DIETARY ASSESSMENT OF DOCOSAHEXAENOIC ACID (DHA) INTAKE IN PREGNANT WOMEN OF SOUTHWEST MONTANA

by

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ABSTRACT

Docosahexaenoic acid (DHA; 22:6n-3) is imperative for prenatal development and is found primarily in the flesh of marine life. Previous research has indicated that pregnant women do not meet current DHA recommendations of 200 mg per day. Much of the research has been conducted in coastal communities with greater access to marine sources and may not reflect non-coastal communities. PURPOSE: The purpose of this study was to describe maternal DHA intake in pregnant women from Southwest Montana and to determine changes in dietary DHA intake over time. METHODS: Thirty-nine participants were asked to complete a non-consecutive 3-day diet record and a DHA focused food frequency questionnaire (FFQ) at weeks 18, 28 and 35 (± 1 week). Maternal fasted plasma and red blood cell samples were obtained via venipuncture during the same data collection periods. Fetal cord blood samples were collected at delivery. Due to time restrictions, all blood lipid parameters were undetermined. After delivery, participants completed a post-partum survey. RESULTS: Participants' overall mean (MEAN ± *SD* (min. – max.)) DHA intake was 248 ± 321 mg/d (8 – 1,836 mg/d). According to those who reported using DHA supplements in their FFQ interviews at weeks 18 (n = 12), 28 (n = 13) and 35 (n = 11), the mean DHA intake was 272 ± 380 mg/d (147 – 1,490 mg/d). The mean DHA intake among non-supplement users at weeks 18 (n = 22), 28 (n = 20) and 35 (n = 21) was 147 ± 139 mg/d (8 – 555 mg/d). Dietary DHA intake from weeks 18, 28 and 35 did not differ significantly (F (2, 62) = 0.220, p = 0.803). CONCLUSION: Dietary DHA intake did not differ over time. The mean dietary DHA intake for non-supplement users was below the current prenatal recommendation. Therefore, pregnant women in Southwest Montana are not meeting current DHA recommendations through dietary means alone and should consider DHA supplementation as a method to meet fetal and maternal DHA needs during pregnancy

CHAPTER 1

INTRODUCTION

Adequate maternal nutrition during pregnancy is imperative for proper fetal development. Dietary fats, especially poly unsaturated fatty acids (PUFAs), are essential in maintaining fetal membrane fluidity and permeability. The PUFA, docosahexaenoic acid (DHA; 22:6n-3), is quickly integrated into fetal retinal and brain lipids, especially during the third trimester of pregnancy, and is associated with fetal neurological development (Innis, 2003). Inadequate maternal DHA intake throughout pregnancy and decreased DHA concentrations *in utero* have been shown to impair neurogenesis and visual acuity post-partum (Innis, 2003; Innis & Friesen, 2008).

The role of PUFAs during pregnancy is currently under investigation. Docosahexaenoic acid is the most abundant omega-3 (n-3) fatty acid (FA) in the mammalian brain and selectively accumulates during fetal brain development (Innis, 2000, 2007a). Research suggests that DHA functions to influence neurogenesis, neurotransmission, and protection against oxidative stress (Innis, 2007a). *In-utero*, lipid-bound DHA may play a role in the development of the lipid-bilayer, providing flexibility and direct interaction with membrane proteins and impacting the speed of signal transduction and neurotransmission (Innis & Elias, 2003). Unesterified DHA influences gene expression, ion channel activities, and provides neuro-protective metabolites in the brain (Innis & Elias, 2003). Intervention studies (Innis & Elias, 2003; Innis & Friesen, 2008) suggest

an adequate maternal intake of dietary DHA will positively influence development *in utero* and post-partum.

Mammalian cells do not have the enzymes needed to insert a double bond at the n-3 position of a FA. Therefore, all of the n-3 PUFAs accumulated in fetal tissue must originate from the maternal diet (Innis & Elias, 2003). Alpha-linolenic acid (ALA; 18:3n-3) is an example of an essential fatty acid (EFA), which must come from outside dietary sources. In the liver, ALA can be converted into long-chain PUFAs (LCPUFAs), such as DHA, but the efficiency of ALA conversions and the amount of DHA produced from ALA is unclear (Innis & Elias, 2003). Thus, pregnant women should consume DHA directly in the form of n-3 PUFA fortified foods, marine n-3 PUFAs or supplementation in order to ensure proper maternal and fetal nutrition (Koletzko, 2008; Koletzko, Cetin, & Brenna, 2007).

Pregnant women are advised to meet current DHA recommendations. The Perinatal Lipid intake (PERILIP) Working Group suggests that pregnant women consume at least 200 mg of DHA per day throughout the pregnancy (Koletzko et al., 2007; Koletzko et al., 2008). In the third trimester, DHA accretion occurs rapidly in the fetal central nervous system and fetal needs have been estimated at 50 – 70 mg of DHA per day (Innis & Friesen, 2008; Troxell et al., 2005). Thus, maternal DHA intake should well exceed 70 mg/d after the 28th week of gestation for positive fetal development.

Research has indicated that higher intakes of DHA and n-3 PUFAs are associated with positive birth outcomes. High maternal n-3 PUFA intakes are related to longer gestational length and higher birth weights (Hadders-Algra, 2008; I. B. Helland et al., 2001). Although there has been literature that acknowledges the importance of DHA for fetal development, much of the research has been limited to pregnant women living in coastal communities with greater accessibility to marine foods (Grandjean, Bjerve, Weihe, & Steuerwald, 2001; Olsen & Secher, 2002). Further investigations are needed to assess DHA consumption among pregnant women in non-coastal communities to determine if individuals with less access to marine n-3 PUFAs are obtaining the recommended amount of DHA for their growing fetus.

Statement of Purpose

The purpose of this study was to determine DHA status in pregnant women and their fetuses from Southwest Montana. The specific aims of this research included: (a) to describe DHA intake and to determine if our population was consuming the recommended ≥ 200 mg of DHA/d; (b) to determine if there was a difference in maternal DHA intake between weeks 18, 28 and 35; (c) to determine if there was a relationship between maternal DHA intake and maternal DHA blood lipid concentration; and (d) to determine if there is a correlation between maternal DHA blood lipid concentrations and fetal DHA blood lipid concentrations. Maternal dietary DHA intake was established in 39 pregnant women. Maternal and fetal DHA lipid status was suppose to be investigated in

the blood parameters of 14 pregnant women and their fetuses; however, results were undetermined due to the time restrictions of this project.

Hypotheses

Hypothesis 1: There is no change in DHA consumption from weeks 18, 28, and 35.

$$H_0: \mu_{18} = \mu_{28} = \mu_{35}$$

$$H_a: \mu_{18} < \mu_{28} < \mu_{35}$$

Hypothesis 2: There is no correlation between maternal DHA consumption and maternal DHA RBC and plasma status at week 35.

$$H_0: |r_{DRBC}| = 0; |r_{DP}| = 0$$

$$H_a: |r_{DRBC}| > 0; |r_{DP}| > 0$$

Hypothesis 3: There is no relationship between maternal RBC DHA concentrations at week 35 and fetal RBC concentrations at delivery.

$$H_0: r_{MFRBC} = 0$$

$$H_a: r_{MFRBC} > 0$$

Hypothesis 4: There is no relationship between maternal plasma DHA concentrations at week 35 and fetal plasma DHA concentrations at delivery.

H_0: $r_{MFP} = 0$

H_a: $r_{MFP} > 0$

Where: μ_{18} is dietary consumption at week 18

μ_{28} is dietary consumption at week 28

μ_{35} is dietary consumption at week 35

$|r_{DRBC}|$ is the absolute value of the correlation coefficient between maternal dietary DHA intake and maternal RBC DHA status

$|r_{DP}|$ is the absolute value of the correlation coefficient between maternal dietary DHA intake and maternal plasma DHA status

r_{MFRBC} is the correlation coefficient between maternal DHA RBC status and fetal RBC status

r_{MFP} is the correlation coefficient between maternal DHA plasma status and fetal plasma status

Limitations

1. The limitations of this study included a small sample size of 39 pregnant women from Gallatin and Park County, Montana. Ethnic and socio-economic variations among subjects were modest and may not reflect all non-costal communities.

Delimitations

1. The scope of this study was delimited to pregnant women in Gallatin and Park Counties, Montana.

2. The study population was delimited to pregnant women who were prior to their 18th week of gestation.

3. Subjects were accepted if they were pregnant with only one fetus, a non-smoker and not previously diagnosed with type 1 or type 2 diabetes mellitus.

Assumptions

1. This study assumed that the participants complied with the required procedures for dietary and blood data collection at weeks 18, 28 and 35.

2. The study assumed that FFQ interviews accurately reflected the DHA intake throughout the pregnancy.

Operational Definitions

Fatty Acid (FA):	A molecule with a hydro-carbon chain that has a methyl (CH_3-) and carboxyl (-COOH) end. Fatty acid molecules can vary in length and in the number of carbon - carbon double bonds.
Polyunsaturated Fatty Acids (PUFA):	A fatty acid molecule, which has more than one carbon-carbon double bond.
Long-Chain PUFA (LCPUFA):	Fatty acid molecules with a carbon chain > 18 carbons.
Essential Fatty Acid (EFA):	A fatty acid molecule that cannot be constructed in mammalian cells and must be consumed from an outside dietary source.
Alpha-linolenic Acid (ALA; 18:3n-3):	An essential n-3 fatty acid that is found in vegetable seeds and oils, such as flax and canola.
Docosahexaenoic Acid (DHA; 22:6n-3):	An n-3 PUFA found primarily in marine life.
Food Frequency Questionnaire (FFQ):	A questionnaire that contains 107 food choices, which are focused on extracting n-3 PUFA consumption over the past month in which the questionnaire has been administered.
3-day diet record:	A measurement (in grams) of food and beverages consumed during three days (two weekdays and one weekend) in a seven day period.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

Proper fetal growth and development relies on adequate maternal nutrition. Observational studies (Innis & Elias, 2003; Stark et al., 2005) have determined that pregnant women in areas with greater access to marine omega-3 (n-3) poly-unsaturated fatty acids (PUFAs) do not meet the current prenatal recommendation of at least 200 mg of docosahexaenoic acid (DHA; 22:6n-3) per day. However, intervention studies (Bergmann et al., 2008; Innis & Friesen, 2008) have determined that ≥ 200 mg of DHA can induce positive visual and neurological effects on the fetus during development and postpartum (Koletzko et al., 2007). Little is known about DHA intake in women of Southwest Montana as compared to other populations. A prospective observational study will allow researchers and health professionals to establish maternal dietary n -3 PUFA intake in pregnant women living in a non-coastal population with less accessibility to marine n -3 PUFAs.

Lipid Metabolism

Dietary lipids provide energy, build numerous cellular components and play a major role in molecular and metabolic processes. Fatty acids (FA) differ in size and can be obtained from an outside dietary source or synthesized from acetyl-CoA (Groff & Gropper, 2000). Cellular acetyl-CoA also can be used to produce energy in the form of adenosine-tri-phosphate (ATP) from degraded lipids. In the mitochondrion, energy production occurs via the β-oxidation cycle, which cleaves two acetyl-CoA molecules at the –COOH end (2000). Acetyl-CoA molecules enter the Krebs Cycle in the mitochondrial matrix to form carbon di-oxide (CO_2), water, and 12 ATP (2000). The process is reversible and can be used to form long-chain FA.

During pregnancy, maternal fuel metabolism shifts from carbohydrate (CHO) to FA utilization. Stored lipids are degraded into free fatty acids (FFA), which helps meet the necessary demands of the growing fetus (Homko, Sivan, Reece, & Boden, 1999). Maternal lypolysis causes an up-regulation of plasma FFA that generates glycerol, a substrate used for gluconeogensis. Increased levels of plasma FFAs drive the β-oxidation process to break down maternal adipose fats into acetyl-CoA (1999). A greater amount of FFA in blood plasma causes lypolytic hormone levels to increase and contributes to a decrease in maternal insulin sensitivity, which allows for CHO preservation for the fetus (1999). The β-oxidation process and lypolysis contributes to a large number of the FFA available for maternal energy during pregnancy. Biochemically

increasing FFA and decreasing insulin sensitivity in pregnant women ensures that the mother and the developing fetus are obtaining enough energy to meet their metabolic needs.

Omega 3 Fatty Acid Metabolism

Essential fatty acids (EFA) are required for proper cellular growth and development; however, they cannot be synthesized *in vivo* and must be consumed from an outside dietary source. Alpha-linolenic acid (ALA; 18:3n-3), which is found in unsaturated vegetable oils, such as flax and canola, is an example of an EFA formed in plant tissue but not mammalian tissue. In the liver, ALA can be converted to DHA or used as a precursor for other LCPUFAs (Innis, 2003).

