4 n-6 and 22:5 n-6 in phosphatidylcholine of adult rat forebrain. Results are reported as LSMEANS±SE. An overall diet effect ($P<0.0001$), and an overall Hg effect for 22:4 n-6 ($P=0.0294$) were found. Bars with different letters are significantly different ($P<0.05$).

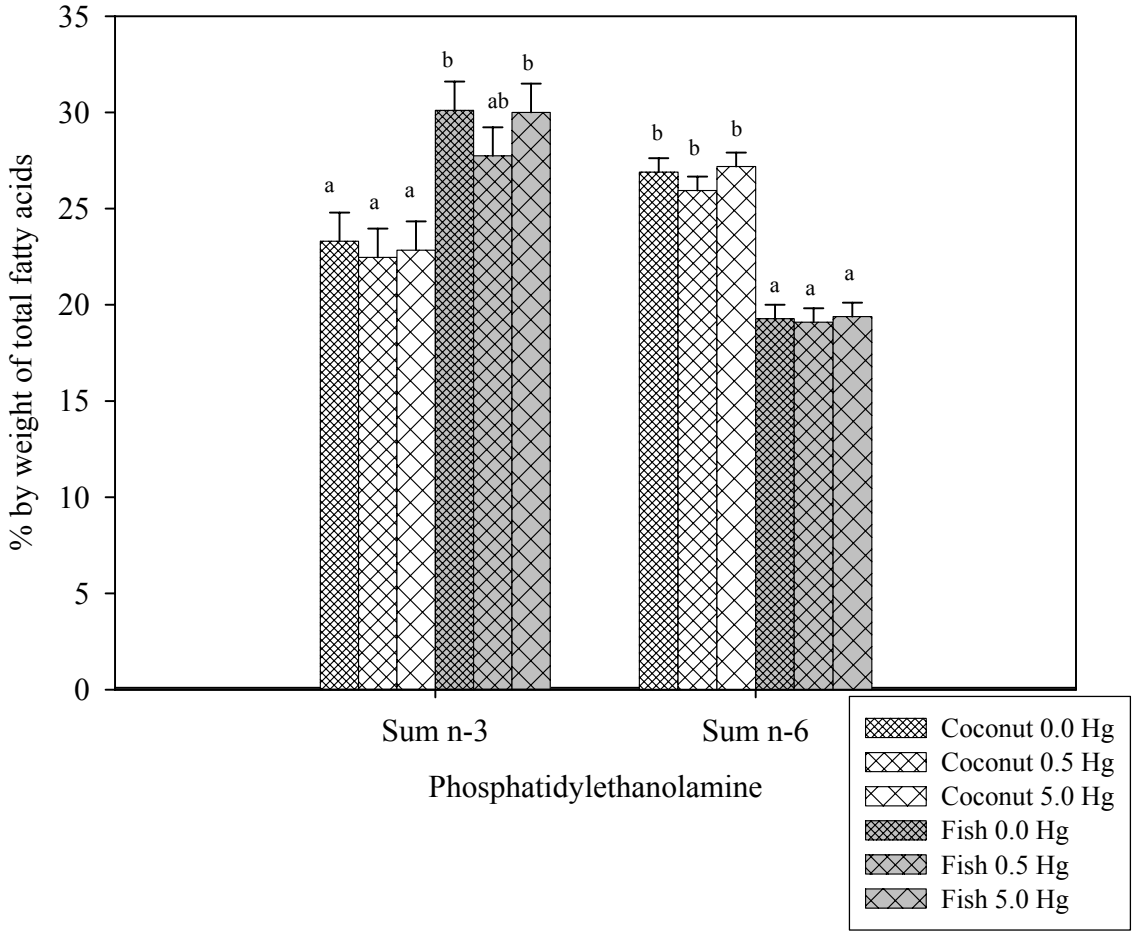

Figure 10. A reciprocal effect of total n-3 and total n-6 fatty acids occurs in phosphatidylethanolamine in adult rat forebrain. Results are reported as LSMEANS±SE. Overall diet effects ($P<0.0001$) were found for both n-3 and n-6 fatty acids. Bars with different letters are significantly different ($P<0.05$).

