Estimating intake of omega-3 fatty acids during pregnancy

Rebecca Me Filipowicz

Iowa State University

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Estimating intake of omega-3 fatty acids during pregnancy

by

Rebecca Mary Elizabeth Filipowicz

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Nutritional Sciences

Program of Study Committee:
Christina Campbell, Major Professor
Sarah Nusser
Ruth Litchfield

Iowa State University
Ames, Iowa
2010

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ABSTRACT

The essential omega 3 (n-3) fatty acid alpha-linoleic acid (ALA) and the n-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) are important structural components of cell membranes. During pregnancy a steady supply of n-3 fatty acids is required to support positive fetal development (1-4) and maternal health (5-9). Over the past decade, extensive clinical research has focused on the role of DHA during pregnancy because it is incorporated into fetal brain grey matter and retina cell membranes more than any other n-3 fatty acid (4). Previous clinical research shows DHA intake during pregnancy supports positive fetal visual acuity and motor skills. No known studies have the primary objective of determining the association of the food frequency questionnaire (FFQ) estimated intakes with some other dietary assessment tool and blood biomarkers for total and individual n-3 fatty acids in pregnant women from non-coastal communities. The objective of the present study was 1) to determine the ability of the semi-quantitative FFQ to estimate intake of total n-3, ALA, EPA, DPA, and DHA and 2) to examine the relationship between the semi-quantitative FFQ and weighed 3-day diet record (3dDR) intake with plasma phospholipids (PLs) and RBC esterified fatty acids (phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) for total and individual n-3 fatty acids (ALA, EPA, DPA, and DHA) in pregnant women from two non-coastal communities. It was hypothesized that 1) a significant association between the FFQ and 3dDR for total and individual n-3 fatty acid estimated intakes would be found and 2) total and individual n-3 fatty acid intakes estimated from the FFQ would show strong correlations with RBC PE and PC total and individual n-3 fatty acid compositions. Intake of total and individual n-3 fatty acids were estimated using the FFQ and weighed 3dDR in 136 healthy, pregnant women between 15-34 weeks gestation during a prospective observational study. A subset of subjects of 64 pregnant women (25-31 weeks of gestation) were used to
determine the associations between the FFQ and weighed 3dDR and blood biomarkers. Spearman’s correlations indicated a significant positive association between the FFQ and weighed 3dDR for intake of total n-3 (rho=0.98), ALA (rho=0.27), EPA (r=0.56) and DHA (r=0.66) (P<0.01). Spearman’s correlations coefficients between the intakes estimated from the FFQ and plasma PL for EPA and DHA (rho=0.33 and rho=0.46, respectively), and RBC PE and PC for total n-3 (rho=0.39 and rho=0.27, respectively), EPA, (rho=0.45 and rho=0.36, respectively) and DHA (rho=0.56 and rho=0.47, respectively) were significant. DHA intake obtained from the 3dDR exhibited a significant positive association for plasma PL (rho=0.34) and RBC PE and PC (rho=0.40 and rho=0.42, respectively). The 3dDR DPA estimated intake was not associated with blood biomarkers. In conclusion, the semi-quantitative FFQ is an appropriate dietary assessment tool to estimate intake of total n-3, ALA, EPA and DHA and correlates well with the weighed 3dDR and blood biomarkers.
CHAPTER 1: General Introduction

Consumption of omega 3 (n-3) fatty acids during gestation has been shown to benefit mother and baby. The essential n-3 fatty acid alpha-linolenic acid (ALA) and the n-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) support maternal (1-5) and fetal health (6-9). DHA is a major component of brain grey matter and retinal phospholipids (6). Clinical research shows DHA intake during pregnancy supports positive fetal visual acuity and motor skills (6-9). The Adequate Intake (AI) for ALA for pregnant women is 1.4 g/d and is routinely met in the American diet (10). Clinical research shows DHA intake during pregnancy supports positive fetal visual acuity and motor skills development (6-9). Maternal intake of at least 200 mg DHA per day throughout gestation is recommended to support a healthy pregnancy, to prevent maternal depletion of DHA and to meet the increased fetal demands of 67 mg DHA per day during the third trimester, a period of rapid fetal growth (11-13). At this time there are no dietary recommendations for EPA and DPA intake during pregnancy. Common sources of ALA include soybean, canola oil, and flaxseed oil (12). Dietary sources of DHA are fatty fish, seafood, meat, and eggs (13); EPA and DPA are routinely found in the same dietary sources as DHA (14-15). Fortified foods and supplements are also consumable sources of ALA, EPA and DHA.

Studies reporting dietary intake of n-3 fatty acids in pregnant women are limited (15-20). Accurate assessment of n-3 fatty acid intake during pregnancy may be useful in clinical settings and during nutrition related research. Possible dietary assessment tools include the weighed diet record, 24-hour recall, and the food frequency questionnaire (FFQ). The weighed diet record and 24-hour recall measure mean usual intake (i.e. intake of nutrients consumed nearly every day) for a group (21). Unless these tools are administered in multiple instances, they do not quantify episodic intake or non-typically consumed nutrients.
such as n-3 fatty acids (22). The weighed diet record and 24-hour recall have been used as reference tools when developing an FFQ to assess fatty acid intake in non-pregnant individuals (23-28). The weighed diet record has been more commonly used as a reference method than the 24 hour recall to assess dietary fatty acid intake in non-pregnant individuals (23-27). The semi-quantitative FFQ is cost effective and has been used to measure episodic intake from 30 days to 5 years in a wide variety of populations (29-31). The FFQ has been used to measure n-3 fatty acid intake in non-pregnant individuals (23-28). No known studies exist that have the primary objective of determining the association of the FFQ with some other dietary assessment tool when intake of ALA, EPA, DPA, and DHA is measured in healthy pregnant women. The semi-quantitative FFQ may be the most suitable dietary assessment tool to measure n-3 fatty acid intake in pregnant women because EPA and DHA are episodically consume nutrients. Each of these tools has strengths and limitations that are discussed thoroughly in the following literature review.

Dietary assessment tools have also shown agreement with biological measurements (i.e. adipose and blood biomarkers) which measure nutrient status (33-36). Biological measurements provide a relatively accurate assessment of fatty acid status because they are objective, do not rely on memory or compliance of the participant, and can be used to assess short and long term status (33-36). Maternal fatty acid status has been previously measured using red blood cell (RBC) esterified fatty acids (i.e. phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) and plasma phospholipids (PLs) (33). Both RBC and plasma fatty acid fractions have shown positive correlations with n-3 fatty acid intake measured by an FFQ in non-pregnant individuals (26-27). Fatty acid compositions of RBC and plasma fractions may be useful in determining the association between maternal fatty acid status and dietary intake of fatty acids during pregnancy.
When validating a dietary assessment tool to assess the intake of a particular nutrient for specific population it is necessary to have a dietary reference method and to know the strengths and limitations both tools. Additionally, it is beneficial to compare both dietary assessment tools to a more objective method like biological measurements.

I. Specific Aims (Figure 1)

Specific Aim #1: To characterize a semi-quantitative FFQ and weighed 3dDR using descriptive statistics for their ability to estimate total n-3 fatty acids and individual n-3 fatty acids, including DHA, ALA, EPA, and DPA.

Specific Aim #2: To determine the association between the FFQ and the 3dDR when intake of total n-3 and individual n-3 fatty acids (ALA, EPA, DPA, and DHA) are estimated in women during the 2nd and 3rd trimester of pregnancy.

Null Hypothesis: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the FFQ is not associated with the total n-3 and individual n-3 fatty acid (DHA, ALA, EPA, and DPA) intake collected via the 3dDR in women during the 2nd and 3rd trimester of pregnancy.

Alternative Hypothesis: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the FFQ is associated with the total n-3 and individual n-3 fatty acid (DHA, ALA, EPA, and DPA) intake collected via the 3dDR in women during the 2nd and 3rd trimester of pregnancy.

Specific Aim #3: To determine the association between the total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intakes estimated via the FFQ and 3dDR with the total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status determined by blood biomarkers (RBC and plasma) in women during the 2nd and 3rd trimester of pregnancy.

Null Hypothesis 1: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the FFQ is not associated with total n-3 and
individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of RBC biomarkers (PE, PC) in women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy.

Alternative Hypothesis 1: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the FFQ is associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of RBC biomarkers (PE, PC) of in women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy.

Null Hypothesis 2: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA, and DHA) intake estimated via the FFQ is not associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of plasma blood biomarker (PL) of in women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy.

Alternative Hypothesis 2: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the FFQ is associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of plasma blood biomarker (PL) of in women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy.

Null Hypothesis 3: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the 3dDR is not associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of RBC biomarkers (PE, PC) of in women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester of pregnancy.

Alternative Hypothesis 3: The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the 3dDR is associated with total n-3 and
individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of RBC biomarkers (PE, PC) of women during the 2nd and 3rd trimester of pregnancy.

**Null Hypothesis 4:** The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the 3dDR is not associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of plasma blood biomarker (PL) of women during the 2nd and 3rd trimester of pregnancy.

**Alternative Hypothesis 4:** The total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) intake estimated via the 3dDR is associated with total n-3 and individual n-3 fatty acid (ALA, EPA, DPA and DHA) status measured by the analysis of plasma blood biomarker (PL) of women during the 2nd and 3rd trimester of pregnancy.

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**Figure 1. Specific Aims Flow Chart.**
Thesis Organization

This thesis begins with a general introduction and specific aims. The literature review focuses on omega 3 fatty acids, dietary assessment tools, and biomarker assessment tools. Third, the research methods are provided. The papers Dietary intake of total and individual n-3 fatty acids by pregnant women: associations between the semi-quantitative food frequency questionnaire and weighed diet record and Validation of a semi-quantitative food frequency questionnaire with blood biomarkers in pregnant women for total and individual n-3 fatty acids will be submitted to the Journal of American Dietetic Association. A general conclusion is included following the papers.
CHAPTER 2: Literature Review

I. Omega-3 Fatty Acids

a. Stereochemistry and nomenclature (37-38)

Fatty acids are a class of lipids. Stereospecific numbering (sn) is used to describe fatty acid molecules and can easily be explained by using the triacylglycerol (TAG) form of a fatty acid as an example. Triacylglycerols are composed of a three carbon glycerol backbone. Drawn in a Fischer Project, the first carbon that appears on the top of the glycerol backbone and that is bound to a hydroxyl group is labeled sn-1. The second carbon, located below the sn-1 carbon and typically bound to a carboxyl group, is labeled sn-2; the third carbon, labeled sn-3, is also bound to a hydroxyl group. More vaguely described, a fatty acid is of a hydrophilic carboxylic group followed by a series of methylene linked groups which form a hydrophobic tail.

Fatty acids are classified by the number of carbons within a hydrocarbon chain. Short chain fatty acids contain between 3 to 7 carbon atoms while medium chain fatty acids contain 8 to 13 carbon atoms; long chain fatty acids contain 14 to 22 carbon atoms and very long chain fatty acids contain more than 22 carbons. Most naturally occurring fatty acids are in the cis-configuration (Figure 2). Nomenclature of fatty acids is determined by the length of the hydrocarbon chain and the number and location of double bonds with the chain. There are various methods for naming fatty acids. Systematic naming of fatty acids is determined by the number of carbon atoms the molecule contains, the location of the first double bond from the carboxylic group of the fatty acid and the location of the remaining carbon-carbon double bonds with in the hydrocarbon chain. For example, the systematic name of DHA is cis-4, cis-7, cis-10, cis-13, cis-16, cis-19 docosahexaenoic acid. As a result of this lengthy and cumbersome name, fatty acids are often referred to in shorthand or the “n minus” naming system. Using this identification method, the first number listed defines
the number of carbons within the chain, the second number indicates the number of double bonds and “n minus” indicates the location of the double bond closest to the methyl end of the molecule. Using DHA as an example again, it has 22 carbons within its hydrocarbon chain and 6 double bonds with the first double bond located at the omega 3 (n-3) position: 22:6n-3.

![Figure 2. Structure of docosahexaenoic acid (DHA).](image)

b. Digestion, absorption, metabolism, and compartmentalization of fatty acids

Dietary fatty acids include non-esterified fatty acids (NEFA), TAG, plasma phospholipids (PLs), and cholesterol. Digestion of dietary fat begins in the stomach through the activation of lingual and gastric lipases. It is continued in the small intestine via phospholipase A2, colipase, cholesterol esterase, and pancreatic lipase. The end products of the digestive process are lipid moieties such as NEFAs, monoacylglycerides, diacylglycerides, and TAGs.

Lipid moieties are absorbed into the enterocyte of the small intestine. Within the enterocyte, dietary fatty acids are emulsified by bile salts to form micelles which then form nascent chylomicrons. Nascent chylomicrons are immature or functionally incomplete chylomicrons composed of TAG, PL, cholesterol and apolipoproteins specifically apolipoprotein 48 (apo 48). Apolipoproteins are fat binding proteins.

When the chylomicron is released from the enterocyte into portal and then systemic circulation it becomes ‘mature’ or a lipoprotein through the addition of apo C and apo E proteins (37). It is the plasma PL found in the lipoprotein molecule that is measured to
assess short term fatty acid status like DHA. Typically, DHA is measured in the sn-2 position of plasma PL of circulating lipoproteins (Figure 3).

As the apolipoproteins of the lipoprotein come in contact with lipoprotein lipase of extrahepatic tissues (adipose and muscle), the TAG rich particle shrinks in size as it donates monounsaturated fatty acids, polyunsaturated fatty acids, long chain polyunsaturated fatty acid (LCPUFAs), saturated fats, and trans fats to the tissues (36). Once delivered to the cell these lipids play a variety of functions (e.g. cell signaling and membrane structure). Unlike extrahepatic tissues, RBC does not use lipoprotein lipase to acquire fatty acids. Albumin transports plasma fatty acids into RBC membranes via acylation-deacylation. Red blood cell membranes have a mosaic bilayer membrane made of cholesterol, protein, and phospholipids (glycerophospholipids and sphingolipids) (36). Sphingolipids are comprised of a de novo synthesized sphingoid base backbone amide linked to a long chain fatty acid (36). Sphingomyelins are the sphingolipids typically found in the outer membrane of the RBC. Glycerophospholipids are comprised of carbon backbone made of one to three fatty acid esters. There are classified into two groups-phosphoglycerides and glycosylglycerides (36). Two phosphoglycerides (i.e. phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) are commonly found within the RBC lipid bilayer and are acyl-linked at sn2 position fatty acid glycerol backbone. Within the RBC lipid bilayer PC favors the outer membrane and PE favors the inner membrane (37). While most fatty acids transferred into the RBC membrane are preferentially acyl-linked to PE, both phosphoglycerides are used to quantify fatty acid status when using the RBC (Figure 3) (38).
c. Omega 6 and omega 3 fatty acid metabolism

The two essential fatty acids (EFAs) linoleic acid (LA) and alpha-linolenic acid (ALA) cannot be synthesized by human tissue and must be obtained from the diet for normal development of specialized cells throughout the body (40). This is because humans lack the delta (Δ) 12- and Δ 16- desaturase enzymes needed to introduce carbon-carbon double bonds between the tenth carbon and the methyl end of the fatty acid chain of stearic acid to endogenously form LA or ALA (37-38).

