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Select Dietary Fatty Acids Modulate Brain Long Chain Omega-3 and Omega-6 PUFA Content in Mice

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DIETARY ENHANCEMENT OF FATTY ACIDS: INFLUENCE OF BRAIN FATTY ACID PROFILES

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TITLE: SELECT DIETARY FATTY ACIDS MODULATE BRAIN LONG CHAIN OMEGA-3 AND OMEGA-6 PUFA CONTENET IN MICE

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Traumatic brain injury (TBI) is a leading cause of mental and physical disability and death in the general population. Neuronal dysfunction and behavioral decline associated with TBI can result from membrane instability in neurons, synaptic junctions, glia, endothelial cells and intracellular organelles. Membrane integrity, and reaction to extrinsic insult, are highly dependent upon dietary intake of polyunsaturated fatty acids (PUFAs), due to brain accretion of long-chain omega-3 PUFAs. The present study was designed to examine the influence of various dietary fatty acids on brain long-chain omega-3 PUFA content. Mice were fed one of five diets: 1) a diet containing corn oil, 2) conventional soybean oil, 3) SDA-enriched soybean oil, 4) flaxseed oil, or 5) fish oil for 9 weeks. At the end of the study, brains were removed and frozen for subsequent fatty acid analysis via gas chromatography (GC). GC data were used to determine the percent area under the curve for key fatty acids, and these values were used to perform statistical analyses (ANOVA). SDA Soy Oil and Flaxseed Oil diets lowered arachidonic acid (AA) relative to all plant oil groups, but not as low as fish oil *($p<0.05$)*. Conversely, SDA soy and flaxseed oil increased docosahexaenoic acid (DHA) relative to all other plant oil groups, but not as high as fish oil *($p<0.05$)*. These changes were reflected in the n6:n3 ratios, where the results were consistent with the AA findings: SDA Soy oil and flaxseed oil produced lower n6:n3 ratios compared to the other plant oil groups, but not as low as fish oil *($p<0.05$)*. In the present study, SDA-enriched soybean oil and flaxseed oil may represent a

sustainable and efficient alternative to marine-based n-3 PUFAs, and could have novel therapeutic uses regarding brain related pathologies.

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CHAPTER 1

INTRODUCTION

Dietary importance of polyunsaturated fatty acids (PUFAs) has been a continuous area of research concerning various disease states and health outcomes since the early 21st century. Nutrient consumption of PUFAs can play an important role in the way the body operates and responds under infection, injury, or stress. A factor that contributes to many human disease states is inflammation; which is a bodily response under unfamiliar conditions. Clinical studies suggest that a balance of select polyunsaturated fatty acids in the diet can help control inflammatory processes in the body; therefore possibly mediating the severity of disease. Numerous conditions exist that have an inflammatory component such as arthritis, Crohn's disease, atherosclerosis, traumatic brain injury (TBI), type I and II diabetes, and obesity (2). The general population is lacking effective treatment strategies for inflammation. As a result, the human diet is receiving much attention for new interventions; particularly adequate and balanced PUFA consumption. The brain relies on PUFAs to help carry out necessary roles that the body needs. Brain injuries increase the importance need for PUFAs to assist with inflammation control and restoring normal function (1). In addition, considering 1/3 of adults and 17% of children in the United States are currently classified as obese (7). This epidemic alone contributes to the vast amount of inflammatory related pathologies today; thus, making it clear that the modern diet is lacking its true potential.

Two subsets of polyunsaturated fatty acids include omega-3 and omega-6. These two classes of PUFAs are considered essential nutrients as they need to be acquired through dietary consumption or supplementation (8). The types of omega-3 PUFAs discussed here will include alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and

