MATERNAL SUPPLEMENT USE DURING PREGNANCY

BY

C2012

Mallory Bratton

Submitted to the graduate degree program in Dietetics and Nutrition and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Master's of Science.

Committee Chair: Susan Carlson, PhD

Committee Members:

Debra Sullivan, PhD, RD

Holly Hull, PhD

Date Defended:

The Thesis Committee for Mallory Bratton

Certifies that this is the approved version of the following thesis:

MATERNAL SUPPLEMENT USE DURING PREGNANCY

_____

Chairperson Susan Carlson, PhD

Date Approved: _____

ABSTRACT

Background: There is little consensus regarding the need for vitamin and mineral supplementation during pregnancy. The composition and use of supplements among pregnant women varies greatly. Toxicity or inadequacy of nutrients could have health effects on the mother and fetus. A recent study reported inadequate micronutrient consumption in United States pregnant women (1), supporting the need for supplementation.

Objective: The purpose of this project is to determine the frequency of supplementation among a sample of low to middle-class pregnant women in the Kansas City metropolitan area and determine if consumed supplements favor the Institute of Medicine's Recommended Daily Allowance (RDA) guidelines.

Design: A subset of women (n=231) enrolled in a phase III clinical trial from 2006-2010 provided information regarding use of supplements. Nutrient intakes during pregnancy are reported as means ± SD. One way ANOVAs and Pearson Chi Square tests were also used for other analyses.

Results: Women reported consuming a nutritional supplement before, during or after pregnancy were 50%, 100%, and 35%, respectively. Supplement use before pregnancy was impacted by previously known cofactors. Mean micronutrient intake from supplements was below the RDA or AI for vitamin D, calcium, and iron, while vitamin C

and folic acid were found to be excessive. Median intakes of choline and iodine were zero.

Conclusions: Supplementation during pregnancy was common in this cohort but individual formulations varied. As a public health message, it would be beneficial for formulations of prenatal supplements to be standardized to ensure women consume nutrients vital to the developing fetus.

ACKNOWLEDGEMENTS

My deepest gratitude to my mentor Susan Carlson, PhD, for all of her knowledge and support. I feel that it is a true privilege to have worked with such an intelligent, motivated, hard-working woman. I also appreciate the assistance of Debra Sullivan, PhD, RD, and Holly Hull, PhD, for serving on my committee.

This project would not have been possible without the assistance and perseverance of Elizabeth Kerling, MS, RD. Words will never be able to express my full appreciation of her help.

And to Susan Scholtz, MS for assisting me in my statistical analyses. I greatly appreciate the time you took to assist me in this project and I definitely could not have done it without you.

My parents, family and friends were an amazing support system that listened to my countless worries, thank you for lending an ear and always believing in my full potential. To my brother Andrew, and sister-in-law Leslie, thank you for giving me a beautiful niece this winter. I am so happy to be able to spend more time with her now.

Table of Contents

List of Tables and Figures

Chapter 1

INTRODUCTION

During pregnancy the sole source of nutrition for the fetus is through the mother's nutrient stores and daily dietary intake. Therefore, it is imperative the mother provide adequate nutrients for the developing fetus. Requirements for fourteen of the twenty-one essential micronutrients are higher during pregnancy; and on the advice of their obstetricians, many women complement their dietary intake with supplements throughout pregnancy (2). However, the use of prenatal supplements among pregnant women varies greatly depending upon how frequently they take the supplements; the length of time they take the supplements; how closely they adhere to dosing instructions; and the formulation of supplements. Little research has been conducted in the United States regarding what supplements women are taking during pregnancy and most of the existing publications are quite old. Published findings do examine factors affecting compliance of supplement intake, but there is no current publication on what women are taking during pregnancy and how this affects their nutritional status. While supplementation of prenatal vitamins and minerals is not recommended by the Institute of Medicine for every pregnant woman, emerging research shows the benefits of supplementation (3).

Statement of purpose

The purpose of this project is to determine the intake of key nutrients recognized as commonly inadequate in the diet of US pregnant women by evaluating both the composition and frequency of supplement intake.

Research questions

Primary research question:

How much vitamin C, vitamin D, vitamin E, folic acid, calcium, iron, iodine, and choline did women receive from prenatal supplements during pregnancy?

Secondary research questions:

1. In addition to prenatal vitamins, what other supplements are being consumed?
2. What demographic differences exist between women who took supplements before pregnancy and those who did not?
3. Is post-natal supplementation related to breastfeeding?
4. Is there a difference between over-the-counter prenatal formulations and prescription prenatal formulations?

Chapter 2

LITERATURE REVIEW

Dietary supplement intake in pregnancy

Publications regarding prenatal supplement use date back to early 80's and often only examine factors affecting capsule compliance. Advances in medicine and pharmacology as well as maternal education regarding prenatal care suggest changes in supplementation are to be expected but none are documented. It is believed that most healthcare providers recommend consuming a prenatal multivitamin and multimineral during pregnancy although there is little evidence to support the need for supplementation in developed countries such as the United States. Prenatal multivitamin use did decrease the risk for fetal malformations in one study suggesting benefits may accrue to at least some women and their offspring (4).

In 1988, the National Maternal and Infant Health Survey (NMIHS) found that 26% of women surveyed in the United States were taking a multivitamin supplement prior to pregnancy (5). A contemporary study culturally diverse, low-income women in the United States reports 16% were taking some multivitamin prior to conception, but not necessarily a prenatal supplement (6). In 2003, a study in Melbourne, Australia evaluated pregnant women between 36-38 weeks gestation to examine the vitamin use before and during pregnancy. The study found 11% of the women took a pre-pregnancy multivitamin and 36% started on the multivitamin without doctor's recommendation, while 30% were recommended to do so by their primary care physician. During pregnancy, 34.7% of this cohort was taking prenatal multivitamins, although a variety of brands and doses were reported by subjects (7).