Linoleic acid, an omega 6 (n-6) fatty acid, is the metabolic precursor to dihomo-y-linolenic acid (DGLA). It forms DGLA through a series of enzymatic reactions involving delta (Δ) 6-desaturase and elongase (Figure 4). The function of a desaturase enzyme is to form
carbon-carbon double bonds and the function of an elongase is to lengthen fatty acid chains by the addition of two carbon units. Dihomo-y-linolenic acid is further desaturated to arachidonic acid (ARA) via \( \Delta 5 \)-desaturase. Arachidonic acid is the precursor for many pro-inflammatory prostaglandins and leukotrienes (39). If ARA is not used for the formation of pro-inflammatory molecules it undergoes further enzymatic reactions modulated by elongase and \( \Delta 6 \)-desaturase and then \( \beta \)-oxidation (40). The end product of this series of reactions is n-6 DPA.

Alpha-linolenic acid, an n-3 fatty acid, is the precursor to endogenously formed EPA. The formation of EPA from ALA is dependent on the same enzymes (\( \Delta 6 \)-desaturase, elongase, and \( \Delta 5 \)-desaturase) used to convert LA to ARA. Eicosapentaenoic acid is a precursor for many anti-inflammatory prostaglandins, leukotrienes, and resolvins (41); it is also the metabolic intermediate preceding DHA during n-3 fatty acid metabolism. Elongase adds a two carbon unit to EPA to form n-3 DPA. Similar to ARA conversion to n-6 DPA, n-3 DPA undergoes a series of reactions catalyzed by elongase and \( \Delta 6 \)-desaturase and finally \( \beta \)-oxidation to form DHA (Figure 4).

The conversion of ALA to EPA, DPA, and DHA primarily occurs in the endoplasmic reticulum of the liver (39). According to a review of literature conducted by Child et al. (42) in 2008, the conversion efficiency of ALA to EPA, DPA and DHA has been shown to be greater in women than men (43-44). The estimated conversion of ALA to EPA, DPA, and DHA in women of reproductive age was 21%, 6% and 9%, respectively (44). However, it is worth noting the dietary factors influencing the rate of conversion during gestation are not yet fully understood. Two major hypotheses exist about the conversion of ALA to EPA, DPA and DHA. The first hypothesis states the conversion of ALA to long chain polyunsaturated fatty acids (LCPUFAs) is dependent on n6:n3 ratio in the diet (45). For example, diets rich in LA and lacking in ALA result in greater \( \Delta 6 \) desaturase activity in the n-6 pathway (greater
conversion of LA to ARA). Similarly, diets low in LA and high in ALA would favor the metabolic pathway that produces EPA and possibly DHA (Figure 4). The second hypothesis states the conversion of ALA to LCPUFAs is influenced by the absolute amounts of ALA and LA in the diet. A tracer study conducted by Goyens et al. (46) indicated an increase in dietary ALA results in an increase of DHA synthesis and a decrease in dietary LA intake results in an increase in EPA synthesis. However, placental transfer of EPA, n-3 DPA, and DHA endogenously formed from ALA is limited during pregnancy (47). In conclusion, the consumption of preformed EPA, DPA, and DHA are preferred over endogenous formation to meet metabolic demands (48).

**Figure 4:** Long Chain PUFA Biosynthesis Pathways. Adapted from Gurr et al. (36).
According to Institute of Medicine (IOM) of the National Academies the Adequate Intake (AI) for LA of 12 g/d for non-pregnant women and 13 g/d for pregnant women are routinely met in the United States (10). The average US population intake of ALA reported by the NHANES III database is 1.33 g/d (49). Additionally, women tend to consume more ALA than men (48). Sources of LA include nuts (e.g. almonds, Brazil nuts, and peanuts), terrestrial animal products (e.g. ham and chicken egg), and vegetable oils such (e.g. soybean, safflower, and corn) (11). The AI for ALA for non-pregnant women is 1.1 g/day and 1.4 g/d for pregnant women; both AIs are routinely met in the American diet (10). The major sources of ALA include soybean, canola oil, and flaxseed oil (11).

The NHANES III database reports only 25% of the population reported an intake of EPA and DHA; the average US population intake of EPA and DHA is 0.04 g/d (0.02 %kcal/d), and of DHA is 0.07 g/d (0.03 %kcal/d) (49). No value was reported for DPA intake. There is no AI for EPA, n-3 DPA, or DHA. However, the International Society for the Study of Fatty Acids and Lipids recommends at least 200 mg DHA per day throughout gestation (10). Additionally, the American College of Obstetricians and Gynecologists and the United States Department of Agriculture Dietary (USDA) Guidelines Committee currently recommend 1-2 servings (20-50 g) of fish per week during pregnancy (5-10). Main dietary sources of EPA, n-3 DPA, and DHA are fatty fish, seafood, fish oil supplements, algae supplements, and fortified foods (11) (e.g. Silk soy milk and Smart Balance Omega Plus Buttery Spread) (Table 1). Meat and eggs also contain EPA, n-3 DPA, and DHA (12).
Table 1. Food and supplement sources of ALA, EPA, n-3 DPA and DHA. Adapted and modified from Nutritionist Nutritionist Pro Version 4.2.2 (2009) dietary intake analysis software (Axxya Systems; Stafford, TX) and the USDA Nutrient Database for Standard Reference.

<table>
<thead>
<tr>
<th>Food</th>
<th>Serving</th>
<th>ALA (g)</th>
<th>EPA (g)</th>
<th>n-3 DPA (g)</th>
<th>DHA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon, Atlantic Farmed 3 oz</td>
<td>0.189</td>
<td>0.977</td>
<td>0.393</td>
<td>1.252</td>
<td></td>
</tr>
<tr>
<td>Tuna, light canned in water 3 oz</td>
<td>0.002</td>
<td>0.040</td>
<td>0.018</td>
<td>0.190</td>
<td></td>
</tr>
<tr>
<td>Nordic Natural Omega 3 fish oil supplement</td>
<td>1 g capsule</td>
<td>0.140</td>
<td>0.330</td>
<td>Not reported</td>
<td>0.220</td>
</tr>
<tr>
<td>Chicken breast, meat only raw 4 oz</td>
<td>0.011</td>
<td>0.000</td>
<td>0.004</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Silk Soy milk 8 oz</td>
<td>0.184</td>
<td>Not reported</td>
<td>Not reported</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>Smart Balance Spread Omega Plus Buttery Spread 1 T</td>
<td>0.320</td>
<td>0.016</td>
<td>Not reported</td>
<td>0.016</td>
<td></td>
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<tr>
<td>Large Egg 1 item</td>
<td>0.052</td>
<td>0.015</td>
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e. Omega 3 fatty acids during pregnancy

i) Maternal Health

It is hypothesized that women who consume n-3 fatty acid during pregnancy may prevent preeclampsia and postpartum depression. It is important to note the results highlighted in the following text are still controversial and further investigations are underway.

Preeclampsia affects at least 5-8% of all pregnancies in the United States (US) (52). The symptoms of preeclampsia (edema, hypertension, and proteinuria) are associated with increased cardiovascular risk later in life for the mother (2). The essential n-3 fatty acid ALA and the n-3 fatty acids EPA, DPA, and DHA prevent preeclampsia (1-2). It was previously acknowledged in this review of literature that n-3 fatty acids are metabolic precursors of eicosanoids. The eicosanoids thromboxane B3 and prostacyclin I3 have been hypothesized to prevent pre-eclampsia via vasoconstriction and platelet aggregation, respectively (53).

Secher, Sereger, and Olsen conducted a series of studies (54-57) that used various levels of n-3 fatty acid supplementation and placebo oils during pregnancy to detect any change in
eicosanoid production and incidence of preeclampsia. The results of these studies indicate that n-3 fatty acid supplementation was more effective at preventing preeclampsia than placebo supplementation. For example in one of the studies by this research group (57) 2.7 g of fish oil, a source of n-3 fatty acids or a control regimen of olive oil or no supplement was given to each participant (n=47) between 30-37 week of gestation. At week 37, a significantly greater increase in the eicosanoids thromboxane B3 and prostacyclin I3 was observed in the experimental group. This increase in eicosanoids is believed to prevent hypertension during pregnancy via vasoconstriction (57-59).

Postpartum depression occurs in roughly 13% of U.S. women after they have given birth (60). Symptoms include sadness, fatigue, insomnia, appetite changes, reduced libido, crying episodes, anxiety, and irritability (60). Postpartum depression has been linked to DHA depletion during gestation (4). Recently, it has been hypothesized the increased risk of DHA depletion during the third trimester may trigger depression before delivery and/or set the stage for postpartum depression (4-5). The relationship between maternal status of DHA and the onset of depression before delivery was shown by the Rees et al. (4). During the third trimester of pregnancy women with no known prior diagnosis of depression underwent psychological evaluation. Each woman was tested for current depression using the Edinburgh Depression Scale. The psychological evaluation indicated 16 of the 22 women enrolled in the study were depressed. Analysis of plasma phospholipids indicated significantly lower levels of DHA and total n-3 fatty acids in the depressed women. Additionally, higher levels of DHA and total n-3 fatty acid measured in the non depressed women were associated with significantly lower odds of depression. The association of depleted n-3 fatty acid levels with postpartum depression was examined by De Vriese et al. (5). Significant depressed levels plasma PL and RBC PE fatty acid composition were observed in women who developed a postpartum depression as compared to the control.
mothers. They were unable to calculate the correlation between depression diagnosis and fatty acid compositions because onset of depression between women varied.

ii) Fetal Health

DHA is a major component of brain grey matter and retinal phospholipids (6). Clinical research shows n-3 fatty acids, specifically DHA, intake during pregnancy supports positive fetal neurodevelopment, motor skills and visual acuity (6-9). Hibbeln et al. (61) measured the impact of maternal n-3 fatty acid consumption from fish during gestation on infant and adolescent neurodevelopment and motor skills development during the Avon Longitudinal Study of Parents and Children. Infant/child cognition and motor skills were examined at several time points after birth. Greater maternal intake of n-3 fatty acids during gestation was associated with significantly lower risk of suboptimum verbal IQ in the offspring at 8 year old (1-340 g fish/wk vs >340 g fish/wk: OR=1.43 and no fish intake vs >340 g fish/wk: OR=2.16, P<0.0001). Additionally, greater maternal intake of n-3 fatty acids was associated with lower suboptimal risk for poorly developed motor skills in the infant at 42 months of age (no fish intake vs >340 g fish/wk: OR=1.68, P<0.0001). These results along with two well conducted longitudinal studies (62-64) indicate that consumption of foods containing DHA during pregnancy may prevent suboptimal neurodevelopment and poor motor skill development in offspring.

The retina has also been shown to contain high amounts of DHA at birth (63). Malcolm et al. (65) examined the role of maternal supplementation of DHA on infant development by measuring visual evoked potential maturation (VEP) and infant cord RBC DHA compositions. A significant correlation was observed between VEP peak latencies and decreased levels of DHA composition in infant cord RBCs (β=0.38, P<0.001). These findings are similar to results provided by other well respected labs (66-67) and indicate that
maternal DHA intake during pregnancy does support healthy retina development in neonates.

In conclusion, n-3 fatty acids support a healthy pregnancy. Women consuming n-3 fatty acid during pregnancy may prevent preeclampsia and postpartum depression. Furthermore, those neonates born to mothers with poor n-3 fatty acid status can be born with a dysfunctional CNS, poor cognitive function, impaired vision, growth retardation, and reproductive failure, fatty liver and dry skin (68).

II. Dietary intake assessment tools

Accurate assessment of n-3 fatty acid intake during pregnancy may be useful in clinical settings and during nutrition related research. Possible dietary assessment tools include the FFQ, weighed diet record, and 24-hour recall.

a. The food frequency questionnaire

The FFQ is a dietary assessment tool commonly used because it is non-invasive and easy to analyze for a variety of population types and sizes over various lengths of time (one month to five years) (29-30). The primary objective of the FFQ in clinical research is to discriminate between high and low consumption of episodically or non-typically consumed foods and/or nutrients like n-3 fatty acids (29). The FFQ can be designed to quantify episodic intake over time by assessing portion size and frequency. It can be administered through an interview or by one’s self. The FFQ has been used to estimate n-3 fatty acid intake in non-pregnant individuals (26-27). No known studies exist that have the primary objective of determining the association of the FFQ with some other dietary assessment tool when intake of ALA, EPA, DPA, and DHA is measured in healthy pregnant women. However, the semi-quantitative FFQ may be the most suitable dietary assessment tool for measuring episodically consumed nutrients like dietary n-3 fatty acid intake in pregnant women. The validity of the FFQ is determined by exploring the association between the
FFQ and other intake and status reference methods (i.e. other dietary assessment tools and biomarkers, respectively) (29).

b. Dietary reference tools for the validation of the FFQ

Choosing a reference method depends on funding availability and study design. The weighed diet record and 24-hour recall have been used as a reference method when developing an FFQ to assess fatty acid intake in non-pregnant individuals (23-28).

i) The weighed diet record

The weighed diet record does not rely on memory and it can assess mean usual intake (i.e. intake of nutrients consumed nearly every day) for a group (29). However, unless it is administered in multiple instances, it cannot always quantify episodic intake (22). The measurements obtained from the weighed diet record have shown to be superior to memory recall of portion sizes used during the FFQ and 24 hr recall (28-29). A known pitfall of weighed records is possible decreased compliance over time when an individual is aware of the weighed amount of food he/she is consuming and the cumbersome act of weighing each individual food consumed (29-30). The FFQ has demonstrated agreement with the weighed diet record for assessing dietary intake of multiple nutrients, including DHA (23).

Sullivan et al. (26) determined the association between a semi-quantitative FFQ and a weighed diet record when LCPUFA intake was estimated in healthy male and female participants (n=53). Repeatability of the FFQ was also tested 4 to 6 weeks later by re-administration of the same FFQ. The 28-item self administered semi-quantitative FFQ was designed to assess LCPUFA intake from the previous three months. Frequency was assessed using ten options ranging from never to intake per day, week, and month. Open ended questions allowed the subject to clarify fortification, brands, and types of meat. Serving size was established by comparing estimated portion sizes to a regular size dinner plate. The weighed food record was completed over two week days and one weekend day
by recording weight and food description. Forty-five subjects completed the FFQ and weighed diet record. Thirty-three subjects participated in the reproducibility of the FFQ. The mean intakes of total LCPUFA, EPA, DPA, and DHA from the two FFQs did not differ significantly. Spearman’s correlation coefficients between FFQ1 and FFQ2 were also significant for total LCPUFA (rho=0.88), EPA (rho=0.88), DPA (rho=0.90), and DHA (rho=0.87) (P<0.05). Spearman’s correlation coefficients between the FFQ and weighed diet record for total LCPUFA, EPA, DPA, an n-3, and DHA intake were rho= 0.75, rho=0.64, rho=0.62, and rho=0.72, respectively. Bland-Altman plots also showed that no systematic variation existed between the two methods for assessing total LCPUFA, EPA and DHA. The Bland-Altman plots showed no systemic bias existed between the two dietary assessment methods when PUFA, EPA, and DHA were measured. More specifically the Bland-Altman plots showed homoscedasticity or constant scatter of differences across the range of means (68). Finally, data were classified into quintiles based on intake estimated by the FFQ and diet record; 49% of subjects were classified into the same quartile when intakes of n-3 LCPUFAs were estimated. These results indicate an FFQ designed to assess specific nutrient intake, the measurements of intake can be characterized as valid, repeatable and shows agreement with the weighed diet record. Furthermore, the unbiased distribution of the Bland-Altman plots indicates the days in which the weighed diet record was collected episodically consumed nutrients were captured.