docosapentaenoic acid (DPA). Omega-6 PUFAs will include linoleic acid (LA), and arachidonic acid (AA). Both subsets of polyunsaturated fatty acids contain characteristics that affect inflammatory properties within the body. The omega-3 PUFAs consist of anti-inflammatory agents whereas omega-6 PUFA's contribute to pro-inflammatory responses. Certain food sources can promote the accretion of these nutrients. Sources of omega 3 PUFAs (EPA/DHA) are found in marine-based food such as specific fish and fish oils as well as plant-based foods (ALA) including green leafy vegetables, flaxseed, canola, and soybean (24). Omega-6 dietary sources are linked to plant oils such as sunflower, safflower, and corn oils. Additionally, animal fat, whole-grain breads, and cereal grains contribute to the supply of the omega-6 intake (8). The significance of consuming adequate amounts of omega-3 PUFAs is associated with anti-inflammatory responses on overall health. Specifically, intake of EPA and DHA has contributed to a reduced risk of metabolic disease, dyslipidemia, insulin resistance, and inflammation itself. Positive health benefits have also been linked with EPA and DHA consumption in relation to coronary artery disease (24). DHA specifically has exhibited a prime function in retina and brain development during early stages of pregnancy (12). The typical Westernized diet is excessive in omega-6 intake and deficient in omega-3.With the help of the agricultural revolution, humans now consume vast amounts of cereal grains which in turn make up a large staple in the human diet (15-17). "The 2010 Dietary Guidelines for Americans recommend consuming at least two (4 oz. servings) of fish per week to achieve an average of 250 mg per day of long chain omega-3 fatty acids" (13). According to the Nutrition Journal and data from the National Health and Nutrition Examination Survey (NHANES), 2003-2008, results indicated that " a large percentage of the U.S. population is not meeting recommendations for omega-3 fatty acid consumption" (14).

In addition to the population failing to meet these recommendations, alternative plant-based sources such as flaxseed, canola, and soybean as discussed before do not efficiently provide optimal tissue levels of EPA and DHA omega-3 PUFA's. Although they offer higher amounts of Alpha Linolenic Acid (ALA), in order to acquire any amount of EPA and DHA from these plant based sources, the multistep conversion of ALA to SDA to EPA/DHA must occur. This conversion process in the body is not extremely cost effective with a low rate of 0.01%-8% in males and 21% in females (24). However, there is potentially a more effective way for this conversion to occur. Recently, Stearidonic acid (SDA) has been produced through biotechnology and given the Generally Recognized as Safe (GRAS) status by the FDA. In comparison to marine-based sources, supplementation of SDA through enriched soybean oil is highly cost effective and viable. It has 6-7 times the amount of omega-3 content and 2-3 times less the amount of ALA when compared to conventional soybean oil. Furthermore, the substitution of SDA for ALA could be a more resourceful way to create end products of EPA and DHA levels in tissue. Ultimately, we need to find alternatives to relatively limited supplies of long chain omega-3 PUFA sources. SDA enriched soybean oil has been shown to be an easily convertible substrate for the synthesis of long chain omega-3 PUFA's (12, 19).

Research has shown diets containing ideal amounts of oily fish intake or fish oil supplementation to be the optimal choice for the deposition of long chain (LC) n-3 PUFA in tissues (18). Evidence shows that populations or cultures who consume high amounts of fish, such as Japanese, have reduced risk of cardiac events (20). According to Calder (2), positive anti-inflammatory effects result from both animal models and patients with rheumatoid arthritis with fish oil supplementation. In agreement with Calder, there is a substantial amount of data (33) to support the beneficial role fish oil plays on various health outcomes. Although research

has found the optimal source, reasons such as food preferences, beliefs concerning the environment, and non-fish alternatives for fish feed suggest marine sources are not sufficient to meet the required American needs (21) for EPA and DHA. SDA enriched soybean oil has been viewed as a plausible option. In 2010, a study published in the American Journal of Nutrition examined SDA enriched soybean oil in a randomized, placebo controlled, double blind study with 252 overweight adults aged 21-70 years old (in good health). Subjects were given one treatment of either soybean oil (control), EPA, or soybean oil enriched with SDA over a 12 week period. Comparison of SDA enriched soybean oil versus EPA and conventional soybean oil was the focus. Results indicated that SDA enriched soybean oil significantly increased omega-3 levels according to the erythrocyte EPA concentrations (22). Additionally, Kawabata et al. presented a study looking at a variety of SDA diets on rats during a four week testing period. Data collection revealed increased EPA amounts found in serum and liver triacyglycerol levels (23). A further study in 2013 by Casey et al. examined lean and obese Zucker rats fed either a (control) westernized, flaxseed, fish (menhaden), or SDA oil diet. Conclusions of metabolic effects from these diets after 12 weeks were made in addition to the consistent supporting results of increased n-3 PUFA levels in erythrocyte and tissue levels from SDA enriched soybean oil. Liver tissue was enriched with n-3 PUFAs while simultaneously decreasing n-6 PUFA. The fish oil diet represented the most EPA and DHA content as would be expected (24). That being said, we can conclude there is current data supporting liver accretion of n-3 PUFAs. Studies examining brain fatty acid levels by means of dietary manipulation are few and are in need of further investigation. Long chain omega 3 polyunsaturated fatty acids can make an impact on nutrition and health; thus, sufficient dietary sources and recommendations are essential.