Synthesis of DHA begins with the desaturation of the ALA (18:3n-3) molecule, by delta (Δ) 6 desaturase into 18:4n-3 (Innis, 2000; Koletzko et al., 2008). The next step is elongation with the addition of two carbons to 20:4n-3 (Koletzko et al., 2008). Desaturation by Δ5 desaturase forms eicosapentenoic acid (EPA; 20:5n-3) (Innis, 2000). The formation of EPA with the Δ5 desaturase and the following steps are pathways found only in animal cells and not plant cells, which is why DHA and other LCPUFAs are not found in plant sources (Innis, 2003). The translocation and regulations of DHA conversion from EPA remains unclear (Innis, 2007b). The current view states that there is an addition of two carbon units to form 22:5n-3, another elongation to form 24:5n-3 and a potential second Δ6 desaturation to form 24:6n-3. Eventually, a partial β-

oxidation cleaves the newly formed 24-carbon molecule into a 22-carbon DHA molecule (22:6n-3) (Innis, 2000; Innis, 2003). Once the DHA molecule is produced it can be used as a structural lipid that enriches certain phospholipid membranes of the nervous system and retina (Innis, 2003).

18:3n-3 (ALA)
↓ Δ 6 Desaturase
18:4n-3
↓ Elongase
20:4n-3
↓ Δ 5 Desaturase
20:5n-3
↓ Elongase
22:5n-3
↓ Elongase
24:5n-3
↓ Δ 6 Desaturase
24:6n-3
↓ Partial β-oxidation
22:6n-3 (DHA)

Figure 2.1. The schematic representation of the desaturation and elongation of ALA to DHA (Innis, 2000).

Maternal consumption of DHA from an outside source is more beneficial in meeting the rapid cellular demands of the growing fetus than consuming only ALA. Research suggests that less than 1 – 4% of dietary ALA is converted to DHA (Innis & Elias, 2003). An increase in maternal DHA intake has been associated with higher DHA levels in maternal and fetal phospholipid tissue and a faster accumulation of fetal DHA rather than consuming ALA alone (Innis & Elias, 2003). Moreover, ALA has not been determined to serve any essential cellular function other than to act as a precursor for EPA and DHA (Innis, 2003).

In fact, most of the ALA consumed is β-oxidized to acetyl-CoA, which further forms cholesterols, various FAs, or can be metabolized into CO_2 and expelled from the body (Innis, 2003). Thus, consuming DHA directly, either in the form of marine n-3 PUFAs or supplementation is more beneficial to the mother and developing fetus.

Recommendations and Sources of DHA During Pregnancy

Polyunsaturated fatty acids are a necessary component to the human diet. Among adults in the United States, PUFAs should provide at least 7% of the total energy intake and 19-22% of actual fat energy intake (Kris-Etherton et al., 2000; Nelms, Sucher, & Long, 2007). The major omega-6 (n-6) and n-3 PUFAs in the diet are linolenic acid (LA; 18:2n-6) and ALA, which comprise of 84-89% and 9-11%, respectively of the PUFAs consumed (Kris-Etherton et al., 2000). The Institute of Medicine (IOM) recommends that pregnant women consume about 13 g/d of n- 6 PUFAs, such as LA, and 1.4 g/d of n-3PUFAs, such as ALA (Simopoulos, 2000).

The Perinatal Lipid intake (PERILIP) Working Group and the Early Nutrition Programming Project (EARNEST) have suggested that pregnant women consume at least 200 mg of DHA/d or at least 2 portions of fatty fish per week (Koletzko, 2008; Koletzko et al., 2007); however, an intake of around 1 g/d of DHA has been used in clinical trials without adverse side effects (Freeman & Sinha, 2007; Helland, Smith, Saarem, Saugstad, & Drevon, 2003; Koletzko et al., 2007; Olsen et al., 1992; Szajewska, Horvath, & Koletzko, 2006). Furthermore,

fetal development and DHA accretion is greatest during the third trimester of pregnancy. Fetal needs have been estimated at 50 – 70 mg of DHA per day (Troxell et al., 2005). Recently, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) has adopted the PERILIP recommendations (International Society for the Study of Fatty Acids and Lipids [ISSFAL], 2006; Koletzko et al., 2007) and recognizes the importance of adequate maternal DHA intake during pregnancy.

The primary source for DHA is the flesh of marine fish, crustaceans, and shell fish and to a much lesser extent, meat, poultry, eggs and dairy products (Cleland, James, & Proudman, 2006; Gebauer, Psota, Harris, & Kris-Etherton, 2006; Innis, 2007b). Marine-derived n-3 PUFAs are excellent sources of protein, vitamins, and minerals during pregnancy (Gebauer et al., 2006); they contribute a significant amount of fats to the phospholipid membranes of the fetal brain and eyes. Plant-based foods, such as nuts, seeds, and derived oils, are high in ALA content, but are void of DHA (Gebauer et al., 2006). Mothers are advised to increase DHA intake, not in the form of ALA, during pregnancy in order to increase blood lipid levels of DHA and to ensure adequate DHA transfer to fetus (Innis, 2007b). For a complete list of dietary and miscellaneous sources of DHA see Tables 2.1 – 2.3.

Table 2.1. Marine Sources of DHA (Gebauer et al., 2006).

Fish (100 g Portion = 3.5 oz)	DHA (g)
Salmon, Atlantic, cooked, dry heat	2.147
Herring, Atlantic, cooked, dry heat	2.014
Mackerel, Pacific and jack, mixed species, cooked, dry heat	1.848
Anchovy, European, raw	1.449
Sardine, Atlantic, Canned in oil, drained with bone	0.982
Trout, mixed species, cooked dry heat	0.936
Shark, mixed species, raw	0.843
Swordfish, cooked, dry heat	0.819
Mussel, blue, cooked, moist heat	0.782
Sea bass, mixed species, cooked dry heat	0.762
Flatfish (Founder and Sole), cooked, dry heat	0.501
Pollock, Atlantic, cooked, dry heat	0.542
Spiny lobster, mixed species, cooked, moist heat	0.48
Halibut, Atlantic and Pacific, cooked, dry heat	0.465
Carp, cooked, dry heat	0.451
Oyster, eastern, farmed, cooked, dry heat	0.440
Crab, Alaska King, cooked, moist heat	0.413
Tuna, skipjack, fresh, cooked, dry heat	0.328
Perch, mixed species, cooked, dry heat	0.324
Shrimp, mixed species, cooked, moist heat	0.315

Table 2.1. Sources of DHA, Continued.

Fish (100 g Portion = 3.5 oz)	DHA (g)
Octopus, common, cooked, moist heat	0.314
Clam, mixed species, cooked, moist heat	0.284
Tuna, white, canned in oil, drained	0.244
Eel, mixed species, cooked, dry heat	0.189
Scallop, mixed species, cooked, breaded and fried	0.180
Catfish, farmed, cooked, dry heat	0.177
Cod, Atlantic, cooked, dry heat	0.158
Pike, northern, cooked, dry heat	0.137
Tuna, light, canned in oil, drained	0.128
Conch, baked or broiled	0.120

Table 2.2. Sources of DHA From Fish Oil (Gebauer et al., 2006).

Fish Oil Supplements (1 Tbsp)	DHA (g)
Salmon	2.48
Cod-Liver	1.49
Sardine	1.379
Herring	0.872

Table 2.3. Miscellaneous Sources of DHA (Gebauer et al., 2006).

Other sources of DHA	DHA (g)
Expecta, maternal supplements (1 capsule)	0.200
L'il Critters omega-3 supplements (2 fish)	0.200
Omega-3 Fortified eggs	0.075

Consumption of farm-raised or wild fatty fish during pregnancy supplies an ample amount of DHA to a developing fetus. Eight ounces or about 227 g/week of cooked farmed Atlantic salmon provides an average of 400 mg of DHA daily (Santerre, 2008). Eight ounces of wild coho or sockeye salmon contains around 350 mg – 400 mg (2008). There is a minimal distinction between the nutritional benefits between wild and farm-raised salmon and women can chose to consume either or both during their pregnancy.

The 10 most popular marine life consumed in the United States (ordered from greatest to least consumption) include: shrimp, canned tuna, salmon, Pollock, tilapia, catfish, crab, cod, clams, scallops (Santerre, 2008). In a 100 g portion, only salmon exceeds the daily recommended amount of DHA for pregnant women. Thus, if pregnant women consume salmon at least twice per week, which would provide around 400 mg of DHA/d, in addition to the other popular marine n-3 PUFAs, they can meet DHA requirements for adequate prenatal nutrition.

Influences on DHA Intake During Pregnancy

A major concern among pregnant women when consuming fish is contamination. Methyl-mercury (mercury) contamination is associated with adverse effects and injuries to a developing nervous system (Santerre, 2008). Fetal vulnerability to fish toxicity is greater than the mother's due to the immature liver and excretory pathways of the fetus (Genuis, 2008). The fetal liver is unable to efficiently detoxify contaminants because of low levels of fetal binding proteins

(Genuis, 2008). Excreted urinary pollutants are usually recycled into the nose and mouth in amniotic fluid and can be rapidly incorporated into developing organs. In 2004, the U.S. Food and Drug Administration (FDA) advised pregnant women to avoid eating predatory fish, such as shark, swordfish, and king mackerel, and to check with local advisories before eating locally caught fish (Genuis, 2008; Santerre, 2008). A concentration of 1,000 parts per billion (ppb) of mercury in commercial fish is considered the legal limit. Farm-raised and wild salmon have <100 ppb concentration of mercury, which is well below the FDA limit (Santerre, 2008).

Research on birth-weight and contaminant levels found that women who consumed fatty fish more than three times per week had infants that were not affected by seafood contamination (Grandjean et al., 2001). All fish contain some organic mercury in their flesh (Myers, Davidson, & Strain, 2007); however, farm-raised or wild salmon have low levels of methyl and other contaminants. Salmon can be consumed at least once per week and will provide high concentrations of LCPUFAs and low levels of mercury. On the other hand, predatory fish have higher toxicity levels and should be totally avoided or consumed sparingly by pregnant women.

Local fish contamination varies from location. When consuming locally caught fish, some guidelines can be applied. For instance, women are advised to eat smaller sized fish more often or large fish less often. Consuming fish like perch or other non-predator fish will have less toxins but lower values of DHA per 100 g of fish (Thorsdottir, Birgisdottir, Halldorsdottir, & Geirsson, 2004). The

contaminant poly-chloride bi-phyenols (PCBs) are generally found downstream from cities. Individuals are advised to fish upstream to avoid PCB contamination in the fish (2004). Levels of PCBs have decreased in the past few decades and are becoming less of a concern.

Endangered species can influence maternal food choices and pose a moral and ethical threat to maternal marine consumption. Varieties of catfish, carp, trout and other marine life are placed on the United States threatened and endangered species list, daily (U.S. Fish & Wildlife Service, 2009). However, many of these species of carp and trout provide a small amount of DHA per day if consumed only once or twice a week. Farm-raised salmon provides adequate DHA concentrations to the mother (Santerre, 2008). Salmon can be used as an alternative fish choice for pregnant women who are concerned with contributing to the endangerment of wild-life. For a complete list of endangered species, please see Appendix G.

Effects of Marine n-3 PUFA Consumption on Membrane Composition

Previous studies (Denomme, Stark, & Holub, 2005; Innis & Elias, 2003; Stark et al., 2005; Williams et al., 2006) have reported relationships between habitual fish consumption and maternal erythrocyte membrane n- 3 PUFA concentrations. Williams et al. (2006) investigated the relationship between n -3 and n-6 PUFA concentrations in maternal plasma and erythrocytes during early pregnancy as related to habitual fish consumption during the periconceptional period (60 days before to 7 days after conception) (McMillen, Schwartz, Coulter,

& Edwards, 2004; Williams et al., 2006). Researchers indicated that as maternal self-reported fish consumption increased, mean erythrocyte n-3 PUFA content increased (all trend *p*-values were < 0.001). Erythrocyte DHA values were 30.0% higher among women who consumed fish more than twice per week as compared to women who consumed fish less than once a week (Williams et al., 2006). A higher consumption of fish during periconception allowed more DHA to be incorporated into maternal erythrocytes during the prenatal period leading to higher DHA availability for the fetus.

Biochemical analysis of plasma phospholipids may help to validate maternal dietary assessment during pregnancy. Innis and Elias (2003) investigated the influence of dietary PUFA intake on plasma phospholipids in 55 pregnant women. The mean intake of DHA, determined via FFQ interview at weeks 28 and 35 was 160 ± 20 mg/d. Plasma phospholipids of the women at week 35 was 5.0 ± 0.18 g DHA/100 g FA (2003). The mean daily intake of DHA was significantly related to the concentration of DHA in the plasma phospholipids ($r = 0.63$; $p < 0.0001$) and the concentration of DHA increased with a greater intake of fatty fish, such as salmon ($r = 0.61$, $p < 0.0001$) (2003). However, the relationship between intake of lean fish and the concentration of DHA was less significant ($r = 0.29$, $p < 0.03$). The research done by Innis and Elias (2003) highlights the relationship between maternal fish consumption and maternal DHA availability for the fetus. With an increase in maternal intake of fatty fish, DHA concentrations in maternal plasma phospholipids increases, which allows for a greater supply of DHA to be transferred across the placenta.