Similarly, Broadfield et al. (27) customized a previously validated semi-quantitative FFQ to assess dietary fatty acids in healthy adults (n=31, demographics unspecified) using a weighed diet record as the reference method. The FFQ was adapted to collect greater detail about food items that contain fatty acids (e.g. dairy products and fats and oils used for spreading and cooking) consumed over the previous month. The adapted FFQ assessed 129 items by assigning commonly used portion sizes (e.g. slice of bread or teaspoon of
(sugar) and verifying frequency of consumption as follows: seven times per week, 2-3 times per week, or rarely-never. The weighed diet record was collected over seven consecutive days and subjects were instructed to include names of all food and beverages consumed. Pearson and Spearman correlation coefficients were used to assess agreement between the two dietary assessment methods. The mean intake of individual fatty acids assessed by the 7dDR was typically greater than the mean intake of individual fatty acids assessed by the FFQ apart from oleic acid, LA, and total n-6 fatty acid. Pearson correlation coefficients were calculated to determine the relationship between the FFQ and weighed diet record for the parametric data and were as follows for the energy adjusted data: total fat \( r=0.40 \), total SFA \( r=0.80 \), palmitic acid \( r=0.77 \), stearic acid \( r=0.70 \), total MUFA \( r=0.21 \), oleic acid \( r=0.20 \), total PUFA \( r=0.20 \), total n-6 \( r=0.26 \), LA \( r=0.24 \), DGLA \( r=0.62 \), and total n-3 \( r=0.26 \). Spearman correlation coefficient were calculated to determine the relationship between the FFQ and weighed diet record for ARA \( \rho=0.70 \), EPA \( \rho=0.50 \), and DHA \( \rho=0.37 \) because these were the only data to exhibit non normal distribution after being adjusted for energy. These results indicated that an FFQ and weighed diet records show agreement when assessing dietary fatty acid intake. However, the wide range of reported correlations may indicate that further refining of this particular FFQ may be needed.

ii) The 24-hour recall

The primary objective of the 24-hour recall in the clinical setting is to measure mean usual intake or nutrients consumed nearly every day by a group (28). The 24-hour typically recall collects intake from the preceding day, in a short amount of time (15-20 minutes) without altering normal intake (29-30). The 24-hour recall relies on client memory. It is usable in diverse populations and has a relatively less burdensome on the client than the diet record (29). Randomly administered 24 hour dietary recalls reduce the possibility of influencing habitual eating patterns because they can be administered without anticipation.
Like the weighed diet record, when used in multiple instances, the 24-hour recall has an increased chance of measuring episodically consumed nutrients such as n-3 fatty acids. Disadvantages of the 24-hour recall may include difficulty estimating portion size without food models and variance between day to day intakes. The 24-hour recall has been less commonly used as a reference method than the weighed diet record to assess dietary fatty acid intake in non-pregnant individuals.

In 2007, Segovia-Siapco validated the use of a 171-item semi-quantitative FFQ against six randomly assigned 24 hour dietary recalls to assess nutrient intake in two experimental groups: walnut supplemented diet and a control diet (n=87). The FFQ was administered in person and the 24-hour dietary recalls were administered over the telephone. The FFQ assessed the previous six months intake by documenting the following consumption frequencies: 1-3 times per month, 1 time per week, 2-4 times per week, 5-6 times per week, 1 time per day, 2-3 times per day and so on. Mean nutrient intake reported by the FFQ was higher than mean nutrient intake reported by the 24-hour recalls for all nutrients except protein, ALA, cholesterol, and alcohol. As a result of attenuation the following equation was used to determine the relationship between the tools:

\[ r_c = r_o \sqrt{1 + \frac{s_w^2}{s_B^2} / n} \]

Where \( r_c = \) corrected/de-attenuated correlation coefficient; \( r_o = \) uncorrected/de-attenuated correlation coefficient between the FFQ and multiple 24-hour recalls; \( s_w^2 = \) within-person variance of the multiple 24-hour recalls; \( s_B^2 = \) between-person estimate of variance in the reference method (24-hour recalls); \( n = \) number of repeated measures of the dietary recalls. Statistically significant de attenuated correlation coefficients were found between the FFQ and 24-hour diet recall for adjusted total energy (\( r = 0.39 \)), total carbohydrate (\( r = 0.42 \)), vegetable protein (\( r = 0.43 \)), total fat (\( r = 0.51 \)), polyunsaturated fat (\( r = 0.77 \)), LA (\( r = 0.78 \)), ALA
(r=0.79), and total fiber (r=0.60) (P<0.001). Bland-Altman plots showed good agreement between the two tools when assessing ALA. As a result of the variation between person ARA, EPA, and DHA could not be corrected for measurement error; these nutrients also exhibited some of the lowest correlations between the FFQ and 24-hour recalls: ARA (r=0.14), EPA (r=0.20), and DHA (r=0.15). These lower correlations may have resulted from administration of the 24-hour recall on a day(s) episodically consumed nutrients were not eaten. This study suggests that a FFQ and 24-hour dietary recall show agreement for mean usual intake but not episodic intake of nutrients for the group. This is a common limitation when assessing dietary intake.

III. Biological Measurements

Dietary assessment tools have also shown agreement with biological measurements (33-36). Biological measurements (i.e. isotopes and biomarkers) provide a relatively accurate assessment of fatty acid status because they are objective and do not rely on memory or compliance of the participant (33). A disadvantage of biological measurements is that they do not reflect true absolute intake. This disadvantage is a result of the affects of digestion, absorption, uptake, utilization, metabolism, excretion, homeostatic mechanisms, and error associated by the biochemical measures themselves (33).

a. Isotopes

Isotopes have recently become a means of assessing DHA status (32). Isotopes are less expensive to analyze than blood biomarker, highly accurate, and do not require the same special handling as other biological samples (27). The physiological influences of isotopes are not fully understood in humans, let alone pregnancy. Therefore, the use of isotopes as a reference method to determine the best dietary tool to assess DHA status in pregnant women is inappropriate at this time.
b. Biomarkers

Biomarkers used to assess n-3 fatty acid status are adipose tissue and blood (45). When determining the association of a biomarker to dietary intake, the data must be collected in a timeframe that is relative to the biomarker half life ($t_{1/2}$). For example, a FFQ designed to assess dietary intake over a year should be validated using adipose tissue because the estimated $t_{1/2}$ of adipose tissue TAG is between 1 and 2 years (28). Pregnancy occurs over a time frame of ten months that does not correspond with the half life of TAG found in adipose tissue. Additionally collecting adipose biopsies may be perceived as too invasive and diminish participation levels. Therefore, adipose is not a favorable biological reference method for the determining the association of dietary n-3 fatty acid intake and status in pregnant women.

An FFQ designed to measure the previous month’s intake of fatty acids has been associated with RBC and plasma ($t_{1/2} =$50 days and $t_{1/2} =$10 days, respectively) fatty acid status (33). The blood sample needed to assess RBC and plasma fatty status is collected via venipuncture. Compared to an adipose biopsy this procedure is less invasive. Both RBC and plasma fatty acid fractions have repeatedly shown positive correlations with n-3 fatty acid intake measured by an FFQ in non-pregnant individuals (26-27).

i) Blood Biomarkers

Several studies have shown that n-3 intake measured by an FFQ correlates with plasma or RBC fatty acid status. Intake is defined as dietary consumption of a specific nutrient; status is defined as the biological level of a specific nutrient as a result of intake. The use of plasma (PLs) and RBC (PE and CE) fatty acid biomarkers to assess status has become increasingly important because of the association of n-3 fatty acid, specifically DHA, with health.
McNaughton et al. (69) determined the association between intake and status when PUFAs were measured in a subset of randomly selected adult participants (n=43; 18 males and 25 non-pregnant females) from an ongoing randomized controlled trial. Dietary intake was measured by a self-administered FFQ (assessed food intake over the past 6 months) and weighed diet record (two non-consecutive days) collected every 2 months for 12 months. The FFQ consisted of 129 specific foods with nine frequency options ranging from ‘never’ to ‘4+ times per day’. Serving size was specified using household measurements (cups or spoons) and common sizes or portions of food items (1 slice of bread). Cooking methods, take-out meals, added types of fats and oils used for cooking and added to foods, and nutritional supplements were assessed. Non-fasting venous blood samples were collected and immediately processed. Plasma PLs were separated and quantified using gas-liquid chromatography. Mean intakes reported by the FFQ were not significantly different from mean intakes reported by the weighed diet record. Spearman’s correlation coefficients for the weighed diet record vs plasma FA were significant: total n-3 (rho=0.33), total LCPUFA (rho=0.44), ARA (rho=0.35), and DHA (rho=0.43) (P<0.05). Similarly, the FFQ vs plasma PLs correlation coefficients for total LCPUFA (rho=0.38), LA (rho=0.34), and DHA (rho=0.32) were significant (P<0.05). The triad method was used to estimate how well each correlation was related to true, but unknown intake. This measurement was termed validity coefficient (VC) and measured using the following equations:

\[
VC_{QT} = \sqrt{\frac{r_{QR} \times r_{RB}}{r_{QB}}} ;
VC_{RT} = \sqrt{\frac{r_{QR} \times r_{RB}}{r_{QB}}} ;
VC_{MT} = \sqrt{\frac{r_{QR} \times r_{RB}}{r_{QB}}}
\]

Where \( r_{QR} \) = correlation coefficient between the FFQ and weighed diet record; \( r_{QB} \) = correlation coefficient between the FFQ and blood biomarker; \( r_{RB} \) = correlation coefficient between the weighed diet and the blood biomarker. \( VC_{QT} \) = validity coefficient between FFQ
and true intake; $VC_{RT} =$ validity coefficient between weighed diet record and true intake; $VC_{BT} =$ validity coefficient between blood biomarker and true intake. The triad method indicated the validity of the FFQ was highest for total LCPUFA, EPA, and DHA (respectively $r=0.63$, $r=0.62$, $r=0.62$); the validity of the plasma fatty acid was highest for ARA, DHA, and total n-3 FA ($r=1.0$, $r=0.83$, $r=0.78$, respectively). The triad method found validity of the weighed diet record was highest for total n-3 FA, LCPUFA, and DHA ($r=0.43$, $r=0.60$, $r=0.52$, respectively). Additionally, 42% of individuals were classified in the same quintiles when intakes of n-3 LCPUFAs were estimated using the FFQ and weighed diet record. The results of this study indicate a positive relationship between the FFQ, weighed diet record, and plasma fatty acid biomarkers. The weighed diet record was associated with the blood biomarkers more often than the FFQ. These results are expected because the weighed diet record reflects short term intake and plasma PL fatty acid compositions reflect status from days (40) or weeks to months (70). However, it may be more appropriate to use the FFQ and RBC fatty acid compositions to understand the association between long term intake and status because there is a wide variation in plasma PLs over time (74).

Sun et al. (71) examined the relationship between fatty acid intake collected via the semi-quantitative FFQ with plasma and RBC fatty acid biomarkers and in women ($n=306$), ages 43-69 years during the Nurse’s Health Study. Fatty acid intake from the previous year was assessed using a semi-quantitative FFQ (130+ food items) in 1984, 1986, and 1990 to determine mean usual intake for a group. Cooking oil, fat for frying and baking, and the addition of butter or margarine to food were assessed by specifying portion size and the frequency of consumption. Frequency was assessed as follows: never, less than a one per month, and 6 or more times per day. Three FFQ questions targeted nonspecific intake of canned tuna and nonspecific intake of fish (i.e. dark meat fish and other fish). Blood samples were collected via venipuncture, processed and analyzed by gas-liquid
chromatography for plasma and RBC lipid FA content. Fat intake was reported as a percent of total fat consumed. Fatty acid composition of the plasma and RBCs were reported as a percent of total fatty acids. The fractions of plasma and RBCs measured (i.e. PLs, PE, and PC) were not specified. Dietary DHA intake showed the strongest Spearman’s correlation with RBC (rho=0.56) and plasma (rho=0.48) fatty acid levels; EPA was correlated more with RBC fatty acids (rho=0.38) than EPA plasma fatty acids (rho=0.21). Linoleic acid exhibited the third strongest correlation between intake and RBC and plasma fatty acids status (rho=0.25 and rho=0.24, respectively). The correlation coefficients for ARA and DPA were near zero. The correlation coefficient between intake and DHA RBC content in 1984, 1986, 1990 was rho=0.41, rho=0.43, and rho=0.56, respectively. The correlation coefficient between intake and EPA RBC content in 1984, 1986, 1990 and cumulative time was respectively rho=0.30, rho=0.37, rho=0.38, and rho=0.41. Similar correlation coefficients for DHA were found between plasma FAs and intake in 1984, 1986, 1990 and cumulative time (rho=0.33, rho=0.36, rho=0.48, and rho=0.44, respectively). Average total plasma and RBC FA content were closely correlated (rho=0.72). Overall, this study illustrates a correlation between dietary fatty acid intake and fatty acids composition in RBC and plasma; correlations were stronger for RBC fatty acids than for plasma acids. These results suggests that RBC fatty acid analysis for n-3 content may be appropriate for long-term intake measurements collected via a FFQ (69). Further investigation may warrant using different time variations (e.g. every 6 months vs. every 30 days) for assessing intake to determine if RBC fatty acid analysis is more appropriate to plasma fatty acid analysis when validating a FFQ because of the difference in each biomarker’s t1/2.

Similarly, Sullivan et al. (22) validated the use of a semi-quantitative FFQ to assess LCPUFA intake against RBC and plasma fatty acid levels. The FFQ was previously validated against a weighed diet record and tested for repeatability (21). Fifty-three
subjects (20 males and 33 females) completed the FFQ. Blood samples were collected via venipuncture, processed and analyzed for RBC and plasma fatty acid compositions (fractions not specified) using gas-liquid chromatography and the Folch method (73), respectively. Statistically significant Spearman’s correlation coefficients were reported between EPA and DHA intake and the RBC percent FA content (rho=0.40 and rho=0.39, respectively) (P<0.01). Total LCPUFA intake also statistically correlated with the RBC total LCPUFA fatty acid composition content (rho=0.50, P<0.001). Plasma fatty acid content was positively correlated with total dietary LCPUFA (rho=0.54), EPA (rho=0.54) and DHA (rho=0.48) (P<0.001). These results suggest that RBC and plasma fatty acids are possible reference methods for validation of an FFQ for total and individual LCPUFA intake.