Accordingly, the present study was designed to examine the influence of various dietary fatty acids on brain long-chain omega-3 PUFA content in mice.

CHAPTER 2

METHODS

Experimental methods for the current study were comparable to those from Casey et al. (24). Male mice 3-4 weeks of age were allowed free access to one of five experimental diets for 9 weeks: 1) conventional soybean oil (Soy); 2) SDA-enriched soybean oil (SDA); 3) a diet containing corn oil (Corn); 4) flaxseed oil (Flax); or 5) fish oil (Fish). Mice were fasted overnight before being fed *ad libitum* during the experimentation. The housing environment consisted of wire cages in a temperature controlled room with a 12 hour light-dark cycle. All experimental protocols applied for animal care and use were approved by Animal Care and Use Committee of Southern Illinois University Carbondale, IL.

Experimental diet compositions were adapted from Monsanto US 17 Rodent Diets and are listed in Table 1. The diets were designed to be isocaloric and isonitrogenous. The CON diet was prepared to resemble the typical Westernized diet with a high omega-6 PUFA and omega-3 PUFA ratio. SDA enriched soybean oil, corn, flaxseed, and fish diets were modified for omega-6 PUFA and omega-3 PUFA content. A percentage variation of each diet was used in order to establish consistent saturated and monounsaturated fat content. Thus, the PUFA to saturated fat ratio was close to 1.0 for all diets. Brains and livers were harvested and frozen for fatty acid analysis. Results are shown in Tables 2-4.

Tissue Fatty Acid Analysis

Experimental procedures regarding tissue fatty acid analysis were utilized from Casey et al (24). “For this liver and brain tissue samples were measured to 500 mg and put into glass test tubes (16×200 mm) with Teflon-lined screw caps, stored at -80°C for 6h, freeze-dried, and then methylated using the NaOCH3 and HCl two-step procedure as published previously [30].

Methylated fatty acids were then analyzed for fatty acids using a Shimadzu GC-2010 gas chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD) equipped with a flame ionization detector and a Supelco 100-m SP-2560 fused silica capillary column (0.25 mm i.d. × 0.2 μm film thickness). The helium carrier gas was maintained at a linear velocity of 23 cm/s. The oven temperature was programmed for 135°C for 5 min, then increased at 5°C/min to 165°C, held there for 80 min, then increased at 3°C/min to 180°C, then increased at 5°C/min to 245°C and held there for 9 min. The injector and detector temperatures were set at 255°C. Peaks were identified by comparing the retention times with those of corresponding standards (Nu-Chek Prep, Elysian, MN; Supelco, Bellefonte, PA; and Larodan Fine Chemicals, Malmo, Sweden). Heptadecanoic acid (C17:0) was added to all samples as an internal standard".

Statistical Analysis

Statistical analyses were performed to test for differences between the means for each of the five diet groups. One-way ANOVA using Prism 6.07 for Windows (GraphPad Software, La Jolla California USA) was used as well as Tukey's post-hoc test to compare all groups. For all tests, $p<0.05$ was considered significant.

