Omega-3 Supplementation and Body Weight in Healthy Young Women

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved July 2012 by the
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ARIZONA STATE UNIVERSITY

May 2013

ABSTRACT

Objective: The purpose of this randomized parallel arm trial was to demonstrate the effects of daily fish oil supplementation (600mg per day for eight weeks) on body composition and body mass in young healthy women, aged 18-38, at a large southwestern university.

Design: 26 non-obese (mean BMI 23.7±0.6 kg/m^2), healthy women (18-38y; mean, 23.5±1.1 y) from a southwestern Arizona university campus community completed the study. Subjects were healthy, non-smokers, consuming less than 3.5 oz of fish per week according to self-report. Participants were randomized to one of two groups: FISH (600 mg omega-3 fatty acids provided in one gel capsule per day), or CON (1000 mg coconut oil placebo provided in one gel capsule per day). Body weight, BMI, and percent body fat were measured using a stadiometer and bioelectrical impedance scale at the screening visit and intervention weeks 1, 4, and 8. 24-hour dietary recalls were also performed at weeks 1 and 8.

Results: 8 weeks of omega-3 fatty acid supplementation did not significantly alter body weight (p=0.830), BMI (p=1.00), or body fat percentage (p=0.600) as compared to placebo. Although not statistically significant, 24-hour dietary recalls performed at the beginning and end of the intervention revealed a trend towards increased caloric intake in the FISH group and decreased caloric intake in the CON group throughout the course of the study (p=0.069). If maintained, this difference in caloric intake could have physiological relevance.

Conclusions: Omega-3 fatty acids do not significantly alter body weight or body composition in healthy young females. These findings do not refute the

current recommendations for Americans to consume at least 8 oz of omega-3-rich seafood per week, supplying 250 mg EPA and DHA per day. More research is needed to investigate the potential for omega-3 fatty acids to modulate daily caloric intake.

DEDICATION

I would like to dedicate this work to my parents, Beverly and Jose Teran. Thank you for instilling in me the value of passion and tenacity, and for giving me the confidence and strength to pursue my dreams.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my mentor, Dr. Carol Johnston, who has been an incredible inspiration. Thank you for sharing your extensive knowledge and infectious passion for science. I am also grateful to have had such a wonderful friend and co-investigator, Megan Gutierrez, to tackle every step of this project with. I could not have completed this work without her tremendous organization skills, positive attitude, and constant motivation. I would also like to express my warm and sincere thanks to Ginger Hook for her patience and support in the lab throughout the course of the study. Likewise, thank you Alyssa Dukes, Elizabeth Sussman, and Emily Medved for devoting your time to helping us with our 24-hour recalls and data entry. I would also like to give a special thank you to Dr. Punam Ohri-Vachaspati and Melinda Johnson for taking time to be on my thesis committee.

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Chapter 1

INTRODUCTION

American adults use omega-3 fatty acid supplements more than any other non-vitamin, non-mineral natural product available. In a 2007 survey, 37% of adults who had used natural products within the previous thirty days had used an omega-3 supplement (National Center for Complementary and Alternative Medicine, 2009). Omega-3 fatty acids are widely acclaimed for their anti-inflammatory properties (Calder, 2006), and health organizations such as the American Heart Association and US Food and Drug Administration have recommended Americans include omega-3-rich foods such as fish as part of a well-balanced diet (Flock & Kris-Etherton, 2011; Kris-Etherton, Harris, & Appel, 2002). Yet as obesity levels rise in the United States (Carroll & Surveys, 2010), the effects of increased omega-3 fatty acids on body composition in healthy people are unknown.

The ability of omega-3 fatty acids to inhibit the production of proinflammatory prostaglandins has been demonstrated to attenuate the severity of chronic inflammatory diseases such as coronary artery disease (Kris-Etherton et al., 2002) and rheumatoid arthritis (Calder, 2006). The omega-3 fats found in fish oil are also believed to have beneficial effects in autoimmune disorders, asthma, inflammatory bowel diseases (Calder, 2006), and some forms of cancer (Calder, 2006; Connor, 2000; Teitelbaum & Allan Walker, 2001), all of which are related to chronic inflammatory states (Calder, 2006).

Despite the fact that obesity is a chronic inflammatory condition plaguing over 60% of American adults (Wyatt, Winters, & Dubbert, 2006), studies aiming

to demonstrate a link between fish oil intake and adiposity are inconclusive. Interestingly, omega-3 fatty acids have been used to increase body mass in patients with cancer cachexia (Colomer et al., 2007; Giacosa & Rondanelli, 2008; Grimble, 2003). Furthermore, one recent study demonstrated a down-regulation of thermogenic activity in brown adipose tissue in mice after inhibition of inflammatory prostaglandin production, suggesting omega-3 fatty acids may increase body mass and reduce basal metabolic rate (Vegiopoulos et al., 2010). Alternatively, a correlational study observed an inverse relationship between plasma omega-3 levels and body weight (Micallef, Munro, Phang, & Garg, 2009), and some studies have demonstrated enhanced fat loss by incorporating omega-3 supplementation into a weight loss intervention (Hill, Buckley, Murphy, & Howe, 2007; Kabir et al., 2007; Noreen et al., 2010). However, others have found no significant changes in body mass after fish oil supplementation (Defina, Marcoux, Devers, Cleaver, & Willis, 2011; Fontani et al., 2005; Moore et al., 2006; Noreen et al., 2010), or have had difficulty demonstrating these effects in women (Thorsdottir et al., 2007).

In addition to these mixed results, the sample selections for most human interventions have made it difficult to generalize results to relatively healthy populations. Most clinical trials have employed subjects who are already obese or chronically inflamed (Hill et al., 2007; Kabir et al., 2007; Kunesová et al., 2006; Moore et al., 2006; Thorsdottir et al., 2007; Warner, Ullrich, Albrink, & Yeater, 1989), and some have paired omega-3 fatty acid supplementation with a weight loss intervention rather than observing its effects independently (Hill et al., 2007; Kunesová et al., 2006; Moore et al., 2006; Thorsdottir et al., 2007; Warner et al.,

1989). Moreover, only a few human interventions have tested these effects in young adults (Couet, Delarue, Ritz, Antoine, & Lamisse, 1997; Noreen et al., 2010). One of these studies, conducted by Couet, et al., demonstrated an increase in fat oxidation and decrease in body fat over the course of three weeks when visible dietary fat was substituted with 6 grams of fish oil per day. However, the sample size was relatively small (seven total subjects), and only one female subject was used (Couet et al., 1997).

Whether omega-3 fatty acids modulate body composition in healthy young women has yet to be investigated, despite the current recommendations for Americans to regularly consume sources of omega-3 fatty acids. More research is needed to determine if there are any unintended consequences of the current fish intake recommendations for young, healthy adult women, and how increased omega-3 intake may alter the susceptibility of this population to becoming overweight.

Purpose of Study

The primary objective of this randomized parallel arm trial was to demonstrate the effects of daily fish oil supplementation (600mg per day for eight weeks) on body composition and body mass in young healthy women, aged 18-40, at a large southwestern university.

Research Aim & Hypothesis

It was hypothesized that fish oil supplementation (600 mg per day) would have no effect on body weight or body composition by the end of the eight week period.

Definition of Terms

- BMI: [weight (in pounds)/height (in inches)^2] x 703; underweight is <18.5 kg/m^2, normal is 18.5-24.9 kg/m^2, overweight is 25.0-29.9 kg/m^2, obese is >30 kg/m^2(American Dietetic Association, 2011)
- Omega-3 fatty acids: polyunsaturated fatty acids, including alpha-linolenic acid (ALA; 18:3 n-3), docosahexaenoic acid (DHA; 22:6 n-3), and eicosapentaenoic acid (EPA; 20:5 n-3) (Connor, 2000)
- Prostaglandins: lipid mediator involved in the inflammation and immune response (Tilley, Coffman, & Koller, 2001)
- Regular smoker: use of 10 or more cigarettes per day (Moran, 2004)
- Training athlete: participating in purposeful, moderate to vigorous exercise more than 5 times per week

Delimitations

Subjects were women aged 18-38 years with no unresolved health issues. The participants attended a large university in the southwestern U.S. Exclusion criteria included regular smoking, BMI of less than 18.5 or more than 30 kg/m^2, and regular intake of omega-3 supplements and/or prescription medications that may interfere with body weight or inflammatory state (such as corticosteroids or non-steroidal anti-inflammatory drugs). Any subjects consuming more than 1 serving of fish or other omega-3-rich food per week, vegetarians, and subjects trying to lose or gain weight were also excluded. Furthermore, competing and/or training athletes and women who were pregnant or lactating were not included.

Limitations

- Dietary omega-3 intake could alter inflammatory prostaglandin production in both the fish oil and control group. This limitation was minimized using a validated food frequency questionnaire to exclude study participants who regularly meet or exceed the American Heart Association's fish intake recommendations (3.5oz of fish twice per week).
- Natural fluctuations in body weight may occur due to hormonal cycles in women. To reduce this limitation, measurements were taken at a similar point in each woman's cycle (every 4 weeks).
- External factors such as lack of sleep or psychological stress may impact inflammatory state and body weight.
- The short time period of the study may not have reflected significant changes in body weight or body composition. It is possible that a longer study period would be necessary to obtain valid and reliable results.
- The subject pool was relatively small (n=26). A larger study using more subjects may be needed to obtain significant results.

Chapter 2

LITERATURE REVIEW

Adiposity

Obesity Prevalence. Over two-thirds of all American adults are either overweight or obese according to results from the 2007-2008 National Health and Nutrition Examination Survey (NHANES) (Carroll & Surveys, 2010), making a reduction in obesity prevalence one of the top national health objectives for 2010 (Pisarik, 2005). Body mass index (BMI, equal to weight in kilograms divided by height in meters squared), is most frequently used to classify obesity (BMI ≥ 30 kg/m^2) and overweight (BMI 25-29.9 kg/m^2) (Carroll & Surveys, 2010). Overweight and obesity are the result of a positive energy balance. These conditions are characterized by an increase in body weight, particularly in adiposity, and are positively correlated with morbidity, mortality, and decreased longevity (Pisarik, 2005). More specifically, obesity has been linked to increased risk of a myriad of chronic diseases, including type 2 diabetes, coronary heart disease, and some forms of cancer (Pisarik, 2005; Wellen & Hotamisligil, 2003). It is estimated that the United States spends nearly $93 billion annually due to obesity and its comorbidities, which is nearly 10% of the nation's yearly health costs (Pisarik, 2005). The negative medical consequences linked to obesity could arise from the chronic, low-grade inflammatory state that seems to originate primarily in white adipose tissue under obesogenic conditions, ultimately leading to metabolic disruptions (Todoric et al., 2006; Wellen & Hotamisligil, 2003).

White Adipose Tissue. One of the chief metabolic roles of white adipose tissue (WAT) is energy storage (Trayhurn & Beatie, 2001). Lipolysis (the

breakdown of stored fats) or lipogenesis (the synthesis of triacylglycerols for storage) occur depending on the body's energy balance and corresponding hormonal and neural signals. For instance, during periods of negative energy balance and low blood glucose, a decrease in insulin and increase in glucagon and epinephrine stimulates the release of free fatty acids (FFAs) from WAT (Ahima & Flier, 2000; Gropper, Smith, & Groff, 2009). Once in circulation, FFAs may then be oxidized to generate usable energy in the form of adenosine triphosphate (ATP) (Ahima & Flier, 2000).

WAT is primarily composed of lipid-containing cells called adipocytes, but also contains collagen, blood vessels, and immune cells, and functions as an important endocrine organ (Ahima & Flier, 2000). Even under healthy conditions, WAT produces potent hormones and proinflammatory molecules, termed adipokines, that play important roles in energy homeostasis (Wellen & Hotamisligil, 2003). Adiponectin and leptin are examples of adipokines released by WAT.

The synthesis and release of the adipokine leptin is positively correlated with adiposity (Drevon, 2005). When blood glucose is elevated or when adipocytes are full, leptin is secreted from WAT. Leptin works to restore energy homeostasis by decreasing appetite and stimulating cellular glucose uptake by increasing insulin sensitivity (Gropper et al., 2009). Leptin takes action in the arcuate nucleus of the hypothalamus by stimulating anorexigenic hormones such as melanocyte stimulating hormone (MSH) (Gropper et al., 2009; Ronti, Lupattelli, & Mannarino, 2006). It also increases lipid oxidation in myocytes by inhibiting malonyl CoA, which is one of the first intermediates produced in fatty

acid synthesis. During periods of high glucose levels, malonyl CoA is elevated and inhibits lipid oxidation. By blocking malonyl CoA, leptin is able to increase rates of lipid oxidation and thus decrease lipid storage. As adipocyte size decreases, leptin production also decreases, and appetite inhibition is reduced (Gropper et al., 2009; Ronti et al., 2006).

Adiponectin is also expressed in WAT, and appears to be protective against coronary heart disease and insulin resistance. Evidence suggests that adiponectin is able to increase insulin sensitivity by promoting lipid oxidation, which leads to reduced triglyceride content in hepatocytes and myocytes (Díez & Iglesias, 2003). Research has demonstrated a synergistic effect of adiponectin and leptin, as the two hormones seem to work together in order to increase insulin sensitivity (Ronti et al., 2006). Interestingly, adiponectin levels are lower in obese as compared to healthy populations, and levels seem to increase with weight loss. Moreover, the action of adiponectin is inhibited by inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α), which are characteristic of obesity-related inflammation (Ronti et al., 2006).

Brown Adipose Tissue. The presence of brown adipose tissue (BAT) is well known in human infants and small mammals. Its function is extremely different from that of WAT, as it is used by newborns and small mammals to generate heat and normalize core body temperature (Calder, 2006; Surette, 2008). Studies observing cell differentiation in rodents have demonstrated a close relationship between BAT and muscle cells. These studies have observed that BAT expresses genes generally thought to be uniquely characteristic to myocytes, whereas WAT does not express these genes (Cypess et al., 2009).

Moreover, a 2008 study demonstrated that myogenic precursors in rodents expressing a particular myogenic transcription factor either developed into muscle cells or BAT, but not WAT (Seale et al., 2008).

BAT is a strong contributor to basal energy expenditure due to its high expression of uncoupling protein 1 (UCP1)—a potent thermogenic mediator (Petrovic et al., 2010). As a proton transporter, UCP1 uncouples oxidative phosphorylation from ATP synthesis at the inner mitochondrial membrane. It is able to do so by allowing hydrogen ions that had been pumped out of the mitochondrial membrane by the electron transport chain to return to the mitochondria rather than going through ATP synthase. This decreases the concentration of hydrogen ions in the inner membrane space. Without this proton gradient, the efficiency of ATP synthesis is reduced and energy is transferred from food directly into heat rather than useable ATP (Celi, 2009; Gropper et al., 2009; Klingenberg, 2001; Seale et al., 2008). Food intake and exposure to cold temperatures are external stimuli that may stimulate BAT thermogenesis. BAT is tightly innervated by the sympathetic nervous system, allowing it to respond to environmental changes such as low temperatures (Zingaretti et al., 2009).

Previously, it was believed that the presence of BAT in adult humans was rare and metabolically insignificant (Celi, 2009). However, recent research has demonstrated otherwise (Cypess et al., 2009; Seale et al., 2008). Studies have shown the presence of brown fat in the muscles of the subclavicular region, neck, chest, and abdomen of adult humans using computerized tomography and the introduction of a radioactive form of glucose (FDG) into tissues. These

techniques enable researchers to characterize the metabolic activity of the tissues (Cypess et al., 2009). Virtanen, et al. used these techniques to identify BAT in healthy volunteers on two occasions: once in room temperature, and the other in a chilled environment. All subjects demonstrated increased FDG uptake in subclavicular adipose. Tissue biopsy confirmed the presence of BAT in varying amounts in each subject. One subject was found to have 63g of BAT, which the authors predict would burn the caloric equivalent of over 8 pounds of white adipose tissue in just one year (Virtanen et al., 2009).