Prenatal supplementation recommendations during pregnancy

Whether it is a multivitamin, folic acid, iron, or iodine supplementation, current recommendations of prenatal supplementation are not consistent. The Institute of Medicine (IOM) recommends general, prenatal supplementation only for women who smoke, abuse alcohol/drugs, have iron deficiency anemia, have poor quality diets, are vegans, and women with ≥2 fetuses (2, 8). The Centers for Disease Control and Prevention (CDC) and the IOM recommends all women of child bearing age who are capable of becoming pregnant, consume 0.4 mg/d folic acid. Once the pregnancy is confirmed, the CDC recommends oral, low dose iron, 30 mg/d beginning with the first prenatal visit (9). Conflicting recommendations from the World Health Organization (WHO) instruct iron supplementation of 60 mg/d for 6 months, or if 6 months of treatment cannot be achieved, continuation during postpartum or an increased dosage of 120 mg/d (10). It is important to note that WHO emphasizes their recommendations to poverty-stricken populations where access to good nutrition and iron-rich foods is limited. The American Thyroid Association recommends that all pregnant women living in North America consume 150 µg/d of iodine (11).

Supplementation outcomes on birth

Different types of supplementation have been associated with various birth outcomes and are often inconsistent. Scholl et al. (12) found that multivitamin use prior to conception has little influence on pregnancy outcome. A retrospective case-control study showed that use of a multivitamin from three months prior to conception through the third month of pregnancy decreased the risk of heart malformations in the fetus (4). In a randomized, controlled placebo-like trial with 4,862 subjects, antenatal multivitamin

supplementation was found to decrease the incidence of cardiovascular malformations ((3, 13).

Iron supplementation throughout pregnancy increased birth weight by 100-200 g, and when compared with a placebo, decreased the risk of low birth weight births (14). Iron supplementation during pregnancy increased birth weight by a mean of 103 g in a Zimbabwe study (15). A study conducted in Nepal, found iron supplementation decreased low birth weight by 16% (16)**.**

When multimicronutrient supplementation is compared to iron-folic acid only supplementation, multimicronutrient supplementation has been found to reduce the incidence of low birth weight babies by 17%. A meta-analysis found that newborn birth weight was 54 g higher among women supplemented with a multimicronutrient supplementation versus iron-folic acid supplementation only. However, the type of supplement did not change the risk of preterm or small-for- gestational-age birth (17).

Scholl et al (18) found that low and marginal folate status was associated with increased risk of preterm delivery and low birth weight. Low maternal zinc status was also associated with low birth weight and preterm delivery.

Supplementation outcomes on mothers

The Camden Study examined maternal nutrition in Camden, New Jersey, one of the poorest cities in the United States. Prenatal supplements were associated with better prognostic indicators including better weight gain for gestation and less bleeding and nausea, etc. The Camden Study also found that the use of prenatal multivitamins during the 1st and 2nd trimesters resulted in a twofold reduction in the risk of preterm delivery; twofold reduction of a low birth weight baby; and decreased risk of a very

preterm delivery and a very low birth weight baby. Serum nutrient concentrations were also examined for ferritin, zinc and folate levels. At study entry, women who supplemented prior to conception differed little in nutrient status from women who did not supplement. By week 28, status of numerous micronutrients differed. Red cell and serum folate as well as ferritin were increased in the preconception supplement users. Zinc levels did not differ at week 28 between the preconception supplement users and the nonusers (12).

In regard to folate status levels in particular, adequate serum folate is associated with appropriate gestational weight gain and duration of pregnancy (12).

Current use of supplementation in the United States and abroad

The categories of nutrient supplements consumed during pregnancy may be divided into four main categories: multivitamins, folic acid, iron and other which includes single nutrient supplementation such as vitamin C, zinc, calcium and pyridoxine.

Folic acid is recommended to decrease the risk of congenital malformations including neural tube defects during pregnancy. One study in the United States targeted women who were attempting to conceive. Of the women who became pregnant, 44% took folic acid 3 months prior to conception. In the same study, 93% of the women were supplementing with folic acid, at 11 weeks gestation (19). In the previously mentioned Australian study, 29% took folic acid during the periconception period. Greater than 50% reported that it was recommended by their primary care physician, while 19% self-prescribed, and 5% used supplementation at the recommendation of friends. During pregnancy, 80% of this population took folic acid (7).

Iron is another supplement often prescribed during pregnancy. National Health and Nutrition Examination Survey III (NHANES III) found 74% of pregnant women were taking iron supplements as a standalone nutrient. The mean iron intake from supplements was 78.4 ± 6.6 mg/d (20), which is greater than the IOM upper limit of 45 mg/d (21). Fifty-one percent of the women in the Australian study took an iron supplement during pregnancy. Of the women who used an iron supplement, 46% did so to combat anemia, 14% used it to enhance energy, and 33% were otherwise recommended to do so by their physicians (7).

Other single nutrient supplements reported to be taken pre-pregnancy in the Australian study included calcium, pyridoxine, and zinc. Calcium was taken by 44% of the subjects and was recommended primarily by physicians, but 21% of the women reported taking the supplement spontaneously. Pyridoxine was taken by 14% of the women, of which 48% reported taking it for morning sickness and nausea. Zinc was also supplemented in 7% of the women, taking the supplement for their own health and the health of the fetus (7). In 1988, the NMIHS found that expectant mothers took calcium, zinc, vitamin C, and vitamin A at least 3 times a week during the last three months of their pregnancy (5).