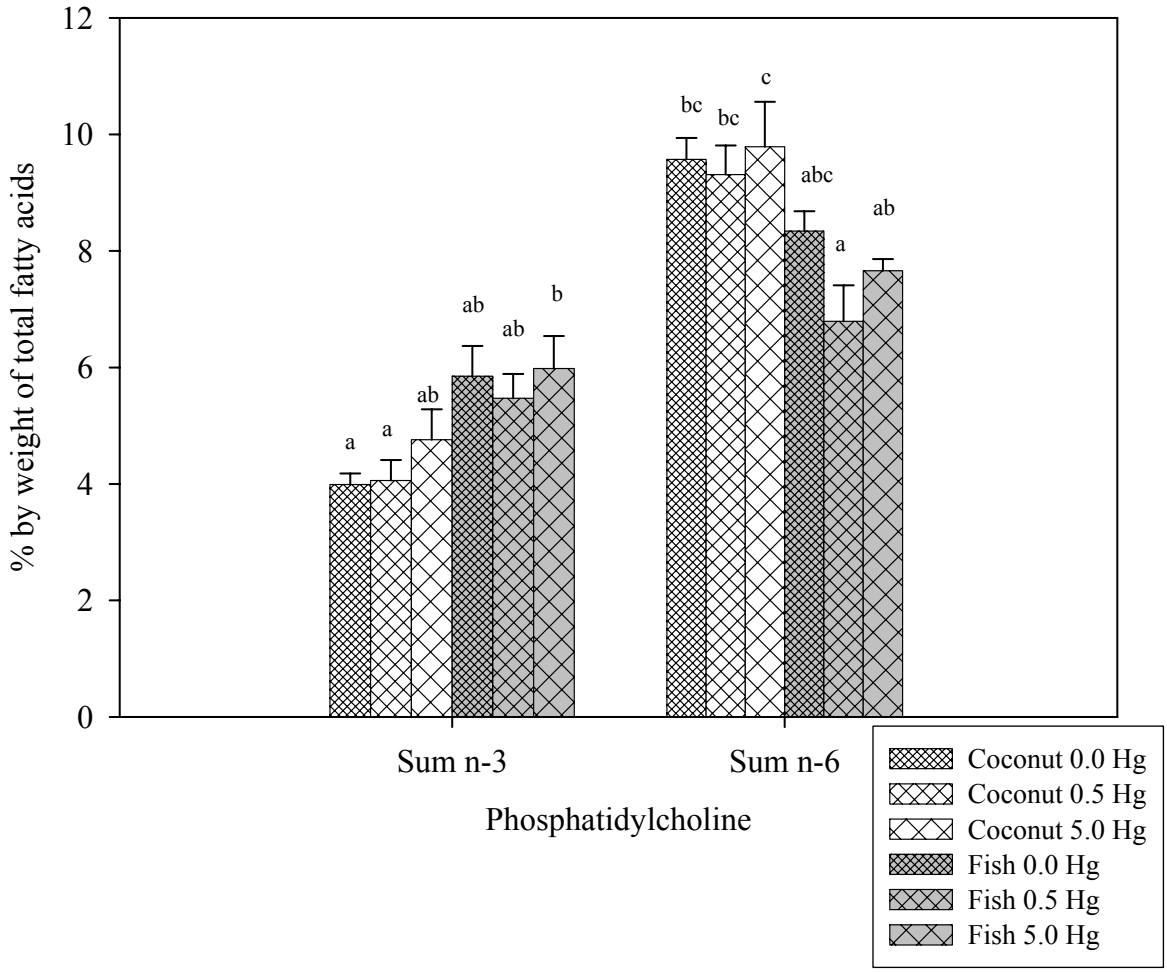

Figure 11. A reciprocal effect of total n-3 and total n-6 fatty acids occurs in phosphatidylcholine in adult rat forebrain. Results are reported as LSMEANS±SE. Overall diet effects (Sum n-6: $P<0.0001$; Sum n-3: $P=0.0002$) were found. Bars with different letters are significantly different ($P<0.05$).

CHAPTER V. DISCUSSION

Chronic MeHg exposure did not result in significant alterations of adult rat brains by decreasing concentrations of docosahexaenoic acid (DHA) or other long chain polyunsaturated fatty acid (LCPUFA) in membrane phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). DHA exhibited greater concentrations in groups receiving fish oil compared with groups receiving coconut oil, but there were no significant differences in DHA among MeHg exposure groups.