Not only have studies established the presence of BAT in adults, but they have also demonstrated correlations between quantities of BAT and gender, age, and body fatness. Using FDG and positron emission tomography-computed tomography (PET-CT), Cypess, et al. demonstrated that the prevalence of BAT in women (BAT activity was detected in 7.5% of women in the study) was more than in men (3.1% of males in the study showed BAT activity), concluding that women were three times more likely to have substantial BAT than men. Furthermore, the authors noted that subjects under 50 years of age had the most detectable BAT ($p<0.001$), and that the amount of BAT was inversely correlated with obesity. It is believed that even more BAT would have been detected had participants been subjected to a BAT stimulus, such as a cold atmosphere (Cypess & Kahn, 2010; Farmer, 2009). Similar results were seen in a study by Zingaretti, et al., in which investigators analyzed adipose tissue samples from the necks of 35 patients. Zingaretti and colleagues found that 1/3 of the samples had regions expressing high levels of UCP1. These regions, termed "BAT islands," were also much more densely innervated than WAT regions. The

highest levels of BAT were detected in the youngest and the leanest of the 35 subjects; the mean BMI for subjects with UCP1 expression was 23± 1 kg/m^2, whereas those with no UCP1 expression had a mean BMI of 26± 1 kg/m^2 ($p<0.03$). However, the study design did not allow the authors to separate leanness and age in order to deduce if it was just one of these variables or a combination of both that influenced UCP1 expression (Zingaretti et al., 2009).

Omega-3 and Polyunsaturated Fatty Acid Overview

Dietary Sources and Metabolism. Polyunsaturated fatty acids (PUFAs) are a broad classification of fatty acids that contain two or more carbon-carbon double bonds. They can be further classified into omega-3 (n-3 FAs) or omega-6 fatty acids (n-6 FAs) depending on the location of their first double bond (Teitelbaum & Allan Walker, 2001). Although both are imperative in normal metabolism, n-3 and n-6 FAs must be obtained from dietary sources since humans lack the enzymes necessary for their *de novo* synthesis (El-Badry, Graf, & Clavien, 2007). Thus, n-3 and n-6 FAs are considered essential fatty acids (EFAs). EFAs have numerous biochemical roles, including energy storage, cell signaling, cell membrane structure, and inflammatory mediation (El-Badry et al., 2007; Surette, 2008).

Omega-3 fatty acids occur in foods in various structures depending on their original source. All n-3 FAs contain between 18 and 22 carbon atoms with their first double bond located at the third carbon from the methyl end of the carbon chain (Calder, 2006). However, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are the most biologically available

sources of dietary n-3 FAs, and are abundant in some animal foods. EPA and DHA are long chain n-3 FAs in comparison to alpha linolenic acid (ALA, 18:3 n-3), the common short chain n-3 FA. High fat fish such as salmon and tuna are particularly rich in EPA and DHA (Molendi-Coste, Legry, & Leclercq, 2011), but limited amounts of n-3 FAs are also found in some cuts of beef, poultry, and eggs depending on the feeding patterns of the animal. ALA is found in plant oils, including walnut and flaxseed oils (**Table 1**) (A. P. Simopoulos, 2001a). Despite large disparities in n-3 FA content between fish species, marine foods generally have between 5 and 15 times the amount of n-3 FAs as poultry or other animal meats (Howe, Meyer, Record, & Baghurst, 2006). Enriched foods have also been developed to enhance levels of EPA and DHA in foods, or to add them to non-animal foods (Mantzioris et al., 2000).

Table 1. Dietary Sources of PUFAs[1]		
Marine Sources		
Food Source	**EPA + DHA (g/4oz)[2]**	**Omega-6 FA (g/4 oz)[2]**
Salmon, wild, Atlantic, Chinook, or Coho	1.2-2.4	0.3-0.5
Anchovies, Herring, or Shad	1.6-2.7	0.1-0.3
Tuna, Bluefin, fresh	1.3-1.7	0.1
Tuna, Albacore, canned in water	1.0	0.1
Swordfish	0.9	0.05
Salmon, Pink, farmed	0.4	0.1
Crab, Blue or Snow	0.4-0.5	0.08
Tuna, light, canned in water	0.3	0.1
Cod, Alaskan	0.2	0.03
Shrimp	0.07	0.02
Eggs, Meat, and Poultry		
Food Source	**EPA + DHA (g/4oz)[2]**	**Omega-6 FA (g/4 oz)[2]**
Egg, fresh	0.03[3]	0.8[3]
Chicken, broilers or fryers, no skin or bone	0.006	0.4
Beef, flank steak	0.003	0.3
Beef, grass fed, strip steaks,	0.002	0.1

lean only		
Beef, ground, 70% lean meat, 30% fat	0.000	0.7
Plant oils		
Food Source	**ALA (g/Tbsp)** [4]	**LA (g/Tbsp)** [4]
Flaxseed oil, cold pressed	7.3	0.004
Canola oil	1.3	2.7
Walnut oil	1.4	7.2
Soybean oil	0.9	6.9
Corn oil	0.2	7.3
Olive oil	0.1	1.3
Safflower oil	0.0	10.1
Sunflower oil	0.0	3.9
Coconut oil	0.0	0.2

[1]Source: U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, 2012, USDA National Nutrient Database for Standard Reference, Release 24, Available at: http://ndb.nal.usda.gov/.
[2]Values based on 4oz raw
[3]Based on 1 large egg (50g)
[4]1 Tbsp oil = 13.6 g

Marine sources of n-3 FAs are distinct from plant sources, such as nuts, seeds, and soy, which supply alpha linolenic acid (ALA, 18:3 n-3) (A. P. Simopoulos, 2001a). ALA is believed to be one of the least usable forms of n-3 FAs since it must be converted to the longer chain n-3 FAs (EPA and DHA) in the endoplasmic reticulum of hepatocytes before it can be used. The rate at which ALA is converted to longer chain n-3 FAs is dependent on the ratio of n-6 to n-3 FAs available in the diet (Arterburn, Hall, & Oken, 2006). Delta-6 desaturase is the rate-limiting enzyme in the conversion of ALA to EPA, and adds a double bond to the original ALA structure. Elongases then add carbon units to the molecule, and delta-5 desaturase finally adds another double bond to form EPA. DHA is synthesized next via delta-6 desaturase and beta-oxidation (G. C. Burdge & Calder, 2005).

The conversion of ALA to EPA and DHA is relatively inefficient in humans (G. C. Burdge & Calder, 2005), but evidence has shown that women are more efficient in this conversion than men. Conversion rates of ALA to EPA and DHA have been shown to be up to 21% and 9%, respectively, in females, as compared to less than 8% and less than 4% in males (Arterburn et al., 2006). It is possible that the increased efficiency of this process in females may be related to the high demands for DHA during pregnancy (G. C. Burdge & Calder, 2005). The conversion rate of ALA to EPA may be further altered by dietary n-6 FA levels, since metabolism of linoleic acid (an n-6 FA) competes with ALA for the rate-limiting enzyme delta-6 desaturase.

Linoleic acid (LA) represents the n-6 FAs and is found in high levels in vegetable oils such as safflower, sunflower, and corn oil (A. P. Simopoulos, 2001b). Once in the body, LA is efficiently converted to arachidonic acid (AA). LA contains an 18-carbon chain, while AA contains a 20-carbon chain. Both have *cis* double bonds beginning at the sixth carbon from the methyl end of the carbon chain (El-Badry et al., 2007). In order to convert LA to AA, LA is first converted to gamma-linoleic acid (18:3 n-6) by delta-6 desaturase. Elongases then add carbon units to form dihomo-gamma-linoleic acid (20:3 n-6). Delta-5 desaturase completes the process by adding another double bond to form AA (Wall, Ross, Fitzgerald, & Stanton, 2010). Like EPA, AA is a critical component of cellular plasma membranes, and plays a major role in cell signaling and inflammatory processes (El-Badry et al., 2007).

Both ALA and LA metabolism utilize the same desaturases and elongases, but the resulting metabolites are very different (Calder, 2006). Activity of these

enzymes is regulated by numerous factors, including feedback inhibition related to dietary intake (El-Badry et al., 2007). Igarashi and colleagues provided rodents with a diet very low in n-3 FA for 15 weeks and compared expression and activity of elongases and delta-5 and 6 desaturases. Rats fed inadequate n-3 FA showed significantly increased expression of delta-5 and 6 desaturases and elongases 2 and 5 in hepatocytes ($p<0.05$), suggesting transcription of these enzymes may be upregulated during periods of low n-3 FA intake in order to convert ALA to EPA and DHA (Igarashi, Ma, Chang, Bell, & Rapoport, 2007).

Desaturase activity appears to change in fasting and fed states, as insulin may stimulate delta-6 desaturase and glucagon may decrease both delta-5- and 6 desaturase activity (El-Badry et al., 2007). Brenner and colleagues found that fasting decreased delta-6 desaturase activity, and glucose refeeding increased activity in rodents (Brenner, 1981). Similarly, investigators observed that diabetic rats had overall low delta-6 desaturase activity, but this activity was increased when insulin was administered (Brenner, 1981). This hormonal regulation appears to hold true in humans as well. Medeiros and colleagues observed delta-5 and delta-6 desaturase activity with respect to insulin levels in obese women. A positive correlation was found between insulin levels and desaturase activity in women with hyperinsulinemia, but a negative correlation was found between desaturase activity and obesity (El-Badry et al., 2007; Madeiros, Liu, Park, Chang, & Smith, 1995).

Interestingly, desaturase activity in males tends to be lower than that of females (Arterburn et al., 2006). A study by Burdge and Wootton showed that conversion of ALA to EPA was 2.5 times greater in young women (mean age of

28 years) than men of a similar age, and conversion of ALA to DHA was over 200 times greater in young women as compared to men (G. C. Burdge & Calder, 2005; G. C. Burdge & Wootton, 2002). Estrogen is believed to lead to increased lipid oxidation, which is needed in the last step of the conversion of ALA to DHA. Thus, higher estrogen levels in women as compared to men could allow for increased DHA formation. This theory was supported by a study comparing ALA to DHA conversion rates in women receiving oral contraceptives (a source of estrogen) to women not taking contraceptives. Those on contraceptives demonstrated 62% greater DHA synthesis compared to the control group, suggesting estrogen may play a role in increasing the ALA to DHA conversion pathway (G. Burdge, 2004).

Current versus Historical Intakes. It is estimated that Americans currently consume an average of one 3.5 oz serving of seafood per week (National Center for Complementary and Alternative Medicine, 2009). The 2010 Dietary Guidelines for Americans recommend Americans increase seafood intake to approximately 8 oz per week (supplying 250mg EPA and DHA per day), citing in particular its importance in cardiac health (Flock & Kris-Etherton, 2011). The American Heart Association confirms these recommendations, suggesting Americans consume at least two 3.5 oz servings of fish per week (Kris-Etherton et al., 2002). The current adequate intake (AI) for n-3 FAs for adults aged 19-30 years is 1.6 g/d for males and 1.1 g/d for females. Despite the need for ALA to be converted into EPA and DHA, this level does not distinguish between ALA, EPA, and DHA intakes (Trumbo, Schlicker, Yates, & Poos, 2002).

Anthropological and epidemiological studies have demonstrated disparities between the typical Western diet and the hunter-gatherer diets of our Paleolithic ancestors approximately 2.5 million-10,000 years ago (Kuipers et al., 2010; A. Simopoulos, 2006). The modern Western diet is generally high in LA-rich foods, including refined vegetable oils and grains, and relatively low in fiber and phytochemicals from unprocessed plant foods (Cordain et al., 2005). Conversely, the Paleolithic diet was high in ALA-rich, unprocessed plant foods such as nuts and seeds, and low in cereal grains and high-fat proteins (Kuipers et al., 2010).

Kuipers, et al., modeled Paleolithic diets using a database of Eastern African foods and knowledge of typical hunter-gatherer food collection strategies. Investigators found that our Paleolithic ancestors consumed significantly more n-3 FAs and less n-6 FAs than those consuming the modern Western diet (Kuipers et al., 2010). The estimated n-6 FA to n-3 FA ratio of the Paleolithic diet is believed to have been close to 1:1, whereas the ratio in the typical Western diet is approximately 16:1 (A. Simopoulos, 2006).

Perhaps the most significant changes to the food supply have occurred within the last two centuries with changes in food technology and agricultural practices. For instance, livestock was primarily grass-fed prior to the 19th century. This practice changed when the development of technologies such as the steam engine and railroad allowed for increased grain harvest, which could then be used as animal feed (Cordain et al., 2005). Grain-based diets led to changes in the fatty acid composition of meats, as grain-fed animals supply higher levels of

saturated and n-6 FAs than free-ranging, grass-fed animals (A. Simopoulos, 2006).

Furthermore, oil-seed processing led to stark increases in consumption of n-6 FA-rich vegetable oils in the 20th century (Cordain et al., 2005). For instance, soybean oil constituted approximately 0.006% of total calories consumed by Americans in 1909. By 1999, soybean oil consumption was over 1,000 times greater, comprising 7.38% of energy consumption (Blasbalg, Hibbeln, Ramsden, Majchrzak, & Rawlings, 2011). This increase in processed food availability is believed to have displaced n-3 FAs in the modern diet, causing an overall increase in n-6 FA and decrease in n-3 FA consumption (Blasbalg et al., 2011; Cordain et al., 2005).

Despite the discrepancies between Paleolithic and modern PUFA intake, the human genome has only changed minimally, possibly contributing to the rise in chronic inflammatory conditions (Kuipers et al., 2010). The effect of this overabundance of n-6 FAs on biochemical processes such as gene transcription and regulation are unclear. Furthermore, the surplus of n-6 FA allows for increased production of AA metabolites, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). These potent inflammatory cytokines have been found to lead to a prothrombotic, proaggregatory state, likely contributing to the development of chronic inflammatory conditions such as cardiovascular disease and autoimmune disorders (A. Simopoulos, 2006).

Observed changes in dietary n-6 to n-3 FA ratios could also explain why recent research has shown the Mediterranean diet to be particularly effective in reducing inflammatory diseases such as asthma and cardiovascular disease (A.

Simopoulos, 2006; A. P. Simopoulos, 2001a). Both the Paleolithic and Mediterranean diets are relatively high in plant foods, nuts and seeds, and protein sources such as fish, thus supplying significantly higher levels of mono- and polyunsaturated fats than the typical Western diet (A. P. Simopoulos, 2001b). A crossover-study by Ambring, et al., demonstrated a statistically significant decrease in serum n-6 to n-3 phospholipid ratio when subjects were on a Mediterranean diet when compared to a Swedish diet. The total number of leukocytes was decreased by 10% ($p<0.05$), and the total number of platelets was decreased by 15% ($p<0.001$) after 4 weeks on the Mediterranean diet, suggesting a decreased risk for coronary artery disease. Investigators also observed a significant inverse correlation between DHA consumption and lymphocyte count regardless of diet (Swedish or Mediterranean), suggesting a link between DHA and decreased risk of coronary heart disease (Ambring et al., 2006).

Interestingly, a study by Kang, et al., demonstrated the capability of rodent cardiomyocytes to express a gene allowing for the conversion of n-6 FAs to n-3 FAs in order to bring the ratio closer to 1 (Kang et al., 2001). Although humans lack the enzymes necessary to perform this conversion, it is interesting that when present, these enzymes return EFA levels to a ratio similar to that believed to be optimal based on evolutionary studies (Kang et al., 2001).

Biological Functions of PUFAs

PUFAs as Energy Sources. Dietary lipids are energy dense, supplying 9 kilocalories per gram (Brown, Isaacs, & Wooldridge, 2007). All dietary lipids,

whether saturated or unsaturated, undergo mitochondrial beta-oxidation (lipid oxidation) to generate ATP. However, evidence suggests that unsaturated fatty acids are more readily oxidized than saturated fatty acids of equal chain length (DeLany, Windhauser, Champagne, & Bray, 2000; Piers, Walker, Stoney, Soares, & O'Dea, 2002). There is also a positive linear relationship between the number of double bonds and oxidation rate of fatty acids, suggesting that saturated fatty acids are more likely to be stored in adipose tissue than unsaturated fatty acids (DeLany et al., 2000).