Dietary supplement adherence in pregnancy

Multiple factors have been associated with adherence to supplement intake by pregnant women. Studies have consistently shown that supplement intake during pregnancy is affected by ethnicity and race (5, 22). A study conducted by Suitor and Gardner (6) in a culturally diverse, low-income population found that white, non-Hispanic pregnant women had higher rates of supplement usage (23%) compared to

Hispanic women (10%). Within the 1988 NMIHS, Yu et al. (5) found black mothers to be 17% less likely than white mothers to take a multivitamin. Cultural differences in the non-Hispanic blacks may explain adherence differences when supplements are prescribed. Common misconceptions about the effects of supplements included increased weight gain, fear of complex labor due to macrosomia, fear of developing too much blood, and fear of deformities in babies (5). Lack of adherence to iron supplements is a particular concern in non-Hispanic blacks because they have a greater risk of iron deficiency anemia (22).

Education and age are positively associated with supplement usage. Sixty-seven percent of women with ≤ 8 years of formal education used multivitamins during pregnancy compared to 89.9% of women with formal education ≥16 years, a 22.9% difference in use. Additionally, women ≤20 years old had a 65.5% adherence rate compared to women , 20-34 years old (82.4%) and those ≥35 years old (86.3%) (5).

Socioeconomic status is also a common factor associated with supplementation use (6). The National Health Institute Survey (NHIS) found that women in households >200% above the poverty level were more likely to take supplements during pregnancy (23). NHANES III found women with socioeconomic status ≤130% poverty level were less likely to consume nutrient supplements during pregnancy (3). Women who received Medicaid for prenatal care were less likely to take supplements than women not receiving Medicaid (assumed to be insured or self-paying). Scholl et al. (12) reported 85% of mothers on Medicaid did not use prenatal multivitamins. The United States Department of Agriculture 1985 Continuing Survey by Individuals, found that 40% of

women ≤130% below poverty level were taking supplements regularly or occasionally (6).

The NHIS found that former smokers were more likely to use supplements (23). Yu et al. (5) found that smokers were more likely to take supplements during pregnancy, suggesting that this population may be potentially more mindful about adhering to health care advice to balance the harmful effects of smoking. White women in the iron supplementation study conducted by Jasti et al. (22) were found to adhere more closely to the regimen if they were current smokers. Jasti et al. (22) suggest that the higher adherence is due to an increased concern about pregnancy outcomes.

Conclusion

Supplementation during pregnancy varies greatly, and new recommendations are continually published based on emerging research. The current recommendations of prenatal supplementation vary among national organizations, as well as primary care providers, with little evidence supporting the use of prenatal supplementation, particularly in developed countries. It is important to examine the barriers to supplement intake adherence and how barriers such as race and ethnicity, education level, socio-economic status, maternal age, and smoking status of prenatal care can be overcome. More research is also necessary to examine what pregnant women are taking for prenatal supplements and the outcomes of supplementation on birth in healthy mothers, particularly in the United States. It is not ethical to conduct a randomized trial among subjects with the limited evidence regarding supplementation outcomes on birth.

Chapter 3

METHODS

Overview

Maternal supplement use during pregnancy is a secondary outcome of the KU DHA Outcome Study (KUDOS) conducted from 2006 to 2011 at the University of Kansas Medical Center in Kansas City, KS. The primary KUDOS study examined the efficacy and safety of docosahexaenoic (DHA) supplementation (600 mg/day) in pregnant women (NCT00266825). Supplementation was given for approximately the last two trimesters of pregnancy. Primary aims of the study were to: 1) determine whether maternal RBC PL DHA can be significantly increased by supplementation, 2) assess the effect of DHA supplementation on duration of gestation, 3) evaluate adverse events in women and infants in the treated and placebo groups, 4) evaluate the effect of maternal DHA supplementation on visual evoked potential acuity in infancy and 5) evaluate the effect of DHA supplementation on the development of fundamental measures of cognitive function in infancy.

The Office of Dietary Supplements supported a study to examine what dietary supplements women take during pregnancy and the amounts of specific nutrients women receive during pregnancy from supplements. This analysis examined not only traditional prenatal vitamin and mineral supplement but also other non-traditional supplements. Supplement intake before, during and after pregnancy was examined as well as factors related to supplement intake.

Sample

Women between the ages of 16 and 35.99 years who were between 8 and 20 weeks gestation were eligible to participate in the study. Study subjects agreed to consume capsules (DHA or placebo) from enrollment in the study until delivery of the infant and to be available by telephone. Participants also agreed to return to the study centers for delivery and postnatal infant follow-up visits.

Exclusion criteria included any potential participants with serious health conditions likely to affect the growth and development of their fetus; the postnatal growth and development of their newborn; or the health of the mother during pregnancy. Serious health conditions included, but were not limited to, cancer, lupus, hepatitis, diabetes mellitus (Type I, Type II, or gestational), or HIV/AIDS. Multiple births were excluded due to the increased risk of preterm and low birth weight delivery. Women with morbid obesity (BMI ≥40) or elevated blood pressure (systolic ≥140 mmHg) were excluded. As not all postnatal developmental tests were standardized for different cultural groups; non-English speaking patients were also excluded.

Research setting

Women enrolled in the study (n=350) were primarily recruited from the University of Kansas Medical Center (Kansas City, Kansas), Truman Medical Center (Kansas City, Missouri), and St. Luke's Hospital (Kansas City, Missouri). Some women enrolled were self-identified from clinicaltrials.gov, myKUMC email blast, and by word of mouth. Potential participants enrolled from clinics were approached at a prenatal appointment and the background, purpose, procedures, and risks of the study were explained.

All of the postnatal follow-up visits occurred at the University of Kansas Medical Center beginning on August 30, 2006. Women returned to the study center for follow-up of their infants when they were at 6 weeks, and 4,6,9,10,12, and 18 months.