The placental transport of DHA to the fetus relies on adequate maternal DHA plasma lipid concentrations. Elias and Innis (2001) measured unrestricted maternal FA intake and determined maternal and fetal plasma phospholipid concentrations. The average maternal DHA intake was not reported because this project was looking at *trans*-FA intake and the influence of birth outcomes. Maternal and infant plasma phospholipid DHA concentrations, measured in percent by weight of total FA were 5.02 ± 0.21% (2.84 – 8.11%; n = 44) and 7.67 ± 0.25% (4.38 – 10.71%; n = 44), respectively, and significantly differed ($p < 0.05$) (2001). Correlation analysis showed maternal and fetal DHA plasma phospholipid concentrations were significantly related ($r = 0.48$; $p < 0.001$). Thus, maternal plasma DHA values may be related to the concentration in infant plasma at delivery; moreover, fetal accretion of n-3 PUFAs may partially be determined by n- 3 PUFA concentrations in maternal plasma lipids (Elias & Innis, 2001).

During the third trimester of pregnancy, LCPUFA transport across the placenta is selective. Bohles, Arndt, Ohlenschlager, Beeg, Gebhardt and Sewell (1999) investigated DHA erythrocyte phospholipid concentrations in 43 women and infants at delivery. The researchers determined that the mean DHA percentage of total FA in maternal and newborn erythrocyte phospholipids was 3.2 ± 2.2% and 3.4 ± 2.3%, respectively, but did not differ significantly between mothers and newborns ($p > 0.05$) (1999). However, there was a correlation between the erythrocyte phospholipid DHA concentrations in newborns and their mothers ($r = 0.63$; $p < 0.001$). This study (1999) provides evidence that maternal

DHA concentrations in the later stages of pregnancy influence fetal cord blood DHA concentrations at delivery.

Epidemiological (Olsen & Secher, 2002; Thorsdottir et al., 2004) and intervention studies (Helland et al., 2001; Helland et al., 2003; Olsen et al., 1992) show that marine consumption and/or fish oil supplements during pregnancy is positively associated with positive fetal outcome. Consuming at least 200 mg of DHA/d during pregnancy may be used to reduce prenatal complications (Koletzko et al., 2008). More research is needed to determine how much marine n-3 PUFAs are being consumed during pregnancy and how they influence maternal and fetal blood lipid parameters.

Placenta Transfer of DHA During Pregnancy

Essential fatty acids cannot be converted to DHA in the developing fetal liver (Innis, 2005, 2007b); therefore, ALA conversion to DHA must occur in the maternal liver. Dietary DHA or to a lesser extent, DHA formed from its precursor ALA, is provided to the fetus via placental transfer from the maternal blood (Cleland et al., 2006; Ruyle, Connor, Anderson, & Lowensohn, 1990). High concentrations of lipoprotein lipase and triacylglycerol hydrolase, two enzymes that hydrolyze triglycerides, are found in the placenta (Stark et al., 2005). These findings suggest that transfer across the placenta may be limited to non-esterified fatty acids and that triacylglycerols are the major source of maternal fatty acids available for fetal nutrition (Stark et al., 2005).

Placental transfer of nutrients involves the umbilical arteries that bring fetal blood into the placental and the umbilical veins, which return enriched blood to the fetus (Ruyle et al., 1990). The exchange of fetal and maternal blood in the placenta allows for facilitated or simple diffusion, or active transport to occur, which provides the growing fetus with appropriate energy stores (Ruyle et al., 1990). Little is known about the actual mechanism by which DHA is transported across the placenta. Speculation is made that FFAs, FA binding proteins, and transport proteins are released into fetal circulation. Free fatty acids are reesterified with the preferential incorporation of the LCPUFA, DHA, into fetal neural cells (Innis, 2007a). Intervention and observational studies have indicated that higher dietary maternal intake of DHA (200 mg/d) during pregnancy results in higher maternal-to-fetal transfer of DHA (Innis, 2007a). Increasing fetal resources for DHA supply can ensure proper DHA incorporation into numerous components of fetal development.

Ruyle et al. (1990) studied the effects of plasma vs. erythrocytes in the transport of LCPUFAs from mother to fetal tissue. At delivery, the umbilical fetal cord was double clamped and blood was drawn from the venous and arterial circulation of the cord (n = 26). Maternal blood was drawn from the antecubital vein (n = 21). Maternal and fetal RBC DHA composition in the vein, as expressed in percent by weight, was 3.7 ± 0.9% and 5.6 ± 1.6%, respectively, and the maternal – fetal venous difference was statistically significant ($p \leq 0.001$) (1990). Concentrations of DHA in the fetal veins and arteries were significantly higher ($p \leq 0.02$) than all other LCPUFAs, with the exception of arachidonic acid

(AA; 20:4n-6) (1990). These findings provide evidence for the importance of high DHA concentrations in fetal erythrocytes and that DHA may play an important role in prenatal development.

Proper fetal development relies on DHA from the maternal diet. Previous research suggests that 50 – 70 mg DHA/d accumulates by the fetus during the third trimester of pregnancy (Innis & Elias, 2003; Troxell et al., 2005). Innis & Elias (2003) administered a food frequency questionnaire to 55 pregnant Canadian women during the 28th and 35th week of gestation. The mean intake of DHA was reported to be 160 ± 20 mg/d (Innis & Elias, 2003), suggesting that Canadian women living in a coastal region may not be obtaining the recommended amount of DHA during their third trimester of pregnancy. The mean daily intake of DHA was significantly related to DHA concentrations in the plasma phospholipids ($p < 0.001$) (Innis & Elias, 2003). As DHA consumption increased among the women, DHA levels in the plasma also increased, allowing for a greater availability for fetal transfer during the third trimester.

Summary

Data assessing maternal DHA consumption and DHA concentrations in maternal and fetal blood lipid parameters is limited. Further investigations need to be performed on a variety of populations in order to provide an adequate recommendation to future mothers. Increased DHA consumption positively enhances fetal development; however, less is known about women who do not have a consistent supply of marine n-3 PUFAs due to where they live.

Therefore, investigating the amount of DHA consumption and the effects of fetal transfer is imperative to better the understanding of dietary needs for healthy pregnant women in a non-coastal community.

CHAPTER 3

METHODOLOGY

Participants

Forty-one pregnant women between the ages of 21 and 39 were recruited from Gallatin and Park Counties in Southwest Montana via flyers, newspaper advertisements and word-of-mouth. Eligibility criteria included singleton pregnancy, at or before week 18 of gestation, and a non-smoker. Minor individuals (under 18 years of age) or individuals who were unable to have a full-term pregnancy were ineligible.

Potential volunteers were initially screened to determine health status, stage of pregnancy, and eligibility for participation. Upon initial acceptance, participants signed a Montana State University Institutional Review Board (IRB) approved Subject Consent Form (see Appendix A). Prior to the first data collection period, participants completed a medical history questionnaire (see Appendix B). Medical providers were faxed an additional subject consent form in order to approve the eligible participant, as well (see Appendix C).

Procedures

The project was a prospective observational study, which took place over the course of the participant's pregnancy. Data was collected any day during the 18th, 28th and 35th (±1 week) week of gestation. Anthropometric information, venipuncture fasted blood samples (no food or beverages for 8 – 12 hours prior to blood draw), and dietary intake were obtained during weeks 18, 28, and 35 of gestation. At the time of birth, a health care provider (e.g., physician, mid-wife, nurse, etc.) collected fetal cord blood from the placenta. After delivery, participants were required to complete a post-partum. Nutritionist Pro software program (Axxya Systems, Stafford, USA) was used to analyze dietary docosahexaenoic acid (DHA) consumption during pregnancy. Fatty acid (FA) analysis from red blood cells (RBC) and plasma samples was conducted at the University of British Columbia. Statistical analysis was performed using SPSS for Windows (Version 17; SPSS Inc, Chicago, USA).

Anthropometric Data

During weeks 18, 28, and 35 (± 1 week) of gestation, participant's height and weight were measured by an investigator and recorded on the appropriate food frequency questionnaire (FFQ) sheet. In the Nutrition Research Lab (NRL), weight was obtained using a calibrated scale and measured to the nearest 0.1 kg. When FFQ interviews were conducted outside of the NRL, a traveling scale was used and the weight was recorded in whole numbers. Height was

determined using a stadiometer and taken to the nearest 0.1 cm at week 18 (± 1 week). All measurements were taken from the participants wearing light clothing and no shoes.

Diet Record and Food Frequency Questionnaire

At each data collection point, participants were provided with a food log and a Precision Electronic Cuisinart scale (model SA-110A; Cuisinart, Newark, NJ) for one week. The participants were required to weigh (in grams) and record all food and beverages consumed during three days (one weekend day and two weekdays) in a seven-day period. An investigator provided each participant with explicit written and verbal instructions on how to weigh and record all foods and beverages using the food scale and dietary food log (see Appendix D).

At the same data collection period, participants took part in an hour-long interview using the specifically designed FFQ (see Appendix E). The FFQ was developed by a graduate student to determine the amount of omega-3 (n-3) polyunsaturated fatty acid (PUFA) consumption during the past month of pregnancy. The correlation between dietary DHA from the 3-day diet record and the FFQ interview was developed and used for a separate project (currently, r = 0.77 on May 3, 2009). Investigators were trained how to administer the FFQ to participants in order to obtain the most detailed descriptions of food products and portion sizes.

Maternal Blood Collection

Venipuncture blood samples were collected by a phlebotomist. Blood samples were obtained at the same data collection points (week 18, 28 and 35) as the dietary information. If participants were unable to fast before the blood draw, they were instructed to eat a low-fat snack (e.g., dry toast). A total of 14 mL whole blood was collected directly into 2 ethylenediamine tetraacetic acid (EDTA) vacutainer tubes and immediately placed on ice or in the refrigerator until centrifugation was possible. An investigator processed the blood sample within one hour of collection. The second tube was taken to the Bozeman Deaconess Hospital (BDH) for a complete blood count (CBC) and a lipid profile panel. Participants' data from BDH was faxed directly to the main office in the Department of Health and Human Development in Herrick Hall. Participants' CBC and lipid profiles were used for another project.

Plasma was separated from whole blood via centrifugation. The EDTA vacutainer tube was placed directly into the centrifuge (Fisher Marathon 2100 R, Ontario, Canada), and spun at gravitation acceleration (g) of 2500 for 10 minutes at 4 degrees Celsius (C˚). After centrifugation, the plasma was pipetted and collected into 1.5 mL labeled cryovial tubes and placed into the freezer at -80 C˚. The red blood cells (RBCs) and the white buffy layer were not disturbed. The RBCs were washed using a saline/EDTA buffer solution that contained 9 g/L of sodium chloride and 1.14 g/L of sodium EDTA in water. The amount of the buffer solution added to the RBCs was at an equal volume to the amount of the plasma

that was removed. The RBCs and buffer mix was then inverted 10 times and spun again at 2500 g for 10 minutes at 4 C˚. After centrifugation, the buffer solution was pipetted out of the tube and discarded until only RBCs and the thin buffy coat layer remained. Again, the saline/EDTA mixture was added to the RBCs at an equal volume to the previously discarded buffer solution. Centrifugation occurred for 10 minutes at 2500 g at 4 C˚. The saline/EDTA mixture was pipetted out of the tube and discarded along with the thin buffy layer. The RBC sample was pipetted into a 1.5 mL labeled cryovial tube for storage. All plasma and RBC tubes were labeled according to the date (e.g., 1/1/09), subject's initials (e.g., LW), number assignment (e.g., 5), week of gestation (18^{th} (A), 28^{th} (B), 35^{th} (C), or fetal cord blood (D)), and blood component ((RBC) or plasma (P)). All samples were stored in the freezer at -80 C˚ in the NRL until shipment to Dr. Shelia Innis' lab could occur.

Fetal Cord Blood

Prior to delivery, medical professionals were provided with explicit verbal and written instructions on cord blood collection and handling. After the delivery of the newborn, medical providers clamped the umbilical cord and collected the sample from the placenta. Cord blood was drained into a sterilized 4 mL EDTA vacutainer tube and gently inverted 10 times. At the Bozeman Birth Center, a midwife drew blood directly from the placenta into the EDTA vacutainer tube via a 25 gauge syringe. All samples were placed on ice or in the refrigerator and a medical provider contacted an investigator as soon as possible. The cord blood

samples were processed exactly as maternal blood samples previously described. The plasma and RBC samples were labeled and stored in the NRL freezer at -80 C˚ until shipment to the University of British Colombia was possible.

Post-Partum Survey

Participants completed a post-partum survey after delivery (see Appendix F). The survey was a generalized questionnaire that the participants filled out by hand and sent into the NRL. Questions on the survey included risk of pregnancy, labor procedures and newborn anthropometric data. Other questions included exercise trends, fish consumption, and supplementation use throughout pregnancy. For the purpose of this study, the post-partum survey was used to determine fish consumption and supplementation use.