Before fatty acid oxidation begins, long chain fatty acids must first be attached to carnitine by carnitine palmitoyl transferases (CPT) so that they may be transported into the mitochondrial matrix (Buckley & Howe, 2009). Here, they are activated to form fatty acyl-CoA (Eaton et al., 1996). The process of beta-oxidation occurs in the mitochondria, generating energy in the form of FADH2, NADH, and acetyl-CoA (Eaton et al., 1996; Gropper et al., 2009).

Effects on Gene Expression. PUFAs, including n-3 and n-6 FAs, may act as ligands for nuclear factors, which directly interact with DNA to regulate gene transcription (Bordoni, Di Nunzio, Danesi, & Biagi, 2006; Ferré, 2004). For instance, PUFAs are able to modulate their own metabolism by acting as ligands for peroxisome proliferator-activated receptors (PPARs), a family of transcription factors involved in inflammation and energy homeostasis (Molendi-Coste et al., 2011).

PPARs occur in three known isoforms: PPARα, PPAR β/δ, and PPARγ, each of which is transcribed from different genes. PPARα is prevalent in hepatic, renal, cardiac, and skeletal muscle tissue (Ferré, 2004), and regulates expression of

genes involved in fatty acid transport and oxidation, inflammation, and cell proliferation (Stienstra, Duval, Müller, & Kersten, 2007). Because PPARα increases fatty acid oxidation, its activation may lead to decreased fat storage. PPAR β/δ is a regulator of lipid metabolism, wound healing, and development, and is prevalent in numerous tissues throughout the body (Ferré, 2004). Finally, PPARγ occurs in four known isoforms which are widely expressed in white and brown adipose tissue, and to a lesser amount in immune cells (Bordoni et al., 2006). In addition to lipid metabolism, PPARγ plays a role in inflammation, adipocyte differentiation, and cell cycle regulation (Bordoni et al., 2006).

Studies demonstrating the ability of PPARs to reduce transcription of pro-inflammatory genes in adipocytes and hepatocytes have called attention to their antiobesogenic potential (Ferré, 2004). Moreover, studies have demonstrated that PPARs may act to decrease hypertrophy of adipocytes (Bordoni et al., 2006), and influence the differentiation of preadipocytes in order to form fewer proinflammatory, macrophage-type cells (Stienstra et al., 2007). All unsaturated fatty acids, including EPA, DHA, AA, and LA, have been shown to activate PPARα, leading to increased expression of genes encoding enzymes needed for lipid oxidation (Ross, Moses, & Fearon, 1999). This leads to a consequential decrease in lipid accumulation in tissues (Ferré, 2004). Activation of PPARγ by AA, DHA, EPA, and other PUFAs may also decrease inflammatory cytokine production in vasculature (Stienstra et al., 2007), and increase adipocyte secretion of antiobesogenic hormones such as leptin and adiponectin (Ferré, 2004).

Long chain PUFAs, including both the n-3 and n-6 FAs, may also control lipid homeostasis by regulating the activation of sterol regulatory element-

binding proteins (SREBPs) (Deckelbaum, Worgall, & Seo, 2006). SREBPs are a family of transcription factors important to lipid synthesis. SREBPs must be activated posttranscriptionally, but PUFAs have been shown to inhibit their activation, leading to decreased lipid synthesis (Deckelbaum et al., 2006; Eberle, Hegarty, Bossard, Ferre, & Foufelle, 2004).

Incorporation into Plasma Membranes. Lipids are also incorporated into cellular plasma membranes, particularly in erythrocytes, platelets, and neutrophils, allowing them to play important roles in cell signaling (A. P. Simopoulos, 1999). Plasma membrane composition is reflective of dietary lipid intake, as n-3 and n-6 FAs compete with each other for assimilation into the phospholipid bilayer (Wall et al., 2010). Studies have demonstrated changes in erythrocyte morphology and composition in rodents after providing diets rich in either n-6 FAs or n-3 FAs. Increased n-3 FA consumption leads to an increased n-3 to n-6 FA ratio in the plasma membrane, and a consequential increase in n-3 metabolite production (Surette, 2008; Teitelbaum & Allan Walker, 2001).

The presence of long-chain PUFAs (including n-3 and n-6 FAs) in cell membranes enhances membrane fluidity. Multiple double bonds found in PUFAs contribute to a more disorganized plasma membrane structure. Because DHA is longer and contains more double bonds than EPA and AA, it is believed to create an even more fluid and disorganized plasma membrane structure (Gorjão et al., 2009). This allows for improved cell-signaling by making membrane-bound receptors, enzymes, and transporters more accessible to signaling molecules such as G-proteins, kinases, and ion channels (Ross et al., 1999).

The PUFA (including both n-3 and n-6 FAs) content of cell membranes may modulate sodium and calcium channel ion pump function by stimulating their activity (Ross et al., 1999). Membrane-associated ion transport, which drives cellular processes such as metabolism, is a major contributor of the basal metabolic rate of mammals. Animals with high basal metabolic rates have been shown to have higher levels of PUFAs in their cell membranes, suggesting PUFAs may act as stimulators of enzymes needed in active transport and cell-signaling, such as calcium ATPases and sodium-potassium ATPases (Hulbert, Turner, Storlien, & Else, 2005). Infante, et al., demonstrated that high levels of PUFA (particularly n-3 FA) in the membranes of muscle cells led to increased activity of calcium ATPase, a major contributor to metabolism (Infante, 1987).

Eicosanoid Synthesis. Essential fatty acids incorporated into the plasma membrane are also used to produce cell-signaling molecules called eicosanoids. Eicosanoids such as leukotrienes, prostaglandins, and thromboxanes act as cell mediators to modulate numerous processes such as inflammation, immunity, and thrombogenesis. Both EPA and AA may be used to synthesize eicosanoids, but the specific type of eicosanoid produced varies depending on the availability of free EPA and AA (Harizi, Corcuff, & Gualde, 2008). Because n-6 FAs are usually present in higher quantities in cell membranes, eicosanoids derived from AA are typically formed more readily (Harizi et al., 2008).

Unsaturated fatty acids used for eicosanoid synthesis come from the phospholipids of inflammatory cell membranes. Physiological stimuli (such as elevated epinephrine or antigen-antibody complexes), or pathological stimuli may act on a specific tissue to stimulate eicosanoid production. In response,

phospholipase A2 (PLA2) acts on cell membranes to liberate AA or EPA from the phospholipid bilayer (Gropper et al., 2009; A. Simopoulos, 2006). Specifically, PLA2 cleaves the ester bond linking the fatty acid to the second carbon on the glycerol backbone, releasing a fatty acid (Gropper et al., 2009).

AA can be converted to the 2 series of prostaglandins and the 4 series of leukotrienes and thromboxanes, while EPA is the precursor to the 5 series of leukotrienes and the 3 series of prostaglandins (Calder, 2010; Das, 2005). EPA and DHA may also be converted to E-series and D-series resolvins, respectively, which are important in inflammation suppression (C. N. Serhan et al., 2002; Charles N Serhan, Gotlinger, Hong, & Arita, 2004).

The pathways converting free EPA and AA to their respective eicosanoids utilize many of the same major enzymes, yet those generated from EPA have slightly different structures and presumably weaker effects than those from AA (Harizi et al., 2008). If present in large quantities, AA-derived eicosanoids generally promote an inflamed, prothrombotic physiologic state. Conversely, EPA-derived eicosanoids typically have either weakly pro-inflammatory or anti-inflammatory properties. Thus, it is believed that high dietary consumption of n-6 FA and low n-3 FA consumption can promote inflammatory conditions (Calder, 2006).

Arachidonic Acid-Derived Eicosanoids. Three different classes of enzymes may act on free AA to produce bioactive eicosanoids: cyclooxygenases (COXs), lipoxygenases (LOXs) or P-450 epoxygenases (Calder, 2006). COX exists in different isoenzymes as either COX-1 or COX-2. COX-1 is a constitutive enzyme, meaning it is not controlled by induction or repression and is present at all times.

Conversely, COX-2 is an inducible enzyme, so the level of its expression depends on physiological conditions (Calder, 2006). AA-derived eicosanoids include leukotriences, lipoxins, prostaglandins, thromboxanes, and hydroxy fatty acids (Harizi et al., 2008). The COX pathways produce prostaglandins PGD2, PGE2, and PGF2a, prostacyclin, and the thromboxanes TXA2 and TXB2 (A. Simopoulos, 2006). PGE2, an AA-derived eicosanoid, has received special attention for its powerful abilities to increase inflammation, resulting in effects such as vasodilation, edema, pain, and fever (Stanke-Labesque et al., 2008). The LOX pathway is responsible for converting AA into various leukotrienes, hydroxyeicosatetraenoic acids (HETEs), and lipoxins. P-450 epoxygenases synthesize HETEs and epoxides (Calder, 2006). The AA-derived eicosanoids, such as PGE2, generally have strong inflammatory potential. However, some evidence has shown that the lipoxins may actually decrease inflammation (Das, 2005) and stop neutrophil migration and adhesion (Chiang, Arita, & Serhan, 2005).

EPA and DHA-Derived Eicosanoids. Metabolism of EPA and DHA are affected by dietary n-3 FA intake, as n-3 FAs compete with n-6 FAs for both incorporation into cell membranes and interaction with COX and 5-LOX (Calder, 2006). This effect has been demonstrated in humans; for instance, fish oil supplementation has been shown to cause an increase in the production of certain leukotrienes, such as LTB5 and LTE5, and a decrease in LTB4 and prostaglandin E2 (PGE2) (Teitelbaum & Allan Walker, 2001; Thies et al., 2001). In contrast with AA-derived eicosanoids, EPA-derived eicosanoids, such as LTB5, exert weak inflammatory or even anti-inflammatory effects (Stanke-Labesque et al., 2008; Teitelbaum & Allan Walker, 2001). Moreover, EPA-derived eicosanoids

may antagonize the actions of AA-derived eicosanoids, ultimately limiting their inflammatory potential (Calder, 2010). For instance, Tull, et al., demonstrated through an *in vitro* study that generation of EPA-derived eicosanoids inhibited the recruitment and migration of neutrophils (Tull et al., 2009). Expression of leukocyte adhesion molecules, which is typically stimulated by cytokines, was suppressed after DHA supplementation in endothelial cells. As a result, inflammatory markers, including IL-1 and IL-6, were significantly decreased in the cells that received DHA supplementation (DeCaterina, Liao, & Libby, 2000). Observations such as these have led to the belief that increased n-3 FA intake weakens the inflammatory response (Molendi-Coste et al., 2011).

EPA and DHA may also be used in the COX-2 pathway to produce the E-series and D-series resolvins, respectively. Resolvins are anti-inflammatory mediators that have been shown to block generation of inflammatory markers such as TNF-α and IL-6, ultimately decreasing leukocyte recruitment and pro-inflammatory cytokine generation (Das, 2005). Low cellular concentrations of resolvins may increase adherence of macrophages to endothelial cells, leading to endothelial injury that is characteristic of chronic inflammatory diseases such as coronary artery disease (Das, 2005).

Inflammation

Acute & Chronic Inflammation. Inflammation is a natural and essential response to physical, chemical, or biological stressors, such as injury and infection (Stienstra et al., 2007). In acute inflammation, leukocytes, macrophages, and eicosanoids are recruited to destroy injurious stimuli (Calder,

2006). If successful, a process of tissue repair and inflammatory cell apoptosis follows, and homeostasis is reestablished (Jernås et al., 2006). If the harmful agent is not eliminated, however, chronic inflammation persists through systemic recruitment of macrophages and inflammatory mediators such as interleukins 6 and 8 (IL-6 and IL-8), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) (Monteiro & Azevedo, 2010), potentially damaging surrounding tissues (Wall et al., 2010).

Anti-Inflammatory Effects of Omega-3 Fatty Acids. Omega-3 fatty acids have been found to attenuate the effects of some chronic inflammatory conditions, including rheumatoid arthritis and atherosclerosis (Monteiro & Azevedo, 2010). Several mechanisms have been proposed to explain the ability of n-3 FAs to decrease systemic inflammation both indirectly and directly.

Because n-3 and n-6 FAs compete for assimilation into plasma membranes, n-3 FA intake decreases the concentration of AA in tissues (Calder, 2006). This leaves less substrate available for production of AA-derived eicosanoids, thereby indirectly suppressing the inflammatory response (Calder, 2010). Furthermore, by competing with AA for metabolism through the COX and LOX pathways, n-3 FAs are able to decrease production of AA-derived, pro-inflammatory eicosanoids (Tull et al., 2009; Wall et al., 2010). Instead, higher levels of less-potent eicosanoids, and possibly even anti-inflammatory eicosanoids, are produced (Calder, 2010).

In addition to antagonizing AA-derived eicosanoid inflammation, resolvins and lipoxins produced from EPA and DHA have the potential to decrease inflammation directly by blocking cytokines and decreasing neutrophil migration

(Charles N Serhan et al., 2004; Wall et al., 2010). The ultimate systemic results of these mechanisms are evidenced by suppressed leukocyte chemotaxis and decreased production of reactive oxygen species and adhesion molecules (Calder, 2010).

Moreover, n-3 FAs may induce anti-inflammatory effects on a genetic level (Calder, 2009, 2010; Wall et al., 2010). PPARs are known to antagonize the effects of nuclear factor kappa B (NFkB), which is a transcription factor that regulates the production of adhesion molecules and pro-inflammatory mediators (including IL-6 and TNF-α). As ligands for PPARs, PUFAs (particularly n-3 FAs) are able to indirectly suppress NFkB activity (Wall et al., 2010). Furthermore, some evidence suggests EPA may inhibit NFkB activity by altering its activation. Under normal conditions, NFkB is activated through phosphorylation, yet cells cultured with n-3 FAs demonstrated less phosphorylation of NFkB as compared to cells cultured with n-6 FAs (Babcock et al., 2003). Thus, n-3 FAs seem to reduce the ability of kinases to activate NFkB (Calder, 2009).

Current Literature Studying n-3 FA Intake and Body Composition

Animal and In Vitro Studies. Although limited, some evidence suggests n-3 FAs may decrease basal metabolic rates in rodents, which could lead to decreased body mass over time. Vegiopoulos, et al., studied the role of COX-2 (an enzyme critical to eicosanoid synthesis) in BAT activity in mice. Investigators found that inhibition of COX-2 led to decreased BAT thermogenesis and a reduction in basal metabolic rate, ultimately leading to increased adiposity. Proinflammatory AA-derived eicosanoids are believed to increase mitochondrial

thermogenesis by up-regulating UCP1. This suggests that n-3 FAs, which compete with AA for metabolism by COX-2, may lead to a reduction in thermogenesis and a consequential increase in adiposity (Vegiopoulos et al., 2010).

Other studies suggest dietary n-3 FA decreases adiposity. An *in vitro* study by Kim, et al., demonstrated antiadipogenic effects of DHA. Preadipocytes exposed to DHA displayed decreased lipid accumulation. Furthermore, DHA led to increased adipocyte apoptosis. Investigators reasoned that DHA was incorporated into cellular plasma membranes (leading to decreased concentration of AA in membrane phospholipids), thus decreasing production of AA-derived eicosanoids, which are typically associated with cell proliferation. Lipolysis in mature, fully differentiated adipocytes, was also stimulated after introduction of DHA (Kim, Della-Fera, Lin, & Baile, 2006). Thus, n-3 FAs may encourage adipocyte apoptosis, discourage accretion of lipids in adipocytes, and increase lipolysis to make fewer lipids available for adipocyte uptake.

Animal studies have found similar results. Ruzickova, et al., found that when a portion of a high fat diet was replaced with EPA and DHA, rodents accumulated less epididymal fat and displayed less weight gain when compared to rodents on a control high fat diet. Observed differences could not be explained by variances in caloric intake, suggesting EPA and DHA may have played a role in the rodents' metabolism. Based on these results, however, it is predicted that humans consuming 100g of fat per day would need to replace 11g of dietary fat with EPA/DHA in order to see a decrease in obesity (Ruzickova et al., 2004). This

would be equivalent to approximately 5-7 servings of oily fish per day, which far exceeds the current dietary recommendations (Flock & Kris-Etherton, 2011).