In the current study, the rats were exposed chronically to MeHg by receiving 0.5 ppm and 5 ppm MeHg chloride in drinking water (40, and 400 microgram/kg body weight/day MeHg). Previous studies revealed that rats bind considerably more mercury in the blood because rats have a greater density of red blood cells with higher blood to brain ratios of mercury concentration for rats than mice, guinea pigs, dogs, monkeys and humans. Therefore, MeHg exposure in rats should be greater to have the same effect as in other animals (88-89).

Newland and Reile found that when female rats were exposed to 0, 0.5 and 6.4 ppm MeHg in drinking water, there was a non-linear accumulation of MeHg in the brain of the offspring (90). MeHg may not have significantly affected fatty acid concentrations in the forebrain in the current study because the doses of MeHg may not have been toxic enough. Very low concentrations of some fatty acids are hard to interpret, and overall

statistical significance with no individual significant effects may be the result of a loss of sensitivity with decreased percentages.

Dietary composition may affect the toxicity of MeHg. Dietary factors, such as selenium, zinc, cysteine, proteins, fats, fibers, and vitamins have been shown to modulate mercury toxicity (91). Recent studies have shown that diets differing in composition also have an effect on mercury deposition in tissues.

Højbjerg et al, investigated the effect of dietary lipids on whole-body retention and organ distribution of mercury in mice. A single dose of MeHg was given to female NMRI mice fed semi-synthetic diets containing varying amounts (5, 10, 20, or 50%) of energy derived from lipid (cod liver, soya oil, or coconut). Different amounts of energy (5 and 50%) from cod liver oil did not affect the whole-body retention of mercury after administration. However, mice fed a diet containing 50 percent of energy from coconut oil retained significantly higher amounts (65.39%) of MeHg than did mice fed a diet containing 5 percent of energy from coconut oil (39.20%). There was a significant reduction of whole body retention of mercury in mice fed a diet containing 50% cod liver oil (24.46%) compared with mice fed a diet containing 50% coconut oil. Conversely, the amount of mercury retention in total brain tissue did not vary greatly when comparing coconut and cod liver oil. The dietary treatment containing 50 percent of energy from coconut oil had the lowest concentration of MeHg retention in brain tissue (0.99%) (92).

Jin et al, conducted a study investigating the effects of different types of dietary fats on target tissue retention and toxicity of MeHg in rats. Weanling male Sprague Dawley rats were administered semipurified casein-based isocaloric diets containing soy oil, seal oil, DHA, fish oil, or lard for 28 days. Rats were then gavaged with 0, 1, or

3 mg MeHg/kg body weight per day and fed the same diet for 14 consecutive days. On day forty-three, the liver and spleen were removed, fixed, and examined for pathological changes along with blood, feces, liver, and brain which were analyzed for total mercury and/or MeHg contents. Total mercury contents in all tissues measured increased with dose. Many of the effects of MeHg were diet-dependent. In rats fed the lard diet with 3 mg MeHg/kg, body weight significantly increased relative liver and spleen weight as compared with vehicle control; whereas in rats fed the fish oil, soy oil, seal oil, or DHA, this effect of MeHg was less obvious or absent, suggesting a protective effect of these diets. MeHg at 3 mg/kg body weight significantly decreased serum albumin levels in all except DHA dietary groups, implying a protection by the DHA diet on this parameter. This suggests that dietary fats may be a determining factor in mercury partitioning between tissues and points to an effect of dietary fat on the metabolism and/or disposition of MeHg in tissues (93). These studies demonstrated that diet composition is of major importance to the toxicokinetics of methylmercury. The lack of significant effect of the highest dose we used in our study (5.0 ppm) on rat forebrain fatty acid concentrations is not fully understood. In our current study, we only saw minor effects of MeHg in the brain, and these effects were almost exclusively occurring within the coconut oil group.