CHAPTER 3

RESULTS

Body and Tissue Weight

As represented in Table 2, no significant differences were reported in final body weight or brain weight amongst the diet groups. Liver weights were greater in SDA Soy vs. Soy, Corn, Flaxseed, and Fish *(p<0.05).*

Brain Fatty Acid Profile

Results from brain fatty acid profiles demonstrated several significant interactions after completion of our statistical analysis as shown in Table 3. Soy and SDA Soy were significantly higher in brain LA compared to Corn, Flax, and Fish diets *(p<0.0001).* We observed no significant difference in LA between Soy versus SDA Soy. There were no significant differences amongst diet groups on ALA profiles. SDA deposition was significantly higher in Soy compared to Corn *(p<0.0001)*, Flax *(p=0.0007)*, and Fish *(p=0.0010)*. SDA Soy was also significantly higher when compared to Corn *(p=0.0006)*, Flax *(p=0096)*, and Fish *(p=0.9998)* diets. When comparing Soy versus SDA Soy, we again observed no significant difference. The corn diet revealed the highest levels of AA amongst diet groups while Fish revealed the lowest *(p<0.0001).* DPA was significantly higher in Soy or Corn compared to SDA Soy, Flax, and Fish *(p<0.0001).* All other diet comparisons for DPA revealed no significance. We observed no significant differences in EPA profiles between diet groups. Fish demonstrated the highest DHA amongst all diets groups *(p<0.0001)*. Flaxseed was significantly higher in DHA compared to diet groups, but not as high as Fish *(p<0.0001).*

Overall, SDA Soy and Flax diets lowered brain AA relative to all plant oil groups, but not as low as fish oil *(p<0.05).* Conversely, SDA Soy and Flax increased brain DHA relative to all

other plant oil groups, but not as high as fish oil *(p<0.05).* These changes were reflected in the n6:n3 ratios, where the results were consistent with the AA findings: SDA Soy and Flax produced lower n6:n3 ratios compared to the other plant oil groups, but not as low as Fish ($p<0.05$).

Liver Fatty Acid Profile

Numerous significant differences were revealed after we statistically analyzed the liver fatty acid profiles as Shown in Table 4. For example, Soy was significantly higher in liver LA compared to Fish *(p=0.0105).* Fish was significantly lower in LA compared to Corn *(p=0.0359).* All other diet comparisons revealed no significant differences in liver LA. We observed no significant differences between diet groups regarding ALA profiles. SDA Soy was significantly higher in SDA when compared to all diet groups *(p<0.0001).* All other diet comparisons for SDA revealed no significance. Flax was significantly lower in AA compared to Corn *(p=0.0195)* and Fish *(p=0.0294).* All other diet comparisons for AA revealed no significance. Liver EPA was significantly highest in Flax compared to all diet groups *(p<0.0001).* SDA Soy was significantly higher in EPA compared to Soy and Corn *(p<0.0001).* Fish revealed significantly higher EPA when compared to Soy, Flax, and Corn *(p<0.0001)*, but not as high as Flax or SDA Soy. DPA deposition was significantly higher in Fish compared to Soy and Corn *(p<0.0001).* SDA Soy was less than Fish but higher in DPA versus Soy and Corn *(p<0.0001).* DHA in the liver was highest in Fish and SDA Soy compared to all diet groups *(p<0.0001).* DHA was significantly lower in Corn versus all diet groups except Soy *(p<0.0001).*

Overall, Flax decreased liver AA compared to Corn and Fish diets *(p<0.05).* Regarding DHA and DPA, Fish and SDA Soy revealed higher levels *(p<0.05).* Soy produced higher n6:n3 ratios compared to all diet groups except Corn *(p<0.05).*

CHAPTER 4

DISCUSSION

Dietary intake of long chain omega-3 PUFAs offer nutrition related benefits, as a variety of dietary sources are studied today. As expected, our data suggest fish oil had the greatest impact on lowering AA and increasing DHA in the brain as represented in Figure 1 *($p<0.0001$).* This relationship was consistent with the findings in liver (Figure 2), where DHA was also significantly higher in fish-oil fed mice compared to all other diet groups *($p<0.0001$).* Conclusions from the SDA Soy treatment group revealed AA in brain content was significantly lower relative to all diet groups, but not as low as flaxseed oil *($p<0.0001$)*; Additionally, SDA Soy increased DHA in the brain versus Soy and Corn diets, but not as high as flaxseed oil and fish oil *($p<0.0001$)*. DHA content in liver was significantly higher in SDA Soy compared to all diets except for Fish Oil *($p<0.0001$).* Although no significant findings were reported from brain EPA levels, liver EPA was highest in flaxseed and SDA Soy *($p<0.0001$).* With previous research findings and the data we observed, replacing high omega-6 oils in the diet such as corn, sunflower, and conventional soybean oil with omega-3 rich oils like flax and fish oil can assist with achieving more balanced ratio of PUFA's in the tissue. These findings support additional dietary alternatives such as SDA enriched soybean oil that could offer direction to the modern diet.