Belzung, et al., observed a dose-dependent decrease in epididymal and peritoneal adipose tissue accretion in rodents consuming EPA and DHA as part of a high-fat diet. Investigators suggested that EPA and DHA may play a role in limiting adipocyte hypertrophy (Belzung, Raclot, & Groscolas, 1993). In a study by Raclot, et al., not only did investigators find that dietary n-3 FA led to decreased adipocyte hypertrophy in rodents, but they also found that expression of metabolic enzymes, including fatty acid synthase and lipoprotein lipase, decreased in rodents receiving EPA and/or DHA (Raclot, Groscolas, Langin, & Ferré, 1997). This suggests that n-3 FAs may play a role in expression of enzymes needed for lipogenesis and lipid oxidation (Raclot et al., 1997).

Omega-3 fatty acids may also have implications in appetite regulation. A 2006 study showed that rats fed EPA had increased levels of leptin (an anorexigenic hormone) and decreased caloric intake (Pérez-Matute, Pérez-Echarri, Martínez, Marti, & Moreno-Aliaga, 2007). Furthermore, a study by Takahashi and Ide also demonstrated decreased intake and a consequential reduction in adiposity in mice fed an EPA/DHA-enriched diet (Takahashi & Ide, 2000).

Human studies showing weight gain. Although the mechanism is elusive, several studies in humans have demonstrated that frequent consumers of fish tend to weigh more (**Table 2**). Iso, et al., distributed food frequency questionnaires to healthy female registered nurses between the ages of 34 and 59 and followed nearly 80,000 of them for 14 years. Although the primary

objective of the study was to observe stroke risk as related to fish consumption, investigators noted that women who reportedly consumed over two servings of fish per week (approximately 0.5 g n-3 FA per day) were more likely to be overweight than non-fish eaters (Iso, 2001). Because the study was a prospective cohort study, however, no definitive conclusions could be drawn about the specific effects of fish consumption.

Fish oil supplementation (>1.5g/day, or >5 servings of fish per week) has been shown to be effective in treatment of cancer cachexia, a condition characterized by hypermetabolism, inflammation, decreased appetite, and marked decreases in body weight (particularly lean body mass) secondary to certain forms of cancer (Colomer et al., 2007). EPA and DHA found in fish oil are believed to decrease levels of proinflammatory cytokines, possibly leading to greater lean body mass accretion, although no conclusive mechanistic explanations have been drawn (Colomer et al., 2007; Fearon et al., 2003; Grimble, 2003). Fearon, et al, randomized 200 men and women with unresectable pancreatic cancer to either a n-3 FA supplement group (consuming approximately 2.2 g EPA per day) or placebo group for eight weeks. Those in the experimental group demonstrated increased dietary intake as compared to control subjects during the intervention. A significant positive correlation was also observed between supplement intake and gains in total body weight ($r = 0.5$, $p < 0.001$) and lean body mass ($r = 0.33$, $p < 0.05$) only in the experimental group (Fearon et al., 2003).

Similarly, a double-blind, placebo-controlled trial by Irving, et al., studied the effects of fish oil supplementation (1.7 g DHA plus 0.6 g EPA per day) on

body weight and appetite in Alzheimer's patients. Subjects receiving fish oil supplementation (n=89) had gained an average of 0.7 ± 2.5 kg 6 months into the study (p=0.02), whereas the placebo group (n=85) showed no significant weight change. Those in the intervention group also showed improved appetite after 12 months (p=0.01) based on caregivers' ratings. Investigators also examined C-Reactive Protein (CRP) levels (an inflammatory marker), noting that weight gain increased as CRP levels decreased, indicating inflammation and appetite and/or weight gain may be correlated in Alzheimer's disease patients. These effects were seen despite the fact that Alzheimer's patients typically lose weight as the disease progresses (Irving et al., 2009).

A similar trend was observed in a study that looked at the impact of dietary PUFAs on cardiovascular disease risk markers. The 24-week study randomly assigned overweight men and women to either the control group or one of four intervention groups varying by type of fish consumed (either oily fish supplying approximately 4.5 g EPA + DHA per week, or white fish supplying approximately 0.7 g EPA + DHA per week), and types of visible fats used (high in sunflower oil providing LA, or high in rapeseed oil, which is low in LA) for the duration of the study. Subjects in the oily fish/sunflower intervention group unexpectedly showed weight gain, while those in the white fish/sunflower group demonstrated weight loss, despite the fact that no significant differences in calorie intake were observed (Moore et al., 2006). Because the effects of n-3 FAs on weight were not the primary objectives of this study, however, it is difficult to determine whether observations resulted because of the addition of dietary fish oils, or because of confounding variables, such as physical activity.

The above studies observed the effects of fish oil on older subjects or subjects with chronic disease and/or inflammation. However, Damsgaard, et al., provided fish oil supplements to 66 young (19-40 years old), healthy men in a randomized, double-blind study for 8 weeks. Investigators measured cardiovascular disease risk markers, including body weight. Investigators showed that subjects receiving fish oil (1.8 g EPA + 1.1 g DHA per day provided in capsules) and consuming visible fats high in linoleic acid gained an average of 0.7 kg over the course of the study ($p<0.05$). However, this trend was not seen in any other subjects, including those receiving fish oil supplements and consuming visible fats low in linoleic acid (Damsgaard, Frøkiaer, Andersen, & Lauritzen, 2008).

Human Studies Showing Weight Loss. Some intervention studies also suggest a relationship between n-3 FAs and decreased body weight (**Table 2**). In a cross-sectional study, Micallef, et al., measured plasma n-3 FAs of 124 adult men and women (aged 18-70, healthy, overweight, and obese). Higher plasma n-3 FA levels were associated with healthier anthropometric measurements (BMI, waist circumference, and hip circumference), and obese subjects had significantly lower plasma n-3 FA levels when compared to healthy-weight individuals (Micallef et al., 2009). Although no definitive conclusions may be drawn from this study, the results suggest plasma n-3 FA levels may be inversely related to BMI.

Hill et al. observed the effects of fish oil supplementation paired with exercise on body composition and cardiovascular health in adults at risk for cardiovascular disease. 75 subjects with a BMI >25 kg/m^2 and hypertension,

elevated cholesterol, or high triacylglycerol levels were randomized to one of three intervention groups: fish oil supplementation alone (supplying ~1.9 g n-3 FA per day), fish oil supplementation plus exercise, sunflower oil supplementation alone (control), or sunflower oil plus exercise. Over the course of the 12 week study, percent body fat was reduced only in the exercise groups, but those in the fish oil plus exercise group lost approximately 1.5 kg fat, which is significantly more than those in the sunflower oil plus exercise group (Hill et al., 2007).

Kunesova, et al., studied the effects of n-3 FA supplementation (2.8 g EPA + DHA per day) and a very low calorie diet on fat oxidation in obese women. After the 21-day intervention, women in the placebo group lost significantly less weight than those receiving the fish oil, and showed lower levels of beta-oxidation as measured by serum beta-hydroxybutyrate levels. There was also an inverse relationship between phospholipid DHA change and BMI change ($r= -0.595$, $p<0.008$). Investigators concluded that the efficacy of a very low calorie diet might be enhanced when paired with fish oil supplementation. However, the sample size was relatively small, ($n=20$), and the study was performed in an inpatient setting, making it difficult to generalize results to a broader population (Kunesová et al., 2006).

In a high-constraint crossover study by Couet, et al., 6 g/day of visible dietary fats were replaced by fish oils for three weeks, and consequential changes in metabolic rate, substrate oxidation, and body composition as measured by dual-energy X-ray absorptiometry (DXA) were studied. Subjects were young and healthy (aged 23 ± 2y; BMI 21.9 ± 1.6 kg/m^2). All meals were

prepared and measured by a dietitian to ensure that caloric intake was accurately recorded. Although no significant difference in weight loss was observed, investigators observed an increase in resting metabolic rate, increase in fat oxidation, and a decrease in body fat mass (-0.88 ± 0.16 kg, $p<0.05$) during the fish oil intervention. Although significant, it is difficult to generalize these results to a broad population since it was such a high-constraint study. Furthermore, the sample size was relatively small (n=6), and only one subject was female, making it difficult to draw conclusions about the effects of n-3 FA supplementation on female body composition (Couet et al., 1997).

Human Interventions Showing No Effect or Mixed Results. Not all studies have found a significant relationship between fish oil supplementation and alterations in body weight or body composition (**Table 2**). For instance, a 10-week parallel-arm study randomized 32 healthy, sedentary adult males to one of four groups: control (usual diet and physical activity), fish oil supplementation (approximately 4g n-3 FA per day), exercise, or fish plus exercise group. No significant change in body fat percentage was found in any of the intervention groups (Brilla & Landerholm, 1990).

Similarly, DeFina, et al randomized sedentary obese or overweight males and females (aged 30-60 years) to take either a fish oil intervention group (receiving 12.5 g EPA + 2.5 g DHA divided into 5 capsules per day) or placebo control group (1:1 ratio of soybean and corn oil in 5 capsules per day) in a double-blind parallel arm trial. Subjects in both groups received lifestyle counseling to promote weight reduction. Although subjects in both groups lost weight, there were no statistically significant differences in weight reduction

between the two groups as indicated by body weight, BMI, body fat percentage, or waist circumference. There were also no significant differences in resting metabolic rate between the two groups (Defina et al., 2011).

Thorsdottir, et al, placed 324 overweight men and women on a weight loss diet and randomly assigned them to consume either 3 servings/week oily fish (supplying 2.1 g/d EPA + DHA), 3 servings/week lean white fish (supplying 0.26 g/d EPA + DHA) 6 capsules of fish oil/day (supplying 1.3 g/d EPA + DHA), or 6 placebo oil capsules/day and no seafood. All of the men consuming seafood (either lean or oily fish) or taking fish oil capsules lost an average of 1 kg more weight than those in the placebo group. No significant differences were seen in women, however. The authors theorized that this gender difference could be related to a difference in reaction to marine foods in men an women. One possibility for this is that women have an enhance ability to convert ALA to DHA as compared to men, so supplementation of n-3 FAs may not have had as profound of an effect on women (Thorsdottir et al., 2007).

Krebs, et al., compared a weight loss intervention paired with a fish oil supplement (1.3 g EPA + 2.9 g DHA per day) to the same intervention with a placebo oil supplement (2.8 g LA + 1.4 g oleic acid per day) in overweight, hyperinsulinemic women. Despite seeing decreased inflammatory markers and lipid profiles in fish oil subjects, no significant difference in weight loss was observed between the two groups, suggesting fish oil is effective in treatment of chronic inflammation and clinical manifestations associated with obesity (such as hyperlipidemia), but not necessarily in weight loss itself (Krebs et al., 2006).

Others still have only found fish oil supplementation to be effective in weight loss promotion only when paired with a weight loss regimen. For instance, Warner, et al., conducted a randomized controlled parallel-arm study and assigned 34 hyperlipidemic subjects to either a corn oil supplement, fish oil supplement, fish oil plus exercise, or control (no exercise or supplement) group. Over the course of the 12-week study, a significant reduction in body fat was observed only in those taking fish oil and exercising. However, the amount of fish oil supplemented per day was extremely high (14g EPA and 10g DHA/day), potentially introducing the risk of prolonged bleeding times, and making it difficult to determine whether lower levels of supplementation would be effective in reducing body fat (Warner et al., 1989).

Table 2. Available Literature. Studies in humans observing the relationship between omega-3 fatty acids and changes in body weight and/or body composition.

Authors and year	**Study Design**	**Intervention**	**Population**	**Outcome**
Irving, et al, 2009	Random-ized, double-blind, placebo-controlled, parallel-arm trial; results analyzed at 6 months and 12 months	0.6 g EPA + 1.7 g DHA (experimental) or 0.6 g LA (control) per day	Males and females with Alzheimer's disease; mean age = 72.6 years; n=204	Fish oil group gained significantly more weight 6 months into the study as compared to control subjects
Micallef, et al, 2009	Cross-sectional	N/A	Males and females aged 18-74 years; healthy, overweight, and obese	Higher plasma n3 FA was related to healthier anthropo-metric measure-

				ments; obese subjects on average had lower n3 FA plasma levels
Damsgaard, et al, 2008	Randomized, double-blind, placebo-controlled, parallel-arm trial; 8 week intervention	Subjects received 1.8 g EPA + 1.1 g DHA per day or 5 mL olive oil (placebo) in capsules and consumed dietary fats either high or low in LA	Healthy young males, 19-40 years; n=64	Only subjects receiving fish oil and consuming dietary fats high in LA gained weight
Hill, et al, 2007	Randomized, double-blind, placebo-controlled, parallel-arm trial; 12 week intervention	0.36 g EPA + 1.56 g DHA per day (experimental) or 6 g sunflower oil per day (control) provided in capsules; also assigned to either a regular physical activity program or no physical activity program	Males and females aged 25-65 years; overweight or obese (BMI > 25 kg/m^2) with either hypertension, elevated cholesterol, or elevated triacylglycerol levels; n=75	Subjects on an exercise regimen and fish oil supplement lost significantly more fat as compared to those receiving sunflower oil and exercise
Krebs, et al, 2006	Randomized, double-blind, placebo-controlled, parallel-arm trial; 24 week intervention	Subjects assigned to either weight loss program plus either 3 g EPA + 2.9 g DHA or 2.8g LA + 1.4g oleic acid per day provided in capsules (experimental),	Overweight, hyperinsulinemic adult females; n=116	Decreased inflammatory markers and improved hyperlipidemia was observed, but there were no significant differences in weight loss between the

		or no weight loss program plus 2.8 g LA + 1.4 g oleic acid per day		two groups
Kunesova, et al, 2006	Random-ized, double-blind, placebo-controlled, parallel-arm trial; 3 week intervention	2.8 g EPA + DHA per day (experimental) or saline (control) provided in capsules plus a very low calorie diet	Severely obese adult females; n=20	Subjects on a very low calorie diet and taking fish oil lost more weight and showed increased lipid oxidation as compared to the control group (very low calorie diet plus placebo)
Moore, et al, 2006	Random-ized, placebo-controlled, parallel-arm trial; 24 week intervention	4 intervention groups consumed either 2 servings of oily fish per week (providing 4.5 g EPA + DHA per week) or 2 servings of white fish per week (providing 0.7 g EPA + DHA per week) and replaced dietary fats with ones high in either n-6 FAs or n-3 FAs. Control group received no intervention.	Males and females aged 35-65 years; overweight or obese (BMI 25-40 kg/m^2) with no other diagnosed chronic conditions; n=142	Oily fish consumers demonstrated increased weight gain
Fearon, et al, 2003	Random-ized, double-blind,	2 cans of a protein and energy supplement	Weight-losing males and females with unresectable	Positive correlation between supplement

	placebo-controlled trial; 8 week intervention	providing 2.2 g EPA (experimental) per day or 0 g EPA per day (control)	pancreatic cancer; n=200	intake and gains in lean body mass and total body weight
Iso, et al, 2001	Prospective cohort study	N/A	Healthy adult females, 34-59 years	Women consuming >2 servings fish/wk were more likely to be overweight than non-fish eaters
Couet, et al, 1997	Crossover study	6 g total n-3 FA per day provided in capsules in order to replace 6g/d visible dietary fat	Healthy, non-obese young adults (5 males, 1 female; age = 23 ± 2 years; BMI = 21.9 ± 1.6 kg/m^2); n=6	Increased resting metabolic rate and decreased fat mass deposition during fish oil intervention
DeFina, et al, 2011	Random-ized, placebo-controlled, parallel-arm trial; 6 month intervention	Subjects assigned to either fish oil (12.5 g EPA + 2.5 g DHA per day) or placebo capsules (soybean and corn oil); all subjects received lifestyle modification counseling to promote weight reduction	Sedentary overweight and obese males and females, aged 30-60 years; n=81	n-3 FA supplemen-tation did not lead to weight loss as compared to placebo
Thorsdott-ir, et al, 2007	Random-ized, placebo controlled, parallel-arm trial; 8 week	Subjects instructed to follow weight loss regimen and detailed meal plan;	Overweight men and women (20-40 years, BMI = 27.5-32.5 kg/m^2); n=324	Men consuming fish oil capsules or lean or oily fish lost an

	intervention	randomized to one of four diets: no seafood (control), 3 servings/week lean white fish (providing 0.26 g/d n-3 FA), 3 servings/week oily fish (providing 2.1 g/d n-3 FA), 6 fish oil capsules/day (providing 1.3 g/d n-3 FA)		average of 1 kg more weight after 1 month than the control; no significant differences were seen in women
Brilla & Landerholm, 1990	Random-ized, parallel-arm trial; 10 week intervention	Subjects assigned to either an aerobic exercise regimen or no exercise regimen; experimental group consumed 4 g total n3 FA per day in capsules	Sedentary adult males, aged 19-34 years; n=32	No significant differences in body weight or composition in any groups (with or without exercise intervention)
Warner, et al, 1989	Random-ized, placebo-controlled, parallel-arm trial; 12 week intervention	14 g EPA + 10 g DHA or 500 mL corn oil per day provided in capsules plus an exercise regimen or no exercise regimen	Males and females with hypertriglyceridemia, aged 27-63 years; n=34	Only subjects taking fish oil and exercising lost weight

Chapter 3

METHODOLOGY

Subjects & Study Design. Healthy, non-obese women at Arizona State University between the ages of 18 and 38 were recruited through email using Arizona State University ListServs. Interested subjects were referred to an online survey (Appendix B) to determine eligibility based on diet, physical activity, smoking habits, brief medical history, and self-reported BMI. Eligible subjects were contacted to schedule a screening visit (study visit 1).