Fatty Acid Analysis

All RBC and plasma FA analysis were performed in Dr. Shelia Innis' laboratory at the University of British Columbia, Canada. Total lipids from RBC were obtained using a 2.2 mL isopropyl alcohol and 1.4 mL chloroform, followed by a series of centrifugations. Evaporation of the solvents occurred with nitrogen and lipid class separations. Plasma lipids were extracted using a modified Folch extraction procedure (Folch, Lees, & Sloane-Stanley, 1957), which used a two part sample in saline, three parts methanol, and six parts chloroform. Vortex and centrifugation occurred at 1000 g for 10 minutes. Following directly after, the

lower organic layer was collected and the aqueous phase was washed with additional chloroform. The organic layer was then collected and pooled with the first layer. The samples were dried under nitrogen to obtain a total lipid extraction. Each lipid class (phospholipids, cholesterol esters, and triglycerides) was separated using high performance liquid chromatography (HPLC) with evaporated light scattering detection (ELSD) (Innis & Dyer, 2002). Lipid fractions were recovered and FAs were converted to their respective methyl esters for separation by gas chromatography using a flame ionization via Agilent 6850 gas chromatography (Agilent Technologies Inc., Santa Cara, CA) with an Agilent HP-88 capillary column (30 m x 0.25 mm internal diameter x 20 μm film thickness). Software accompanying Agilent quantified the peak areas, which calculated the percent weight of each fatty acid based on the peak areas (Innis & King, 1999). The inter- and intra-assay coefficient of variance for DHA analysis conducted at UBC was 3.1 and 2.1%, respectively.

Statistical Analysis

Descriptive statistics (mean ± standard deviation (*SD*) and range (min. – max.)) were used to describe participants' age, weight, economic and educational status and dietary intake of DHA. The sources of fish and supplementation consumed during pregnancy were determined from the post-partum survey. Repeated measures one-way analysis of variance (ANOVA) was used to test for differences in the mean intake of DHA calculated from the FFQ at weeks 18, 28 and 35. All analyses were done with SPSS for WINDOWS (version

17.0; SPSS Inc, Chicago, USA). The level of significance was set with an alpha value (α) $p < 0.05$.

CHAPTER 4

RESULTS

Introduction

The results for this project will focus on dietary docosahexaenoic acid (DHA) intake. Dietary intake was determined using the food frequency questionnaire (FFQ) interview at weeks 18, 28 and 35 (± 1 week). Maternal and fetal blood lipid parameters were undetermined due to time restrictions. Therefore, hypotheses two, three and four will not be addressed in chapter four. Expected results for hypothesis two, three and four will be discussed in the chapter five.

Subjects

Subject Adherence to the Project

Forty-one participants responded to recruitment efforts and consented to be in the study. Prior to the 18th week of gestation, two participants dropped out due to a miscarriage (n = 1) or a lack of commitment to the project's timing (n = 1). Three-day diet records were completed by 39, 37 and 35 individuals during weeks 18, 28 and 35, respectively. Reason for incomplete 3-day diet data included a loss of interest in the project (n = 1) and unavailability at week 28 (n = 1) and pre-term labor (n = 1) and relocation (n = 1) at week 35.

Food frequency questionnaire interviews were administered to 34, 33, and 32 participants during weeks 18, 28 and 35, respectively. The FFQ was created and implemented after the first five participants enrolled. Thus, 34 FFQ interviews were conducted at week 18. Reasons for lost FFQ data at week 28 included enrollment prior to the FFQ design (n = 2), scheduling conflicts (n = 2), loss of interest in the project (n = 1) and unavailability (n = 1). Lost FFQ data at the 35th week of gestation was due to pre-term labor at week 35 (n = 1) and relocation (n = 1). Overall, 32 participants had complete dietary FFQ data and were used for DHA intake statistical analyses.

The post-partum survey was provided to participants at week 35. Thirty-six participants completed the post-partum survey after delivery. Reasons for incomplete surveys included birth complications (n = 1), loss of interest in the project (n = 1), and relocation (n = 1).

Week 18 blood samples were collected from 38 of the 39 participants. The first study participant began the project prior to blood collection. At week 28, blood samples were collected from 37 participants. The reasons for incomplete data was due to loss of interest (n = 1) and unavailability (n = 1) at week 28. Week 35 blood samples were taken from 36 participants. Loss of data at week 35 was due to relocation (n = 1), pre-term labor (n = 1), and a scheduling conflict (n = 1). Cord blood samples were collected from 36 out of 39 participants. Samples were not taken from three participants due to birth complications (n = 1), relocation (n = 1) and loss of interest in the project (n = 1). For a diagram of participant adherence to the project see Appendix H.

Demographic and Anthropometric Data

The average age of participants was 30.4 ± 4.5 years (21 - 39 years) and mean household income was $47,564 ± $22,880 ($10,000 – $75,000). Participants were predominantly Caucasian and educated at the college level or above. Ninety-five percent of Gallatin County is white and 63% of the Bozeman population (≥ 25 years of age) has some form of college education (Bozeman Population Overview, 2000). Therefore, our study population was representative of Bozeman, Montana. Summary statistics for participant demographics are shown in Table 4.1.

Table 4.1. Summary statistics for participant demographics (n= 39).

Demographic	Sample Size
Education (years)	
≤ 12 (High School)	3
13 – 16 (College)	23
≥ 17 (Graduate)	13
Number of Pregnancies	
Primigravida	23
Multigravida	16
Marital Status	
Single	2
Married	37
Ethnicity	
Caucasian	35
Hispanic	1
Asian	1
Asian/Caucasian	1
Native American	1

Participants' mean height and pre-pregnancy weight was 166.0 ± 4.6 cm (155.0 – 177.8 cm) and 62.7 ± 10.8 kg (45.5 – 102.3 kg), respectively. Mean body mass index (BMI) for participants prior to pregnancy was 23.2 ± 4.2 kg/m^2 (18.2 – 39.1 kg/m^2). At weeks 18, 28 and 35, participants mean BMI was 25.0

kg/m^2, 26.8 kg/m^2, and 28.0 kg/m^2, respectively. For a detailed description of participants' weight at weeks 18, 28 and 35 please see Table 4.2.

Table 4.2. Participants' weight at weeks 18, 28, and 35.

Variable	Sample Size	Mean	Standard Deviation (SD)	Range (min. – max.)
Wk 18 Weight (kg)	39	68.3	11.6	49.7 – 102.3
Wk 28 Weight (kg)	37	73.7	12.7	52.7 – 110.0
Wk 35 Weight (kg)	35	76.7	13.4	56.4 – 110.0

Maternal Dietary DHA Intake

The FFQ interview was used to determine DHA intake of the pregnant women over a 30 day period. Since fish and seafood are not typically consumed on a daily basis, estimation of DHA intake was better represented from a method that covered a greater scope of reporting time (e.g., a one-month time period). The mean intake for participants, calculated from the average of the 3 FFQ assessment points, was determined to be 248 ± 321 mg (8 – 1,839 mg). For group summary statistics of DHA consumption at weeks 18, 28 and 35, see Table 4.3.

Table 4.3. Participants' summary statistics DHA intake at weeks 18, 28 and 35 derived from FFQ.

DHA (mg)	Sample Size	Mean	Standard Deviation (SD)	Range (min. – max.)	Median
Wk 18 DHA (mg)	34	246	323	14 – 1,791	155
Wk 28 DHA (mg)	33	253	324	8 – 1,839	209
Wk 35 DHA (mg)	32	246	326	13 – 1,681	190

Participants' overall median intake was 186 mg. According to FFQ interview data, the participants that consumed at least 200 mg of DHA during weeks 18, 28 and 35 were 15 (out of 34), 17 (out of 33) and 16 (out of 32), respectively. For a detailed plot of total DHA intake per subject at weeks 18, 28 and 35 please see Figure 4.1. For a detailed description of participants' DHA intake in relation to current recommendations, please see Table 4.4.

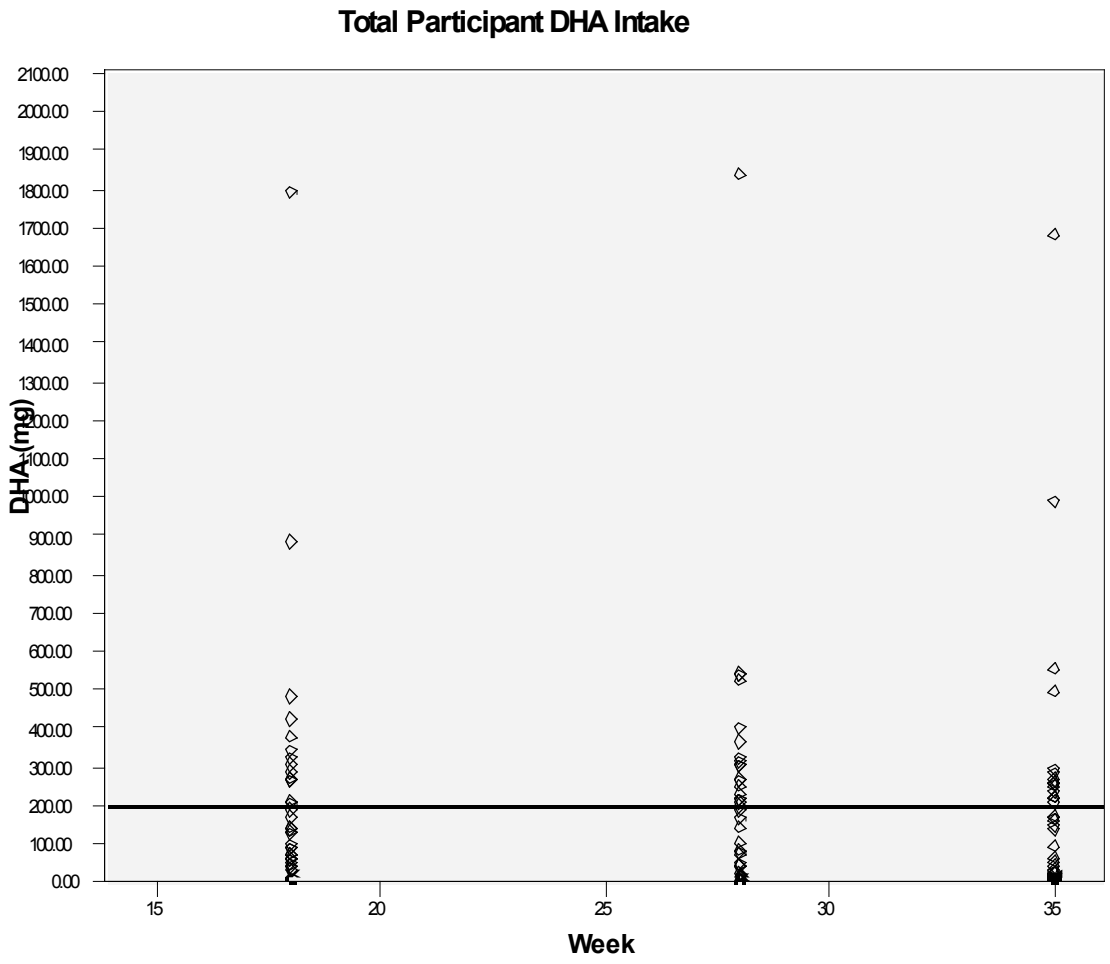


Figure 4.1. Total DHA intake at weeks 18, 28 and 35 for each participant (divided by the line, which represents 200 mg of DHA).

Table 4.4. The number of participant above and below current DHA recommendations derived from the FFQ interview at weeks 18, 28 and 35.

DHA Intake	Week 18 n = 34	Week 28 n = 33	Week 35 n = 32
≥ 200 mg	15	17	16
70 – 199 mg	10	8	6
< 70 mg	9	8	10

There was no statistical difference in DHA intake from weeks 18, 28 and 35 (F (2, 62) = 0.220, p = 0.803). Dietary DHA intake was based on complete FFQ data from 32 participants at weeks 18, 28 ad 35. The Mauchly's test of sphericity, which is used to validate repeated measures factors and within subject effects was not significant (p = 0.08). Therefore, the null hypothesis, which stated that there would be no change in DHA intake from weeks 18, 28 and 35, must not be rejected.

Sources of DHA Among Participants

Based upon weeks 18, 28 and 35 FFQ interviews, there were 12, 13 and 11 participants reported using DHA supplements, respectively. At week 18 the mean daily DHA intake from supplementation was 252 ± 400 mg (15 – 1,492 mg). The average daily DHA intake for weeks 28 and 35 were 284 ± 370 mg (53 – 1,492 mg) and 280 ± 405 mg (78 – 1,492 mg), respectively. The average median DHA intake from daily supplementation taken from weeks 18, 28 and 35 was 180 mg. According to the post-partum survey, 16 women used DHA

supplementation during their pregnancy; their mean daily DHA intake was 340 ± 373 mg (78 – 1,492 mg) and the median was 200 mg/d.

Among participants who did not consume DHA supplementation, the mean DHA intake for week 18 was 145 ± 117 mg (14 – 424 mg; n = 22). At week 28 and 35, the mean DHA intake was 144 ± 79 mg (8 – 541 mg; n = 20) and 152 ± 94 mg (13 – 555 mg; n = 21), respectively. The median value at weeks 18, 28 and 35 was 92 mg, 79 mg, and 94 mg, respectively.