Previous research studies (26, 29, 30, 32, 31, 25) have been performed in various models to examine the influence of dietary SDA and LC n-3 PUFA content in blood and tissue levels. A naturally occurring oil containing SDA known as echium oil has most recently become a popular area of focus in research. Before discussing various animal studies, a recent human study was performed in 2016 comparing echium oil with linseed oil (also known as flaxseed oil). Kuhnt et

al. concluded echium oil to better increase amounts of EPA and DPA by 25% in plasma, red blood cells (RBC), and peripheral blood mononuclear cells (PBMC) versus linseed oil. A lower ratio of AA/EPA in plasma, RBC, and PBMC was also revealed. This study may well suggest echium oil is a better alternative than flaxseed oil for increasing EPA and DPA in the blood in humans (26). In regards to animal models, broiler chickens SDA-rich echium oil was more efficient at increasing EPA and DPA in the blood versus ALA feed (29). These conclusions also support substitution of SDA for ALA is a more resourceful way to create end products of n3 PUFAs in blood and tissues. Examining this effect in the animal model may suggest similar results in humans. In addition, research from Miller et al. (30) demonstrated similar conclusions in Atlantic salmon. Echium oil increased the biosynthesis elongation products in muscle and carcass; suggesting SDA to be a better precursor of LC n-3 PUFA's. Research from Bharadwaj et al. (32) examined LC n-3 PUFA's in hybrid sea bass fed diets consisting of Fish (CON), ALA, or SDA oils at three different levels (.5, 1, and 2%). DHA was significantly higher in fish fed SDA diets versus ALA. Thus, proposing a SDA enriched diet may increase DHA concentrations in muscle of the hybrid sea bass. Further models such as lamb and rats have been researched as well. A 4 week study was conducted by Kitessa et al. (31) examining LC n-3 PUFA's in lamb tissues from stomach infused treatments of saline (CON), echium oil (Low), echium oil (High), linseed oil (Low) or linseed oil (High). Results suggested that both echium oil and linseed oil increase EPA and DPA in lamb muscle tissues, but not DHA. Furthermore, rats fed a 12 week diet of echium oil or fish oil saw increased DPA in tissues (especially in the heart) from echium oil and increased levels of DHA in the heart from fish oil; where researchers from the study presented SDA oils can increase n-3 PUFA levels in plasma and tissue in rats (25).

Further exploration is needed to verify if SDA oils are as strong an influence as fish oils on inflammatory response. In addition, very few studies have observed brain LC n-3 PUFA content. With marine-based sources being costly and under consumed, new strategies for better intake of these vital nutrients are needed. Research has shown LC omega-3 PUFA consumption is linked to health benefits such as anti-inflammatory responses; aiding in prevention and potential treatment of various disease states. In the present study, SDA-enriched soybean oil and flaxseed oil may represent a sustainable and efficient alternative to marine-based n-3 PUFAs, and could have novel therapeutic uses regarding brain related pathologies.

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APPENDIX A

Table 1: Composition of experimental diets*

Ingredients *(g/kg)*	**SOYBEAN**		**SDA**		**CORN**		**FLAXSEED**		**FISH**	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Casein, Sodium	200	800	200	800	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12	3	12	3	12
Corn Starch	240	960	240	960	240	960	240	960	240	960
Maltodextrin 10	75	300	75	300	75	300	75	300	75	300
Sucrose	100	400	100	400	100	400	100	400	100	400
Cellulose	50	0	50	0	50	0	50	0	50	0
Flaxseed Oil	0	0	0	0	0	0	150	1350	0	0
Menhaden (Fish) Oil	0	0	0	0	0	0	0	0	140	1260
Corn Oil	0	0	0	0	150	1350	0	0	0	0
Soybean Oil	150	1350	0	0	0	0	0	0	10	90
SDA Soybean Oil	0	0	150	1350	0	0	0	0	0	0
Protein	23.2	21.0	23.2	21.0	23.2	21.0	23.2	21.0	23.2	21.0
Carbohydrate	48.6	44.0	48.6	44.0	48.6	44.0	48.6	44.0	48.6	44.0
Fat	17.1	35.0	17.1	35.0	17.1	35.0	17.1	35.0	17.1	35.0
Total		100.0		100.0		100.0		100.0		100.0

*Adapted from Monsanto US17 Rodent Diets (Research Diets, Inc)

Table 2: Brain, liver, and final body weight in mice fed experimental diets for 9 weeks.