**FFQ and blood biomarker associations in pregnant individuals**

To date the number of FFQs validated to assess n-3 FAs against biomarkers in pregnant women is limited (16-20). It is critical to document this population’s n-3 intake because of the role DHA plays during pregnancy (increased fetal/infant cognition and visual acuity) (6-9) and to clearly define DHA needs during pregnancy. Otto et al. (16) assessed essential fatty acid (EFA) status (ALA and AA) using a FFQ in pregnant women (n=24) to investigate the influence of EFA intake prior to pregnancy and at week 10 of gestation. Plasma and RBC phospholipid fatty acid composition was determined prior to conception and at 6, 8, and 10 weeks of gestation using gas chromatography; EFA intake was assessed prior to conception and at 10 weeks gestation using a FFQ previously validated in pregnant women. A significant increase in plasma phospholipids (palmitic acid, ARA, DHA, n-6, and n-3) from pre-pregnancy to week 10 of pregnancy was observed (P<0.0001). Red blood cell phospholipids stearic and LA significantly declined during this time frame (P<0.0001). No significant differences in dietary fatty acid intake were reported by the FFQ between preconception and week 10 of gestation. Significant Spearman’s correlation
coefficients were found between pre-pregnancy dietary DHA intake and DHA plasma phospholipids (rho=0.64, P=0.009) and DHA RBC phospholipids (rho=0.65, P=0.007). At wk 10 of gestation, only dietary LA intake was significantly correlated with LA plasma phospholipids (rho=0.67, P=0.004) (9). The lack of significant correlations between dietary fatty acid intake and biomarker levels during pregnancy may be a result of the repartitioning of fatty acids to the fetus for development. The physiological changes during pregnancy are complex and necessary to fulfill the needs of the growing fetus. The results of this study indicate some ability to detect fatty acid status in early pregnancy when using a FFQ. However, because of the changes in metabolism throughout pregnancy it is desirable to look at the association between intake and status at different points of gestation through a single study or through multiple studies to fully fatty acid needs in pregnant women.

De Vriese et al. (74) aimed to describe the relationship between dietary intake of LCPUFA and LCPUFA compositions in maternal and umbilical plasma PLs and CEs at delivery. Thirty pregnant women completed the same FFQ (previously validated in pregnant women (16)) between 6-22 wks gestation and between 32-40 wks gestation to assess fat intake from the previous month. There was no significant difference in dietary intake of fatty acids between the FFQs; the FFQ measurements were averaged for comparison against the biomarkers. At birth maternal and umbilical venous blood samples were collected and analyzed for fatty acid compositions using the Folch method (71). Pearson’s correlation coefficients were measured. Significant correlations were found between maternal dietary fatty acid intake and maternal plasma PLs for LA PL (r=0.53), ALA CE (r=0.53), EPA PL (r=0.48), DHA PL (r=0.52), EPA + DHA PL (r=0.53) and total n-6 PL (r=0.51) and DHA CE (r=0.39), EPA + DHA CE (r=0.44) (P<0.05). Significant correlations between average maternal fatty acid intake and umbilical plasma PL fatty acids were only found in EPA PL
regardless of the fact that maternal blood samples were not collected at the time the FFQs were administered, the findings of this study suggest an FFQ designed for a specific target population to assess dietary intake of LCPUFAs correlates with plasma fatty acids in the mother and infant at birth.

Parra et al. (15) compared PUFA dietary intake and RBC status in pregnant women during the third trimester of pregnancy. Dietary intake and RBC fatty acid compositions were analyzed from a subset of an ongoing study (n=35). A semi-quantitative FFQ including 104 items was used to collect dietary intake of n-3 PUFAs over the previous year. Specific food groups were targeted because of the known fat composition of these foods (eggs, fish, oils, milk, and terrestrial meat). Intake was assessed over the past year; frequency ranged from never up to 6 times per day. Red blood cells were analyzed using liquid gas chromatography (analyzed fraction of RBCs not defined). Pearson correlation coefficients between dietary intake and RBC were significantly associated when ALA (r=0.32), EPA (r=0.36), and DHA (r=0.35) were measured (P ≤ 0.05). A linear regression model demonstrated that RBC PUFA analysis was predicted by dietary intake of ALA (β=0.52), DHA (β=0.30), and AA (β=0.49). This study suggests that a FFQ may be a reliable method of documenting PUFA intake in pregnant women in comparison to RBC fatty acid levels. However, further research is needed to document specific PUFA intake (e.g., DHA and EPA) and to assess agreement between plasma and RBC fatty acid status.

In conclusion, fatty acid compositions of RBC and plasma fractions may be useful biological measurements to determine the association between maternal fatty acid status and dietary intake of fatty acids during pregnancy. The rapidly increasing body of research centered on DHA intake during pregnancy necessitates a validated assessment tool to ensure accurate reporting of DHA intake and status. Literature suggests the FFQ might be
the most appropriate tool to assess long term DHA status in pregnant women. The validation of the FFQ as a DHA assessment tool can be achieved by assessing its agreement between another dietary assessment tool (weighed diet record) and blood biomarkers (erythrocyte and plasma fatty acid compositions).
CHAPTER 3: Methods

A prospective observational study was conducted over two years in Bozeman, Montana and Ames, Iowa. Participants were recruited via newspaper advertisements, flyers, word of mouth, physician’s offices, and Craig’s list postings. Inclusion criteria were between the ages of 18-45 years, non-smoker, and singleton pregnancy. Exclusion criteria were history of type 2 diabetes, hypertension, heart disease, chronic renal disease, and not residing in surrounding the communities. Eligible participants included in the convenience sample signed an informed consent form approved by the Montana State University (MSU) Institutional Review Board (IRB) or by the Iowa State University (ISU) IRB according to the location they participated in the study. Subjects were enrolled between weeks 15-35 of gestation. All participants completed a demographic survey to collect age, annual income, highest grade of education completed, number of parities, pre-pregnancy weight, and marital status. Self reported height was documented in feet and inches. A portable Sunbeam analog scale (Boca Raton, FL) was used to record weight to the nearest tenth of a pound.

To fulfill the needs of Specific Aim #1 and 2, subjects were recruited and enrolled between weeks 15-35 of gestation (n=136). A subset of these participants (n=63) was used to address Specific Aim #3.

![Specific Aim Flow Chart](image)

Figure 4. Subject Population and Specific Aim Flow Chart.
I. Assessment of dietary intake

Diet data were collected by trained interviewers using a semi-quantitative FFQ and weighed 3-day diet record (3dDR). Subjects were randomly assigned to one of two orders to avoid bias in the data collection: FFQ prior to weighed 3dDR or 3dDR prior to FFQ.

a. Food frequency questionnaire

A semi-quantitative FFQ was developed by the principal investigator and several graduate research assistants. The FFQ included 107-food items and estimated dietary intake of total n-3 fatty acids, ALA, EPA, n-3 DPA, and DHA from the previous 30 days. The FFQ was administered through a face-to-face interview and took approximately 45 minutes to complete. All research staff members were trained how to administer the FFQ prior to collecting data through the following steps: 1) observed at least two FFQ interviews; 2) completed an FFQ interview as a mock research subject; 3) completed a FFQ interview as the interviewer with a mock research subject; and 4) completed their first two FFQ interviews with actual research subjects under supervision of a previously trained staff member. Frequency of food intake was assessed using the following ranges: 1-4 times per day, 1-7 times per week or 1-3 times per month. Food models and measuring cups were used to estimate portion sizes. Food categories on the FFQ included milk products, yogurt/ice cream, fats and oils, poultry/meat, fish, eggs, vegetables, fruits, snacks, grains, fast food, and supplements. When assessing poultry, meat, and fish consumption the interviewer further inquired about specific meat cuts (e.g. skinless boneless chicken breast vs chicken thigh with skin) and types of fish (white tuna canned in water vs raw albacore tuna). Duplicate assessment of cooking fats was avoided by documenting food items as raw or unprepared and by recording butter and margarine spread when used for cooking and spreading, but not for baked goods. Researchers indirectly inquired about supplements and foods commonly fortified with ALA, EPA, and DHA to avoid influencing the subjects from seeking out and consuming n-3 fatty acid fortified foods and supplements. If a
subject reported consuming a supplement on only one of the dietary assessment tools the interviewer confirmed its consumption or lack of consumption over the phone with the research subject.

b. Weighed diet record

Participants received detailed instruction on how to complete a weighed 3dDR using a Cuisinart SA-110A scale (Cuisinart, East Windsor, NJ). Subjects were given seven days to record three days of diet records: two week days and one weekend day diet. These days were not required to be consecutive. After data collection was complete, research staff reviewed the dietary log with the subject present to ensure complete record taking and to clarify any discrepancies that may exist on the record.

II. Diet analysis

Diet data was analyzed using Nutritionist Pro Version 4.2.2 (2009) dietary intake analysis software (Axxya Systems; Stafford, TX) by trained research staff. Before staff members were allowed to analyze dietary data they completed a standardized diet record developed by the principal investigator using Nutritionist Pro and were required to be within ± 2% of the average kilocalories, carbohydrate, fat, and protein content as determined by data outputs of previously trained staff. The FFQ and 3dDR for each subject were entered into the nutrient database by the same research staff to ensure consistent food selections between records. Whenever possible, food items selected during the analysis process were from the USDA Nutrient Database for Standard Reference because brand name foods within the nutrient database rarely contain complete dietary information for dietary fatty acids. Fortified foods and supplements containing ALA, EPA, and DHA reported via the FFQ and 3dDR were manually added to the nutrient database. Dietary information for fortified foods was determined via food labels, internet searches and through direct contact with the food manufacturer. Dietary intake of DPA was analyzed using the USDA Nutrient Database for Standard Reference website (75) because Nutritionist Pro does not include DPA composition of food. Dietary analysis output was
further reviewed by the first author to ensure the food with the most complete fat composition was selected and to ensure that the DHA and/or DHA + EPA supplements choices matched between the FFQ and weighed diet record.

III. Fatty acid blood biomarker measurements

a. Maternal blood sample collection

A fasted (no food or calorie containing beverages for at least eight hours) maternal venous blood sample was collected in an EDTA vacutainer (Becton Dickenson, Franklin Lakes, NJ) to coincide with dietary data collection. Vacutainers containing blood were placed on ice immediately after collection to maintain the integrity of the sample. Once the blood samples were returned to the MSU Nutrition Research Lab or the ISU campus they were centrifuged at 2000 g for 10 minutes at 4 degrees C to separate the plasma from the RBC. Plasma was stored at -80 degrees C. The remaining RBCs were washed with saline/EDTA (9 g/Liter sodium chloride and 1.14 g/Liter sodium EDTA in deionized water) and centrifuged at 2000g for 10 min at 4 degrees C. The buffy layer was removed and the wash step was repeated one more time to ensure all plasma had been washed away from the RBC. The RBCs were also stored at -80 degrees C.

b. Fatty acid analysis

Frozen plasma and RBCs samples were shipped on dry ice to The University of British Columbia, Nutrition Research Lab for analysis in November 2008, March 2009, October 2009 and January 2010. This is a well established lab that has conducted fatty acid analysis for over 25 years.

Plasma lipids were extracted using a modified Folch (26) extraction procedure using 2 parts sample in saline, 3 parts methanol and 6 parts chloroform. After vortexing and centrifugation at 2500 g for 10 minutes, the lower organic phase was collected. The aqueous phase was washed with additional chloroform and again the organic phase was collected, pooled with the first organic phase then dried under nitrogen to obtain a total lipid extract.
Red blood cell lipid classes were separated using TLC. Lipid fractions were recovered and converted to their respective methyl esters by gas chromatography with flame ionization using an Agilent 6850 gas chromatogram (Agilent Technologies Inc., Santa Clara, CA) with an Agilent HP-88 capillary column (30 m x 0.35 mm internal diameter x 20-µ film thickness). Agilent Chemstation software (Agilent Technologies Inc., Santa Clara, CA) was used to quantify the fatty acid peaks as a percent weight (g/100 g fatty acid) of each fatty acid based on the area under the curve.

IV. Statistical analysis

Specific Aim #1: Descriptive statistics were calculated for the total n-3 fatty acid and individual fatty acid intake including ALA, EPA, and DHA. These statistics included mean, median, mode, range, and standard deviation. Additionally, a Kolmogorov–Smirnov (K-S) test was used to test for a normal distribution of n-3 fatty acids intakes collected via the FFQ and 3dDR.

Specific Aim #2: Spearman’s Correlation coefficients determined the relationship between the daily intakes of total n-3 fatty acid and individual fatty acid estimated by the FFQ and the weighed 3dDR. Data were classified into three groups based on DHA intake estimated by the 3dDR. The lowest group, <70 mg DHA/day, was chosen as a reference to the fetal DHA needs of 67 mg DHA/day during the third trimester (7). The highest group, ≥ 200 mg DHA/day was based on the daily recommendation for maternal intake of 200 mg DHA/day throughout gestation (11).

Specific Aim #3: Descriptive statistics were calculated for the total DHA content of the RBC and plasma. A K-S test was performed to test for a normal distribution of n-3 fatty acids for blood biomarkers. Spearman’s correlation coefficients determined the relationship between the daily intakes of total n-3 fatty acid and individual fatty acid estimated by the FFQ and 3dDR and the fatty acid blood biomarkers.
Data were analyzed using Predictive Analytical Software (PASW) 18.0.0 (SPSS Inc, Chicago, IL). Statistical significance was set at P<0.05.
CHAPTER 4: Dietary Intake of total and individual n-3 fatty acids by pregnant women: associations between the semi-quantitative food frequency questionnaire and weighed diet record

A paper to be submitted to the *Journal of the American Dietetic Association*

Rebecca Filipowicz, Christina G. Campbell, PhD, RD.

Abstract

Introduction

Omega 3 (n-3) fatty acids are beneficial to maternal and fetal health. No known studies have the primary objective of determining the association of the food frequency questionnaire (FFQ) with some other dietary assessment tool when total and individual n-3 fatty acids intakes are estimated in pregnant women from non-coastal communities.

Objective

To determine the ability of the semi-quantitative FFQ to estimate intake of total n-3, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) in pregnant women from non-coastal communities.

Methods

Intake of total and individual n-3 fatty acids were estimated using the FFQ and weighed 3dDR in 136 healthy, pregnant women between 15-34 weeks gestation during a prospective observational study. Estimated intakes of total n-3, ALA, EPA, DPA, and DHA collected by FFQ and 3dDR were analyzed using Nutritionist Pro (Axxya Systems 9.2.2). Spearman’s Correlation coefficients were conducted to determine if the FFQ and 3dDR were associated. Data were classified into three groups based on DHA intake estimated by the 3dDR to determine if the dietary assessment tools agreed when <70 mg DHA/day, 70-199 mg DHA/day, or ≥200 mg DHA/day was consumed.

Results
Spearman’s correlations indicated a significant positive association between the FFQ and weighed 3dDR for intake of total n-3 (rho=0.98), ALA (rho=0.27), EPA (r=0.56) and DHA (r=0.66) (P<0.01). Agreement between estimated intake of DHA between the FFQ and 3dDR was found to be 58% for all levels of intake. The FFQ was better at determining episodic intake of DHA, 13 individuals reported consuming <70 mg DHA/day on the 3dDR and ≥200 mg DHA/day on the FFQ.