During the screening visit, subjects completed a written consent (Appendix C). Next, subjects completed a brief medical history questionnaire (Appendix D) and validated omega-3 fatty acid food frequency questionnaire (FFQ) (Appendix E). Anthropometric measurements, including weight, height, and percent body fat, were also be measured. Height and weight were measured using a stadiometer and calibrated scale. BMI and percent body fat were determined using a bioelectrical impedance scale (Tanita).

Exclusion criteria included regular smoking (use of >10 cigarettes per day), BMI >30 or BMI <18.5, regular use of omega-3 or fish oil supplements, vegetarian dietary patterns (excluding all fish, meat, and poultry from the diet), regular consumption of one or more 3.5oz servings of fish per week, and/or use of prescription medications that may interfere with body weight or inflammatory state (such as corticosteroids or non-steroidal anti-inflammatory drugs). Potential subjects taking birth control were excluded if they had been taking the drug for less than three months. Women who were pregnant or lactating, as well as

competing and/or training athletes were excluded. Furthermore, subjects with any unresolved health issues were not used in the study. Finally, anyone who has had the seasonal flu shot was excluded because of the accompanying study.

To determine a sample size, a power analysis was performed using a probability of 0.05 and a power of 0.8, along with a verified sample size calculator (Schoenfeld, 2010). Based on prior human interventions testing the effect of a dietary supplement on body weight, an expected change of 1.9 kg and a standard deviation of 1.3 were used. This calculation suggested that a total of 18 subjects would be adequate for a randomized, parallel arm trial (Appendix F). A total of 35 subjects were enrolled in the study, which allowed for 18 subjects in the fish oil group (FISH) and 17 subjects in the coconut oil placebo group (CON). However, 9 total subjects were lost to attrition throughout the study, leaving 13 subjects in each group (for a total of 26 subjects) that completed the study.

Qualifying subjects entering the 8-week trial were stratified based on age, BMI, weight, and n-3 FA consumption as indicated by the omega-3 FFQ, and randomly assigned to either the experimental (fish oil, FISH) or control (placebo, CON) group. Random assignments to groups were performed by a coin toss.

During the baseline study visit (study visit 2), a 24-hour recall was performed to understand typical dietary patterns and estimate caloric intake. Next, supplements were administered using a double-blind procedure to prevent bias during data collection and analysis. Subjects received either placebo (Puritan's Pride brand, coconut oil softgels, 1000mg each) or fish oil capsules (EnergyFirst brand, USP-certified fish oil softgels, 400mg EPA + 200mg

DHA/capsule), which were similarly-sized gel capsules. Subjects were instructed to ingest one capsule in the morning, preferably with food, and check off each day that capsule was taken on provided calendar (Appendix G). Subjects also received booklets containing validated Godin Leisure-Time Exercise questionnaires to complete weekly (Appendix H) and validated daily cold symptom surveys for accompanying study (Appendix I) during visit 2. Subjects were asked to document alcohol, cigarettes, medications, and supplements used each day at the bottom of each cold symptom survey. An explanation of all questionnaires was provided during subject meetings, and a practice-run was completed before subjects left study visit 2. Height, weight, BMI, and percent body fat measurements were taken during the baseline visit (using a stadiometer and bioelectrical impedance scale, Tanita) and a fasting blood sample was collected for the accompanying study.

Visits at weeks 4 and 8 began with body weight, percent body fat, and BMI measurements. Unused capsules from the previous weeks were returned during these visits using a double-blind procedure, and remaining study materials (booklets containing physical activity log and capsules for weeks 5-8) were distributed during the week 4 visit. A final 24-hour dietary recall and fasting blood sample were also performed during the final visit for the accompanying study (week 8).

Compliance to capsule administration was monitored through capsule counts at weeks 4 and 8. Biweekly emails from a blind master list were also sent to all subjects to check for compliance to study protocol.

Statistical Analysis. SPSS Version 19.0 was used to perform statistical analysis, and Food Processor SQL was used to assess 24-hour recalls. Weekly leisure time exercise was measured in MET-hours per week, which was determined by multiplying the numbers of reported mild, moderate, and strenuous physical activity sessions by 3, 5, or 9, respectively. Unless indicated otherwise, data is reported as means ± standard error (SE). Differences in means of outcome variables were compared using 2-tailed independent t-tests, univariate analysis of variance, or repeated measures analysis of variance. Data were tested for normality, and non-parametric tests were used for data that were not normally distributed. Differences in variables were considered significant at $p \leq 0.05$.

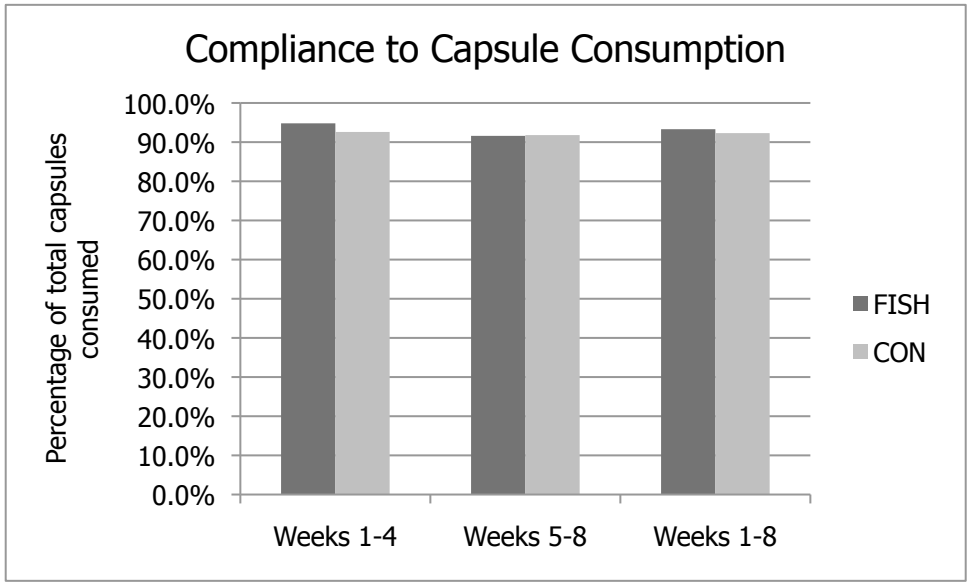
Chapter 4

DATA & RESULTS

Recruitment for this double blind, placebo-controlled, parallel-arm trial took place in January and February 2012. A total of 163 people completed the Internet survey, but only 53 were contacted for a screening visit due to exclusion criteria. A total of 43 females attended the initial screening visit. Of the 43 participants screened, 35 were enrolled in the study. Participants were then stratified by age, body weight, BMI, and n-3 FA intake (as measured by a FFQ administered during the screening visit), and randomly assigned to the intervention (FISH) or control (CON) group. This resulted in 18 subjects in the FISH group and 17 subjects in the CON group. However, 8 participants dropped out of the study before the week 1 visit due to apparent lack of interest, scheduling conflicts, or loss of communication, and one participant dropped out after the week 4 visit due to loss of communication. Thus, 13 subjects in the CON group and 13 subjects in the FISH group completed the study and were used for data analysis. There was an overall 93.3% compliance rate in the FISH group and a 92.3% compliance rate in the CON group based on the number of capsules leftover at the mid-point (4 weeks) and end (8 weeks) of the intervention. Although average compliance decreased in the FISH group between the first and second half of the study, compliance remained above 91% for both groups throughout the course of the study (**Table 3, *Figure 1***).

Table 3. Compliance. Average percentage of capsules taken based on number of capsules returned [1,2]			
	Weeks 1-4	Weeks 5-8	Weeks 1-8
FISH	94.8 ± 0.02 %	91.6 ± 0.03%	93.3 ± 0.02%
CON	92.6 ± 0.02%	91.8 ± 0.03%	92.3 ± 0.03%
ALL	93.7 ± 0.02%	91.7 ± 0.02%	92.8 ± 0.02%
[1]FISH - fish oil group, 400mg/d EPA + 200mg/d DHA; CON - placebo group, 1000mg/d coconut oil; ALL – All subjects [2]Values reported as means ± SE			

Figure 1. Compliance to capsule administration as measured by average percentage of capsules taken.



Independent t-tests showed that subjects in the FISH and CON groups were similar upon enrollment in the study (**Table 4**). The mean age of each group was similar (24.0 ± 1.6 years for FISH and 23.0 ± 1.7 years for CON) and ranged from 18-38 years in both groups. Baseline body weight, BMI, and percent body fat recorded at the screening visit did not differ significantly between groups. Omega-3 fatty acid intake as indicated by the FFQ completed at the screening visit (study visit 1) was also similar between the two groups. The mean baseline n-3 FA intake in the FISH group was 0.7 ± 0.1 g/d and 0.4 ± 0.07

g/d in the CON group (p=0.137). 24-hour recalls performed at the second study visit (Day 0 for the 8-week intervention) showed that baseline caloric and EFA intake was also similar between the two groups (1664 ± 117 kcal/day for FISH and 1742 ± 137 kcal/day for CON, p=0.669).

Table 4. Baseline Characteristics of Participants[1,2]

	All (n=26)	FISH (n=13)	CON (n=13)	*P*-value
Age (y)	23.5 ± 1.1	24.0 ± 1.6	23.0 ± 1.7	0.339[3]
Weight (lb)	144.1 ± 4.4	140.7 ± 6.1	147.6 ± 6.5	0.424[4]
BMI (kg/m^2)	23.7 ± 0.6	23.2 ± 0.8	24.2 ± 0.9	0.424[4]
Body Fat Percent	29.2 ± 1.3	28.4 ± 2.0	30.1 ± 1.8	0.520[4]
Daily n-3 FA intake FFQ (g)	0.6 ± 0.08	0.7 ± 0.1	0.4 ± 0.07	0.137[3]
Linolenic Acid (g/d) 24h recall[5]	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	0.573[3]
Linoleic Acid (g/d) 24 h recall[5]	4.9 ± 0.9	4.8 ± 1.2	5.0 ± 1.4	0.778[3]
Daily energy intake (kcal/d) 24h recall[5]	1703 ± 89	1664 ± 117	1742 ± 137	0.669[4]

[1]FISH - fish oil group, 400mg/d EPA + 200mg/d DHA; CON - placebo group, 1000mg/d coconut oil; ALL – All subjects
[2]Values reported as means ± SE
[3]Data not normally distributed; analysis by Mann Whitney test
[4]Data normally distributed; analysis by independent t-test
[5]Data collected immediatley prior to the start of the intervention (study visit 2)

Changes in body weight, BMI, and percent body fat between week 8 and week 1 of the intervention were not strongly correlated with potential confounding variables, including baseline n-3 FA intake, consumption of non-steroidal anti-inflammatory drugs (NSAIDS), prescription medications, alcoholic or caffeinated beverages, and exercise per week (**Table 5**). Baseline energy intake as determined by 24-hour recalls at the start of week 1 correlated

significantly with both body weight and BMI (p=0.048 and p=0.031, respectively). However, the strength of these relationships was only moderately positive (r=0.391 for energy intake and body weight, and r=0.425 for energy intake and BMI), so they were not controlled for during data analysis.

Table 5. Potential Confounding Variables. Correlations between potential confounding variables and main outcome measures, n=26

Variable	Pearson correlation coefficient (r)	*P*-Value	Spearman's rho correlation coefficient (r)	*P*-Value
Age (years)				
Weight difference	-0.390	0.850	-0.129	0.531
BMI difference	-0.053	0.797	-0.148	0.472
Body fat percentage difference	0.081	0.695	0.099	0.630
Weight (lbs) at week 1				
Weight difference	0.086	0.675	-0.056	0.787
BMI difference	0.089	0.666	-0.017	0.935
Body fat percentage difference	-0.009	0.966	-0.020	0.923
Linolenic Acid Intake (g/d) at baseline (FFQ)				
Weight difference	-0.214	0.293	-0.290	0.151
BMI difference	-0.244	0.230	-0.335	0.094
Body fat percentage difference	-0.033	0.873	-0.086	0.675
Baseline Energy Intake (kcal/day, based on week 1 24-hour recall)				
Weight difference	0.391	0.048*	0.325	0.105
BMI difference	0.425	0.031*	0.393	0.047*
Body fat percentage difference	0.298	0.139	0.222	0.276
Change in Energy Intake (week 8 kcal – week 1 kcal, based on 24-hour recalls				
Weight difference	0.201	0.358	0.271	0.211
BMI difference	0.132	0.549	0.190	0.385
Body fat percentage difference	0.149	0.497	0.180	0.412
Capsule Compliance (% of total capsules taken)				
Weight difference	0.040	0.846	0.125	0.544

BMI difference	0.039	0.174	0.136	0.509
Body fat percentage difference	0.174	0.394	0.043	0.834
NSAIDS taken (total days consumed during study)				
Weight difference	0.077	0.708	0.069	0.736
BMI difference	0.068	0.742	0.068	0.741
Body fat percentage difference	0.023	0.911	0.076	0.711
Other prescription (total days consumed during study)				
Weight difference	-0.006	0.976	-0.074	0.718
BMI difference	0.027	0.897	-0.017	0.936
Body fat percentage difference	0.039	0.849	-0.054	0.794
Alcohol (total days consumed during study)				
Weight difference	0.020	0.921	-0.103	0.616
BMI difference	-0.023	0.912	-0.135	0.510
Body fat percentage difference	0.194	0.343	0.251	0.216
Caffeine (Ave. # beverages per day)				
Weight difference	0.176	0.391	0.089	0.664
BMI difference	-0.041	0.844	0.126	0.540
Body fat percentage difference	0.177	0.386	0.071	0.730
Strenuous exercise (Ave. # days/wk)				
Weight difference	0.091	0.657	0.070	0.733
BMI difference	0.056	0.784	0.013	0.949
Body fat percentage difference	0.057	0.782	0.183	0.372
Moderate exercise (Ave. # days/wk)				
Weight difference	-0.103	0.616	-0.211	0.300
BMI difference	-0.128	0.533	-0.185	0.366
Body fat percentage difference	-0.035	0.867	0.018	0.930
Mild exercise (Ave. # days/wk)				
Weight difference	-0.062	0.765	-0.082	0.691
BMI difference	-0.071	0.730	-0.054	0.794

Body fat percentage difference	0.052	0.801	0.033	0.873
Overall physical activity (METS-hours/wk)				
Weight difference	0.097	0.693	-0.006	0.981
BMI difference	0.111	0.652	0.004	0.989
Body fat percentage difference	0.101	0.681	0.183	0.453
*Statistically significant at the 0.05 level				

After 4 weeks of the intervention, univariate analysis did not demonstrate significant differences in body weight or composition measurements between the FISH and CON groups (**Table 6**). Subjects in the FISH group had gained an average of 0.68 ± 0.61 lbs, while the CON group had lost an average of 0.49 ± 0.59 lbs (p=0.180). Despite the weight loss demonstrated in the CON group, body fat percent increased by 0.015 ± 0.24% in the CON group and by 0.29 ± 0.50% in the FISH group. However, this change was not statistically significant (p=0.621).