	SOY	**SDA**	**CORN**	**FLAX**	**FISH**
Final body weight (g)	32.00 ± 1.23	32.76 ± 1.45	31.72 ± 0.81	30.72 ± 0.60	30.77 ± 0.86
Liver weight (g)	1.13 ± 0.04^{A}	1.32 ± 0.05^{B}	1.05 ± 0.03^{A}	1.05 ± 0.04^{A}	1.09 ± 0.03^{A}
Brain weight (g)	0.42 ± 0.01	0.43 ± 0.00	0.43 ± 0.01	0.42 ± 0.01	0.41 ± 0.00

$^{\triangle}$Different letters represent significance between means as determined by one-way ANOVA and Tukey's post hoc test *($p < 0.05$).*

Table 3: Brain fatty acid profile in mice fed experimental diets for 9 weeks.

Fatty Acid (% of total)	SOYBEAN	SDA	CORN	FLAXSEED	FISH
LA [18:2(n-6)]	6.21 ± 0.11^{A}	5.84 ± 0.21^{B}	4.90 ± 0.09^{C}	5.29 ± 0.12^{C}	4.99 ± 0.11^{C}
ALA [18:3(n-3)]	6.22 ± 0.68	6.48 ± 0.59	5.77 ± 0.29	6.33 ± 0.37	6.96 ± 0.59
SDA [18:4(n-3)]	0.38 ± 0.04^{A}	0.34 ± 0.02^{A}	0.23 ± 0.01^{B}	0.25 ± 0.01^{B}	0.25 ± 0.01^{B}
AA [20:4(n-6)]	28.48 ± 0.55^{A}	26.44 ± 0.33^{B}	30.70 ± 0.27^{C}	25.10 ± 0.58^{B}	22.47 ± 0.44^{D}
EPA [20:5(n-3)]	0.61 ± 0.07	0.80 ± 0.04^{A}	0.55 ± 0.03^{B}	0.70 ± 0.05	0.68 ± 0.08
DPA [22:5(n-3)]	9.92 ± 0.44^{A}	6.03 ± 0.18^{B}	9.44 ± 0.27^{A}	$5.65 \pm 0.10^{B,C}$	4.76 ± 0.13^{C}
DHA [22:6(n-3)]	47.36 ± 1.06^{A}	53.50 ± 0.62^{B}	48.46 ± 0.43^{A}	56.26 ± 0.51^{C}	59.64 ± 0.91^{D}
n-6 : n-3	0.53 ± 0.01^{A}	0.49 ± 0.01^{B}	0.56 ± 0.00^{A}	0.44 ± 0.01^{C}	0.38 ± 0.01^{D}

△Different letters represent significance between means as determined by one-way ANOVA and Tukey's post hoc test *($p < 0.05$).*

Table 4: Liver fatty acid profile in mice fed experimental diets for 9 weeks

Fatty Acid (% of total)	SOYBEAN	SDA	CORN	FLAXSEED	FISH
LA [18:2(n-6)]	49.80 ± 10.33^{A}	37.28 ± 3.06	44.86 ± 5.80^{A}	42.85 ± 2.62	20.11 ± 1.91
ALA [18:3(n-3)]	22.06 ± 9.76	16.30 ± 5.13	33.10 ± 6.88	18.04 ± 2.27	7.69 ± 2.80
SDA [18:4(n-3)]	2.04 ± 0.28^{A}	5.35 ± 0.46^{B}	1.83 ± 0.16^{A}	1.81 ± 0.13^{A}	1.46 ± 0.20^{A}
AA [20:4(n-6)]	14.69 ± 2.91	10.72 ± 1.30	15.30 ± 1.72^{A,C}	7.56 ± 1.54^{B}	15.79 ± 0.74^{C}
EPA [20:5(n-3)]	0.84 ± 0.17^{A}	12.88 ± 1.03^{B}	1.19 ± 0.21^{A}	16.45 ± 0.88^{C}	10.47 ± 0.89^{B}
DPA [22:5(n-3)]	1.17 ± 0.23^{A}	3.43 ± 0.34^{B}	0.64 ± 0.16^{A}	2.88 ± 0.37^{B}	3.80 ± 0.12^{B}
DHA [22:6(n-3)]	5.11 ± 0.76^{A}	16.30 ± 1.03^{B}	2.63 ± 0.23^{A}	11.42 ± 1.14^{C}	39.15 ± 2.01^{D}
n-6 : n-3	4.89 ± 1.26^{A}	1.10 ± 0.22^{B}	4.23 ± 1.47	0.88 ± 0.04^{C}	0.57 ± 0.04^{D}