Conclusion

The semi-quantitative FFQ is an appropriate dietary assessment tool to estimate intake of total n-3, ALA, EPA and DHA and correlates well with the weighed 3dDR.

Introduction

Consumption of omega 3 (n-3) fatty acids during gestation has been shown to benefit mother and baby. The essential n-3 fatty acid alpha-linolenic acid (ALA) and the n-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) support maternal (1-5) and fetal health (6-9). DHA is a major component of brain grey matter and retinal phospholipids (6). The Adequate Intake (AI) for ALA for pregnant women is 1.4 g/d and is routinely met in the American diet (10). Clinical research shows DHA intake during pregnancy supports positive fetal visual acuity and motor skills development (6-9). Maternal intake of at least 200 mg DHA per day throughout gestation is recommended to support a healthy pregnancy, to prevent maternal depletion of DHA, and to meet the increased fetal demands of 67 mg DHA per day during the third trimester, a period of rapid fetal growth (11-13). At this time there are no dietary recommendations for EPA and DPA intake during pregnancy.

Common sources of ALA include soybean, canola oil, and flaxseed oil (12). Dietary sources of DHA are fatty fish, seafood, meat, and eggs (13); EPA and DPA are routinely found in the same dietary sources as DHA (14). Fortified foods and supplements are also consumable sources of ALA, EPA and DHA.
Accurate estimation of n-3 fatty acid intake during pregnancy may be useful in clinical settings and during nutrition related research. Possible dietary assessment tools include the weighed diet record, 24-hour recall, and the food frequency questionnaire (FFQ). Dietary intake estimated by a weighed diet record does not rely on memory and is documented over several days. A known limitation of the weighed record is possible decreased compliance over time when an individual is aware of the weighed amount of food he/she is consuming and the cumbersome act of weighing each individual food consumed (16). The 24-hour recall estimates intake from the preceding day, in a short amount of time (15-20 minutes) without altering normal intake. It is usable in diverse populations and has a relatively low burden on the client. A possible limitation of the 24-hour recall is the reliance on memory to estimate intake. The weighed diet record and 24-hour recall measure mean usual intake of food or nutrients consumed nearly every day by a group (16). Unless the weighed diet record and 24-hour recall are administered in multiple instances, neither tool consistently measures episodic intake nor sporadically consumed nutrients, like n-3 fatty acids. The weighed diet record has been more commonly used as a reference method than the 24 hour recall to assess dietary fatty acid intake in non-pregnant individuals (17-21). The strengths and limitations of both tools have been discussed thoroughly elsewhere (22-24).

The semi-quantitative FFQ is cost effective and has been used to measure episodic or sporadic intake from the previous 30 days to 5 years in a wide variety of populations (15-20). The FFQ has been used to estimate n-3 fatty acid intake in non-pregnant individuals (17-21). No known studies exist that have the primary objective of determining the association of the FFQ with some other dietary assessment tool for total and individual n-3 fatty acids in pregnant women from non-coastal communities. However, the semi-quantitative FFQ may be a suitable dietary assessment tool to estimate episodically consumed nutrients such as dietary n-3 fatty acids over long periods of time.
When determining the association between dietary assessment tools measurement error and non-matching data is a limitation. Sources of error may be caused by collecting data on non-consumption days, misreporting frequency of consumption or portion size, and poor food description (25). Measurement error can lead to the over- or underestimation of intake in comparison to the other tool. It is important to note there is no one perfect dietary assessment tool. When validating a dietary assessment it is necessary to have a reference dietary assessment tool and to recognize the strengths and limitations of both tools.

The purpose of the present study was to compare the estimated intakes of total and individual n-3 (ALA, EPA, DPA, and DHA) collected by FFQ and weighed 3-day diet record (3dDR) in women 15-35 weeks pregnant living in a non-coastal community. It was hypothesized that a significant association between the FFQ and 3dDR for total and individual n-3 fatty acid estimated intakes would be found.

Methods

A prospective observational study was conducted over two years in Bozeman, Montana and Ames, Iowa. Participants were recruited via newspaper advertisements, flyers, word of mouth, physician’s offices, and Craig’s list postings. Inclusion criteria were between the ages of 18-45 years, non-smoker, and singleton pregnancy. Exclusion criteria were history of type 2 diabetes, hypertension, heart disease, chronic renal disease, and not residing in surrounding the communities. Eligible participants included in the convenience sample signed an informed consent form approved by the Montana State University (MSU) Institutional Review Board (IRB) or by the Iowa State University (ISU) IRB according to the location they participated in the study. Subjects were enrolled between weeks 15-35 of gestation. All participants completed a demographic survey to collect age, annual income, highest grade of education completed, number of parities, pre-pregnancy weight, and marital status. Self reported height was documented in feet and inches. A portable Sunbeam analog scale (Boca Raton, FL) was used to record weight to the nearest tenth of a pound.
Assessment of Dietary Intake

Diet data were collected by trained interviewers using a semi-quantitative FFQ and 3dDR. Subjects were randomly assigned to one of two orders to avoid bias in the data collection: FFQ prior to weighed 3dDR or 3dDR prior to FFQ.

Food Frequency Questionnaire

A semi-quantitative FFQ was developed by the principal investigator and several graduate research assistants. The FFQ included 107-food items and estimated dietary intake of total n-3 fatty acids, ALA, EPA, n-3 DPA, and DHA from the previous 30 days. The FFQ was administered through a face-to-face interview and took approximately 45 minutes to complete. All research staff members were trained how to administer the FFQ prior to collecting data through the following steps: 1) observed at least two FFQ interviews; 2) completed an FFQ interview as a mock research subject; 3) completed a FFQ interview as the interviewer with a mock research subject; and 4) completed their first two FFQ interviews with actual research subjects under supervision of a previously trained staff member. Frequency of food intake was assessed using the following ranges: 1-4 times per day, 1-7 times per week or 1-3 times per month. Food models and measuring cups were used to estimate portion sizes. Food categories on the FFQ included milk products, yogurt/ice cream, fats and oils, poultry/meat, fish, eggs, vegetables, fruits, snacks, grains, fast food, and supplements. When assessing poultry, meat, and fish consumption the interviewer further inquired about specific meat cuts (e.g. skinless boneless chicken breast vs. chicken thigh with skin) and types of fish (white tuna canned in water vs. raw albacore tuna). Duplicate assessment of cooking fats was avoided by documenting food items as raw or unprepared and by recording butter and margarine spread when used for cooking and spreading, but not for baked goods. Researchers indirectly inquired about supplements and foods commonly fortified with ALA, EPA, and DHA to avoid influencing the subjects from seeking out and consuming n-3 fatty acid fortified foods and supplements. If a
subject reported consuming a supplement on only one of the dietary assessment tools the interviewer confirmed its consumption or lack of consumption over the phone with the research subject.

**Weighed Diet Record**

Participants received detailed instruction on how to complete a weighed 3dDR using a Cuisinart SA-110A scale (Cuisinart, East Windsor, NJ). Subjects were given seven days to record three days of diet records: two week days and one weekend day diet. These days were not required to be consecutive. After data collection was complete, research staff reviewed the dietary log with the subject present to ensure complete record taking and to clarify any discrepancies that may exist on the record.

**Diet Analysis**

Diet data was analyzed using Nutritionist Pro Version 4.2.2 (2009) dietary intake analysis software (Axxya Systems; Stafford, TX) by trained research staff. Before staff members were allowed to analyze dietary data they completed a standardized diet record developed by the principal investigator using Nutritionist Pro and were required to be within ± 2% of the average kilocalories, carbohydrate, fat, and protein content as determined by data outputs of previously trained staff. The FFQ and 3dDR for each subject were entered into the nutrient database by the same research staff to ensure consistent food selections between records. Whenever possible, food items selected during the analysis process were from the USDA Nutrient Database for Standard Reference because brand name foods within the nutrient database rarely contain complete dietary information for dietary fatty acids. Fortified foods and supplements containing ALA, EPA, and DHA reported via the FFQ and 3dDR were manually added to the nutrient database. Dietary information for fortified foods was determined via food labels, internet searches and through direct contact with the food manufacturer. Dietary intake of DPA was analyzed using the USDA Nutrient Database for Standard Reference website (26) because Nutritionist Pro does not include DPA composition of food. Dietary analysis output was
further reviewed by the first author to ensure the food with the most complete fat composition was selected and to ensure that the DHA and/or DHA + EPA supplements choices matched between the FFQ and weighed diet record.

**Statistical Analysis**

Mean and median values for daily total n-3 fatty acid and individual n-3 fatty acid intake were determined from the semi-quantitative FFQ and weighed 3dDR. A Kolmogorov–Smirnov (K-S) test was used to test for a normal distribution of n-3 fatty acid intake data from the FFQ and 3dDR. Spearman’s correlation coefficients were chosen because the data were non-linear and determined the rank relationship between estimated total n-3 and individual n-3 fatty acid intakes measured by the FFQ and weighed 3dDR. Data were classified into three groups based on DHA intake estimated by the 3dDR. The lowest group, <70 mg DHA/day, was chosen as a reference to the fetal DHA needs of 67 mg DHA/day during the third trimester (12). The highest group, ≥ 200 mg DHA/day was based on the daily recommendation for maternal intake of 200 mg DHA/day throughout gestation (11-13). Data were analyzed using Predictive Analytical Software (PASW) 18.0.0 (SPSS Inc, Chicago, IL). Statistical significance was set at P<0.05.

**Results**

**Subject Characteristics**

A total of 142 women (n=64 from MT and n=78 from IA) were recruited for participation. Mean (± standard deviation) age and pre-pregnancy body mass index (BMI) of participants were 30 ± 4 years and 23.4 ± 4.5 kg/m², respectively. The mean week of gestation was 29 weeks and 1 day ± 3 weeks and 4 days. Age, pre-pregnancy BMI, and week of gestation did not differ significantly between participants from MT and IA. Therefore, dietary data were analyzed as one group regardless of location. The majority of participating women (92%) were Caucasian, while the remaining 8% of the study population was comprised of Native American (n=1), Asian (n=6), African American (n=2), or other (n=1). Approximately 41% of women had completed some graduate/professional school, half had completed 1-4 y of college, and less than 10% had only
some degree of secondary education. Slightly more than one-third of the participant’s annual income was greater than $75,000, 47% had an annual income which ranged between $20,001 and $75,000, and approximately 20% identified as students had an annual income less than $20,000. One hundred thirty-two women reported being married, twelve women were single and one woman was divorced. Reported ethnicity, education level, annual income level, and marital status did not significantly differ between participants from either location.

After signing informed consent and reporting demographic data, six women withdrew from the study before collecting any dietary data. Three participants did not complete a weighed 3dDR and were excluded from the statistical analysis. The final data analysis included 136 women.

**FFQ and weighed 3dDR analysis**

Statistical analysis was performed with (n=136) and without (n=109) EPA and DHA supplementation. No significant differences were observed when supplements were included or excluded from statistical analysis of dietary intake data; therefore, dietary data with EPA and DHA supplementation was included in the final analysis. Few individuals consumed fortified food and it was not a statistically significant source of EPA and DHA (data not shown).

Total n-3 fatty acid intake represents the sum of all ALA, EPA, DHA, and DPA fatty acids reported by the dietary assessment tools. A K-S test indicated non-normal distributions for total n-3, ALA, EPA, DPA, and DHA on the FFQ and 3dDR (P<0.01). The FFQ estimated greater mean intakes than the weighed 3dDR for total n-3 fatty acid (2.414 ± 1.970 g/d vs. 2.486 ± 2.067 g/d, respectively), ALA (1.932 ± 1.772 g/d vs. 1.637 ± 0.986 g/d, respectively), EPA (0.050 ± 0.070 g/d vs. 0.047 ± 0.093 g/d, respectively), DPA (0.350 ± 0.616 g/d vs. 0.023 ± 0.045 g/d, respectively), and DHA (0.095 ± 0.104 g/d vs. 0.081 ± 0.140 g/d, respectively). The median and 5-95th percentiles for all participants are shown in Table 1.

The semi-quantitative FFQ and weighed 3dDR had positive associations for total n-3 (rho=0.98), ALA (rho=0.28), EPA (rho=0.56), and DHA (rho=0.66) for all participants (n=136)
(P<0.01). No significant association was found between estimates of n-3 DPA intakes from the FFQ and 3dDR (rho=0.08). Outliers were identified as those individuals whose reported intake on the FFQ was greater than ± 2 standard deviations from the mean. Significant associations were also found for n-3, ALA, EPA, and DHA when outliers were removed from the analysis (Figure 1). Associations between estimated intakes collected via the FFQ and weighed 3dDR without outliers were maintained for total n-3 fatty acid and DHA, increased for ALA and EPA, and decreased for DPA.

Dietary intake data for all subjects (n=136) was divided into groups using DHA intake estimated from the 3dDR. When <70 mg DHA/day was estimated via the weighed 3dDR, 46 individuals reported consuming <70 mg DHA/day on the FFQ, 20 individuals reported consuming 70-199 mg DHA/day, and 12 individual reported consuming ≥200 mg DHA on the FFQ. Within the second group defined by DHA intake on the 3dDR, 70-199 mg DHA/day, 6 individuals reported consuming <70 mg DHA/day via the FFQ, 6 individuals reported consuming 70-199 mg DHA/day on both the FFQ and 3dDR, whereas 5 individuals reported consuming ≥200 mg DHA/ day on the FFQ. Finally, within the highest group (≥200 mg DHA/ day), 9 individuals reported consuming <70 mg DHA/day via the FFQ, 5 individuals reported consuming 70-199 mg DHA/day, and 27 individuals reported consuming ≥200 mg DHA/day. In summary, 58% agreement between the FFQ and the 3dDR was found whereas 42% disagreement was revealed.

**Discussion**

The results of this study demonstrate a good association between estimated intakes of n-3, ALA, EPA, and DHA from a semi-quantitative FFQ and a 3dDR in pregnant women living in non-coastal communities. The mean intake of n-3 fatty acids from the FFQ was higher than those from the 3dDR. For example, during this study some subjects reported consuming foods containing EPA and DHA on the FFQ at least two times per week and when their 3dDR was
reviewed little to no foods containing EPA and DHA were documented. This may be a result of overestimation of frequency and portion size by the FFQ and/or decreased compliance during the collection of the diet record (17, 31). This observation may be magnified by the fact the weighed diet record may report 0 mg of intake for episodically consumed nutrients (e.g. EPA and DHA) (28-29).

No known study conducted in pregnant women has focused on the association between dietary intake of n-3 fatty acids, specifically ALA, EPA, DPA and DHA, estimated by an FFQ with a dietary reference method such as the weighed 3dDR; therefore, it may be more appropriate to compare the findings of this study to those found in non-pregnant individuals assessing n-3 fatty acids. Our findings were significantly associated but to a less extent, specifically for n-3 DPA, than the results from a study conducted by Sullivan et al. (19). A 28-item self administered semi-quantitative FFQ, designed to measure intake from the previous 3 months, and a weighed diet record were used to estimate long chain polyunsaturated fatty acid (LCPUFA) intake in healthy male and female participants (n=53). Spearman’s correlation coefficients between the FFQ and weighed diet record for total LCPUFA, EPA, n-3 DPA, an n-3, and DHA intake were rho= 0.75, rho=0.64, rho=0.62, and rho=0.72, respectively. These results indicate the FFQ and weighed diet record are associated for LCPUFA and individual n-3 fatty acid intakes.