Ethics

Approval for the KUDOS study was obtained by the Institutional Review Board, protocol #10186. The study is currently under an existing protocol. Written informed consent was obtained (see Appendix A) and the welfare of study subjects was upheld according to the Helsinki Declaration. Confidentiality was ensured throughout the study. Names of subjects or other identifying information was not released without written consent. All subjects were assigned a random identification number. Information was secured under lock and key and only approved study protocol members were allowed access to collected data. Information was protected by the Health Insurance Portability and Accountability Act (HIPAA) which is a federal law to ensure privacy of health information.

Procedures and materials

This sub-study design to determine supplement intake was a prospective cohort study. Subjects (n=348) were asked at enrollment what supplements they were currently taking, what they took prior to pregnancy, and what supplements were prescribed (see Appendix B). Data were recorded by the study personnel. Women still enrolled at the first postnatal visit follow-up visit (n=231) were asked again what supplements they took before, during and after pregnancy (see Appendix C). Data were again recorded by the study personnel. Beginning July 2009, subjects (n=76) were also sent a monthly supplement questionnaire during pregnancy (see Appendix D). A total of

178 questionnaires were collected. Information was gathered by reading the questions from the forms. The questionnaires were not standardized.

In order to determine the length of time a supplement was taken, the start date reported on the initial enrollment questionnaire was utilized because it was presumed to be most accurate due to closer proximity to the actual start date. If no start date was indicated on the enrollment questionnaire, the start date from the 6 week follow-up visit was utilized. The stop date used was reported at the 6 week infant visit or if ongoing, subsequent clinic visits. For example, if the mother was still taking the prenatal supplement at 6 weeks, 4, 6, and 9 months, but reported the stop date at the 12 month visit, that date would be used. If no stop date was specified, in the case a subject dropped from the study, the stop date was assumed to be the date of delivery.

Study personnel captured supplement brand names when possible. If a brand name was reported at enrollment, but at the 6 week follow-up the subject was unsure, it was assumed to be the same supplement as reported at enrollment. Some assumptions were made regarding the formulation of the products. For example, if a woman reported taking an over-the-counter prenatal, but was unsure of the name, average over-the-counter nutrient amounts were assigned. The most frequently consumed over-the-counter supplements had identical compositions and therefore were used when the brand name was unknown. If women reported unknown prescription prenatals (n=21) we removed them from the analyses, there was no common formulation for prescription prenatal supplements. In many cases women changed prenatal supplements during pregnancy. If data overlapped, we assumed that the initial prenatal was taken until the second supplement began.

Frequency of supplementation was best assessed from the women receiving the monthly questionnaires. If the reporting varied on the questionnaires, an average was taken. For women without the monthly questionnaires, frequency reported at the 6 week clinic visit was used. When frequency was not reported (n=2), the subject was not included in the analysis. One subject was excluded for nutrient analysis due to excessive frequency report of prescription prenatal supplements.

Supplementation after pregnancy was considered to be any supplement taken at least 30 days after pregnancy ended. Anything less than 30 days did not qualify.

When assessing nutrient intake, eight nutrients were chosen. Vitamin C, vitamin D, vitamin E, folic acid, calcium, and iron were used by recommendation from the R03NR008458-01 NIH-funded study, whose purpose was to develop a profile of common nutritional patterns among pregnant African American women (1). In addition, iodine and choline were analyzed due to emerging interest in pregnancy. DHA was recorded, however, the study selected against women consuming >300 mg DHA/day because it was designed to compare DHA supplementation to placebo.

Statistical analysis

Nutrient intakes during pregnancy are reported as means ± SD. Differences in specific nutrient contents between prescribed and over-the-counter pre-natal vitamins were assessed using a one-way ANOVA. Women electing to take pre-natal vitamins prior to conception were compared to their peers with regard to smoking status, maternal age at enrollment, and race. Differences in smoking status and maternal age were determined using one-way ANOVA, while comparisons of maternal race were analyzed with a Pearson Chi-Square test for categorical variables. One-way ANOVA

was also used to assess differences in length of breastfeeding between women electing to consume and those who refrained from consuming vitamins postnatally. All data were analyzed with SPSS Statistics 17.0 software (SPSS, Chicago, IL), and a *P*-value ≤ 0.05 was considered significant.

Data collection

Data collection was completed via questionnaires previously described and entered in the Microsoft Access® database (Appendices A-D). The type and frequency of supplements was recorded for each subject. An additional database was established with all the supplements taken by subjects as well the amount of nutrients within each supplement type. Prenatal formulations were collected by a combination of internet searches and store visits to determine supplement formulation.

Chapter 4

RESULTS

Nutrient intake during pregnancy from prenatal vitamins

Nutrient intake for nine micronutrients was calculated by total intake during pregnancy and intake per pregnancy day. Length of supplementation and frequency were taken into account in the analysis. Average frequency of supplementation was 0.997 supplements per day. Table 1 summarizes the total nutrient intake during pregnancy. Table 2 summarizes total nutrient intake per pregnancy day. Figures 1-12 outline the individual nutrient intakes per pregnancy and per pregnancy day. Iodine, Choline and DHA were not consumed by many women, thus medians were calculated rather than means.