According to the 34 completed week 35 FFQ interview, 24 participants stated they consumed both meat (red meat and poultry) and fish during their pregnancy. One participant was an ova-lacto vegetarian, two participants avoided meat but ate fish, and five women avoided fish but ate meat. According to the 36 completed post-partum survey, four women claimed to avoid fish due to concerns of mercury contamination. Of these four participants, one participant added nausea as a reason for lack of fish consumption and two added they were concerned about food safety issues. Two participants did not consume fish because of taste. Thirteen participants consumed fish at least once per week. For a detailed description of participants' fish consumption throughout pregnancy see Table 4.5.

Table 4.5. Participants' fish consumption throughout pregnancy derived from the post-partum survey.

Fish Consumption	N
0	5
< 1/month	4
1/month	5
2/month	7
1/week	13
2/week	1
3/week	1

Tuna and salmon were the most consumed types of fish during pregnancy. According to the post-partum survey, the types of fish consumed from most to least included tuna (n = 18), salmon (n = 17), cod (n = 6), albacore (n = 4) and trout (n = 2). Other types of marine food consumed by participants included white-fish, tilapia, walleye, halibut, mahi-mahi, eel, anchovies, sardines, cat-fish, and shell-fish, such as shrimp, crab and scallops. Many of the participants consumed more than one variety of fish and shell-fish.

According to the post-partum survey, the n-3 and DHA fortified foods, which provided a source of DHA included peanut butter, eggs and soy milk. Eight participants stated they consumed DHA fortified soy-milk. Twenty-one participants reported eating DHA enriched eggs. Fortified peanut-butter was consumed by one participant during her pregnancy.

CHAPTER 5

DISCUSSION

Introduction

This study was used to describe maternal docosahexaenoic acid (DHA) intake in a non-coastal community. The purpose of this study was to describe maternal DHA intake, to determine if participants met current DHA recommendations, and to determine if maternal DHA intake differed from weeks 18, 28 and 35. Biochemical analysis was not performed on maternal and fetal plasma and red blood cell (RBC) phospholipids due to time restrictions. Much of the previous prenatal research on maternal DHA intake has taken place in coastal communities (Denomme et al., 2005; Innis & Elias, 2003; Oken, Kleinman, Olsen, Rich-Edwards, & Gillman, 2004). Results from these populations may not reflect pregnant women in Southwest Montana, which have different accessibility to marine omega-3 (n-3) polyunsaturated fatty acids (PUFAs).

Recommendations and Sources of DHA During Pregnancy

Maternal DHA intake was determined using a food frequency questionnaire (FFQ) interview. Previously reported n-3 PUFA intake has been based on either food diaries (Bonham et al., 2008; Denomme et al., 2005) or FFQ interviews (Innis & Elias, 2003; Stark et al., 2005). Food frequency

questionnaires are used because the scope of reporting time is greater than a 3-day diet record. Since marine n-3 PUFAs are not typically consumed on a day-to-day basis, our study used an n-3 focused FFQ, which was created for this project and covered a four week period, in order to determine maternal DHA intake.

Meeting Current Recommendations for DHA Intake Among Participants

Participants' overall mean DHA intake was 248 ± 321 mg. This value shows that as a group, participants were meeting the recommended ≥ 200 mg of DHA/d through out their pregnancy. However, participants' range in overall dietary DHA intake was 1,758 mg/d and their overall median intake was 186 mg/d.

According to FFQ interview data at weeks 18 and 28, 44% and 52% of our participants were meeting the ≥ 200 mg of DHA recommendations, respectively. At week 35, 50% of our population was meeting current recommendations. During week 35, 11 participants consumed ≤ 100 mg of DHA/d and 10 participants did not meet 3rd trimester recommendations of 50 – 70 mg of DHA/d. Therefore, 31% of our participants were not meeting fetal DHA requirements during the 3rd trimester and 50% of our population was consuming less DHA than recommended.

In North America, the estimated average maternal intake of DHA is 40 – 120 mg/d (Denomme et al., 2005; Innis & Elias, 2003; Innis & Friesen, 2008; Stark et al., 2005); however, individual intake can vary widely. Participants in our

study had a DHA intake that ranged from 8 – 1,839 mg/d, which was a greater range than that previously reported. For instance, Innis and Elias (2003) found that pregnant Canadian women have a DHA intake range of 24 – 524 mg/d and Innis and Friesen (2008) found pregnant women had consumed 10 – 760 mg of DHA/d.

The participant with the daily DHA intake of 8 mg/d at week 28 is Caucasian, educated at the graduate level, married and with a household income of $50,000 – $75,000. In her post-partum survey she stated that she consumed prenatal vitamins and 1,000 mg of flax seed oil, daily. At least once per week during her pregnancy, she consumed salmon, walleye or tilapia. According to the USDA Nutrient Data base (USDA Agricultural Research Services, 2009) tilapia has a DHA value of 130 mg per 100 g of fish, walleye contains 288 mg of DHA per 100 g of fish, and wild Atlantic salmon contains 1,429 – 2,147 mg of DHA per 100 g of fish. If tilapia, walleye or salmon was consumed once per week, they would provide 19 mg, 41 mg or 204 –306 mg of DHA/d. Only one 100 g portion of salmon per week provides the recommended amount of DHA.

The Perinatal Lipid Intake (PERILIP) Working Group and the International Society for the Study of Fatty Acids and Lipids (ISSFAL) suggest that pregnant women consume two portions of fatty fish per week (ISSFAL, 2006; Koletzko, 2008). This participant did not take DHA supplementation nor did she consume the recommended amount of fatty fish per week. In fact, in her week 28 FFQ interview, she stated that she had not consumed any fish during the previous month in question. This leads us to believe that a zero fish intake and a lack of

DHA supplements provide a low DHA intake of around 8.0 mg/d. Furthermore, the discrepancy between this participant's FFQ interview and post-partum survey emphasizes the potential for participants to over-report fish or supplementation intake in the post-partum survey.

This participant took 1,000 mg of flaxseed oil, which provides 533 mg of alpha-linolenic acid (ALA) and 0 mg of DHA (USDA Agricultural Research Services, 2009). Others have reported that only 1 – 4% of ALA is converted to DHA (Innis & Elias, 2003). Therefore, consuming n-3 PUFAs in the form of ALA from flaxseed oil does not provide enough DHA to meet maternal dietary DHA intake recommendations during pregnancy.

The participant in question is well-educated and affluent. Our findings suggest that DHA education and intervention may be necessary for women in a variety of socio-economic standings (Stark et al., 2005). Moreover, pregnant women who rely on ALA conversion to form LCPUFAs and limited amounts of fish may not receive the adequate amount of DHA during their pregnancy.

Although DHA values in our study had a range of 1,831 mg, results for the overall mean maternal DHA in our participants are higher than previously reported in other studies. Denomme et al. (2005) found that 20 pregnant Canadian women during their second and third trimester consumed an average of 82 ± 33 mg/d of DHA and did not consume DHA supplements. Innis and Elias (2003) estimated, from food sources, that DHA intake among pregnant women located in the Pacific coastal community of Vancouver was 160 ± 20 mg/d in

2003 and 110 mg/d in 2008 (Innis & Friesen, 2008). Among African-American women, Stark et al. (2005) predicted a mean DHA intake of 68 ± 75 mg/d.

Although women in our study had a greater mean intake, only 16 participants were meeting the ≥ 200 mg of DHA/d recommendation at week 35. An average of 12 participants reported, via the FFQ interviews, using DHA supplementation. These participants had a total mean and median DHA intake value of 272 ± 380 mg/d and 185 mg/d, respectively. According to the post-partum survey, 16 women used DHA supplementation during their pregnancy and had a mean daily DHA intake of 340 ± 373 mg and a median value of 200 mg.

The group of participants who used DHA supplementation met current prenatal DHA recommendations. However, participants who did not consume DHA in the form of supplementation had a total mean DHA value of 147 ± 139 mg and a median value of 88 mg. The mean DHA value among participants who did not consume any form of DHA supplementation is congruent with previous research findings (Innis & Elias, 2003; Stark et al., 2005). Therefore, the mean dietary DHA intake for participants who did not consume supplementation was below the recommended 200 mg of DHA/d.

DHA Intake During the Third Trimester of Pregnancy

Fetal demands for DHA are high and may challenge maternal capacities. About 90% of fetal fat deposits occur during the last 10 weeks of gestation (Hadders-Algra, 2008). Fetal DHA needs during the third trimester has been estimated at 67 mg/d (Denomme et al., 2005; Innis, 2003; Innis, 2007b). Since the fetus represents around 25% of the total weight gained at term pregnancy, maternal intake of DHA should exceed the amount that accumulates in fetal tissue (Innis & Elias, 2003). Pregnant women should consume > 67 mg of DHA/d in order to meet their physiological demands and the demands of the growing fetus.

At week 35, participants in our study consumed an overall mean DHA value of 246 ± 326 mg and a median value of 190 mg. However, among the participants that did not consume DHA supplementation (n = 21) the mean DHA intake value was 152 ± 155 mg/d and the median value was 94 mg/d. Participants that did consume DHA supplementation (n = 11) had a mean DHA intake of 280 ± 405 mg/d and a median value of 200 mg. Thus, participants who consumed DHA supplementation were exceeding the recommended 200 mg. Furthermore, our findings suggest pregnant women in Southwest Montana do not obtain enough DHA via dietary intake for maintaining maternal and fetal needs during the third trimester and pregnant women in the Bozeman community should consider DHA supplementation in order to meet the current DHA recommendations.

Week 35 FFQ interviews were used to investigate the DHA intake of those participants who reported, via post-partum survey, not consuming fish during their pregnancy. Those participants who reportedly avoided fish but consumed DHA supplements had a daily DHA intake of 284 mg and 221 mg. Although these participants (n = 2) did not eat marine n – 3 PUFAs, they were obtaining the recommended amount of DHA for adequate third trimester maternal and fetal needs by consuming soft-gel fish oil capsules or Expecta DHA supplements. Among those participants who did not consume fish or DHA supplementation (n = 3) the daily DHA intake was 24 mg, 26 mg, and 41 mg. The mean daily DHA intake of 30 mg among these participants was insufficient to meet the estimated fetal needs of 67 mg of DHA/d. This data supports evidence that DHA supplementation can be a resourceful way for pregnant women to meet fetal and maternal needs during gestation even if they do not consume fish.

Dietary Source of DHA

According to the 32 completed FFQ interview, at week 35, the majority of the participants (n = 24) consumed meat and fish during their pregnancy. Reasons for lack of fish consumption among participants included mercury contamination and/or food safety concerns (n = 3), mercury contamination and nausea (n = 1), and taste (n = 2). Of the six participants who did not eat fish, three reported taking a DHA supplement throughout pregnancy (200 – 1,000 mg) and three did not take any DHA supplementation.

According to the post-partum survey, many participants in our study did not consume fish on a weekly basis. Assuming that the average FFQ estimation of four ounces of fish per serving was correct, 16 participants in our study had a low habitual fish intake of around 8 ounces or 2 servings per month. Thirteen women did state they consumed around four ounces of fish once per week. This value is comparable to other first nation countries, such as the United Kingdom (UK). In the UK, the average fish consumption per week is 140 g or 5 ounces (Bonham et al., 2008). Among participants that consumed fish during their pregnancy, the major sources of marine n-3 PUFA included tuna, salmon and cod. Other less frequently consumed marine n – 3 PUFAs were albacore, trout, tilapia, halibut, mahi-mahi, and shell-fish. Fortified eggs, milks and other products provided DHA to the maternal diet during pregnancy.

Recommendations for fish intake during pregnancy are based on adequate n-3 PUFAs for the mother and fetus; however, a concern of adverse effects on prenatal mercury exposure has lead to guidelines on maternal fish consumption (Bonham et al., 2008). The United Kingdom's Scientific Advisory Committee on Nutrition recommend that pregnant women consume no more than 10 ounces of oily fish per week and the US Food and Drug Administration (FDA) and US Environmental Protection Agency (EPA) jointly recommend that women consume no more than 12 ounces of low mercury fish per week (Bonham et al., 2008). However, eight ounces of cooked salmon per week sufficiently provides around 400 mg of DHA/d (Santerre, 2008).

Consuming a low mercury fish, such as salmon, provides an adequate amount of LCPUFAs and does not require the mother to exceed the advisory warnings of 10 – 12 ounces of fish per week. Only one participant in our study admitted to consuming fish three times per week. The type of fish that this participant consumed most frequently was salmon; however, others included sardines, anchovies and shell-fish. According to her week 35 FFQ, she consumed salmon twice per week, shrimp once per week, crab once per week, and snapper once during the month. This participant had a daily DHA value of 493 mg at week 35 and did not consume DHA supplementation. Consuming 12 ounces of salmon per week does not exceed the US FDA and US EPA warnings of mercury poisoning and provided around 600 mg of DHA/d. This participant consumed eight ounces of salmon and other marine n-3 PUFAs throughout the week, and was able to exceed maternal and fetal DHA needs for the third trimester. Therefore, obtaining adequate DHA intake for maternal and fetal needs via dietary sources is possible if one consumes at least two portions of fatty fish per week.