$^{\triangle}$Different letters represent significance between means as determined by one-way ANOVA and Tukey's post hoc test *($p < 0.05$).*

APPENDIX B

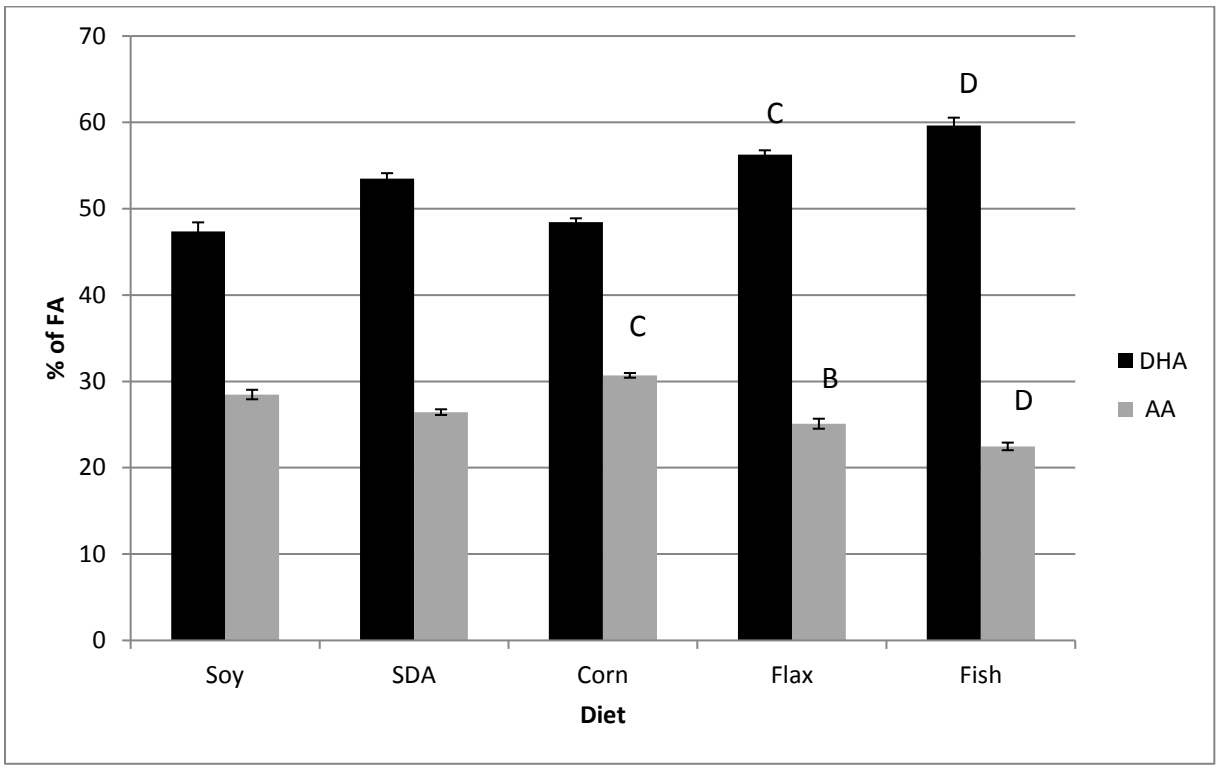

Figure 1: Demonstrates the effects of various dietary fatty acids based diets on brain docosahexaenoic acid (DHA) and arachidonic acid (AA) level. Male mice 3 – 4 weeks of age were allowed free access to one of five experimental diets for 9 weeks: 1) conventional soybean oil (Soy); 2) SDA-enriched soybean oil (SDA), 3) a diet containing corn oil (Corn); 4) flaxseed oil (Flax); or 5) fish oil (Fish). Different letters represent significance between means as determined by one-way ANOVA and Tukey's post hoc test ($P < 0.05$).