TABLE 1: Total Nutrient Intake During Pregnancy (n=203)

	Mean	Standard Deviation	Range
Vitamin C (mg)	25,681	8,749	1,509 - 57,840
Vitamin D (IU)	91,197	33,601	10,057 - 253,000
Vitamin E (IU)	6,423	3,715	616 - 27,500
Folic Acid (µg)	189,703	62,224	17,200 – 385,600
Calcium (mg)	46,549	19,537	0 - 144,600
Iron (mg)	6,254	2,510	704 - 21,330

TABLE 2: Nutrient Intake Per Pregnancy Day (n=203)

	Mean	Standard Deviation	Range
Vitamin C (mg)	94	31	6 - 207
Vitamin D (IU)	333	119	37 - 917
Vitamin E (IU)	23	13	2 - 100
Folic Acid (µg)	693	209	60 - 1,382
Calcium (mg)	170	69	0 - 518
Iron (mg)	23	9	3 - 74

FIGURE 1: Total Vitamin C Intake During Pregnancy

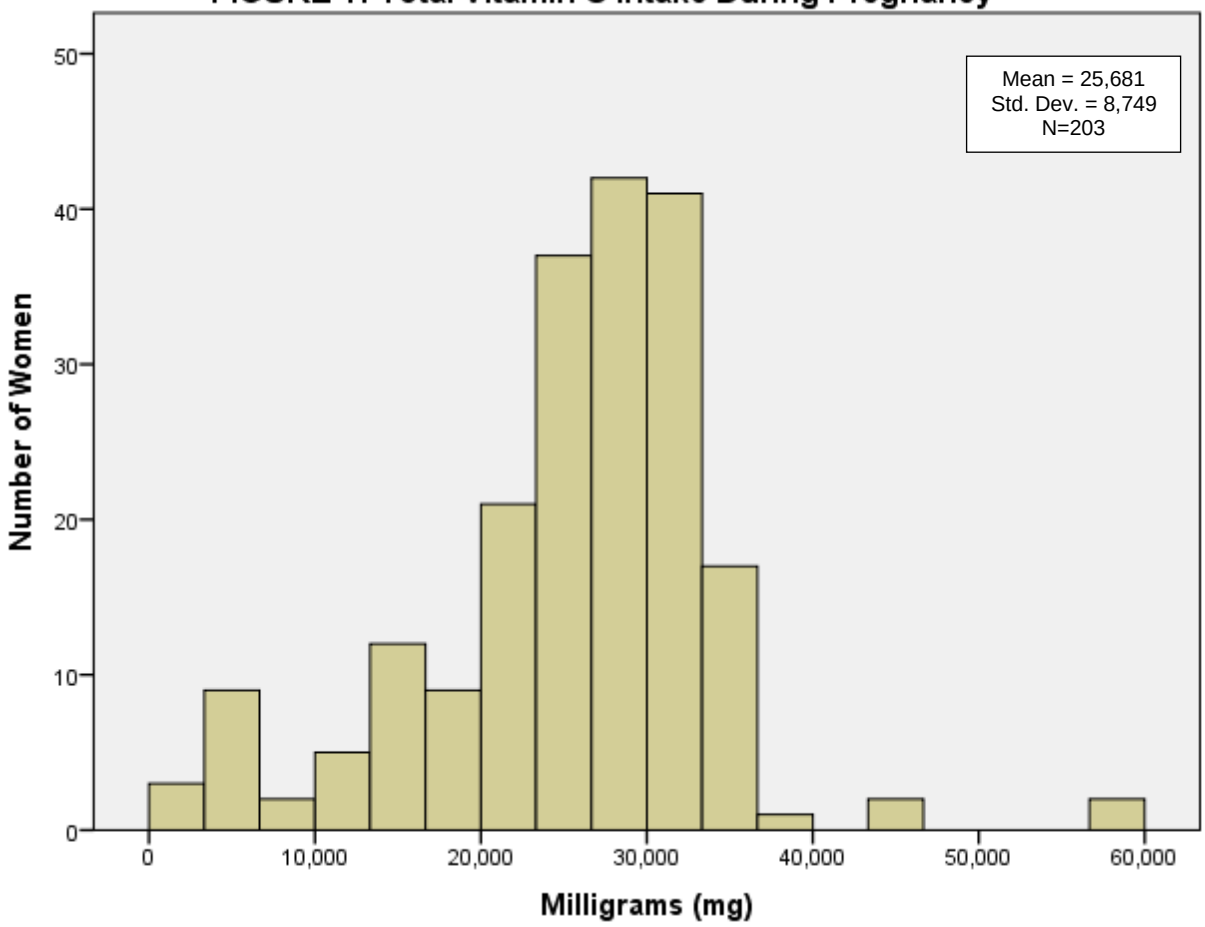


FIGURE 2: Vitamin C Intake Per Pregnancy Day

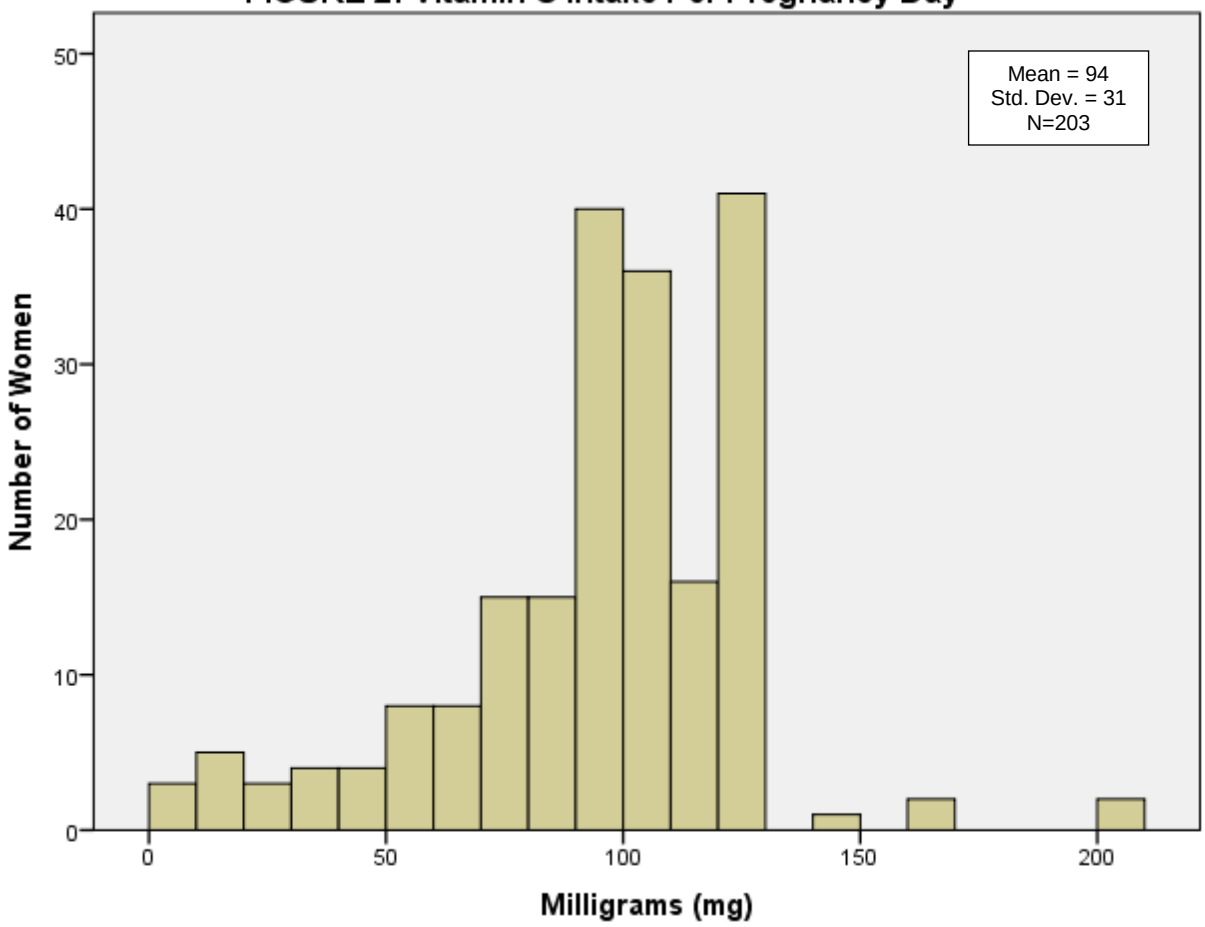


FIGURE 3: Total Vitamin D Intake During Pregnancy

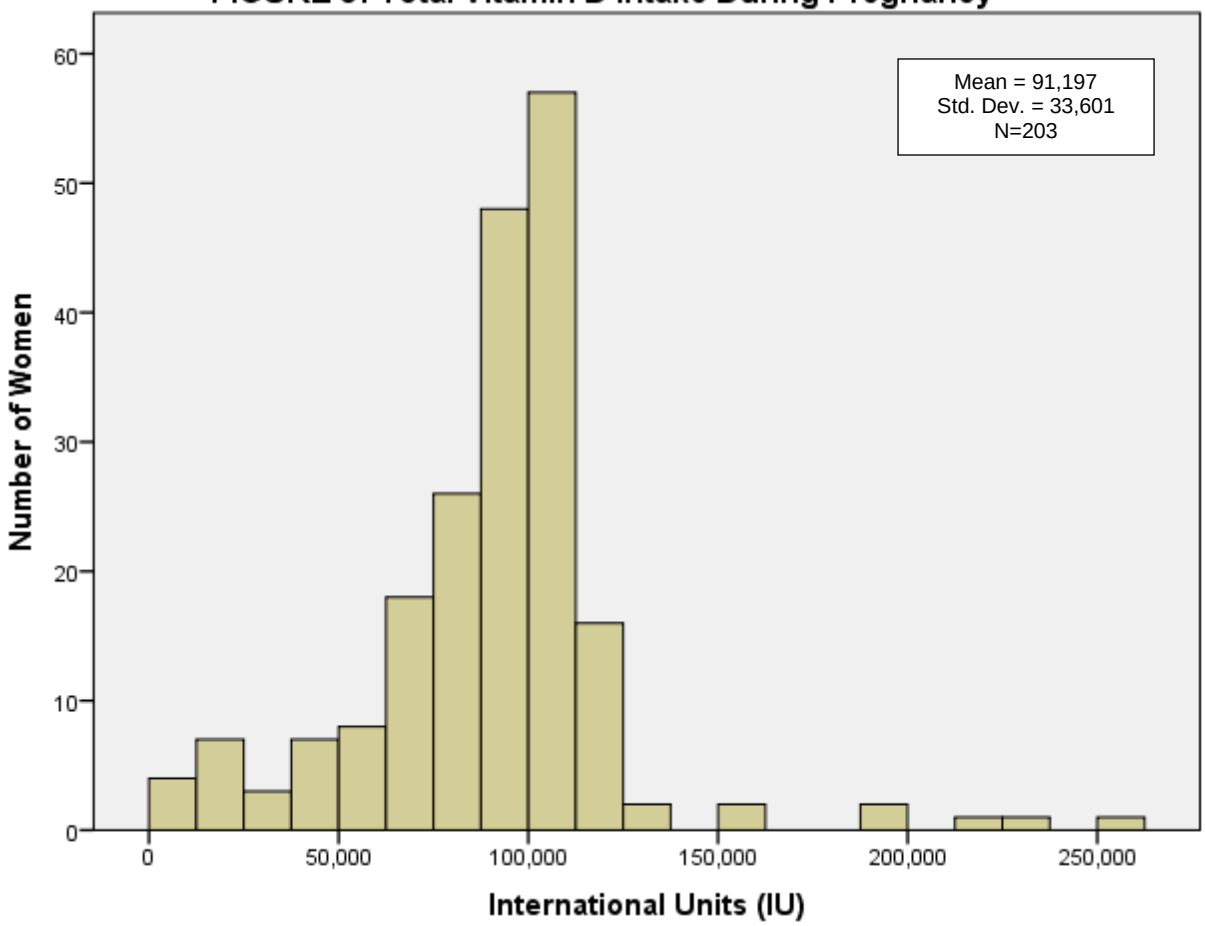


FIGURE 4: Vitamin D Intake Per Pregnancy Day