The associations between the FFQ and 3dDR from this study were greater than those observed in two other studies conducted with non-pregnant individuals (16, 24). Tokudome et al. (17) examined the relationship of an FFQ and the average of four weighed 7-day diet records administered every four months to estimate the fatty acid intake in Japanese female dietitians. Significant Pearson’s correlation coefficients of 0.27, 0.32, 0.39 and 0.43 for total n-3, ALA, EPA, and DHA intakes, respectively (P<0.05) were observed. Similarly, Broadfield et al. (26) customized a previously validated semi-quantitative FFQ to assess dietary fatty acids in healthy
adults (n=31, demographics unspecified) using a weighed diet record as the reference method. Spearman’s correlation coefficients were calculated to determine the relationship between the FFQ and weighed diet record for total n-3 (rho=0.23), EPA (rho=0.50) and DHA (rho=0.37) (P<0.05). Significant correlations were also observed between both dietary assessment tools for total fat, total SFA, palmitic acid, stearic acid, oleic acid, total n-6, and linoleic acid. These results support our findings that an FFQ specifically designed to measure n-3 fatty acid intake is associated with data obtained from a weighed 3dDR.

During the present study over half of the subjects reported consuming DHA on the 3dDR and FFQ within the same groups of intake (<70 mg DHA/day, 70-199 mg DHA/day, and ≥200 mg DHA/day). These findings are similar to those reported by McNaughton et al. (27) who reported classifying 42% individuals in the same quintiles and by Sullivan et al. (19) who reported classifying 49% of subjects into the same quartile when intakes of n-3 LCPUFAs were estimated using the FFQ and weighed diet record. The smaller level of data stratification used in the present study is an obvious difference between this study and those conducted by McNaughton et al. and Sullivan et al. The groups defined during this study add clinical relevance to the estimated intakes. For example, by using these defined groups we were able to indicate that both the FFQ and 3dDR estimated that 52 individuals were not meeting the suggested recommendation of at least 200 mg DHA/ day during the 2nd and 3rd trimester of pregnancy. Additionally, 27 individuals were found to be consuming at least 200 mg/DHA day as estimated by the FFQ and weighed 3dDR. It is also important to note the FFQ was able to estimate DHA intake for 59 subjects that did not report consuming DHA on their 3dDR. For example, 13 individuals reported consuming <70 mg DHA/day on the 3dDR and ≥200 mg DHA/day on the FFQ. This discrepancy between estimated intakes may be a result of collecting the 3dDR on non-consumption days. These findings indicate the FFQ is associated with the 3dDR and it may be better at detecting episodically consumed nutrients like DHA overtime.
The agreement between the FFQ and 3dDR in this study were comparable with results of previous studies in non-pregnant individuals. We conclude our FFQ designed to measure specific fatty acids is an improvement over previously tested tools which only estimated dietary intake of total fat in pregnant women. Furthermore, the results of this study indicate this FFQ, by itself, would be an appropriate tool to estimate an individual's total n-3, ALA, EPA, and DHA intake within the clinical and research setting if the diet data collection and analysis protocols we established are followed. In conclusion, the semi-quantitative FFQ is capable of estimating intake of total n-3, ALA, EPA and DHA and is associated with the weighed 3dDR.
References


5. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sci*. 2003;73:3181–3187.


Table 1. Median (5\textsuperscript{th}-95\textsuperscript{th} percentiles) total and individual n-3 fatty acid dietary intakes estimated via the food frequency questionnaire (FFQ) and weighed 3-day diet record (3dDR) in pregnant women during the 2\textsuperscript{nd} and 3\textsuperscript{rd} trimester (n=136).

<table>
<thead>
<tr>
<th>Dietary fatty acid (^a)</th>
<th>Semi-quantitative FFQ (g/d)</th>
<th>Median (5th-95th percentile)</th>
<th>Weighed 3dDR (g/d)</th>
<th>Median (5th-95th percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>total n-3</td>
<td>1.935 (0.746-5.358)</td>
<td>1.609 (0.595-4.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA</td>
<td>1.542 (0.650-4.081)</td>
<td>1.481 (0.568-3.396)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.023 (0.002-0.183)</td>
<td>0.009 (0.000-0.248)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPA</td>
<td>0.131 (0.004-1.555)</td>
<td>0.006 (0.000-0.134)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>0.056 (0.007-0.371)</td>
<td>0.028 (0.001-0.452)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosaheaxenoic acid (DHA)
Table 2. Agreement between the 3dDR and FFQ for different levels of estimated mean docosahexaenoic acid (DHA) intake (n=136).

<table>
<thead>
<tr>
<th>Group</th>
<th>Agreement between groups</th>
<th>Disagreement between groups</th>
<th>Percent Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.070 g DHA/day</td>
<td>46ª</td>
<td>32</td>
<td>58%</td>
</tr>
<tr>
<td>0.070-0.199 g DHA/day</td>
<td>6</td>
<td>11</td>
<td>35%</td>
</tr>
<tr>
<td>≥0.200 g DHA/day</td>
<td>27</td>
<td>14</td>
<td>66%</td>
</tr>
</tbody>
</table>

ª Number of subjects.
Figure 1. Spearman’s correlation coefficients without outliers for mean estimated intakes of total n-3 (n=135), alpha linolenic acid (ALA) (n=135), eicosapentaenoic acid (EPA) (n=134), docosapentaenoic acid (DPA) (n=134), and docosahexaenoic acid (DHA) (n=134) collected via the food frequency questionnaire (FFQ) and weighed 3-day diet record (3dDR).
CHAPTER 5: Validation of a semi-quantitative food frequency questionnaire with blood biomarkers in pregnant women for total and individual n-3 fatty acids

A paper to be submitted to the Journal of the American Dietetic Association

Rebecca Filipowicz, Christina Campbell

Abstract

Background

Omega 3 (n-3) fatty acids are important structural components of cell membranes. Previous clinical research shows DHA intake during pregnancy supports positive fetal visual acuity and motor skills. Despite the interest in prenatal n-3 fatty acid intake, limited studies have examined the association between total and individual intakes measured by a food frequency questionnaire (FFQ) and plasma or red blood cell (RBC) n-3 fatty acid status during pregnancy.

Objective

We examined the relationship between the semi-quantitative FFQ and weighed 3-day diet record (3dDR) intakes with plasma phospholipids (PLs) and RBC esterified fatty acids (phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) composition for total and individual n-3 fatty acids (alpha-linoleic acid (ALA) and the n-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)) in pregnant women from two non-coastal communities.

Design

Dietary n-3 fatty acid intake was estimated using an FFQ and weighed 3dDR and associated with blood biomarkers in a subset of 64 pregnant women (25-31 weeks of gestation) from ongoing observational. The FFQ was previously validated with the 3dDR for total n-3, ALA, EPA, and DHA intake. Spearman’s correlation coefficients were used to identify the
associations between estimated dietary intake of total and individual n-3 fatty acids from the FFQ and 3dDR and fatty acid compositions of plasma PLs and RBC PE and PC.

**Results**

The results of this study indicate the estimated intakes of total and individual n-3 fatty acids from a semi-quantitative FFQ are more associated with blood biomarkers than estimated intakes of total and individual n-3 fatty acids from a weighed 3dDR in pregnant women living in non-coastal communities. Spearman’s correlations coefficients between the intakes estimated from the FFQ and plasma PL for EPA and DHA (rho=0.33 and rho=0.46, respectively), and RBC PE and PC for total n-3 (rho=0.39 and rho=0.27, respectively), EPA, (rho=0.45 and rho=0.36, respectively) and DHA (rho=0.56 and rho=0.47, respectively) were significant. DHA intake obtained from the 3dDR exhibited a significant positive association for plasma PL (rho=0.34) and RBC PE and PC (rho=0.40 and rho=0.42, respectively). The 3dDR DPA estimated intake was not associated with blood biomarkers.

**Conclusion**

The estimated intakes of total and individual n-3 fatty acids from a semi-quantitative FFQ are more strongly associated with blood biomarkers than estimated intakes of total and individual n-3 fatty acids from a weighed 3dDR in pregnant women living from non-coastal communities. In conclusion, the semi-quantitative FFQ is a more appropriate tool to estimate total and individual n-3 fatty acid intakes in clinical and research settings.

**Introduction**

The essential omega 3 (n-3) fatty acid alpha-linoleic acid (ALA) and the n-3 fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) are important structural components of cell membranes (1). During pregnancy a steady supply of n-3 fatty acids is required to support positive fetal development (1-4) and maternal health (5-9). Over the past decade, extensive clinical research has focused on the role of DHA
during pregnancy because it is incorporated into fetal brain grey matter and retina cell membranes more than any other n-3 fatty acid (4). The Adequate Intake (AI) for ALA (1.4 g/day) is routinely met in the American diet through the consumption of foods containing soybean and canola oil (10). The consumption of preformed EPA, n-3 DPA, and DHA is recommended as a result of the low conversion rate of ALA to EPA, n-3 DPA, and DHA during pregnancy (11). Maternal intake of at least 200 mg DHA per day throughout gestation is recommended to support a healthy pregnancy, to prevent maternal depletion of DHA and to meet the increased fetal demands of 67 mg DHA per day during the third trimester, a period of rapid fetal growth (12-14). At this time there are no dietary recommendations for EPA and DPA intake during pregnancy. Dietary sources of DHA are fatty fish, seafood, meat, and eggs (14); EPA and DPA are routinely found in the same dietary sources as DHA (15). Fortified foods and supplements are also consumable sources of ALA, EPA and DHA.

As a result of the growing body of research which focuses on the affects of n-3 fatty acids on maternal and fetal health, several studies have determined the association between dietary intake of n-3 fatty acids and n-3 fatty acids status (biological measurement/level) during pregnancy (16-19). However, no known studies have the primary objective of validating the use of a food frequency questionnaire (FFQ) to estimate total and individual n-3 fatty acids with a biological reference measurement in pregnant women from non-coastal communities. Dietary assessment tools have been previously validated with biological measurements because they are objective and do not rely on memory or compliance of the participant (20-23). Possible biological measurements that can be used to validate an FFQ include isotopes, adipose biopsy, and blood. Isotopes are highly accurate and do not require the same special handling as adipose and blood samples (21). However, the use of isotopes as a biological reference measure in pregnant women is inappropriate at this time because the physiological influences of isotopes are not fully understood. Adipose tissue has also been used to validate an FFQ designed to assess dietary intake and considered by some researchers be highly reflective of
fatty acid status (22). The half life ($t_{1/2}$) of adipose tissue is between 1 and 2 years and does not correspond with the length of pregnancy (10 months) (22). Additionally collecting adipose biopsies may be perceived as too invasive and diminish participation levels. Therefore, adipose is not a favorable biological reference method for the determining the association of dietary n-3 fatty acid intake and status in pregnant women.

Plasma phospholipids (PL) and red blood cell (RBC) phosphatidylethanolamine (PE) and phosphatidylcholine (PC) fatty acid compositions have been used to determine fatty acid status in pregnant women because they are easy to collect and have low levels of risk for the mothers and their fetus (16-17). Plasma fatty acids fractions may be useful biological measurements to determine the association between short term dietary intake of total and individual n-3 fatty acids maternal fatty acid status because it has a $t_{1/2}$ of 10 days (22). Additionally, RBC fatty acid fractions may be a useful biological measurement to determine the association between long term dietary intake of total and individual n-3 fatty acids maternal fatty acid status because it has a $t_{1/2}$ of 30 days (22). A known disadvantage of blood biomarkers is that they do not reflect true absolute intake because of the affects of digestion, absorption, uptake, utilization, metabolism, excretion, homeostatic mechanisms, and error associated by the biochemical measures themselves (21). Both plasma phospholipids (PL) and red blood cells (RBC) PE and PC have exhibited positive correlations with n-3 fatty acids estimated from an FFQ in pregnant (16-17) and non-pregnant individuals (24).

We have developed a semi-quantitative FFQ that is positively associated with a weighed 3-day diet record (3dDR) for estimated intakes of total n-3, ALA, EPA, and DHA in women (n=136) during the 2nd and 3rd trimester of pregnancy residing in non-coastal communities (data unpublished). The FFQ also estimated intake of ALA, EPA, and DHA from fortified foods and supplements. The usefulness of this FFQ in clinical and research settings would be further supported if the estimated intakes of total and individual n-3 fatty acids from the FFQ and
weighed 3-day diet record were validated with total and individual n-3 fatty acid status during pregnancy.

The aims of this study were: 1) to examine the relationship between total n-3, ALA, EPA, DPA, and DHA estimated intakes from the FFQ and plasma PL, RBC PE and PC; and 2) to examine the relationship between total n-3, ALA, EPA, DPA, and DHA estimated intakes from the weighed 3dDR and plasma PL, RBC PE and PC in women during the 2nd or 3rd trimester of pregnancy residing in two non-coastal communities. It was hypothesized that total and individual n-3 fatty acid intakes estimated from FFQ would show strong correlations with RBC PE and PC total and individual n-3 fatty acid compositions and that total and individual n-3 fatty acid intakes estimated from weighed 3dDR would show strong correlations with plasma PL total and individual n-3 fatty acid compositions

**Methods**

A subset of participants (n=64) from an ongoing prospective observational study was used for the comparison of the FFQ and 3dDR with plasma and RBC fatty acid biomarkers. Participants from Bozeman, Montana and Ames, Iowa were included in the convenience sample because they were between 25-31 weeks pregnant, pregnant with one baby, between the ages of 18-45, non-smokers, and had no previous history of chronic disease (type 2 diabetes, hypertension, heart disease, chronic renal disease). Informed consent was obtained when the subject signed a consent form approved by the Montana State University Institutional Review Board (IRB) or by the Iowa State University IRB according to the location they participated in the study. Age, income, highest grade of education completed, number of parities, pre-pregnancy weight, and marital status were documented. Self-reported height was documented in feet and inches. A portable Sunbeam analog scale (Boca Raton, FL) was used to record weight to the nearest tenth of a pound.

**Dietary Assessment**
Total and individual n-3 fatty acid intake was estimated using a semi-quantitative FFQ and 3dDR over a 7 day period. Subjects were randomly assigned to one of two orders (FFQ prior to weighed 3dDR or vice versa) to avoid bias. The 107-food item semi-quantitative FFQ measured the twelve categories of food: milk products, yogurt/ice cream, fats and oils, poultry/meat, fish, eggs, vegetables, fruit, snacks, grains, fast food, and supplements. Within each category trained interviewers thoroughly inquired about intake of foods including those fortified with n-3 fatty acids, different cuts of meat and types of fish, cooking and spreading fats, eggs, and supplements containing EPA and DHA. Frequency was measured using the following categories: 1-4 times per day, 1-7 times per week or 1-3 times per month. Portion size was estimated using food models and measuring cups. Indirect inquiry was used to estimate consumption of EPA and DHA supplements and n-3 fatty acid fortified foods during the FFQ interview to avoid influencing the subjects from seeking out and consuming fortified foods and supplements. The FFQ interview took approximately 45 minutes to complete. Participants received detailed instructions on how to complete a weighed 3dDR (two week days and one weekend day diet, which were not required to be consecutive) using a Cuisinart SA-110A scale (Cuisinart, East Windsor, NJ). Once the subject returned the weighed 3dDR, a member of the research staff reviewed the dietary log with the subject to ensure complete record taking and to clarify any discrepancies that may exist.