Table 6. Mean Body Composition Differences Weeks 1-4 (difference = wk 4 - wk 0) [1,2]

	All (n=26)	FISH (n=13)	CON (n=13)	*P*-Value[3]	Effect Size[3]
Body Weight (lbs)	0.092 ± 0.43	0.68 ± 0.61	-0.49 ± 0.59	0.180	0.074
BMI (kg/m^2)	0.012 ± 0.073	0.092 ± 0.11	-0.069 ± 0.097	0.280	0.048
Body Fat Percent (%)	0.15 ± 0.27	0.29 ± 0.50	0.015 ± 0.24	0.621	0.010

[1]FISH - fish oil group, 400mg/d EPA + 200mg/d DHA; CON - placebo group, 1000mg/d coconut oil; ALL – all subjects
[2]Values reported as means ± SE
[3]P-value and effect size represent univariate analysis; all data are normally distributed; controlling for baseline energy intake did not alter results

Upon completion of the 8-week intervention, subjects in the FISH group had gained an average of 0.29 ± 0.45 lbs, while those in the CON group had

gained an average of 0.092 ± 0.67 lbs (***Figure2, Figure 3***). The difference in the change in weight between the two groups was not statistically significant (*p*=0.830). Both groups experienced nearly the same overall increase in BMI (0.023 ± 0.11 kg/m^2 in the FISH group and 0.023 ± 0.12 kg/m^2 in the CON group, p=1.00) (***Figure 2, Figure 4***). Body fat percentage also increased in both groups (0.48 ± 0.46% in FISH and 0.22 ± 0.18 in CON), but the difference in the change in body fat percentage between the two groups was not statistically significant (*p*=0.600) (***Figure 2, Figure 5,*** **and Table 7**). Because daily caloric intake has a direct effect on body weight and composition, analysis of the change in body weight, BMI, and body fat percentage was reassessed using multivariate analysis while controlling for the difference in energy intake between the beginning (week 1) and end (week 8) of the intervention. However, there were still no significant differences in body weight or composition changes between the FISH and CON groups (*p*=0.664 for change in body weight, *p*=0.545 for BMI, and *p*=0.508 for body fat percent).

Table 7. Mean Body Composition Differences Weeks 1-8 (difference = wk 8 - wk 1) [1,2]

	All (n=26)	FISH (n=13)	CON (n=13)	*P*-Value[3]	Effect Size[3]	*P*-Value[4]	Effect Size[4]
Body Weight (lbs)	0.19 ± 0.45	0.29 ± 0.63	0.092 ± 0.67	0.830	0.002	0.664	0.010
BMI (kg/m^2)	0.023 ± 0.078	0.023 ± 0.11	0.023 ± 0.12	1.00	0.000	0.545	0.019
Body Fat Percent (%)	0.35 ± 0.24	0.48 ± 0.46	0.22 ± 0.18	0.600	0.012	0.508	0.022

[1]FISH - fish oil group, 400mg/d EPA + 200mg/d DHA; CON - placebo group, 1000mg/d coconut oil; ALL – all subjects
[2]Values reported as means ± SE
[3]P-value and effect size represent univariate analysis; all data are normally distributed; controlling for baseline energy intake did not alter results
[4]P-value and effect size represent multivariate analysis with the change in energy intake (week 8 kcal/d – week 1 kcal/d) as covariate

Figure 2. Changes in main outcome measures throughout the course of the study in the FISH and CON groups.

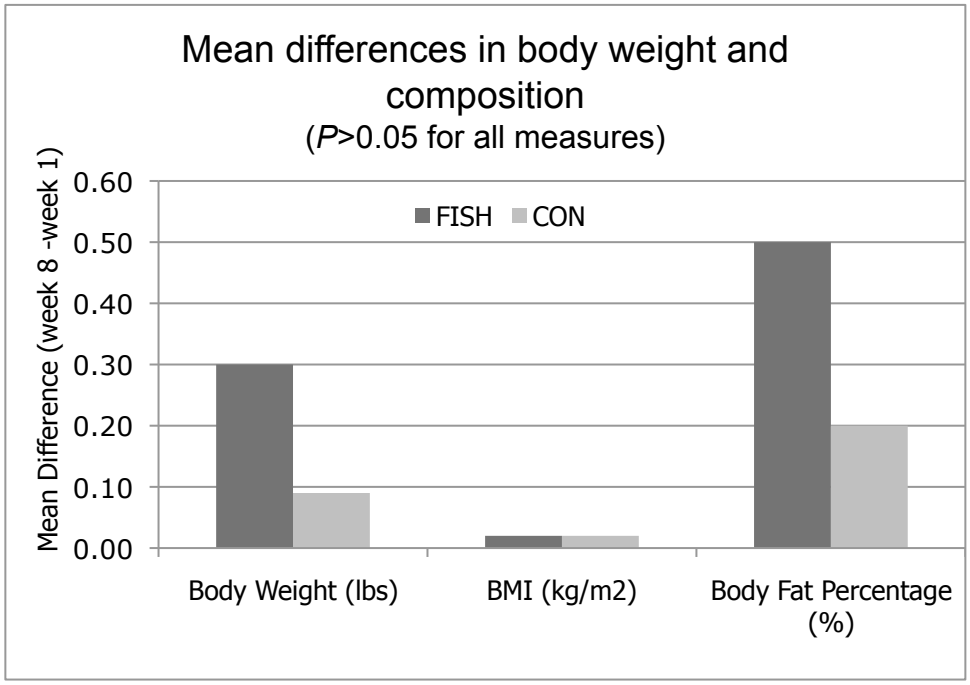


Figure 3. Change in body weight for FISH and CON groups at the beginning, middle, and end of the study.

Figure 4. Change in body mass index for FISH and CON groups at the beginning, middle, and end of the study.

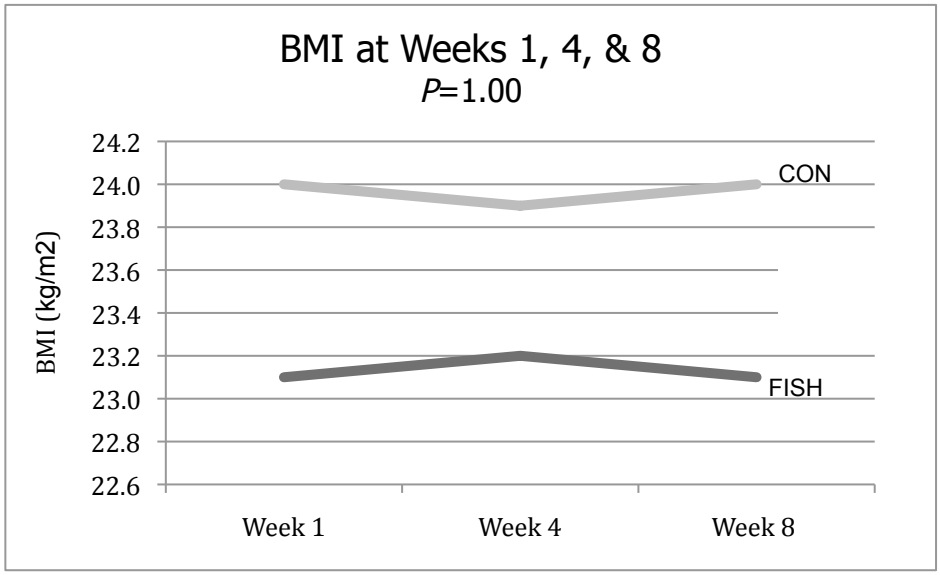
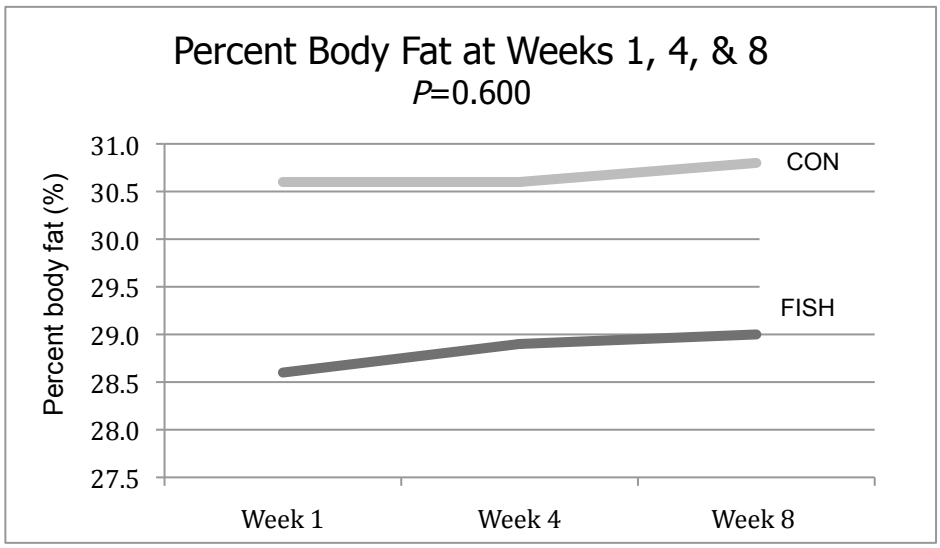


Figure 5. Change in percent body fat for FISH and CON groups at the beginning, middle, and end of the study.



24-hour dietary recalls were performed at weeks 1 and 8 and analyzed using Food Processor SQL ("Food Processor SQL," n.d.) in order to control for potential changes in caloric and EFA intake within and between each group

throughout the study (**Table 8**). The FISH group reported an 11% increased mean energy intake at week 8 as compared to week 1, while the mean caloric intake of the CON group decreased by approximately 13% over the course of the study (***Figure 6***). A boxplot displaying the differences in caloric intake between weeks 1 and 8 of the intervention is shown below (***Figure 7***). Although the changes in caloric intake differed between the FISH and CON groups, this trend did not achieve statistical significance when analyzed using repeated measures multivariate analysis of variance (p=0.069). These differences were analyzed again using week 1 body weight as a covariate, which caused the trend to further approach statistical significance (p=0.055). There were no statistically significant differences in linolenic or linoleic acid intake between the FISH and CON groups or within each group at weeks 1 and 8. Linolenic acid intake was also assessed using a validated FFQ, but no statistically significant differences between FISH and CON groups throughout the course of the study were observed.

Table 8. Dietary Data at Weeks 0, 1, and 8[1,2]						
	Week 0	Week 1	Week 8	*P*-Value, Repeated Measures	Effect Size[3]	*P*-Value, Mann-Whitney Nonpara-metric Test
Total energy intake (kcal), 24 hour recall						
FISH	ND	1664 ± 117	1853 ± 161	0.069[4]	0.131	N/A
CON	ND	1742 ± 137	1520 ± 123			
ALL	ND	1703 ± 89	1687 ± 105			
Linolenic acid intake (g/d), 24 hour recall						
FISH[5]	ND	0.48 ± 0.1	0.62 ± 0.2	N/A	0.058	0.975
CON[5]	ND	0.53 ± 0.2	0.48 ±0.1			
ALL	ND	0.50 ± 0.1	0.55 ± 0.1			
Linoleic acid intake (g/d), 24 hour recall						
FISH	ND	4.7 ± 1.2	5.09 ± 1.3	N/A	0.004	0.778
CON[5]	ND	4.9 ± 1.4	4.53 ± 1.1			
ALL	ND	5.2 ± 0.9	4.81 ± 0.8			
Linolenic acid intake (g/d), FFQ						
FISH[5]	0.68 ± 0.2	ND	0.58 ± 0.1	N/A	0.017	0.174
CON[5]	0.42 ± 0.1	ND	0.63 ± 0.2			
ALL	0.55 ± 0.1	ND	0.60 ± 0.1			

[1]FISH - fish oil group (n=13), 400mg/d EPA + 200mg/d DHA; CON - placebo group (n=13), 1000mg/d coconut oil; ALL – all subjects (n=26); ND – No data; N/A – Not applicable

[2]Values reported as means ± SE; values do not include additional 0.6 g/d fat provided by fish oil supplement for FISH subjects

[3]Effect size determined using repeated measures multivariate analysis of variance

[4]*P*=0.055 when controlling for body weight (lbs) at week 1

[5]Data are not normally distributed

Figure 6. Comparison of mean energy intake for FISH and CON groups as determined by 24-hour recalls performed at the beginning and end of the study.

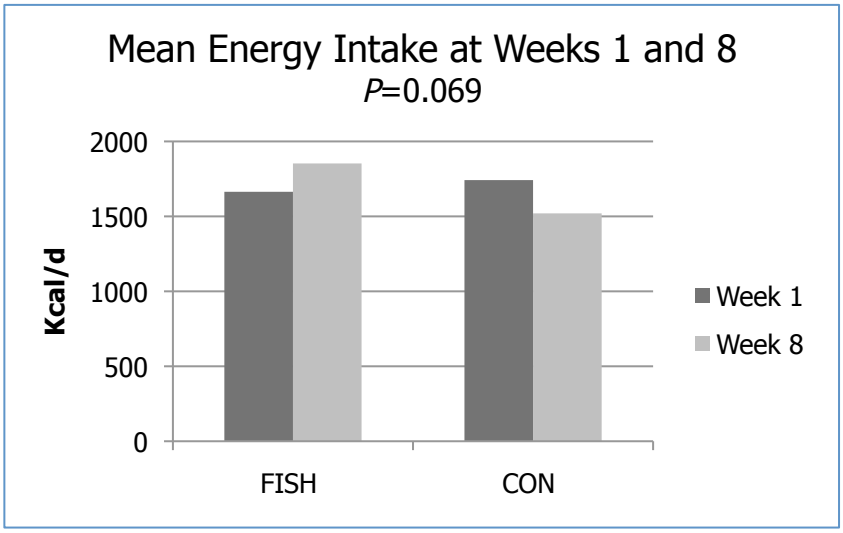
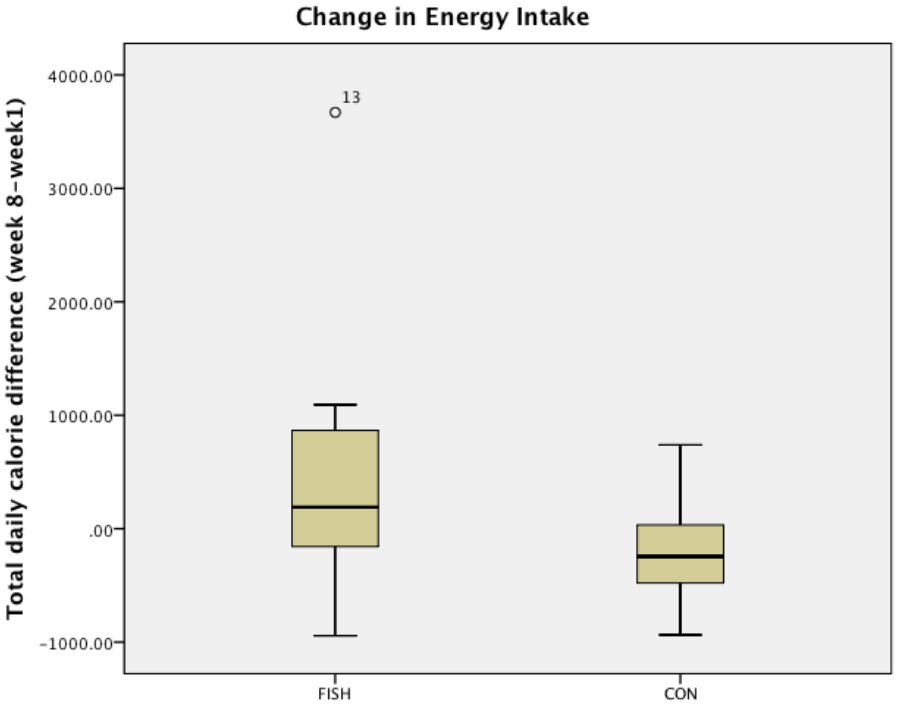


Figure 7. Boxplot displaying the change in energy intake for subjects in the FISH and CON groups as determined by 24-hour recalls performed at the beginning and end of the study.