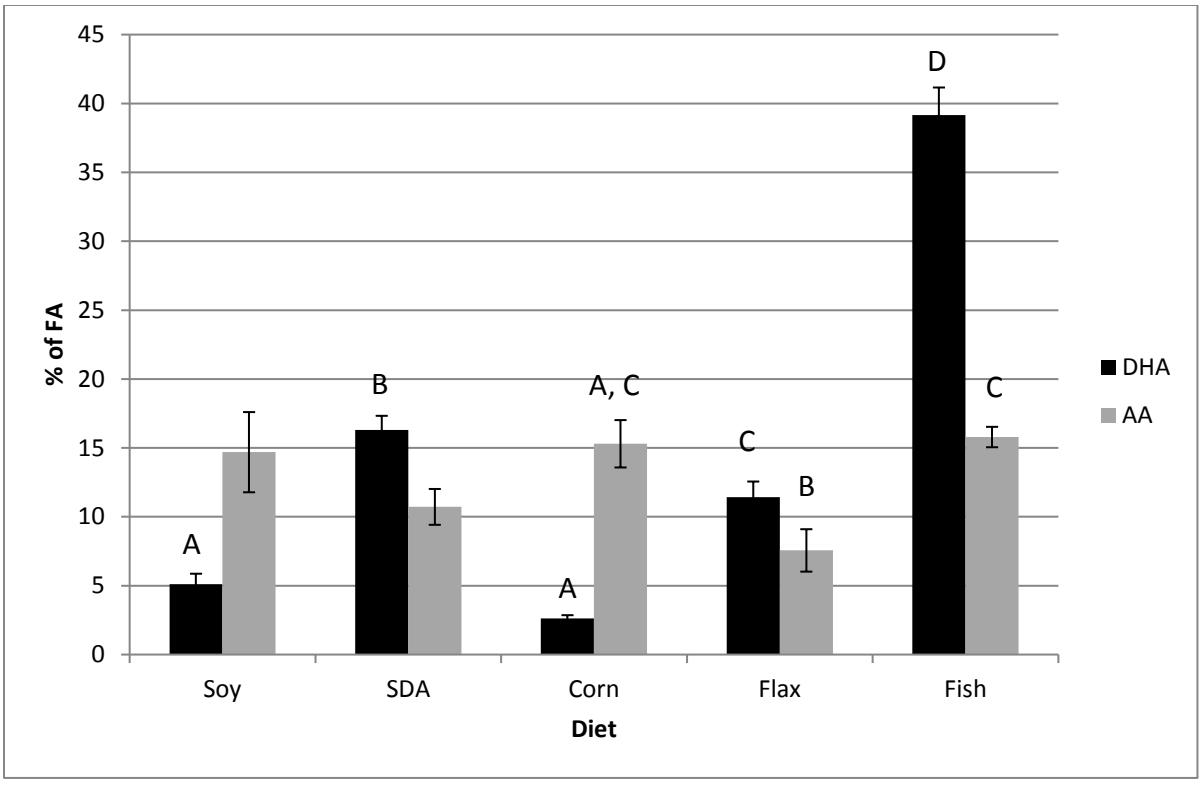

Figure 2: Demonstrates the effects of various dietary fatty acids based diets on liver docosahexaenoic acid (DHA) and arachidonic acid (AA) level. Male mice 3 – 4 weeks of age were allowed free access to one of five experimental diets for 9 weeks: 1) conventional soybean oil (Soy); 2) SDA-enriched soybean oil (SDA), 3) a diet containing corn oil (Corn); 4) flaxseed oil (Flax); or 5) fish oil (Fish). Different letters represent significance between means as determined by one-way ANOVA and Tukey's post hoc test ($P < 0.05$).

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Major Professor: Dr. William Banz