Sources of Error in Maternal Dietary Intake

Many factors influence maternal dietary intake during pregnancy. Sources of error in this project could have included under-reporting during data collection of the 3-day diet record and the FFQ interview. During the FFQ interview, participants may have had difficulty estimating portion sizes and/or remembering foods that were less frequently consumed during the month in question.

Moreover, 16 women reported using DHA supplementation during their pregnancy; however, only 12, 13 and 11 participants reporting using DHA supplements during weeks 18, 28 and 35, respectively. The inconsistency between the post-partum survey and the FFQ interview could potentially be attributed to the forgetfulness of the participants during the FFQ interviews and 3-day diet records or an over-reporting of maternal DHA supplementation on the post-partum survey.

Due to a large reporting time, the FFQ interview can be a more reliable method of obtaining maternal DHA intake during pregnancy. The 3-day diet record was unable to capture all maternal fish consumption during the week in question, since many participants did not report eating fish more than once per week. The correlation between DHA intake from maternal 3-day dietary information and the FFQ interview is currently at $r = 0.77$ (May, 2009). Therefore, future studies can use the FFQ interview to determine maternal dietary DHA intake, which reduces the amount of work a participant has to do and may introduce participation in a greater socioeconomic demographic.

The value of maternal DHA intake did not differ significantly from weeks 18, 28 and 35. This suggests that three data points at weeks 18, 28 and 35 may not be necessary for future projects because participants' DHA intake does not differ throughout the pregnancy. Other researchers have collected dietary data from week 28, 35 or 36 only, and others used both week 28 and 35 (Bonham et al., 2008; Innis & Elias, 2003; Innis & Friesen, 2008; Stark et al., 2005). Therefore, reducing dietary data collection to one or two time points may

increase participation while maintaining an actual representation of n-3 PUFA intakes during pregnancy.

Effects of Marine n – 3 PUFAs on Membrane Composition

The maternal and fetal blood parameters were undetermined in our study. Due to a lack of results, a regression analysis could not be used to determine the correlation between maternal DHA intake and maternal blood lipid parameters at week 35. The relationship between maternal blood parameters and fetal blood parameters could not be investigated, as well. Nevertheless, a discussion is provided to demonstrate an understanding of what was expected in maternal and fetal blood parameters.

Relationship Between Maternal DHA Intake and Maternal Blood Lipid Parameters

Maternal metabolic changes are expected to occur throughout pregnancy. In the early stages of pregnancy, maternal fat stores increase and insulin sensitivity decreases (Lain & Catalano, 2007). Insulin is a lypogenic substance that favors maternal fat accumulation in preparation for the rise in energy needs associated with the rapidly growing fetus and placenta (Homko et al., 1999). By the third trimester, insulin resistance increases in order to favor a rise in maternal free fatty acid (FFA) concentrations, which allows for a greater carbohydrate (CHO) and fatty acid (FA) availability for the growing fetus (Homko et al., 1999).

Available maternal FFAs for fetal development can arise from the catabolism of maternal adipocyte cells or directly from maternal dietary intake.

Our second hypothesis stated that there is no correlation between maternal DHA intake and maternal RBC and plasma DHA concentrations at week 35. However, previous research has shown that an increase in maternal DHA intake can increase maternal DHA lipid profiles. Innis and Freisen (2008) administered 400 mg of DHA/d from weeks 16 to 36 (n = 68) and observed an increase of 32% in RBC DHA concentrations in the intervention group ($p < 0.05$).

Among Canadian women, Innis and Elias (2003) determined that the mean daily intake of DHA is significantly related to the concentration of DHA in the plasma phospholipids ($r = 0.63$; $p < 0.0001$). Plasma phospholipids DHA concentrations were measured at 5.0 ± 0.18 g DHA/100 g fatty acid (Innis & Elias, 2003). They further determined that an increase in fatty fish consumption was related to an increase in DHA plasma phospholipid concentrations ($r = 0.61$, $p < 0.0001$) (Innis & Elias, 2003). Therefore, an increase in maternal fatty fish intake or DHA supplementation can increase DHA concentrations in maternal RBCs and plasma phospholipids, which provides higher DHA concentrations to the developing fetus.

Previous research has shown that an increase in maternal dietary DHA will increase maternal blood lipid parameters. Therefore, our research expected to see a direct relationship between maternal DHA intake and DHA blood lipid concentrations. However, due to the lack of analyzed data, we can neither reject nor fail to reject our second null hypothesis.

Relationship between Maternal and Fetal Blood Lipid Parameters

Hypothesis three and four stated that there is no relationship between maternal plasma and red blood cell (RBC) DHA concentrations at week 35 and fetal plasma and RBC concentrations at delivery. Elias and Innis (2001) described how DHA concentrations in infant plasma lipids were significantly related to maternal plasma lipid concentrations ($r = 0.48$; $p < 0.001$). The mean maternal and infant plasma phospholipid DHA concentrations, measured in % by weight of total FA were 5.02% and 7.67%, respectively (Elias & Innis, 2001). Others found that maternal and newborn plasma phospholipid concentrations at delivery were 3.66 ± 0.06% and 5.82 ± 0.09%, respectively ($p < 0.001$) (Vlaardingerbroek & Hornstra, 2004).

The concentration of DHA has been shown to be around 300 – 400 times greater in fetal compared to maternal plasma phospholipids (Innis, 2003). Research has shown that the concentration of DHA in maternal plasma phospholipids declines towards the end of gestation (Bergmann et al., 2008; Haggarty, 2004; Vlaardingerbroek & Hornstra, 2004). Plasma contains a high concentration of FFA, which provides direct energy stores for fetal accretion. At delivery, higher fetal DHA plasma phospholipid concentrations would be expected compared to maternal DHA concentrations at week 35 or delivery. Fetal needs for FFAs containing DHA are greater than maternal needs for higher plasma DHA FFA concentrations. Increased plasma FFA during late stage pregnancy has been shown to be induced by insulin resistance and aid in

inhibiting glucose uptake, which provides an increase in nutrients transported to the fetus (Homko et al., 1999).

Research on the difference between maternal and infant DHA RBC concentrations is conflicting. Bohles et al. (1999) did not observe a significant difference between maternal and infant DHA erythrocyte concentrations at delivery, although fetal RBC DHA concentrations were slightly greater ($p > 0.05$). The mean maternal and fetal DHA RBC phospholipid concentrations were 3.2% and 3.4%, respectively ($r = 0.63$; $p < 0.001$). However, others found that at delivery the percent of total DHA in maternal and fetal RBC and plasma phospholipid were 3.85 ± 0.05% and 4.73 ± 0.05%, respectively and significantly differed ($p < 0.001$) (Vlaardingerbroek & Hornstra, 2004).

The turn over of a RBC is around 100 – 120 days, or 3 – 4 months. Maternal DHA RBC concentrations are expected to be higher since maternal RBCs are older than fetal RBC and reflect the maternal diet prior to and during pregnancy. The fetus begins producing RBC around the 16th week of gestation (FortuneCity, 1999). If maternal DHA intake and DHA blood lipid concentrations are low during the first trimester of pregnancy, then the fetal RBC collected at birth is a reflection of maternal DHA status during the earlier stages of pregnancy and not the later stages of pregnancy and may be low, too. Therefore, determining maternal and fetal transfer of DHA using erythrocytes may be less of an indicator of DHA availability at the time of collection than plasma concentrations. Thus, if our results were congruent with other research, we would have suspected that maternal plasma and RBC DHA concentrations were

positively related to fetal plasma and RBC DHA blood lipid parameters at delivery. However, our results were undetermined and we can neither reject nor fail to reject the null hypotheses for hypotheses three and four.

Possible Sources of Error and Improvements

The study population may not be reflective of the greater Southwest Montana population or other non-coastal communities. Maternal dietary intake of DHA was higher than previously reported (Denomme et al., 2005; Innis & Elias, 2003; Stark et al., 2005). The women in our study were predominantly white, well-educated, and affluent. The mean household income was $47,564, which alludes to the fact that our participants could afford prenatal vitamins, DHA supplements, and marine n-3 PUFAs. All the women in our study had some form health-care and worked with a health-care provider.

Recruitment efforts took place at the Bozeman farmer's market and grocery stores. Flyers were placed in coffee shops, clothing stores and local news papers. This type of recruitment could have targeted educated and affluent women, who shopped locally and participated in local events. Women who were interested in the project but lived in the smaller communities surrounding Bozeman, such as Ennis, Manhattan, and Livingston stated that they could not afford the time and money it would have cost to drive to Bozeman, and were unable to participate.

During recruitment efforts people showed an interest in our study. Women who were interested in our project, but did not follow through with recruitment

efforts, were intimidated with the data collection. Completing a 3-day diet record, an FFQ interview and a venipuncture blood draw was unappealing to many potential recruits. Therefore, our participants were individuals who enjoyed contributing to the growing knowledge of prenatal and maternal healthcare science. Again, these participants may not be reflective of the greater Southwest Montana region, nor can they be used to generalize other non-coastal populations.

In order to obtain accurate and representative results from this community in the future, the project should be repeated with a larger sample size. Bozeman, Montana has a population of around 33,000 people (Bozeman Population Overview, 2000). Our participants represent only 0.12% of the Bozeman population. Obtaining a larger sample can provide a greater representation of the Southwest Montana community. Furthermore, performing this study in communities around the state of Montana, for example in Billings, Missoula and/or Havre, researchers can have a better understanding of the entire Montana population as it relates to DHA intake.

Future studies should address how pregnant women acquire their knowledge about DHA intake and supplementation. Around half of our population was consuming DHA supplementation throughout their pregnancy; however, it is undetermined where participants obtained information regarding n-3 PUFA supplementation. For instance, the participant that consumed 1,000 mg of flax oil/d only consumed 8 mg of DHA/d during the month surrounding the 28th week of gestation. However, the participants that were consuming DHA

supplementation had a mean daily DHA intake that exceeded current recommendations. Pregnant women may think that all n-3 PUFAs are the same or provide the same benefits to the growing fetus. Further investigations regarding participants' knowledge of DHA intake can allow researchers and healthcare providers to understand the consumer perception of FA intake during pregnancy and better suggest sources of DHA to meet maternal dietary DHA recommendations.

CHAPTER 6

CONCLUSION

Dietary patterns of our participants remained consistent over time and docosahexaenoic acid (DHA) intake did not differ significantly. Participants who consumed supplementation did so throughout the pregnancy. The participants who were low marine omega-3 (n-3) polyunsaturated fatty acid (PUFA) consumers (less than once per week) did not change their dietary fish habits by the 35th week of pregnancy. Since dietary patterns did not change over time, one can conclude that future studies can use week 28 and/or 35 to determine DHA status in pregnant women.

Current recommendations suggest that pregnant women among the general population should consume at least 200 mg of DHA per day and even higher dosages can be beneficial, especially during the third trimester of pregnancy (Hadders-Algra, 2008). Our overall population's DHA intake mean was above the current recommendation. However, only half (n = 16) of our population was meeting DHA recommendations, especially during the week 35 data collection period. Among the 16 participants, the daily DHA mean was 418 ± 392 mg and the median was 257 mg/d. The primary source of DHA among the 16 participants was DHA supplementation (n = 11); only 5 participants were meeting current DHA recommendations through dietary means, alone at week 35.

Current recommendations state that pregnant women can obtain adequate DHA concentrations for maternal and fetal needs by consuming at least two portions of fatty fish per week (ISSFAL, 2006; Koletzko et al., 2007). However, only two participants in our study reported consuming marine n-3 PUFAs at least twice per week. More than half of our population consumed marine n-3 PUFAs once a week or less.

Dietary DHA intake from marine n-3 PUFAs is insufficient to meet maternal and fetal needs among pregnant women in Southwest Montana. The most adequate source of DHA for pregnant women in this community is DHA supplementation. Furthermore, n-3 PUFA education is necessary among this population to ensure that maternal DHA intake sufficiently meets current DHA dietary recommendations.

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APPENDICES

APPENDIX A

SUBJECT CONSENT FORM

Subject Consent Form
For
Participation in Human Research
Montana State University

Project Title: Assessment of physical activity levels and dietary intake in pregnant women

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Purpose and Rationale of the Study:
The purpose of this study is to document the physical activity patterns and consumption of healthy fats in pregnant women. This study will assess healthy pregnant women's current fat intake and the amount of fat transferred to the baby during pregnancy.

You have been identified as a possible participant because you are a healthy women living in the communities surrounding Montana State University (Bozeman, Belgrade, Four Corners, and Livingston) who has shown interest in our study by responding to our recruiting efforts. You have been selected to participate based on several criteria including:

- Pregnant (prior to 18th week of pregnancy);
- Not pregnant with multiple babies (e.g. twins); and
- Not a smoker.