Diet Analysis

Diet data was analyzed using Nutritionist Pro Version 4.2.2 (2009) dietary intake analysis software (Axxya Systems; Stafford, TX). The dietary intake analysis software was modified by the first author to include fortified foods and supplements. The FFQ and 3dDR for each subject was analyzed by the same trained research staff to ensure consistent food selections between records. Additionally, USDA Nutrient Database for Standard Reference food choices was chosen over brand name foods because they contained more complete dietary information for dietary fatty acids. Dietary intake of DPA was analyzed using the USDA
Nutrient Database for Standard Reference (25). Dietary analysis output was further reviewed by the first author to ensure the food with most complete fat composition was selected and to ensure that the DHA and/or DHA + EPA supplements choices matched between the FFQ and weighed diet record. The FFQ was previously shown to be significantly associated with a weighed 3dDR for total n-3 (rho=0.98, P<0.01), ALA (rho=0.27, P<0.01), EPA (r=0.56, P<0.01) and DHA (r=0.66, P<0.01) (unpublished data).

Blood collection

A fasted (no food or calorie containing beverages for at least eight hours) maternal venous blood sample was collected in an EDTA vacutainer (Becton Dickenson, Franklin Lakes, NJ) to coincide with dietary data collection. Vacutainers containing blood were placed on ice immediately after collection to maintain the integrity of the sample. Once the blood samples were returned to the MSU Nutrition Research Lab or the ISU campus they were centrifuged at 2000 g for 10 minutes at 4 degrees C to separate the plasma from the RBC. Plasma was stored at -80 degrees C. The remaining RBCs were washed with saline/EDTA (9 g/Liter sodium chloride and 1.14 g/Liter sodium EDTA in deionized water) and centrifuged at 2000g for 10 min at 4 degrees C. The buffy layer was removed and the wash step was repeated one more time to ensure all plasma had been washed away from the RBC. The RBCs were also stored at -80 degrees C.

Fatty acid analysis

Plasma lipids were extracted using a modified Folch (26) extraction procedure using 2 parts sample in saline, 3 parts methanol and 6 parts chloroform. After vortexing and centrifugation at 2500 g for 10 minutes, the lower organic phase was collected. The aqueous phase was washed with additional chloroform and again the organic phase was collected, pooled with the first organic phase then dried under nitrogen to obtain a total lipid extract.

Red blood cell lipid classes were separated using TLC. Lipid fractions were recovered and converted to their respective methyl esters by gas chromatography with flame ionization
using an Agilent 6850 gas chromatogram (Agilent Technologies Inc., Santa Clara, CA) with an Agilent HP-88 capillary column (30 m x 0.35 mm internal diameter x 20-µ film thickness). Agilent Chemstation software (Agilent Technologies Inc., Santa Clara, CA) was used to quantify the fatty acid peaks as a percent weight (g/100 g fatty acid) of each fatty acid based on the area under the curve.

**Statistical Analysis**

Mean and median values were determined for total and individual n-3 fatty acid dietary intake estimated by the FFQ and 3dDR and plasma and RBC fatty acid compositions. A Kolmogorov–Smirnov (K-S) test was used to test for a normal distribution of n-3 fatty acid dietary intake data from the FFQ and 3dDR and the n-3 fatty acid composition measured via plasma PL and RBC PE and PC. Spearman’s correlation coefficients determined the relationship between the total and individual n-3 fatty acid intakes from the FFQ and 3dDR and the plasma and RBC fatty acid composition. Diet and blood associations for EPA and DHA were also examined using Spearman’s correlation coefficient for non-supplement and supplement users. Data were analyzed using Predictive Analytical Software (PASW) 18.0.0 (SPSS Inc, Chicago, IL). Statistical significance was set at P<0.05.

**Results**

*Subject Characteristics*

Dietary data was analyzed as one group because age, gestation length, and pre-pregnancy BMI were previously determined to not differ significantly between subjects from MT and IA (unpublished data). Sixty-four women completed the study; subject characteristics are provided in Table 1. One subject was treated as an outlier because her DHA intake collected via the FFQ was ± 2 standard deviations (SD) from the mean. No other outliers existed for total n-3, ALA, EPA, and n-3 DPA intakes; therefore data from 63 women were included in the final analysis.
Total n-3 fatty acid intake represents the sum of all ALA, EPA, DPA, and DHA fatty acids estimated in the diet. A K-S tested indicated non-normal distributions for total n-3, ALA, EPA, DPA, and DHA intakes estimated by the FFQ and 3dDR intakes (P<0.01). Table 2 presents the median and 5th-95th percentile of total and individual n-3 fatty acid intake as measured by 3dDR and FFQ for all subjects (n=63), supplement users (n=19), and non-supplement users (n=44). The FFQ estimated greater mean intakes in all subjects than the weighed 3dDR for total n-3 fatty acid (2.082 ± 1.435 g/d vs. 1.773 ± 1.158 g/d, respectively), ALA (1.640 ± 1.221 g/d vs. 1.501 ± 0.949 g/d, respectively), EPA (0.094 ± 0.173 g/d vs. 0.089 ± 0.174 g/d, respectively), and DPA (0.220 ± 0.490 g/d vs. 0.022 ± 0.040 g/d, respectively). In all subjects, estimated mean intake of DHA collected by the FFQ was less than the estimated mean intake of DHA collected by the weighed 3dDR (0.144 ± 0.160 g/d vs. 0.161 ± 0.232 g/d, respectively).

Supplement user’s estimated mean EPA intake from the FFQ (0.188 ± 0.170 g/d) and the 3dDR (0.220 ± 0.258 g/d) was greater than the non-supplement users estimated mean EPA intake from the FFQ (0.030 ± 0.044 g/d) and the 3dDR (0.031 ± 0.064 g/d). The supplement users estimated mean DHA intake reported by the FFQ and 3dDR (0.311 ± 0.159 g/d and 0.399 ± 0.270, respectively) was also greater than non-supplement users estimated mean DHA intake reported by the FFQ and 3dDR (0.070 ± 0.095 g/d and 0.057 ± 0.101 g/d, respectively).

Total n-3 fatty acids represent the sum of all ALA, EPA, DPA, and DHA fatty acids detected in the blood. A K-S test indicated non-normal distributions for total n-3, ALA, EPA, DPA, and DHA status for plasma PL and RBC PE and PC (P<0.01). The median and 5th-95th percentile are shown for all subjects, supplement users, and non-supplement users in Table 2. Mean ± SD plasma PL for all subjects (n=63) for total n-3, ALA, EPA, DPA, and DHA was 5.668 ± 1.358 g/100 g, 0.279 ± 0.081 g/100 g, 0.399 ± 0.273 g/100 g, 0.713 ± 0.154 g/100 g, and 4.278 ± 1.060 g/100 g, respectively. Mean (±SD) fatty acid compositions measured in the RBC PC were less than RBC PE in all subjects for total n-3 (2.595 ± 0.671 g/100 g vs. 6.350 ± 1.547
g/100 g), EPA (0.217 ± 0.139 g/100 g vs. 0.275 ± 0.141 g/100 g), DPA (0.373 ± 0.087 g/100 g vs. 1.935 ± 0.468 g/100 g), and DHA (1.761 ± 0.520 g/100 g vs. 3.969 ± 1.093 g/100 g). Mean (±SD) fatty acid compositions measured in the RBC PC were greater than RBC PE in ALA (0.243 ± 0.069 g/100 g vs. 0.167 ± 0.069 g/100 g). The supplement users EPA composition for plasma PL (0.399 ± 0.273 g/100 g), RBC PE (0.275 ± 0.141 g/100 g), and RBC PC (0.399 ± 0.273 g/100 g) was greater than non-supplement users EPA composition for plasma PL (0.307 ± 0.136 g/100 g), RBC PE (0.233 ± 0.009 g/100 g), and RBC PC (0.166 ± 0.064 g/100 g). The supplement users had higher DHA composition than non-supplement users in plasma PL (4.983 ± 1.073 g/100 g vs. 3.937 ± 0.908 g/100 g), RBC PE (4.577 ± 1.360 g/100 g vs. 3.706 ± 0.846 g/100 g), and RBC PC (2.131 ± 0.516 g/100 g vs. 1.601 ± 0.438 g/100 g).

Spearman’s correlation coefficients were calculated to illustrate the association between diet data collected from the 3dDR and FFQ and the plasma PL and RBC PC and PE fatty acid compositions for all subjects, supplement users, and non-supplement users (Table 3). Bivariate plots (Figure 1 A-C) showed that as individual intakes of DHA estimated from the FFQ increased, individual DHA fatty acid compositions measured in plasma PL and RBC PE and PC also increased. Additionally, a smaller degree of heteroscedasticity was observed in the bivariate plots for estimated DHA intake from the FFQ vs. plasma PL, RBC PE and PC DHA (Figure 1: A-C) compared to the bivariate plots for estimated DHA intake from 3dDR vs. plasma PL, RBC PE and PC DHA (Figure 1: D-F).

Spearman’s correlation coefficients for supplement users revealed that as estimated individual DHA intakes measured by the FFQ increased, DHA composition in plasma PL and RBC PC also increased (Table 3). Estimated individual DHA intake measured by the 3dDR and RBC PC DHA compositions followed a similar pattern for supplement users. For supplement users, estimated DHA intakes from the FFQ were associated with RBC PE. The FFQ was associated with RBC PE for DHA. No associations were observed between non-supplement user’s estimated dietary intakes from the 3dDR and plasma PL, RBC PC for EPA and DHA.
Discussion

The results of this study indicate the estimated intakes of total and individual n-3 fatty acids from a semi-quantitative FFQ are more associated with blood biomarkers than estimated intakes of total and individual n-3 fatty acids from a weighed 3dDR in pregnant women living in non-coastal communities. The magnitude of correlation coefficients between estimated dietary fatty acid intakes and blood fatty acid compositions observed in the present study are similar to those previously reported.

The FFQ-plasma correlations were greater than the correlations between intake from the 3dDR-plasma correlations for total n-3 fatty acids, EPA and DHA. In contrast, the 3dDR-plasma correlations were higher than FFQ-plasma correlations for ALA. Both methods showed poor correlations for DPA. These correlations are similar to those observed by Sun et al. (27) between an FFQ and plasma PL for ALA (rho=0.23), EPA (rho=0.21), n-3 DPA (rho= -0.03), and DHA (rho=0.48) in non-pregnant females (n=306). Additionally, the results of the present study are similar to findings of other studies conducted in pregnant women from coastal communities. de Vriese et al. (18) described the relationship between dietary intakes of LCPUFA estimated by a previously validated FFQ (17) and LCPUFA compositions in maternal plasma PL collected at birth in pregnant women (n=30) residing near the coast of Belgium. Significant Pearson’s correlations between maternal dietary fatty acid intake and maternal plasma PLs for EPA PL (r=0.48) and DHA PL (r=0.52) (P<0.05) were greater than those observed in the present study. Similarly, Innis et al. (28) determined the association between dietary estimated intakes of n-3 fatty acids with plasma PL n-3 fatty acid compositions in pregnant women (n=55) residing around Vancouver. Dietary intake of EPA and DHA estimated by the FFQ were significantly related to the compositions of DHA (r = 0.61, P < 0.0001) and EPA (r = 0.55, P < 0.0001) in the plasma PL. No significant associations were found for ALA between dietary and plasma PL.
compositions. These results support our findings that the estimated intakes of n-3 fatty acids from the FFQ are associated with plasma PL n-3 fatty acid composition.

It is more difficult to discuss the association of estimated intake of n-3 fatty acids with RBC PE and PC in relation to previously published works because few studies examine the association between intakes of n-3 fatty acids with these specific RBC fatty acid fractions. More often than not authors report total RBC fatty acid compositions. For example, Parra et al. (29) measured the association between dietary intake of n-3 fatty acids and n-3 fatty acid status during the third trimester of pregnancy (n=35). Pearson correlation coefficients between dietary intake and RBC were significantly associated for ALA (r=0.32), EPA (r=0.36), and DHA (r=0.35) (P≤0.05). A linear regression model demonstrated that RBC ALA and DHA compositions were positively related to dietary intake for ALA (r=0.52) and DHA (r=0.30); these results support our hypothesis that an FFQ may be a more reliable method than the 3dDR for estimating n-3 intakes in pregnant women overtime because it is better at documenting episodic intake of specific n-3 fatty acids like EPA and DHA.

The results of the present study are most suitably compared to those reported by Friesen et al. (30) when dietary intakes of n-3 fatty acids were compared to RBC PE and PC in near-term pregnant women (n=105). Estimated intakes were significantly associated with RBC PE (rho=0.31, P<0.001) and RBC PC (rho=0.37, P<0.001) for DHA. Additionally, estimated EPA intakes were positively associated with RBC PE EPA (rho=0.39, P<0.001). The results reported by Friesen et al. are similar to the results of the present study because specific RBC fatty acid fraction (PE and PC) were measured and because reported PE fatty acid compositions included alkenyl-linked fatty acids.

The present study differs from previously published studies because EPA and DHA supplementation intake was retained in the final analysis. No correlations were observed between EPA intake and blood biomarkers for the supplement user group. However, a
A significantly positive correlation was observed between total DHA intake, RBC PE and PC in the supplement user group. No association was observed between dietary intake and plasma PL, or RBC PE and PC for EPA and DHA in the non-supplement users.

Our study has two limitations. First, metabolism of n-3 fatty acids during pregnancy is dynamic; therefore, compartmentalization of n-3 fatty acids may change throughout pregnancy (16). Additionally, the factors that influence endogenous mobilization of fatty acids during pregnancy differ between individuals and should be taken into full consideration. The second limitation of our study is that despite our meticulous collection and analysis of intake, the dietary assessment tools used to collect diet data always have some degree of measurement error and do not reflect true intake.