Chapter 5

DISCUSSION

In this double blind, randomized, placebo-controlled trial, 8 weeks of n-3 FA supplementation (600 mg EPA + DHA per day) did not change body weight, BMI, or body fat percentage in young healthy women as compared to a placebo (1000 mg coconut oil per day). These findings do not refute the current recommendations for Americans to consume at least 8 oz of fish per week, supplying 250 mg EPA and DHA per day (Flock & Kris-Etherton, 2011).

Although not statistically significant, 24-hour dietary recalls performed at the beginning and end of the intervention revealed a trend towards increased daily caloric intake in the FISH group and decreased daily energy intake in the CON group throughout the course of the study (p=0.069). This trend approached significance when week 1 body weight was controlled for (p=0.055). If maintained, this difference in caloric intake (an average of approximately 150-200 kcal/d) could lead to changes in body weight over time.

Interestingly, other research has looked into the potential for n-3 FAs to alter caloric intake. Previous studies in our lab showed that subjects consuming fish as the sole source of flesh food weighed more and had higher caloric intake as compared to those consuming a vegetarian or regular diet. Some studies have observed a link between n-3 FA intake and levels of appetite-regulating hormones. For instance, leptin is an anorexigenic adipokine, and its synthesis is positively correlated with adipocyte size. Thus, leptin helps to achieve energy homeostasis by decreasing appetite when excess adipose tissue is present (Cave

et al., 2008). Drevon, et al demonstrated a decrease in leptin production after providing rats with high levels of n-3 FA. Investigators also observed an increase in lipid oxidation in rats fed high levels of n-3 FAs, and speculated that increased lipid oxidation may have led to reduced leptin concentration (Drevon, 2005). In an *in vitro* study, cells cultured with DHA and EPA demonstrated decreased leptin mRNA expression. This decrease would theoretically lead to a decline in satiety and increase in energy intake (Drevon, 2005). However, the levels of n-3 FAs provided by these studies are not physiologically relevant for humans, as they would require intake much higher than current recommendations.

Research has also observed a potential link between EPA and DHA intake and ghrelin levels. Ghrelin is an appetite-stimulating hormone produced in the stomach, and appears to occur in lower levels in obese as compared to lean populations. Various factors are believed to influence ghrelin expression, including dietary fat intake. Some evidence suggests low dietary fat intake during periods of fasting leads to decreased ghrelin expression, which translates to a decline in appetite (Ramel, Parra, Martinéz, Kiely, & Thorsdottir, 2009). Thus, it is interesting to think that increased fat intake may lead to increased ghrelin production. In an 8-week randomized, placebo-controlled, parallel-arm trial, 324 overweight adult men and women were placed on calorie-restricted diets with either no seafood (control, 0 g/d n-3 FAs), three servings of lean white fish per week (providing 0.26 g/d n-3 FAs), three servings of oily fish per week (providing 2.1 g/d n-3 FAs), or daily fish oil capsules (providing 1.3 g/d n-3 FAs). Although all groups lost weight, only women in the fish oil supplementation group demonstrated higher ghrelin levels at the end of the intervention. This effect was

not seen in men (Ramel et al., 2009). Moreover, significant differences in weight loss as a result of EPA and DHA consumption were observed only males in the study, while no difference in weight loss was observed in females (Thorsdottir et al., 2007). Although not clear from this study, it is possible that the increased ghrelin observed in the women taking fish oil may have been the reason no significant differences in weight loss were observed. Although the increased ghrelin levels observed in women could have been related to calorie restriction and weight loss during the intervention, it is interesting to note that these effects were not observed in men, who experienced even more weight loss. Moreover, there were no statistically significant differences in weight loss between women in the 4 intervention groups, so it would not be logical that their ghrelin levels would be significantly different.

More research is needed to identify the potential for n-3 FAs to modulate appetite-regulating hormones. The present study protocol only used two 24-hour recalls to characterize caloric intake, so it is difficult to accurately depict trends in energy intake, hunger, and satiety throughout the study. Because there is no gold standard for dietary analysis, it is difficult to draw definitive conclusions about appetite and caloric intake from dietary recalls alone. Furthermore, the validity and reliability of 24-hour recalls is limited by the memory of the participant and the ability of the participant to estimate portion sizes. 24-hour recalls also assume that the previous day accurately characterizes typical caloric and nutrient intake, which may lead to flawed generalizations (Thompson, Subar, Loria, Reedy, & Baranowski, 2010).

Mean caloric intake for the CON group also decreased by nearly 200 kcal/d throughout the course of the study. This was not anticipated, as coconut oil does not supply EFAs and consists primarily of medium chain triglycerides and saturated fatty acids. Thus, 1 g of fat from coconut oil should be a trivial amount, and should not be an effective dose. Moreover, typical daily fat intake for females aged 20-39 years is approximately 67g (US Department of Health and Human Services, 2012).

Interestingly, several studies have observed decreased caloric intake as a result of coconut oil supplementation. For instance, Assunção, et al., found that coconut oil may decrease carbohydrate intake by increasing insulin secretion. However, these effects were observed only when 30mL (approximately 28g) of dietary fats were replaced with coconut oil (Assunção, Ferreira, dos Santos, Cabral, & Florêncio, 2009). The present study provided about 3.5% of the dose provided by Assunção and colleagues. In a 12-week intervention, Han, et al., randomly assigned 40 type 2 diabetic Chinese adults to replace a portion of their dietary fats with either medium chain triglycerides (MCT) from coconut oil (18 g/d) or long chain triglycerides (LCT). MCT consumption was linked to decreased total caloric intake and body weight in men and women (Han et al., 2007). Once again, however, this dose was considerably higher than the one provided by the present study protocol.

In order to obtain more accurate results in future studies, blood concentrations of the appetite-modulating hormones leptin and ghrelin could be tracked. The use of several methods of dietary assessment, such as food records, or more frequent 24 hour recalls, would provide a more accurate

perception of caloric intake as related to fish oil supplementation. Participants could also be given questionnaires to identify perceived changes in satiety, hunger, or food cravings to estimate the effects of these changes in hormone levels, if any.

The direction of weight and BMI change for both the FISH and CON groups changed at week 4 (***Figures 3, 4, and 5***). It is not clear why these changes were observed, as average capsule compliance remained above 90% in both groups for both the first and second halves of the study (**Table 3**). It is possible that the metabolic response to n-3 FA supplementation changed as plasma levels of n-3 FAs increased and leveled off. Arterburn, et al., showed that plasma DHA concentrations equilibrated after 4 weeks of supplementation, which is the same amount of time subjects in this study had been supplementing with n-3 FAs before the change in trend direction was observed. However, this saturation point occurred in the Arterburn study after 1.2 g/d n-3 FA supplementation, which is twice the amount of n-3 FAs provided by this study's protocol (Arterburn et al., 2006).

Another possibility is that dietary n-3 or n-6 FA intake changed at week 4, leading to a consequential change in LA-derived eicosanoid production (depending on the ratio of n-6 to n-3 FAs in cellular plasma membranes) at the mid-point of this study. Changes in dietary intake could have off-set the effects of the supplement, potentially modulating appetite, thermogenesis, or lipid homeostasis, and leading to the difference in weight changes observed between the first and second halves of the study. However, this study design only

collected information on n-3 and n-6 FA intake at the beginning and end of the study, so it is difficult to determine if changes occurred at the mid-point.

In future studies, it would be beneficial to track plasma EPA, DHA, and AA levels at multiple points throughout the study to ensure plasma n-3 FA levels are stable. This could also reveal potential changes in outcome measures once saturation is reached in cell plasma membranes.

It is important to note that subjects in this study were young (23.5 ± 1.1 years) women with a healthy BMI (23.7 ± 0.6 kg/m^2) and no prior health conditions. It is therefore difficult to generalize these results to other populations, such as those with some level of chronic inflammation, older subjects, or overweight and obese populations. It is also not possible to generalize these results to male populations. This is particularly true since women tend to metabolize ALA (a source of n-3 FAs) more efficiently than men, meaning young males may respond differently to the same level of n-3 FA supplementation (Arterburn et al., 2006).

There are a number of reasons why significantly different changes in body weight may not have been observed between FISH and CON groups. Because the women used in this trial were healthy and non-obese (BMI = 23.7 ± 0.6 kg/m^2), baseline inflammation might not have been extreme enough to be manipulated by n-3 FA supplementation. A majority of studies observing significant results studied obese or chronically inflamed populations (Hill et al., 2007; Krebs et al., 2006; Kunesová et al., 2006; Moore et al., 2006; Warner et al., 1989). Thus, there may have been a more pronounced effect of n-3 FAs on suppressing inflammation in these previous studies.

Moreover, it is possible that n-3 FA supplementation may have had multiple contradictory effects on metabolism and appetite, leading to no significant changes overall. Previous studies have hypothesized a number of mechanisms by which n-3 FAs may modulate weight and body composition in a positive or negative direction. These mechanisms include increased lipid oxidation, upregulation of UCP1 to increase thermogenesis, decreased resting metabolic rate due to inflammation suppression, or increased appetite due to increased ghrelin production and decreased leptin production. It is unclear under which conditions and to what degree these metabolic changes occur, so it is possible that multiple changes could have led to an insignificant net weight change.

It is also possible that the n-3 FA supplements altered body composition but not overall body weight. This was seen in the Couet, et al. study, which observed no significant change in body weight but a decrease in body fat accretion when subjects ingested n-3 FAs (Couet et al., 1997). Percent body fat was measured in the present study at weeks 1, 4, and 8 using bioelectrical impedance analysis (BIA), and revealed no significant changes between weeks 1 and 8. BIA is a relatively inexpensive, convenient, and noninvasive method for measuring body fatness, but the precision and validity of these results can be limited by individual variations in body shape and hydration status (Kyle et al., 2004). Dual energy X-ray absortiometry (DXA), which was used in the Couet, et al. study, is generally revered as the gold standard for body composition measurement. However, this method is costly and requires trained personnel. A combination of multiple measures of body composition, such as skin fold tests,

waist circumference measurements, and BIA might have been more effective in detecting body composition changes.

Finally, the amount of n-3 FA provided in this study (600 g/d) was relatively low. Other studies have demonstrated significant changes in body weight after supplementation of more than 2-3 g/d fish oils (Couet et al., 1997; Kunesová et al., 2006) and studies in rodents have used even higher doses (Flachs et al., 2006). It is possible that a subtherapeutic dose, such as the one provided in this study, may be ineffective in altering body weight. However, therapeutic doses seem impractical, as they exceed recommendations from the American Heart Association and USDA. They also likely require administration of multiple pills each day, or consumption of several servings of oily fish per day. For some consumers, this could be unrealistic. Furthermore, therapeutic doses of fish oils could lead to unwanted side-effects such as prolonged bleeding time (Iso, 2001) or gastrointestinal symptoms (National Center for Complementary and Alternative Medicine, 2009).

Limitations. This trial has several limitations. The 8-week intervention was relatively short compared to other studies, especially since weight can fluctuate substantially due to factors such as hydration. The study protocol also did not include testing participants' baseline EFA levels. If the women already had adequate n-3 FA and cells were at saturation, it is possible that supplementation may not have had any profound effects. Additionally, the potential metabolic changes that could incur with decreased inflammation may result in subtle changes in energy balance or adiposity that would not be evident in just a few weeks. A longer intervention may make these subtle changes more apparent.

Despite the goal to enroll 40 females in the trial, only 26 completed the study due to scheduling and recruitment restrictions, which may have made it difficult to observe significant changes.

Strengths. Participants were screened for a number of exclusion criteria prior to enrollment in the study. These criteria included frequent fish consumption, regular smoking, BMI of less than 18.5 or more than 30, and regular intake of omega-3 supplements and/or prescription medications that could have interfered with body weight or inflammatory state (such as corticosteroids or non-steroidal anti-inflammatory drugs). These exclusion criteria helped to ensure subjects were relatively similar upon enrollment in the study, and minimized the potential for prior conditions or lifestyles to interfere with the study protocol. Since physical activity was monitored weekly with the Godin Leisure-Time Exercise questionnaire, change in physical activity was eliminated as a potential confounding variable. Furthermore, the double-blind nature of this trial minimized bias by preventing investigators and participants from knowing what treatment they were receiving.

Conclusion. This randomized controlled trial suggests that daily supplementation of n-3 FA (600 mg/d EPA + DHA) for 8 weeks does not lead to changes in body weight, BMI, or percent body fat in young healthy women as compared to placebo. These results do not refute the recommendation for Americans to increase weekly seafood consumption to approximately 8 oz fish per week. However, further research is needed to investigate the effects of n-3 FAs on appetite, as well as the effects of prolonged supplementation on body weight and body composition.

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APPENDIX A

IRB APPROVAL

ASU ARIZONA STATE UNIVERSITY
Knowledge Enterprise Development

Office of Research Integrity and Assurance

To: Carol Johnston

From: Shannon Ringenbach, Biosci IRB

Date: 01/04/2012

Committee Action: Expedited Approval

Approval Date: 01/04/2012

Review Type: Expedited F2 F7

IRB Protocol #: 1112007220

Study Title: Omega 3 Fatty Acid Supplements and Health

Expiration Date: 01/03/2013

The above-referenced protocol was approved following expedited review by the Institutional Review Board. It is the Principal Investigator's responsibility to obtain review and continued approval before the expiration date. You may not continue any research activity beyond the expiration date without approval by the Institutional Review Board.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.
Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX B

INTERNET SURVEY

*1. Please provide your email address

*2. Please select your gender

- Male
- Female

*3. Please enter your height and weight

Height (inches)	
Weight (pounds)	

*4. Are you a vegetarian and exclude all meat, fish, and poultry from your diet?

- Yes
- No

*5. Do you consume any of the following foods more than once per week? (Please check yes or no):

	Yes	No
Eggs		
Dairy		
Fish		
Beef		
Flax seed or flax oils		
Soy products (e.g., milk, tofu, etc.)		

*6. Do you take any dietary supplements?

- Yes
- No

If yes (please specify)

*7. Please check any of the following foods that you are allergic to (check all that apply):

- Soy
- Fish
- Milk
- Eggs
- Tree nuts and/or peanuts
- None of the above

*8. Are you pregnant, lactating, or do you anticipate becoming pregnant?

- Yes
- No

*9. If you smoke, please select how many cigarettes you smoke per day

- 0
- 1-5
- 6-10
- >10

*10. Do you participate in vigorous, highly intense exercise more than 5 times per week?

- Yes
- No

*11. Do you take any of the following medications: e.g. beta-blockers, ACE inhibitors, diphenhydramine or cyproheptadine (allergy medications), lithium carbonate, corticosteroids, insulin, thiazolidinediones (Actos, Avandia, or Avandamet), sulfonylureas, incretins, sodium valproate, or thyroid replacement therapy?

- Yes
- No

*12. Will you be able to maintain your current diet and physical activity for a consecutive 8 weeks?

- Yes
- No

*13. Are you willing and able to travel to the ASU Downtown Campus to meet with research investigators on four separate occasions?

- Yes
- No

Done

APPENDIX C

INFORMED CONSENT

CONSENT FORM
Nutrient Supplementation and Health Parameters

INTRODUCTION
The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS
Dr. Carol Johnston, Director of the Nutrition Program at Arizona State University, as well as Nutrition graduate students, Megan Gutierrez and Bianca Teran, have invited your participation in a research study.

STUDY PURPOSE
The purpose of the research is to examine the effect of nutrient supplementation in young college females, 18-40 years old, on immune function and overall health.

DESCRIPTION OF RESEARCH STUDY
If you decide to participate, then as a study participant you will join a study to evaluate the effect of ingestion of a supplement daily for 8 weeks on health markers. You will be instructed to complete a one-page questionnaire daily regarding illness. If you are interested in joining the study, you will be asked to come to an initial screening where your height and weight will be measured and you will complete a health history and diet quality questionnaires. If you are eligible for the study, you and the other participants will be randomly assigned in either the control (placebo) or experimental (nutrient supplement) group. Subjects will be asked to visit the research site on 3 occasions at 0, 4, and 8 weeks. At weeks 0, 4, and 8 you will be weighed. At weeks 0 and 8 a fasting blood sample will be drawn. At each blood sampling approximately 4 tablespoons of blood will be collected. At weeks 4 and 8 you will complete a diet quality questionnaire and you will need to bring in your daily survey booklet with your supplement pack.