Study Outline:
If you agree to participate, you will be asked to do the following:

<u>Prior to Beginning</u>
You will be required to receive confirmation that you are healthy enough to participate in this study from your medical provider. The attached consent letter will be sent by the principal investigator to your medical provider and returned to the project director. You will need to provide contact information for your medical provider so that the letter can be sent.

<u>Throughout your pregnancy</u>
You may participate in physical activities that you choose or have been recommended to you by your physician and you feel comfortable doing. You will consume the "diet" of your choice. We will not ask you to modify your current food choices. You will need to fill out various questionnaires related to your medical history. At any time you are invited to discuss any concerns that you have about the study protocol; however, the project director will not make any physical activity or diet recommendations.

At weeks 18, 28 and 35 of your pregnancy

You will be required to meet with a study coordinator or project director at Herrick Hall Room 18 before you begin recording your diet, physical activity, and heart rate.

During each visit you will be given a dietary scale, heart rate monitor, 3-day food log, 3-day physical activity log, and diet questionnaire. You will have a weight measurement taken and you will have a fasted **blood** sample obtained via venipuncture by a Red Cross trained phlebotomist. Consenting to this study allows the investigators to utilize frozen blood samples for future analysis.

The 3-day **food log** requires you to weigh all food and beverages consumed for 3 consecutive days. You will be given detailed instructions on how to properly complete the forms and tips on accurately weighing food. You will be provided with a dietary scale, at no cost to you, for use during the study to facilitate the process. You may perceive this to be a tedious process; however it is the most accurate means of collecting dietary intake information. You will not be given a diet to follow; observations are made on what you typically choose to eat.

During each visit you will complete a **food questionnaire** that will take approximately 15 minutes to complete. This questionnaire provides information about the foods you have consumed during the past month.

You will be provided with an **activity monitor** that is worn on the wrist like a watch. The activity monitor will be worn with a locking wristband for seven days, 24 hours a day to ensure the best possible data collection. The monitor is water resistant and can be worn while showering, washing dishes, and swimming. This wrist activity monitor has been used in the Montana State University Movement Science Lab for previous monitoring studies and it has never been reported to cause discomfort or localized skin irritation.

The 7-day **physical activity log** requires you to record all of your daily activities into a log that will be provided for the same 7 consecutive days you record your food intake, heart rate, and activity monitor. No recommendations for physical activity will be provided to you through this study. All choices for physical activity will be left up to you.

You will be provided with a **heart rate monitor** that consists of a band worn around your chest and a transmitter worn like a watch. You will need to wear the heart rate monitor consistently for 3 days from the time you awake to the time you go to bed. You may remove the heart rate monitor when sleeping and showering. If you choose to swim continue to wear the heart rate monitor. These 3 days of wearing the heart rate monitor will coincide with the 3 days you record your food log and physical activity log during a period of no more than 7 days. You will receive detailed instruction on proper usage.

You will need to arrange a time with a study coordinator or project director to pass in your 3-day food record, 3-day physical activity log, heart rate monitor, and dietary scale at the end of each data collection period.

Delivery

At birth, an additional blood sample will be collected from the umbilical cord. Cord blood will be collected in a routine procedure already done by your physician at birth once the baby is detached from the placenta. The cord blood sample will be collected from the umbilical cord section still attached to the placenta. There is no risk to your newborn associated with this blood collection.

During the 35th week visit you will be provided with a **reminder packet** specific to the delivery of your baby containing the cord blood sample instructions, a follow-up questionnaire and instructions for coordinating the sample pick-up by an investigator. You will be asked to contact a study coordinator when you go into labor and after the delivery so that blood samples and questionnaires can be picked up immediately from the hospital. Consent to participate in this study allows the investigators to contact you following the completion of the study.

Risks:
This is an observational study and it will not provide any risks to you or your fetus. This study is looking at what you are doing and does not require you to make any changes to your daily patterns, therefore there are no risks associated with the observational component of the study.

Approximately 3 tablespoons of blood will be removed by putting a needle in a vein of your arm on 4 occasions. This is a standard method used to obtain blood for tests. There is momentary pain at the time the needle is inserted into the vein but other discomfort should be minimal. In about 10% of the cases, a small amount of bleeding under the skin will produce a bruise. The risk of infection is less than 1 in 1,000.

Benefits:
You will be provided with a summary and explanation of the results from the study. Gifts will be provided throughout the study as a token of our appreciation for your participation in the study.

Funding:
This project is funded by the Department of Health and Human Development and the NIH--INBRE.

Confidentiality:
The data obtained from the study will be regarded as privileged and confidential. Your privacy will be maintained in any future analysis and/or presentation of the data with the use of coded identifications for each participant's data. All data will be stored in a locked file cabinet with access only by the principle investigator and project director. Additionally, any data entered into the computer will be available with restricted password only.

Freedom of Consent:
Participation in this study is completely voluntary. You may withdraw consent in person or by phone with the principal investigator, Christina Campbell at any time. In the event of any physical injury occurring in connection with the study, Montana State University will not provide any special compensation or any medical treatment. We will advise and assist the participant in receiving medical treatment by contacting the appropriate health

care provider as directed by you, the participant.

Please feel free to ask any questions or express your concerns regarding this study. The investigator will attempt to answer all questions. Contact Dr. Christina Campbell at 994-5002. Please address any questions relating to the rights of human subjects to Mark Quinn, Chair, Human Subjects Committee, 994-5721.

Authorization:
I have read the above and understand the inconveniences and risks of this study. I, _____ (*your name*), agree to participate in the project. I understand that I may later refuse to participate, and that I may withdraw from the study at any time. I have received a copy of this consent form for my own records.

Signed: _____

Witness: _____

Investigator: _____

Date: _____

APPENDIX B

MEDICAL HISTORY QUESTIONNAIRE

Medical History Questionnaire

Please answer the following questions to the best of your knowledge. All information provided here is completely confidential. Please ask for clarification if needed.

Subject Name:_____
(Last) (First) (MI)

Phone Number we can reach you at: _____

Email address_____

Address:_____

Age: _____yrs_____mo Date of Birth:_____

Pre-pregnancy weight:_____lbs Height:_____ft_____in

Is this your first pregnancy? Yes No

If no, number of pregnancies_____

Number of children_____

Birth dates of children
_____
mo/day/yr mo/day/yr mo/day/yr mo/day/yr mo/day/yr

Are you planning to breastfeed?

First day of last menstrual period:_____ Due Date:_____

What is the first day of your next week of pregnancy? (circle)

Sunday Monday Tuesday Wednesday Thursday Friday Saturday

In what week of your pregnancy did you find out you were pregnant?_____

Prior to your pregnancy what was your average number of workouts per week?_____

Average duration of workout_____

Type of activity_____

Since you became pregnant what has been your average number of workouts per week?_____

Average duration of workout_____

Type of activity_____

Have you experienced any morning sickness that altered your activity level? Yes No

If yes, please describe_____

Are you following any guidelines regarding exercise during your pregnancy?_____

If yes, please describe_____

Where did you receive the guidelines?_____

Race (circle):

1. American Indian or Alaska Native
2. African American
3. Caucasian
4. Asian
5. Hispanic
6. Other (specify):_____

Marital Status (circle):

1. single
2. married
3. divorced/separated
4. widowed

Education Level

What is the last grade in school that you completed? Please specify if two year school (circle)

1. Elementary 01 02 03 04 05 06 07 08
2. High School 09 10 11 12
3. College 13 14 15 16
4. Graduate/Professional School 17+

Employment:
What is your occupation?_____
If employed how many hours a week do you work?_____

Income:
What is your household annual income (circle)

1. No income
2. $1 to $10,000
3. $10,001 to $20,000
4. $20,001 to $30,000
5. $30,001 to $40,000
6. $40,001 to $50,000
7. $50,001 to $75,000
8. $75,001 or more

Medical History (circle any, and give age at diagnosis):

	Age
1. Diabetes	____
2. Thyroid Disease	____
3. Cirrhosis	____
4. Hepatitis	____
5. Gall Stones	____
6. Kidney Stones	____
7. Nephritis	____
8. Cancer (specify)	____
9. High Blood Pressure	____
10. Angina	____
11. Allergies (specify)	____
12. Goiter	____
13. Cardiovascular Disease	____
14. Depression requiring medication	____
15. Insomnia requiring medication	____

Drug History:

1. Do you currently take any medications on a regular basis?_____
   If yes, please specify_____
2. Have you taken medication regularly in the past?_____
   If yes, please specify_____
   How long ago was medication taken regularly?_____
3. Do you currently take vitamin supplements on a regular basis?_____

If yes, please specify_____

Have you in the past?_____

If so, how long ago?_____

4. Do you currently take herbal supplements on a regular basis?_____

If yes, please specify_____

Have you in the past?_____

If so, how long ago?_____

Diet History:

a. Are you a vegetarian? Yes No

b. If yes, circle one of the following:

i. Lacto-ovo (consume milk, milk products and eggs)
ii. Ovo (consume eggs, but no milk or milk products)
iii. Lacto (consume milk and milk products, but no eggs)
iv. Vegan (consume no animal products)

c. During your pregnancy are you consuming alcohol?_____

If so, how many drinks each week?_____

APPENDIX C

MEDICAL PROVIDER CONSENT FORM



Dear Medical Provider,

_____ has volunteered to participate in an observational study regarding exercise duration, frequency, and intensity in habitually active pregnant women. This study will not be prescribing exercise; it only involves recording what the subject chooses to do on her own. Your patient will wear a heart rate monitor during each bout of exercise, record her daily activities in a log book, and keep a weighed food diet record. Your patient will be keeping these records during the 18th, 28th, and 35th weeks of her pregnancy. At the beginning of each "study" week your patient will be weighed.

We would like you to confirm that _____ is healthy enough to participate in exercise during her pregnancy, and that we will be informed if her health status alters her ability to safely exercise.

Signature of Medical Provider_____

Print Name_____

Date_____

Please return this form via facsimile as soon as possible. Thank you for your help with this project.

Sincerely,

Rebecca Filipowicz
Gita Gelfer
Phone: (406)994-5001
Fax: (406)994-2013

MONTANA

nutrition research laboratory

APPENDIX D

3-DAY DIET FOOD LOG EXAMPLE

For Official Use Only
Subject ID:

Date:_____

Time	Food	Constituents	Description	Weight
9am	Grape Nuts		Post Brand	120g
9am	Sugar		White	3g
9am	Milk		1%	106g
9am	Blueberries		Frozen, unsweetened	50g
9am	Orange Juice		Tropicana, no pulp, calcium added	120g
11:30 am	Almonds		Raw, unsalted, Kirkland brand	60g
1:00pm	Sandwich	Bread	Whole Wheat, Wheat Montana	45g
1pm		Sprouts	Alfalfa	5g
1pm		Cheese	Tillamook Sharp Cheddar	33g
1pm	Apple Juice		From concentrate, Apple Tree brand 100% juice	70g

For Official Use Only
Subject ID:

Date:_____

Time	Food	Constituents	Description	Weight
	Daily Supplements			

APPENDIX E

FOOD FREQUENCY QUESTIONNAIRE

Nutrition Research Lab, Montana State University, Bozeman

Subject _____
Height_____
Weight_____
Week of Pregnancy____
Interviewer_____
Date _____

How would you describe your diet since you became pregnant?

I eat both meat and fish	**____**
I avoid meat, but eat fish	**____**
I avoid fish, but eat meat	**____**
I'm a vegetarian and include dairy and eggs in my diet	**____**
I'm a vegetarian and include dairy but not eggs in my diet	**____**
I'm a vegetarian and include dairy, eggs, and fish in my diet	**____**
I'm a vegan, avoid all animal products	**____**

How would you describe your diet before you became pregnant?

I eat both meat and fish	____
I avoid meat, but eat fish	____
I avoid fish, but eat meat	____
I'm a vegetarian and include dairy and eggs in my diet	____
I'm a vegetarian and include dairy but not eggs in my diet	____
I'm a vegetarian and include dairy, eggs, and fish in my diet	____
I'm a vegan, avoid all animal products	____

How often have you had the following meals per week in the last month?