There is evidence that methylmercury increases lipid peroxidation in membranes and can lead to changes in the fatty acid composition of the membrane (94-95). Ando et al, conducted a study investigating the ability of MeHg to induce lipid peroxidation in rats. One group of 5 rats was assigned to a dietary treatment to which 10 ppm of methylmercury was added while another group of 5 rats was fed a diet containing no MeHg as a control. Samples of expired air and blood were obtained every two weeks and

analyzed for ethane and pentane production and for plasma fatty acid composition, respectively. Oxidative stress may initiate lipid peroxidation that generates ethane. Ethane, at low concentrations, is eliminated by pulmonary exhalation. The amount of expired ethane was generally higher in the MeHg exposure group than in the control group throughout the experimental period. During the last 2 weeks of the experimental period, the difference in the amount of expired ethane between the two groups was statistically significant (95).

When dietary fish oil is consumed, the concentration of DHA increases within the membrane (10, 15-19). This increase in DHA could protect the membrane from peroxidation (74), which could be the reason MeHg was unable to alter the brain composition in groups receiving the fish oil.

When results from the current study were compared with results from the brain composition tables in the literature review, we found similarities in both PE and PC. DHA was comparable in diets supplemented with fish oil, or n-3 adequate (11-29% PE, 0.3-5% PC), and the current study (22-29% PE, 3-5% PC). In the PC fraction, the current study reported ranges of 16:0 that ranged from 38 to 40 percent, and in the brain composition tables values ranged from 13 to 50 percent. However, 22:5 n-6 showed a significant decrease in fish oil supplemented animals in both PE and PC in the current study, this trend was consistent with all but one study (15) reported in the brain composition tables, and was present only in the PE fraction.

In the present study we hypothesized that animals supplemented with fish oil will have an increased concentration of DHA in brain tissue in both the PE and PC fractions. Differences in dietary composition of diets can alter the fatty acid profiles of forebrain

phospholipids (PE and PC). Animals supplemented with dietary fish oil showed a significant increase in DHA and a significant decrease in ARA concentration in both the PE and PC fractions. Eicosapentaenoic acid was only present in the fish oil fed animals and was a much lower concentration than the DHA in both the PE and PC fractions.

Bezard et al (96), reviewed the metabolism of essential fatty acids. These authors outline how dietary ALA inhibits desaturation of LA by competing for the delta-6 desaturase enzyme (2). Moreover, the desaturation products ARA, EPA, and DHA also inhibit desaturation of LA and ALA by delta-6 desaturase and of 20:3 n-6 by the delta-5 desaturase enzyme. The results of our study show that in the PE and PC fraction, there was a significant increase in LA ($P<0.001$) and significant decreases in ARA ($P<0.01$), 22:4 n-6 ($P<0.01$), and 22:5 n-6 ($P<0.01$) with dietary fish oil supplementation, signaling the inhibition of the delta-6 desaturase for the n-6 fatty acids by fish oil.

Aging has been associated with modifications in the composition of the biophysical properties of brain membranes in rats and humans (97-98). Favreliere et al, found the DHA levels were markedly decreased in hippocampus PE whereas DHA levels in frontal cortex were only slightly affected. Age induced effects seemed to differ depending on the specific phospholipids involved and the brain region studied. This may correspond to a different sensitivity of the cognitive processes mediated respectively by these two structures (99). Our results paralleled previous results with neonatal brain data in reference to which fatty acids increased or decreased in response to dietary fish oil. However, the percentage of individual fatty acids in the neonatal brain were considerably lower when compared with adult samples. In the coconut oil group neonatal DHA concentrations for PE and PC were 15.9 and 1.89 percent of total fatty acids (88%

increase) compared with adults 22.8 and 4.12 percent of total fatty acids (82% increase) respectively. In rats, most of the DHA is accumulated in neural tissues after birth, and the brain growth spurt begins in the immediate postnatal period, with the maximum rate of growth occurring at about 10 days of age, which is why neonatal values could have been low compared with the adults. In contrast, the human brain growth spurt extends from the beginning of the third trimester of gestation and continues for about 18 months after birth (21, 31).