If you say YES, then your participation will last for 8 weeks at the Downtown Phoenix campus at Arizona State University. Approximately 40 subjects will be participating in this study locally.

RISKS
There may be a slight chance of gastrointestinal distress when taking the supplement on an empty stomach. This risk is reduced if you ingest the supplement with a meal and consume plenty of water. Blood draws may cause light-headedness or temporary bruising. A nurse or trained phlebotomist will be performing the blood draws.

BENEFITS
Although there may be no direct benefits to you, the possible benefit of your participation is that you will be able to experience what it is like to be a part of a research study that may provide new evidence to support the health of many college women.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but the researchers will not identify you. Your name will not be associated with any data related to the study. In order to maintain confidentiality of your records, you will be assigned to a subject
number by Dr. Carol Johnston, which will be used throughout the course of the study to identify you. Only Dr. Johnston will have access to subject names and their corresponding codes.

WITHDRAWAL PRIVILEGE

It is ok for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

COSTS AND PAYMENTS

The researchers want your decision about participating in the study to be absolutely voluntary, yet they recognize that your participation may pose some costs such as inconvenience and a small time commitment. In order to help defray your costs, you will receive a $10 Target gift card at week 4 and a $15 Target gift card at week 8 visits for a total of $25.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, Principal Investigator and Professor of Nutrition at ASU (480-727-1713), Megan Gutierrez, Graduate Student (440-452-5142), or Bianca Teran, Graduate Student (520-370-2441).

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given (offered) to you.

Your signature below indicates that you consent to participate in the above study.

_____	_____	_____
Subject's Signature	Printed Name	Date

Preferred contact: phone and/or email:

_____

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of Investigator_____

Date_____

APPENDIX D

MEDICAL HISTORY QUESTIONNAIRE

HEALTH /HISTORY QUESTIONNAIRE ID#_____

1. (To be completed by researchers): Height _____ Weight _____

   Percent body fat_____ BMI_____

2. Age: _____

3. Have you lost or gained more than 5 lbs in the last 12 months? Yes No

   If yes, how much lost or gained? _____ How long ago? _____

4. College Status (please circle) Fresh. Soph. Jr. Sr. Grad.

5. Ethnicity: (please circle) Native American African-American Caucasian Hispanic Asian Other

6. Do you smoke? No, never _____

   Yes _____ # Cigarettes per day = _____

   I used to, but I quit _____ months/years (circle) ago

7. Do you take any medications regularly? Yes No *If yes, list type and frequency:*

Medication	Dosage	Frequency

8. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No *If yes, list type and frequency:*

Supplement	Dosage	Frequency

9. Have you ever been hospitalized? _____ If yes, for what? _____

10. Please ANSWER (YES/NO) if **you currently have** or if **you have ever** been diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			Chest Pain		
High Blood Pressure			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorders			Emotional Problems		

Please elaborate on any condition listed above._____
_____

11. How would you rate your lifestyle?

Not active _____ Active _____
Somewhat active _____ Very Active _____

12. Please circle the total time you spend in each category for an average **week.**

Light activities such as:
Slow walking, golf, slow cycling, doubles tennis, easy swimming, gardening
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as:
Mod. Walking, mod. cycling, singles tennis, mod. swimming, mod. weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as:
Fast walking/jogging, fast cycling, court sports, fast swimming, heavy/intense weight lifting
Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

13. How much alcohol do you drink? (average drinks per day) _____

14. Do you have any food allergies? Yes No If yes, explain:_____

15. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Yes No

If yes, explain: _____

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE

Food Frequency Questionnaire

This form asks about your usual dietary intake over the past month. Read each food item. If you have not eaten this food in the past month, mark "none" and move onto the next food item. Indicate whether you think your usual serving size is small (S), medium (M), or large (L) by marking the correct serving size box. Think over the past month. How often do you usually eat each of the following food items? Answer each question as best you can; estimate if you are not sure. **NOTE: A small (S) serving is equal to half (½) the usual serving. A medium (M) is equal to the medium servings listed on the form. A large (L) is equal to one and a half (1 ½) times as much or more of the medium serving.**

Seafood & Fish	Medium Serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Tuna	3 ounces											
Salmon	3 ounces											
Whitefish	3 ounces											
Herring	3 ounces											
Walleye	3 ounces											
Lake trout	3 ounces											
Rainbow trout	3 ounces											
Sablefish	3 ounces											
Mackerel	3 ounces											
Catfish	3 ounces											
Flounder	3 ounces											
Perch	3 ounces											
Atlantic cod	3 ounces											
Atlantic bluefish	3 ounces											
Atlantic sturgeon	3 ounces											
Halibut	3 ounces											
Swordfish	3 ounces											
Mussels	3 ounces											
Scallops	3 ounces											
Oysters	3 ounces											
Shrimp	3 ounces											
Sardines	3 ounces											
Anchovy	3 ounces											
Blue crab	3 ounces											
Northern lobster	3 ounces											

3 ounces is about the size of a deck of cards.

Meat	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Turkey	3 ounces											
Chicken	3 ounces											
Beef	3 ounces											
Pork	3 ounces											

*** 3 ounces is about the size of a deck of cards.**

Eggs	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Regular egg	1 egg											
Omega-3 enriched egg	1 egg											
Eggland's Best egg	1 egg											

Dairy products	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
2% milk	1 cup											
1% milk	1 cup											
Skim milk	1 cup											
Cheddar cheese	¼ cup											
Swiss Cheese	1 ounce											
Mozzarella Cheese	1 ounce											
2% fat cottage cheese	½ cup											
1% fat cottage cheese	½ cup											
Feta cheese	1 ounce											
Yogurt- no fat or low fat	8 ounces											

Nuts/seeds	Medium serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Walnuts	1 ounce											
Pumpkin seeds	1 ounce											
Flaxseeds	1 ounce											
Butternuts	1 ounce											
Cashews	1 ounce											
Hickory nuts	1 ounce											
Beechnuts	1 ounce											
Almonds	1 ounce											
Pistachios	1 ounce											
Pine nuts	1 ounce											
Pecans	1 ounce											
Brazilnuts	1 ounce											
Sunflower seeds	1 ounce											
Sesame seeds	1 Tbsp											
Poppy seeds	1 Tbsp											

Fats and oils	Medium Serving	None	S	M	L	Once a month	Less than once a week	1-2 times a week	3-4 times a week	5-6 times a week	Daily	More than once a day
Miracle whip	1 tsp											
Margarine	1 tsp											
Soybean oil	1 tsp											
Sunflower oil	1 tsp											
Flax oil	1 tsp											
Canola oil	1 tsp											
Olive Oil	1 tsp											
Walnut oil	1 tsp											

APPENDIX F

POWER ANALYSIS

Investigators	Study Design	Total Number of Subjects Used	Standard Deviation	Change in Body Weight (kg)	Calculated Sample Size
Basu, et al.	Parallel	35	0.7	2.5	6
Thom, E	Parallel	40	2.0	3.5	14
Couet, et al.	Crossover	6	0.62	0.7	28
Blankson, et al.	Parallel	60	2.0	0.8	200
Average		**35**	**1.3**	**1.9**	**62**

APPENDIX G

CAPSULE CALENDAR

Please check each day capsule is consumed:

January 2012

Sun	Mon	Tue	Wed	Thu	Fri	Sat
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

February 2012

Sun	Mon	Tue	Wed	Thu	Fri	Sat
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29			

March 2012

Sun	Mon	Tue	Wed	Thu	Fri	Sat
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31

INSTRUCTIONS

1. Check off each day indicating that the capsule was taken. Please take one capsule each morning, preferably with food.
2. Arrive to lab at scheduled times for testing; at weeks 4 and 8 bring booklet and remaining capsules.

Contact the researchers if you have any questions at:

Dr. Carol Johnston: Carol.Johnston@asu.edu, 602-827-2265
Bianca Teran: bmteran@asu.edu, 520-370-2441
Megan Gutierrez: mgutie15@asu.edu, 440-452-5142

APPENDIX H

PHYSICAL ACTIVITY LOG

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times Per Week:
a) STRENUOUS EXERCISE **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
b) MODERATE EXERCISE **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
c) MILD EXERCISE **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	**SOMETIMES**	**NEVER/RARELY**
1.	2.	3.

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).

	Times Per Week:
a) STRENUOUS EXERCISE **(HEART BEATS RAPIDLY)** (e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)	_____
b) MODERATE EXERCISE **(NOT EXHAUSTING)** (e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____
c) MILD EXERCISE **(MINIMAL EFFORT)** (e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)	_____

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN	**SOMETIMES**	**NEVER/RARELY**
1.	2.	3.

APPENDIX I

COLD SYMPTOM SURVEY

Day:	*Date:*	*Time* (To be completed at or near bedtime)	*ID:*

Please fill in one circle for each of the following items:

	Not sick	Very mildly		Mildly		Moderately		Severely
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
In terms of respiratory tract illness only, how sick do you feel **today**?	O	O	O	O	O	O	O	O

Please rate the **average severity of your cold symptoms over the last 24 hours** for each symptom:

	Do not have this symptom	Very mild		Mild		Moderate		Severe
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Runny nose	O	O	O	O	O	O	O	O
Plugged nose	O	O	O	O	O	O	O	O
Sneezing	O	O	O	O	O	O	O	O
Sore throat	O	O	O	O	O	O	O	O
Scratchy throat	O	O	O	O	O	O	O	O
Cough	O	O	O	O	O	O	O	O
Hoarseness	O	O	O	O	O	O	O	O
Head congestion	O	O	O	O	O	O	O	O
Chest congestion	O	O	O	O	O	O	O	O
Feeling tired	O	O	O	O	O	O	O	O

Over the last 24 hours, how much has your cold interfered with your ability to:

	Not at all	Very mildly		Mildly		Moderately		Severely
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Think clearly	O	O	O	O	O	O	O	O
Sleep well	O	O	O	O	O	O	O	O
Breathe easily	O	O	O	O	O	O	O	O
Walk, climb stairs, exercise	O	O	O	O	O	O	O	O
Accomplish daily activities	O	O	O	O	O	O	O	O
Work outside the home	O	O	O	O	O	O	O	O
Work inside the home	O	O	O	O	O	O	O	O
Interact with others	O	O	O	O	O	O	O	O
Live your personal life	O	O	O	O	O	O	O	O

Compared to yesterday, I feel that my cold is: [If you did not have cold symptoms yesterday, please leave blank.]

Very much better	Somewhat better	A little better	The same	A little worse	Somewhat worse	Very much worse
O	O	O	O	O	O	O

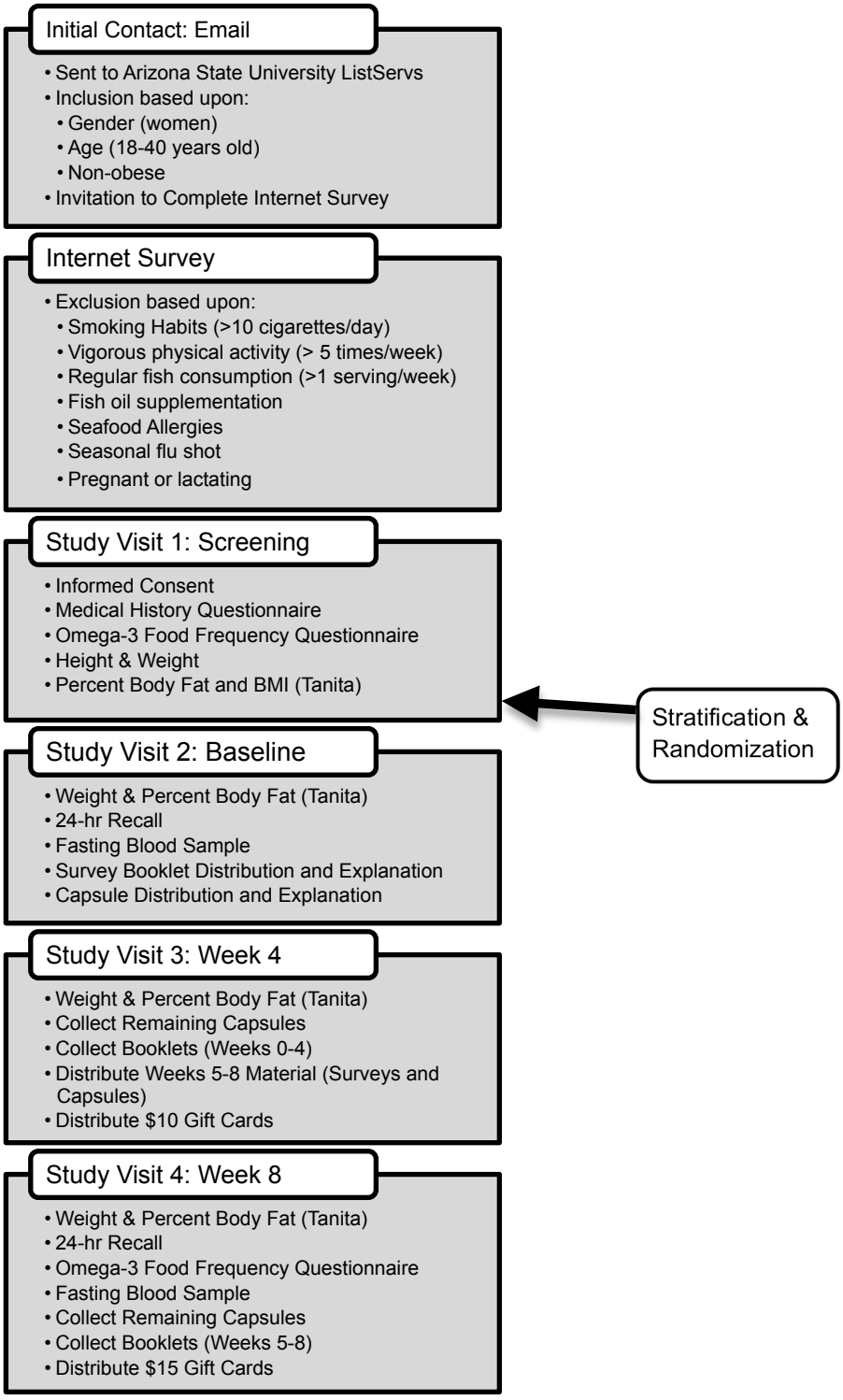
Please list any products (including prescription or over-the-counter medicines, herbal preparations or supplements, and/or lozenges) taken to relieve respiratory symptoms.

Product name:	Dosage:	Time(s) taken:

Did you smoke any cigarettes today? **Yes** _____ **No** _____
Did you drink alcohol today? **Yes** _____ **No** _____
How many caffeinated beverages did you consume today? _____

APPENDIX J

METHODOLOGY TIMELINE


Initial Contact: Email
• Sent to Arizona State University ListServs
• Inclusion based upon:
• Gender (women)
• Age (18-40 years old)
• Non-obese
• Invitation to Complete Internet Survey
Internet Survey
• Exclusion based upon:
• Smoking Habits (>10 cigarettes/day)
• Vigorous physical activity (> 5 times/week)
• Regular fish consumption (>1 serving/week)
• Fish oil supplementation
• Seafood Allergies
• Seasonal flu shot
• Pregnant or lactating
Study Visit 1: Screening
• Informed Consent
• Medical History Questionnaire
• Omega-3 Food Frequency Questionnaire
• Height & Weight
• Percent Body Fat and BMI (Tanita)
Stratification & Randomization
Study Visit 2: Baseline
• Weight & Percent Body Fat (Tanita)
• 24-hr Recall
• Fasting Blood Sample
• Survey Booklet Distribution and Explanation
• Capsule Distribution and Explanation
Study Visit 3: Week 4
• Weight & Percent Body Fat (Tanita)
• Collect Remaining Capsules
• Collect Booklets (Weeks 0-4)
• Distribute Weeks 5-8 Material (Surveys and Capsules)
• Distribute $10 Gift Cards
Study Visit 4: Week 8
• Weight & Percent Body Fat (Tanita)
• 24-hr Recall
• Omega-3 Food Frequency Questionnaire
• Fasting Blood Sample
• Collect Remaining Capsules
• Collect Booklets (Weeks 5-8)
• Distribute $15 Gift Cards