	Number of meals per week							
	7	6	5	4	3	2	1	0
Breakfast	___	___	___	___	___	___	___	___
Snack, morning	___	___	___	___	___	___	___	___
Lunch	___	___	___	___	___	___	___	___
Snack, afternoon	___	___	___	___	___	___	___	___
Dinner	___	___	___	___	___	___	___	___
Snack, after dinner	___	___	___	___	___	___	___	___
Snack, before bed	___	___	___	___	___	___	___	___
Other	___	___	___	___	___	___	___	___

Dairy Products

Milk	**Brand/Type**	**Amount (cups)**	Day	Times per Week	Month
Cow (type)	_____ _____ _____	_____ __	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Soy	_____ _____ _____	_____ __	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Flavored	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Almond	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Hemp	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Cheese	**Brand/Type**	**Amount (ounces)**	Day	**Times per** Week	Month
Cheddar	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Mozzarella	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Parmesan	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
American	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Swiss	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cottage	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cream	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Yogurt	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Plain	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Flavored	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Soy	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Frozen	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Ice Cream	Brand/Type	Amount (cups)	Times per Day	Times per Week	Times per Month
Vanilla	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Flavored	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Eggs	Brand/Type	Amount (items)	Times per Day	Times per Week	Times per Month
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Other	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Sour Cream	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cream in coffee	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Whipped cream	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pudding	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Table and Cooking Fat

	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Butter for spreading	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Margarine for spreading	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Butter for cooking	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Margarine for cooking	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other spread	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Olive oil	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Canola oil	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Flax seed oil	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Salad dressing	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Mayonnaise	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Peanut Butter	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Meat and Poultry

	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Chicken	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Turkey	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Beef	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pork	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Lamb	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Wild Game	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Hot dogs	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
	_____	_____			

Sausage	_____ _____	___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Lunch meat	_____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Bacon	_____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Fish and Shellfish

	Brand/Type	Amount (ounces)	Day	Times per Week	Month
Canned tuna	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Fresh tuna	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Fatty fish	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Lean fish	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Sushi	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Locally caught fish	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Fish sticks	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Breaded fish sandwiches	_____ _____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Shellfish	_____ _____	_____ ___	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Vegetables

	Brand/Type	Amount (cups)	Times per Day	Times per Week	Times per Month
Potatoes	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Leafy greens	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Orange vegetables	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Green Vegetables	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Beans	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Tofu	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Soybeans	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Salsa	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Tomato/pasta sauce	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Avocado	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Olives	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Fruit

	Brand/Type	Amount (cups)	Day	Times per Week	Month
5 most common	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Grains

Cereals	Brand/Type	Amount (cups)	Day	Times per Week	Month
Flax added	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Oatmeal	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cream of Wheat	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Hot whole grain	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Breakfast cereal	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Rice	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Pasta	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Quinoa	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Couscous	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Flax	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other___	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Breads	**Brand/Type**	**Amount (items)**	Day	**Times per** Week	Month
Wheat bread	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
White bread	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
English Muffins	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Bagels	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Buns or rolls	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Tortillas	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____ _____ _____	_____ _____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Baked Goods	Brand/Type	Amount (items)	Times per Day	Times per Week	Times per Month
Muffin	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Donuts	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Waffles	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pancakes	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cookies	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Cakes	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Fruit breads	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pie	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Poptarts	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

\

Snacks	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Crackers	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pretzels	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Potato Chips	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Corn Chips	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Popcorn	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Granola bars	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Energy bars	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Almonds	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Nuts	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Seeds	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Graham crackers	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Candy	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Mixed/Prepared Foods

	Brand/Type	Amount	Times per Day	Times per Week	Times per Month
Lasagna	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Macaroni and Cheese	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Hamburger helper	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Frozen dinners	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Tacos	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Burritos	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Pizza	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Stir-fry	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Soup	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Casseroles	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Rice-a-roni	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____ _____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

Beverages

	Brand/Type	Amount (ounces)	Times per Day	Times per Week	Times per Month
Orange juice	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Other 100% fruit juice	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Sweetened juice	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Vegetable juice	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Soda	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Tea	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Coffee	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Instant breakfast	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Slim fast	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Ensure	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Sports drinks	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
Flavored water	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3
_____	_____	_____	1 2 3 4 5 6+	1 2 3 4 5 6 7	1 2 3

How often do you eat fast food?

Brand/Type	Times per		
	Day	Week	Month
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3

Please list other foods that you have eaten in the last month that you have not been asked about.

Brand/Type	Times per		
	Day	Week	Month
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3
_____ _____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3

Please list and vitamins and supplements that you are taking.

Brand/Type	Times per		
	Day	Week	Month
_____ _____	1 2 3	1 2 3 4 5 6 7	1 2 3

Are there foods that you commonly ate before you became pregnant that you do not eat now?

Brand/Type	Reasons for not eating
_____ _____ _____ _	
_____ _____ _____ _	

APPENDIX F

POST-PARTUM SURVEY

Thank you for your participation in the Blossom Project. Please take the time to complete this short questionnaire and return in the attached self addressed stamped envelope.

Birth Outcome Questionnaire

Date Survey Completed:_____

At the end of your pregnancy you were considered:

□ **High risk**
□ Low risk
□ **No risk**

Why were you considered "at risk"?

How many weeks pregnant were you when you had your baby?

□ **35 weeks**
□ 36 weeks
□ **37 weeks**
□ 38 weeks
□ **39 weeks**
□ 40 weeks
□ **More than 40 weeks**

Other:_____

Due Date

Birth Date of Baby

Sex of Baby (circle one)	**Female**	**Male**

Birth Weight _____ lb._____ oz.

Head Circumference

Length

APGAR score _____ (1 minute) _____ (5minutes)

Your weight prior to Labor _____ lbs

Did you exercise during your first trimester? Yes No

If yes, how often?

□ **1/week**
□ 2-3/week
□ **3-4/week**
□ 4+/week

On average, how long were your bouts of exercise?

□ **Less than 30 minutes**
□ 30-45 minutes
□ **45-60 minutes**
□ More than 60 minutes

Did you exercise less than you expected/desired during the first trimester? Yes No

If yes check as many of the following reasons that apply:
□ Moring sickness
□ **Fatigue**
□ Time limitations
□ **Abdominal discomfort**
□ Physician recommendations
□ **Other:**

What types of exercises did you favor the most during the first trimester?

□ Walking
□ **Running**
□ Biking
□ **Swimming**
□ Yoga
□ **Other:**

Did you exercise during your second trimester? Yes No

If yes, how often?

□ **1/week**
□ 2-3/week
□ **3-4/week**
□ 4+/week

On average, how long were your bouts of exercise?

□ **Less than 30 minutes**
□ 30-45 minutes
□ **45-60 minutes**
□ More than 60 minutes

Did you exercise less than you expected/desired during the second trimester?
Yes No

If yes check as many of the following reasons that apply:
□ Moring sickness
□ **Fatigue**
□ Time limitations
□ **Abdominal discomfort**
□ Physician recommendations
□ **Other:**

What types of exercises did you favor the most during the second trimester?

□ Walking
□ **Running**
□ Biking
□ **Swimming**
□ Yoga
□ **Other:**

Did you exercise during your third trimester? Yes No

If yes, how often?

□ **1/week**
□ 2-3/week
□ **3-4/week**
□ 4+/week

How long were your bouts of exercise?

□ **Less than 30 minutes**
□ 30-45 minutes
□ **45-60 minutes**
□ More than 60 minutes

Did you exercise less than you expected/desired during the third trimester?
Yes No

If yes check as many of the following reasons that apply:
□ Moring sickness
□ **Fatigue**
□ Time limitations
□ **Abdominal discomfort**
□ Physician recommendations
□ **Other:**

What types of exercises did you favor the most during the third trimester?

□ Walking
□ **Running**
□ Biking
□ **Swimming**
□ Yoga
□ **Other:**

Did you stop exercising before the birth of your baby? Yes No
If yes, circle the number of weeks prior to birth 1 2 3 4 5 6 7 8 9
Reason(s) why you stopped exercising?

Type of delivery	**Vaginal**	**Cesarean**

Length of active labor (at least 4cm dilated pushing and contractions)
(hrs, min)

Did you experience any complications during labor?
Yes No

If yes, what where your complications
□ **Breech**
□ Epidural
□ **Induction**

□ Cesarean
□ **Stalled labor after 4cm dilation**
□ Difficulty pushing

Did you use any pain medications during labor?
Yes No
If yes, what type?

□ **Epidural**
□ Narcotics (circle any that apply: Demerol, Nubain, or Stadol)
□ **Local Blocks**
□ Other
□ **Not sure**

Did you have your labor induced? Yes No
If yes:
□ **Pitocin**
□ "Broke your water"

Did your medical provider immediately clamp the umbilical cord?
Yes No Unknown

Will/are you breastfeeding?
Yes No
If yes, did you have difficulty beginning breastfeeding?
Yes No
Why?

Are you using infant formula?
Yes No
If yes, why?
If yes what brand?

Did you take any supplements during pregnancy? Yes No

If yes, what kind?
□ **Prenatal vitamin (Specify Brand):**
□ Herbal supplements
□ **Other**

Did you consume fish during your pregnancy? Yes No
If yes, check average serving frequency that applies:
□ **1/week**
□ 2/week
□ **3/week**
□ Other (Specify amount per/week):

What type of fish did you consume during pregnancy:

□ Tuna
□ **Albacore**
□ Cod
□ **Salmon**
□ Trout
□ **Other (Specify type):**

If you chose not to consume fish during your pregnancy check any reasons that may apply:

□ I don't like fish
□ **I couldn't eat the fish due to nausea**
□ Mercury contamination concerns
□ **Food Safety issues (food poisoning)**
□ Costs too much money
□ **Other (Specify type):**

Did you consume any of the following foods during pregnancy?

□ Soy milk fortified with DHA/Omega-3
□ **Eggs fortified with Omega-3s**

APPENDIX G

LIST OF ENDANGERED SPECIES

Threatened and Endangered Species listed in the U.S. Fish and Wildlife Service

Last updated: May 3, 2009
(US Fish & Wildlife Service, 2009)

- **T = Threatened; E = Endangered**

Inverted Common Name	Listing Status
Catfish, Yaqui	T
Cavefish, Alabama	E
Cavefish, Ozark	T
Chub, bonytail	E
Chub, Borax Lake	E
Chub, Chihuahua	T
Chub, Gila	E
Chub, humpback	E
Chub, Hutton tui	T
Chub, Mohave tui	E
Chub, Oregon	E
Chub, Owens tui	E
Chub, Pahranagat roundtail	E
Chub, slender	T
Chub, Sonora	T
Chub, spotfin	T
Chub, Virgin River	E
Chub, Yaqui	E
Cui-ui	E
Dace, Ash Meadows speckled	E
Dace, blackside	T
Dace, Clover Valley speckled	E
Dace, desert	T
Dace, Foskett speckled	T
Dace, Independence Valley speckled	E
Dace, Kendall Warm Springs	E
Dace, Moapa	E
Darter, amber	E
Darter, bayou	T
Darter, bluemask (=jewel)	E
Darter, boulder	E
Darter, Cherokee	T

Inverted Common Name	Listing Status
Darter, duskytail	E
Darter, Etowah	E
Darter, fountain	E
Darter, goldline	T
Darter, leopard	T
Darter, Maryland	E
Darter, Niangua	T
Darter, Okaloosa	E
Darter, relict	E
Darter, slackwater	T
Darter, snail	T
Darter, vermilion	E
Darter, watercress	E
Gambusia, Big Bend	E
Gambusia, Clear Creek	E
Gambusia, Pecos	E
Gambusia, San Marcos	E
Goby, tidewater	E
Logperch, Conasauga	E
Logperch, Roanoke	E
Madtom, Neosho	T
Madtom, pygmy	E
Madtom, Scioto	E
Madtom, smoky	E
Madtom, yellowfin	T
Minnow, Devils River	T
Minnow, loach	T
Minnow, Rio Grande silvery	E
Pikeminnow (=squawfish), Colorado	E
Poolfish, Pahrump	E
Pupfish, Ash Meadows Amargosa	E
Pupfish, Comanche Springs	E
Pupfish, desert	E
Pupfish, Devils Hole	E
Pupfish, Leon Springs	E
Pupfish, Owens	E
Pupfish, Warm Springs	E
Salmon, Atlantic	E
Salmon, Chinook	T
Salmon, chum	T

Inverted Common Name	Listing Status
Salmon, coho	T
Salmon, sockeye	T
Sawfish, smalltooth	E
Sculpin, pygmy	T
Shiner, Arkansas River	T
Shiner, beautiful	T
Shiner, blue	T
Shiner, Cahaba	E
Shiner, Cape Fear	E
Shiner, palezone	E
Shiner, Pecos bluntnose	T
Shiner, Topeka	E
Silverside, Waccamaw	T
Smelt, delta	T
Spikedace	T
Spinedace, Big Spring	T
Spinedace, Little Colorado	T
Spinedace, White River	E
Springfish, Hiko White River	E
Springfish, Railroad Valley	T
Springfish, White River	E
Steelhead	T
Stickleback, unarmored threespine	E
Sturgeon, Alabama	E
Sturgeon, gulf	T
Sturgeon, North American green	T
Sturgeon, pallid	E
Sturgeon, shortnose	E
Sturgeon, white	E
Sucker, June	E
Sucker, Lost River	E
Sucker, Modoc	E
Sucker, razorback	E
Sucker, Santa Ana	T
Sucker, shortnose	E
Sucker, Warner	T
Topminnow, Gila (incl. Yaqui)	E
Trout, Apache	T
Trout, bull	T
Trout, Gila	T

Inverted Common Name	Listing Status
Trout, greenback cutthroat	T
Trout, Little Kern golden	T
Trout, Paiute cutthroat	T

APPENDIX H

PARTICIPANT ADHERENCE TO THE PROJECT

Participant Adherence to the Project

n = number of participants
n_d = number of participants participating in 3-day diet record
n_f = number of participants participating in food frequency questionnaire interview
n_b = number of participant participating in blood draw