The developing brain readily incorporates dietary fatty acids, while the adult brain is refractory to changes in fatty acid composition. Anderson attempted to localize the time in development when this transition occurs, chicks were fed large amounts of n-3 fatty acids from fish oil beginning at 0, 1, 2, or 3 weeks of age. Control chicks were fed a soybean oil-based diet, as were the experimental chicks before introduction of the fish oil diet. Resistance to diet-induced increases in brain n-3 fatty acid levels began at 2 weeks of age, and was substantial at 3 weeks. Docosahexaenoic acid was particularly resistant to change as the brain matured, increasing by 38 percent when fish oil was fed from time of hatching, but only by 8 percent when fish oil feeding was delayed until 3 weeks of age. Dietary fish oil caused a decrease in brain n-6 fatty acids, and this decrease occurred even at later time points when the rise in brain n-3 fatty acids was much less prominent. These results show that resistance to changes in brain fatty acid composition is evident at a relatively early age, before brain development is complete (100).

CHAPTER VI. CONCLUSIONS

In the current study, chronic methylmercury exposure had no significant effects on the two major long chain polyunsaturated fatty acids (LCPUFA) in the adult rat forebrain. Neither DHA or ARA were affected by exposure to methylmercury (MeHg). However, a significant diet effect did occur in the LCPUFA when animals consumed fish oil with a significant increase in DHA in both PE (23-30% increase) and PC (25-46% increase) fractions along with a significant decrease in ARA in both PE (18-20% decrease) and PC (12-28% decrease) fractions. Thus, on the basis of these findings, there was no evidence showing a relationship between LCPUFA and MeHg toxicity in the adult rat brain.

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APPENDICES

Appendix A

Lipid extraction of tissue homogenates

1. For one sample in duplicate, label four large size (25 x 150 mm) tubes, four medium (16 x 125 mm) and 6 small (13 x 100 mm) tubes with lined Teflon caps. Put Teflon tape on the medium sized tubes.
2. Take forebrain sample out of freezer, weigh (approximate weight 0.35-0.63g), and transfer to glass homogenizer.
3. Add Ultrapure water to sample at a concentration of 0.1 g/1 mL water (e.g. if 0.23 gram sample, use 2.3 mL of distilled water).
4. Homogenize for 5 minutes until homogeneous.
5. Put 1 mL of homogenate in large test tube and add 20 mL solvent (chloroform:methanol = 2:1; methanol containing 50 mg BHT/L).
6. Flush the tube with nitrogen, screw on cap tightly and vortex for 15 minutes.
7. Filter the homogenate using Whatman #4 filter paper into another large test tube. Rinse original tube with 2 mL chloroform: methanol (2:1) solvent and pour through paper.
8. Add 4.6 mL of 0.58% NaCl solution to the test tube, and add 1 mL of lower phase solvent (chloroform: methanol: water; 86:14:1) to the tube.
9. Flush the tube with nitrogen and cap tube; vortex for 5 minutes.
10. Centrifuge the test tubes at 5º C for 10 minutes at 2000 rpm.
11. Use a 10 mL disposable pipette to remove the supernatant (upper phase), and add 2 mL of upper phase (chloroform: methanol: water; 3:48:47).

12. Vortex for 2 minutes, centrifuge at 5º C for 5 minutes at 2000 rpm, and then remove the top layer.
13. Evaporate the lower layer under nitrogen until an oil-like appearance occurs.
14. Weigh small test tube using an analytical balance prior to adding any of the sample.
15. Add 1 mL of dichloromethane (DCM) to the sample and vortex for 5 seconds. Transfer the dissolved sample to small size test tube.
16. Evaporate and weigh the samples.
17. Reconstitute the sample using 1 mg lipid/100 µL DCM.

Appendix B

Thin layer chromatography (Skipsky et al, 85)

1. Bake 20 x 20 cm (cut in half), 250 Microns Silica gel-G # 01011 thin layer chromatography plate at 150º C (Analtech Inc., Newark, DE) for 1 hour.
2. Prepare solvent tank using a chloroform: methanol: acetic acid: water (50:25:6:1.5); add a piece of chromatography paper to tank and seal completely with tape. Let tank equilibrate for 2 hours.
3. Carefully remove one half of the silica gel TLC plate from oven. Spot the TLC plate in a straight line 1 inch from the bottom of the plate with 200 µL of sample from extraction and place into the tank. Allow the solvent front to migrate to within approximately 1.5 cm of the top of the plate.
4. Remove the plate from the tank and let dry. Using a diamond glasscutter, cut approximately 1 inch off the left side of the plate.
5. Take the 1 inch section and spray with 50% H_2SO_4. Heat the sprayed section in the oven until the phospholipid bands can be visualized.
6. Identify bands using authentic standards (Maytrea Inc., Pleasant Gap, PA).
7. Scrape the PC and PE bands of the unsprayed portion of the TLC plate into the appropriately labeled medium (16 x 125 mm) test tubes (with Teflon lined caps and threads covered with Teflon tape).