In summary, this study indicates that the estimated intakes of total and individual n-3 fatty acids obtained from a semi-quantitative FFQ exhibited a stronger association with blood biomarkers than estimated intakes of total and individual n-3 fatty acids from a weighed 3dDR in pregnant women living in non-coastal communities. It was observed that the FFQ and RBC PE and PC were associated for total n-3 fatty acids, EPA, and DHA. The dietary intakes of ALA, EPA, and DHA estimated by the FFQ were associated with plasma PL. Dietary intake of DPA estimated by the FFQ and DPA blood biomarkers showed poor correlations on all accounts. The 3dDR was associated with RBC PE for total n-3 and DHA and with RBC PC for ALA, DPA, and DHA. Finally, the 3dDR and plasma PL were associated for ALA, EPA, and DHA. The more frequent and greater associations observed between the FFQ and blood biomarkers may have resulted from the ability of the FFQ to estimate episodic intake of nutrients overtime. In conclusion, the semi-quantitative FFQ is an appropriate tool to estimate total and individual n-3 fatty acid intakes in clinical and research settings.
Table 1. Anthropometrics and Demographics (n=64).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
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<tr>
<td>Pre-pregnancy BMI (kg/m$^2$)</td>
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</tr>
<tr>
<td>Week of Gestation</td>
<td>28.4 ± 1.3</td>
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<td>Grade Level Completed</td>
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<td>College</td>
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<td>Graduate/Professional School</td>
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<tr>
<td>Income ($ per year)</td>
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<tr>
<td>&lt;20,001</td>
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</tr>
<tr>
<td>Married</td>
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</table>
Table 2. Total and individual omega 3 (n-3) fatty acid dietary intakes from the 3dDR and FFQ and compositions (g/100 g) in red blood cell (RBC) phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and plasma phospholipids (PL) of pregnant women.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Diet (g/d)</th>
<th>Blood (g/100 g fatty acid)</th>
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<tr>
<td></td>
<td>Median (5th-95th percentile)</td>
<td>Median (5th-95th percentile)</td>
</tr>
<tr>
<td></td>
<td>3dDR</td>
<td>FFQ</td>
</tr>
<tr>
<td>All Subjects (N=63)</td>
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<tr>
<td>total n-3</td>
<td>1.467 (0.607-4.724)</td>
<td>1.718 (0.720-5.298)</td>
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<tr>
<td>ALA</td>
<td>1.305 (0.581-3.170)</td>
<td>1.462 (0.531-4.358)</td>
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<tr>
<td>EPA</td>
<td>0.008 (0.000-0.406)</td>
<td>0.019 (0.002-0.376)</td>
</tr>
<tr>
<td>DPA</td>
<td>0.005 (0.000-0.140)</td>
<td>0.048 (0.002-1.402)</td>
</tr>
<tr>
<td>DHA</td>
<td>0.037 (0.003-0.732)</td>
<td>0.058 (0.008-0.522)</td>
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<tr>
<td>Supplement user’s (N=19)</td>
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<td></td>
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<tr>
<td>EPA</td>
<td>0.185 (0.000-0.699)</td>
<td>0.196 (0.003-0.409)</td>
</tr>
<tr>
<td>DHA</td>
<td>0.235 (0.128-0.890)</td>
<td>0.271 (0.000-0.541)</td>
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<td>Non-supplement user’s (N=44)</td>
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<tr>
<td>EPA</td>
<td>0.007 (0.000-0.186)</td>
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<td>DHA</td>
<td>0.024 (0.001-0.217)</td>
<td>0.031 (0.005-0.360)</td>
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</table>

ª For the fatty acids in RBC PE, 23.661 ± 3.344 g/100 g were alkenyl linked.

b alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)
Table 3. Relationship between total and individual n-3 dietary fatty acid intakes as estimated by weighed 3 day diet record (3dDR) and semi-quantitative food frequency questionnaire (FFQ) and total and individual n-3 fatty acid compositions plasma phospholipids (PL), and red blood cell (RBC) phosphatidylethanolamine (PE) and phosphatidylcholine (PC).

<table>
<thead>
<tr>
<th>Fatty Acids b</th>
<th>Spearman's Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FFQ vs PL</td>
</tr>
<tr>
<td>All Subjects (N=63)</td>
<td></td>
</tr>
<tr>
<td>total n-3</td>
<td>0.14</td>
</tr>
<tr>
<td>ALA</td>
<td>0.37**</td>
</tr>
<tr>
<td>EPA</td>
<td>0.33**</td>
</tr>
<tr>
<td>DPA</td>
<td>0.16</td>
</tr>
<tr>
<td>DHA</td>
<td>0.46**</td>
</tr>
<tr>
<td>Supplement user's (N=19)</td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.36</td>
</tr>
<tr>
<td>DHA</td>
<td>0.47**</td>
</tr>
<tr>
<td>Non-supplement user's (N=44)</td>
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</tr>
<tr>
<td>EPA</td>
<td>0.11</td>
</tr>
<tr>
<td>DHA</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Significant at P<0.05; **significant at P<0.01; ***significant at P<0.001.

b Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).
Figure 1. The relationship between estimated docosahexaenoic acid (DHA) intakes from the food frequency questionnaire (FFQ) with DHA plasma phospholipids (PL) and red blood cell (RBC) phosphatidylethanolamine (PE) and phosphatidylcholine (PC).
References


9. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. Life Sci. 2003;73:3181–3187.


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GENERAL CONCLUSION

Omega 3 fatty acids, specifically EPA and DHA, have received a considerable amount of attention because of their positive effects during gestation. Studies have shown an association between estimated n-3 intakes during gestation and maternal health. Additionally, n-3 fatty acids, specifically DHA have been shown to support positive visual acuity and motor skills. The semi-quantitative FFQ may be the most suitable dietary assessment tool to estimate the intake of episodically consumed n-3 fatty acid intake in pregnant women.

The FFQ has shown correlations between other dietary reference methods like the weighed diet record and 24-hour recall in non-pregnant individuals. Additionally, a significantly positive association has been shown for the FFQ with blood biomarkers (plasma and RBC) when fatty acid intake is measured in non-pregnant individuals. The administration of two dietary assessment tools and the assessment of specific blood biomarkers like plasma PL and RBC PE and PC provides a more thorough investigation on how well the FFQ is estimating n-3 fatty acid intake.

Our dietary data was collected and analyzed through an in-depth process to reduce the possibility of systematic bias. Blood analysis was more complete than in previous published works because we reported specific RBC fractions (PE and PC) to provide a more thorough status measurement. Our dietary data showed a significantly, positive correlation between the FFQ and 3dDR when EPA and DHA was assessed. Furthermore, we observed association between all dietary data with all blood biomarkers for DHA. Dietary EPA collected via the FFQ, not the 3dDR, was also associated with blood biomarkers.

In conclusion, the semi-quantitative FFQ is an appropriate dietary assessment tool to estimate intake of total n-3, ALA, EPA and DHA and correlates well with the weighed 3dDR and blood biomarkers. Therefore, this FFQ an acceptable method to assess omega-3 fatty acid
intake would be a useful dietary assessment tool to use within the clinical and research settings in pregnant women
THESIS REFERENCES


5. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sci.* 2003;73:3181–3187.


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58. Makrides M, Duley L, Olsen SF. Marine oil and other prostaglandin precursor


### Appendix A

<table>
<thead>
<tr>
<th>Author (yr)</th>
<th>Population</th>
<th>Statistics</th>
<th>Significant Correlations between FFQ and/or weighed diet record n-3 dietary intakes and Blood Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>McNaughton et al. (2007)</td>
<td>N=43 (18 males and 25 females)</td>
<td>Spearman’s Correlation, the Methods of Triad (not reported)</td>
<td>FFQ Total n3 0.21, LCPUFA 0.38 (P&lt;0.05), ALA 0.00, EPA 0.21, n-3 DPA 0.21, DHA 0.32 (P&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weighed Diet Record Total n-3 0.33 (P&lt;0.05), LCPUFA 0.44 (P&lt;0.05), ALA 0.09, EPA 0.22, n-3 DPA 0.25, DHA 0.43 (P&lt;0.05)</td>
</tr>
<tr>
<td>Sun et al. (2007)</td>
<td>N=308 females</td>
<td>Spearman’s Correlation (average of 3 time points)</td>
<td>FFQ ALA 0.18, EPA 0.38 (P&lt;0.01), n-3 DPA 0.01, DHA 0.56 (P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sullivan et al. (2008)</td>
<td>N=53 (20 males and 33 females)</td>
<td>Spearman’s Correlation</td>
<td>FFQ n-3 0.50 (P&lt;0.001), EPA 0.54 (P&lt;0.001), DHA 0.48 (P&lt;0.001)</td>
</tr>
<tr>
<td>Otto et al. (2001)</td>
<td>N=67 pregnant women</td>
<td>Spearman’s Correlation</td>
<td>FFQ Prepregnancy DHA 0.65, FFQ Wk10 gestation LA 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>De Vriese et al. (2002)</td>
<td>N=30 pregnant women</td>
<td>Pearson’s Correlation</td>
<td>FFQ LA 0.53 (P&lt;0.01), ALA 0.53 (P&lt;0.01), EPA 0.48 (P&lt;0.01), DHA 0.52 (P&lt;0.01), EPA + DHA 0.39 (P&lt;0.05)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Parra et al. (2002)</td>
<td>N=35 pregnant women</td>
<td>Pearson’s Correlation, then linear regression</td>
<td>FFQ ALA 0.52 (P=0.641), DHA 0.30 (P=0.045)</td>
</tr>
<tr>
<td>Innis et al. (2002)</td>
<td>N=55 pregnant women</td>
<td>Pearson’s Correlation</td>
<td>FFQ EPA 0.55 (P&lt;0.0001), DHA 0.61 (P&lt;0.0001)</td>
</tr>
<tr>
<td>Dietary fatty acid</td>
<td>Semi-quantitative FFQ (g/d)</td>
<td>Weighed 3dDR (g/d)</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median (5th-95th percentile)</td>
<td>Mean ± standard deviation</td>
<td>Median (5th-95th percentile)</td>
</tr>
<tr>
<td>total n-3</td>
<td>1.935 (0.746-5.358)</td>
<td>2.527 ± 2.040</td>
<td>1.609 (0.595-4.001)</td>
</tr>
<tr>
<td>ALA</td>
<td>1.542 (0.650-4.081)</td>
<td>1.932 ± 1.772</td>
<td>1.481 (0.568-3.396)</td>
</tr>
<tr>
<td>EPA</td>
<td>0.023 (0.002-0.183)</td>
<td>0.050 ± 0.070</td>
<td>0.009 (0.000-0.248)</td>
</tr>
<tr>
<td>DPA</td>
<td>0.131 (0.004-1.555)</td>
<td>0.350 ± 0.616</td>
<td>0.006 (0.000-0.134)</td>
</tr>
<tr>
<td>DHA</td>
<td>0.056 (0.007-0.371)</td>
<td>0.095 ± 0.104</td>
<td>0.028 (0.001-0.452)</td>
</tr>
</tbody>
</table>
### Table 1. Anthropometrics and Demographics (n=64).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
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</tr>
<tr>
<td>Prepregnancy weight (kg)</td>
<td>65.8±13.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.6±13.5</td>
</tr>
<tr>
<td>Height (kg)</td>
<td>167.6±6.7</td>
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<tr>
<td>Week of Gestation</td>
<td>28.4±1.3</td>
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<th><strong>Grade Level Completed</strong></th>
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<td>Graduate/Professional School</td>
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<tr>
<th><strong>Income ($ per year)</strong></th>
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<tr>
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<tr>
<td>20,001-30,000</td>
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<tr>
<td>30,001-40,000</td>
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<td>7</td>
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<tr>
<td>50,001-75,000</td>
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<td>75,000+</td>
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<th><strong>Race</strong></th>
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<td>Caucasian</td>
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<tr>
<th><strong>Marital Status</strong></th>
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<td>Single</td>
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<tr>
<td>Married</td>
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</table>
Table 2. Dietary intake of total and individual n-3 dietary fatty acid intakes as estimated by weighed 3 day diet record (3dDR) and semi-quantitative food frequency questionnaire (FFQ) among all subjects (n=63).

<table>
<thead>
<tr>
<th></th>
<th>3dDR (g/d)</th>
<th>FFQ (g/d)</th>
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<tbody>
<tr>
<td></td>
<td>Median (5th-95th percentile)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>All Subjects (N=63)</strong></td>
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<tr>
<td>total n-3</td>
<td>1.467 (0.607-4.724)</td>
<td>1.773 ± 1.158</td>
</tr>
<tr>
<td>ALA</td>
<td>1.305 (0.581-3.170)</td>
<td>1.501 ± 0.949</td>
</tr>
<tr>
<td>EPA</td>
<td>0.008 (0.000-0.406)</td>
<td>0.089 ± 0.174</td>
</tr>
<tr>
<td>DPA</td>
<td>0.005 (0.000-0.140)</td>
<td>0.022 ± 0.040</td>
</tr>
<tr>
<td>DHA</td>
<td>0.037 (0.003-0.732)</td>
<td>0.161 ± 0.232</td>
</tr>
<tr>
<td><strong>Supplement user’s (N=19)</strong></td>
<td></td>
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<tr>
<td>total EPA</td>
<td>0.185 (0.000-0.699)</td>
<td>0.220 ± 0.258</td>
</tr>
<tr>
<td>total DHA</td>
<td>0.235 (0.128-0.890)</td>
<td>0.399 ± 0.270</td>
</tr>
<tr>
<td><strong>Non-supplement user’s (N=44)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.007 (0.000-0.186)</td>
<td>0.031 ± 0.064</td>
</tr>
<tr>
<td>DHA</td>
<td>0.024 (0.001-0.217)</td>
<td>0.057 ± 0.101</td>
</tr>
</tbody>
</table>

b Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).
Table 3. Total and individual fatty acid compositions (g/100 g) in red blood cell (RBC) phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and plasma phospholipids (PL) of pregnant women (n=63).

<table>
<thead>
<tr>
<th></th>
<th>PE</th>
<th>PC</th>
<th>PL</th>
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<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td></td>
<td>(5th-95th percentile)</td>
<td>(5th-95th percentile)</td>
<td>(5th-95th percentile)</td>
</tr>
<tr>
<td>All subjects (N=63)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total n-3</td>
<td>6.129 (3.780-9.376)</td>
<td>6.350 ± 1.547</td>
<td>2.527 (1.441-3.973)</td>
</tr>
<tr>
<td>ALA</td>
<td>0.184 (0.052-0.292)</td>
<td>0.167 ± 0.069</td>
<td>0.236 (0.130-0.375)</td>
</tr>
<tr>
<td>EPA</td>
<td>0.230 (0.110-0.560)</td>
<td>0.275 ± 0.141</td>
<td>0.184 (0.094-0.538)</td>
</tr>
<tr>
<td>DPA</td>
<td>1.881 (1.143-2.850)</td>
<td>1.935 ± 0.468</td>
<td>0.361 (0.245-0.579)</td>
</tr>
<tr>
<td>DHA</td>
<td>3.790 (2.371-6.121)</td>
<td>3.969 ± 1.093</td>
<td>1.717 (0.778-2.611)</td>
</tr>
</tbody>
</table>

Supplement user’s (N=19)

|          |                             |                             |                             |                             |
| EPA      | 0.230 (0.110-0.560)         | 0.275 ± 0.141               | 0.184 (0.094-0.538)         | 0.217 ± 0.139               |
| DHA      | 4.831 (1.959-6.411)         | 4.577. ± 1.360              | 2.231 (0.966-2.825)         | 2.131 ± 0.516               |

Non-supplement user’s (N=44)

|          |                             |                             |                             |                             |
| EPA      | 0.223 (0.104-0.497)         | 0.233 ± 0.009               | 0.161 (0.074-0.267)         | 0.166 ± 0.064               |
| DHA      | 3.701 (2.379-5.053)         | 3.706 ± 0.846               | 1.589 (0.759-2.438)         | 1.601 ± 0.438               |

b Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)