Appendix C

Methylation of fatty acids

1. Add 1.0 mL of 10% boron trifluoride (BF_3) solution (Supelco, Bellefonte, PA) into each tube containing the sample. Flush with nitrogen and seal tightly.
2. Place tubes on the heating block at 110º C for 30 minutes. Check the tubes for leakage throughout heating period.
3. Remove the tube from the heating block and cool immediately for 5-10 minutes in an ice bath.
4. Add 1.0 mL ultra-pure water and 2.0 mL of pentane and flush the tube with nitrogen.
5. Vortex for 2 minutes and centrifuge at 5º C for 5 minutes at 2000 rpm.
6. Transfer the supernatant using a syringe to small tube and evaporate under nitrogen.
7. Add 80 µL of dichloromethane and transfer to a 1 mL gas chromatography (GC) vial.
8. Store at 1 º C until GC analysis.

Appendix D

Capillary Gas Chromatography:

Column: 30m x 0.25mm ID
Oven: 50º C to 220º C @ 4º C/min
Injection: 250º C
Carrier: helium 1ml/min
Det: FID, 260 º C
Injection: 1 µl of sample

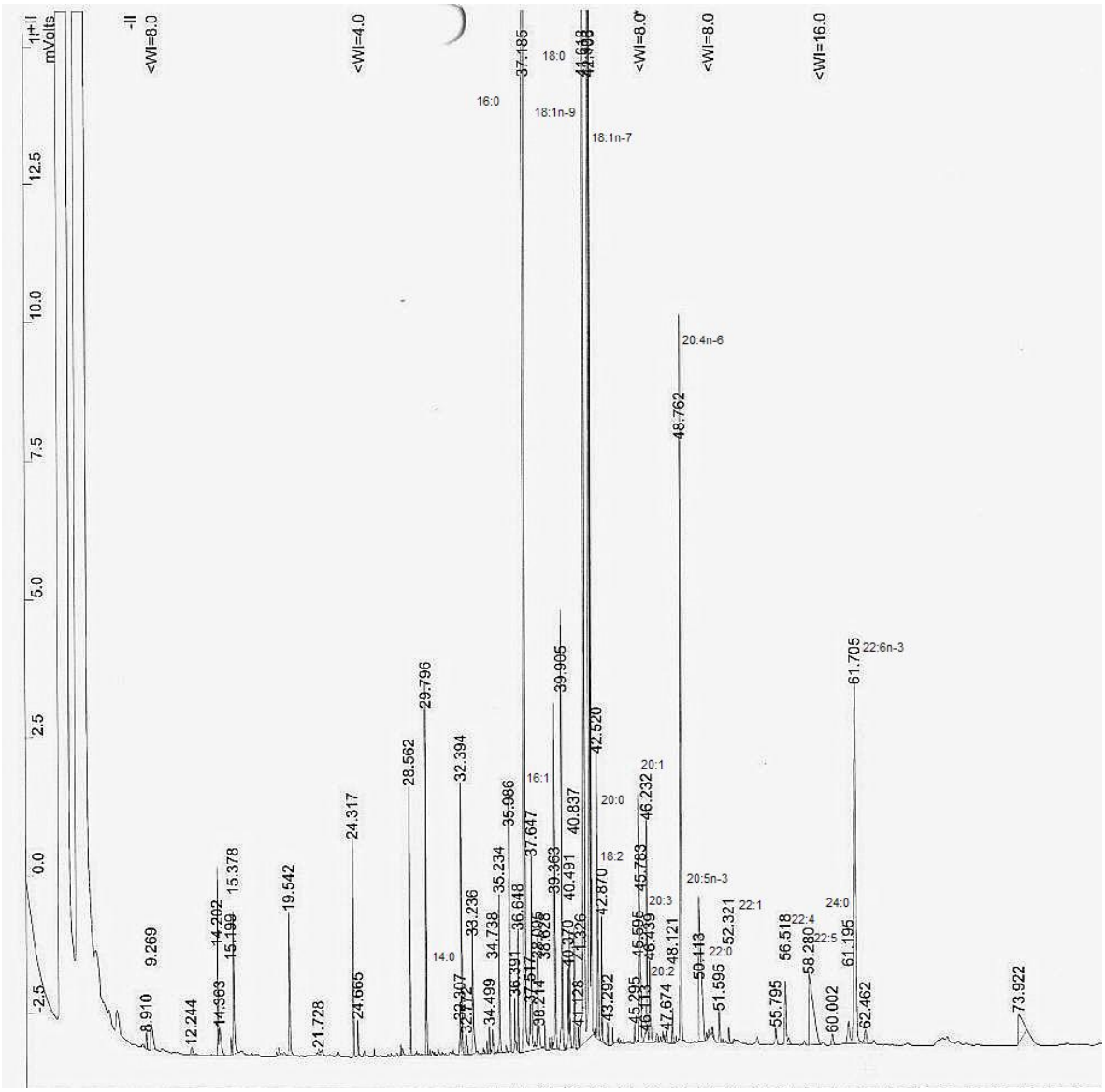

Figure 12. Gas chromatography sample 839 PE

Appendix E

Relative Correction Factors (RCF) based on Standard 85

STD 85

Fatty Acids	% by wt	Area Counts	Area Counts per 1%	RCF
6:0	3.03	246052	81205.28	1.09
8:0	3.03	306019	100996.37	1.35
10:0	3.03	321240	106019.80	1.42
11:0	3.03	317530	104795.38	1.40
12:0	3.03	310769	102564.03	1.37
13:0	3.03	291589	96233.99	1.29
14:0	3.03	270435	89252.48	1.19
14:1	3.03	265081	87485.48	1.17
15:0	3.03	245750	81105.61	1.09
15:1	3.03	246858	81471.29	1.09
16:0	6.06	452518	74672.94	1.00
16:1	3.03	230846	76186.80	1.02
17:0	3.03	209367	69098.02	0.93
17:1	3.03	211424	69776.90	0.93
18:0	3.03	199148	65725.41	0.88
18:1n9	3.03	206399	68118.48	0.91
18:1t	3.03	196560	64871.29	0.87
18:2n6	3.03	204123	67367.33	0.90
18:3n-6	3.03	201827	66609.57	0.89
18:3n3	3.03	199233	65753.47	0.88
20:0	3.03	193033	63707.26	0.85
20:1	3.03	191097	63068.32	0.84
20:2	3.03	189358	62494.39	0.84
20:3n6	3.03	187834	61991.42	0.83
20:4	3.03	187613	61918.48	0.83
20:3n3	3.03	187297	61814.19	0.83
22:0	3.03	190034	62717.49	0.84
22:1	3.03	190565	62892.74	0.84
22:2	3.03	189566	62563.04	0.84
22:6n3	3.03	170309	56207.59	0.75
24:1	3.03	190270	62795.38	0.84

Use of relative correction factors: Sample Calculation

Area Counts (AC) → 452518 → 74672.94 → RCF=1.00 (16:0)

% 6.06 74672.94-Reference Value

Area Counts (AC) → 270435 → 89252.47 → RCF=1.19 (14:0)

% 3.03 74672.94

Table 12. Calculations for sample #839 PE

Fatty Acid	AC	Corrected Area Counts (CAC)	Corrected Percentage	
	Listed on chromatogram	AC/RCF	CAC/Sum of all CAC*100	=
14:0	4519	4519/1.19	(3780.8/ 2156615.49)*100	0.175
16:0	111060	111060/1	(111060/ 2156615.49)*100	5.149
22:6 n-3	609741	609741/.752	(810053.4/ 2156615.49)*100	37.561

Appendix F.

Glossary of selected fatty acids

Fatty Acid	Common Name
16:0	Palmitic Acid
18:2 n-6	Linoleic Acid (LA)
18:3 n-3	Alpha-linolenic acid (ALA)
20:4 n-6	Arachidonic Acid (ARA)
20:5 n-3	Eicosapentaenoic Acid (EPA)
22:5 n-6	Docosapentaenoic Acid (DPA)
22:6 n-3	Docosahexaenoic Acid (DHA)