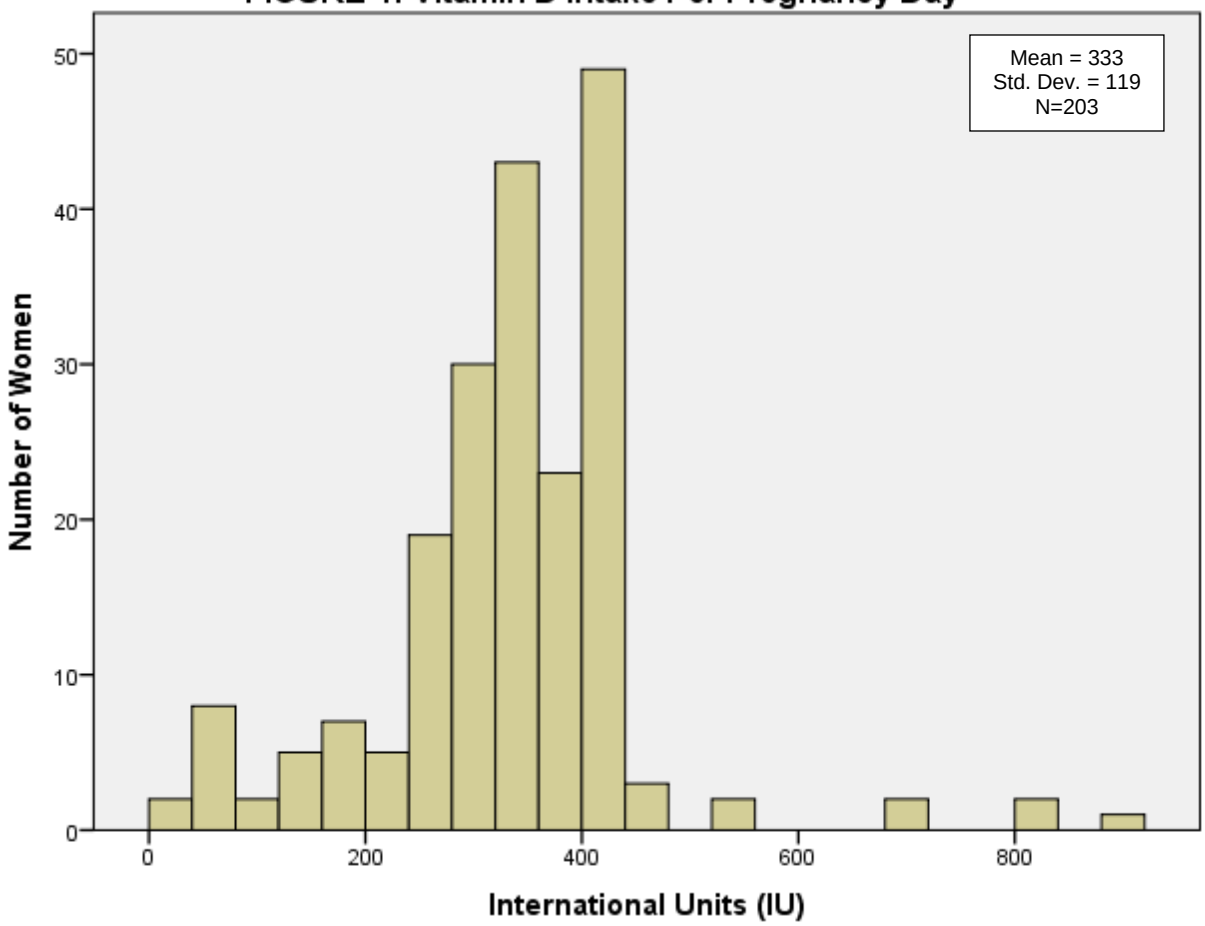


FIGURE 5: Total Vitamin E Intake During Pregnancy

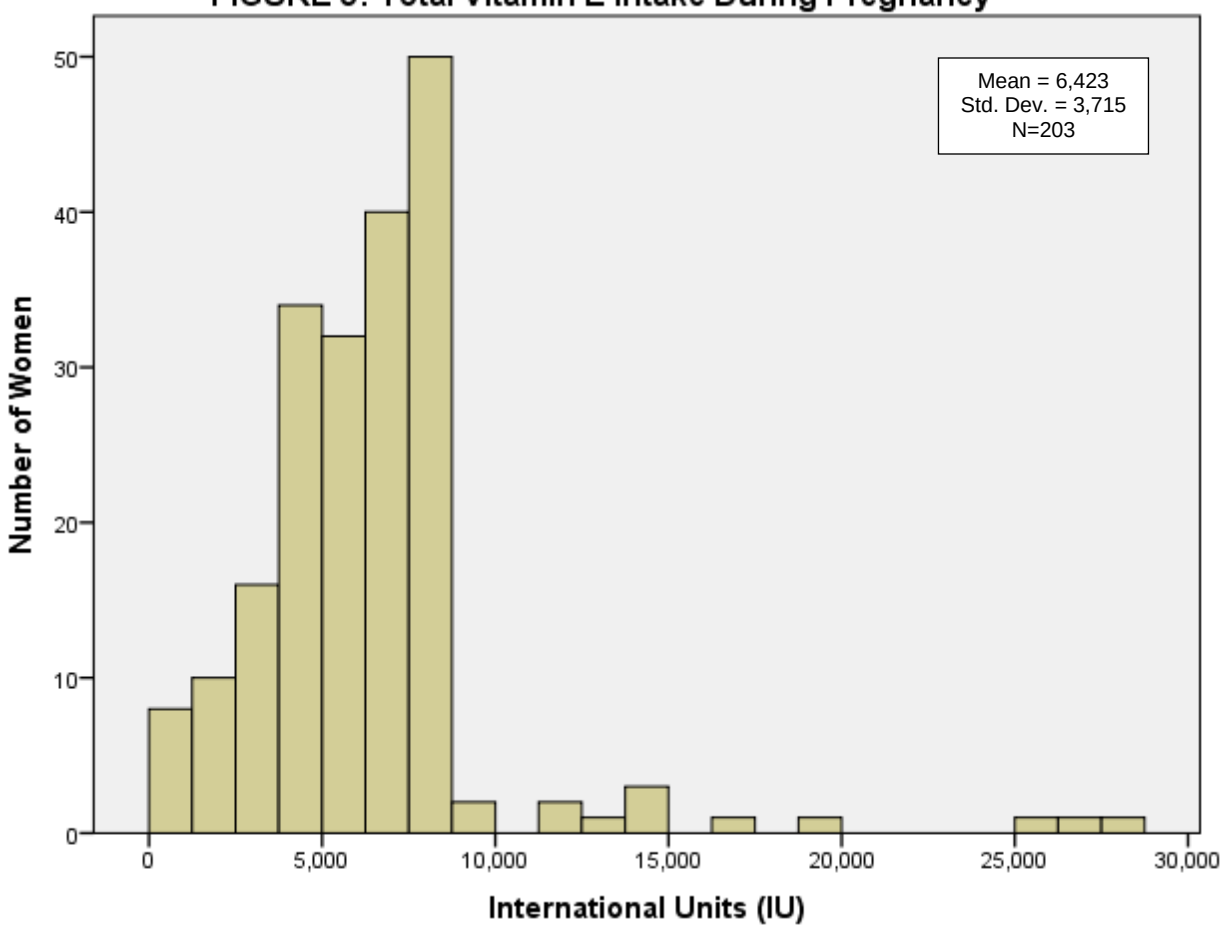


FIGURE 6: Vitamin E Intake Per Pregnancy Day

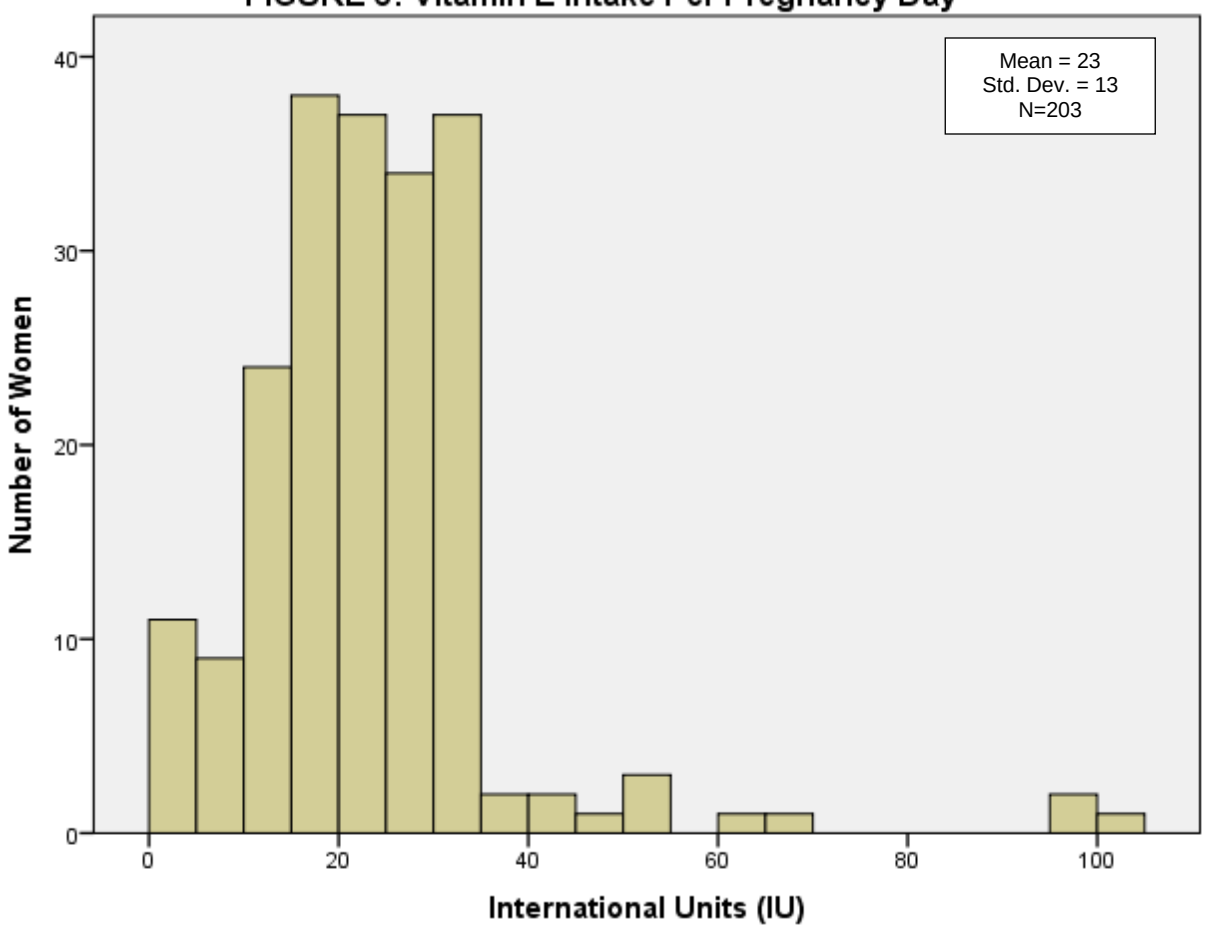


FIGURE 7: Total Folic Acid Intake During Pregnancy

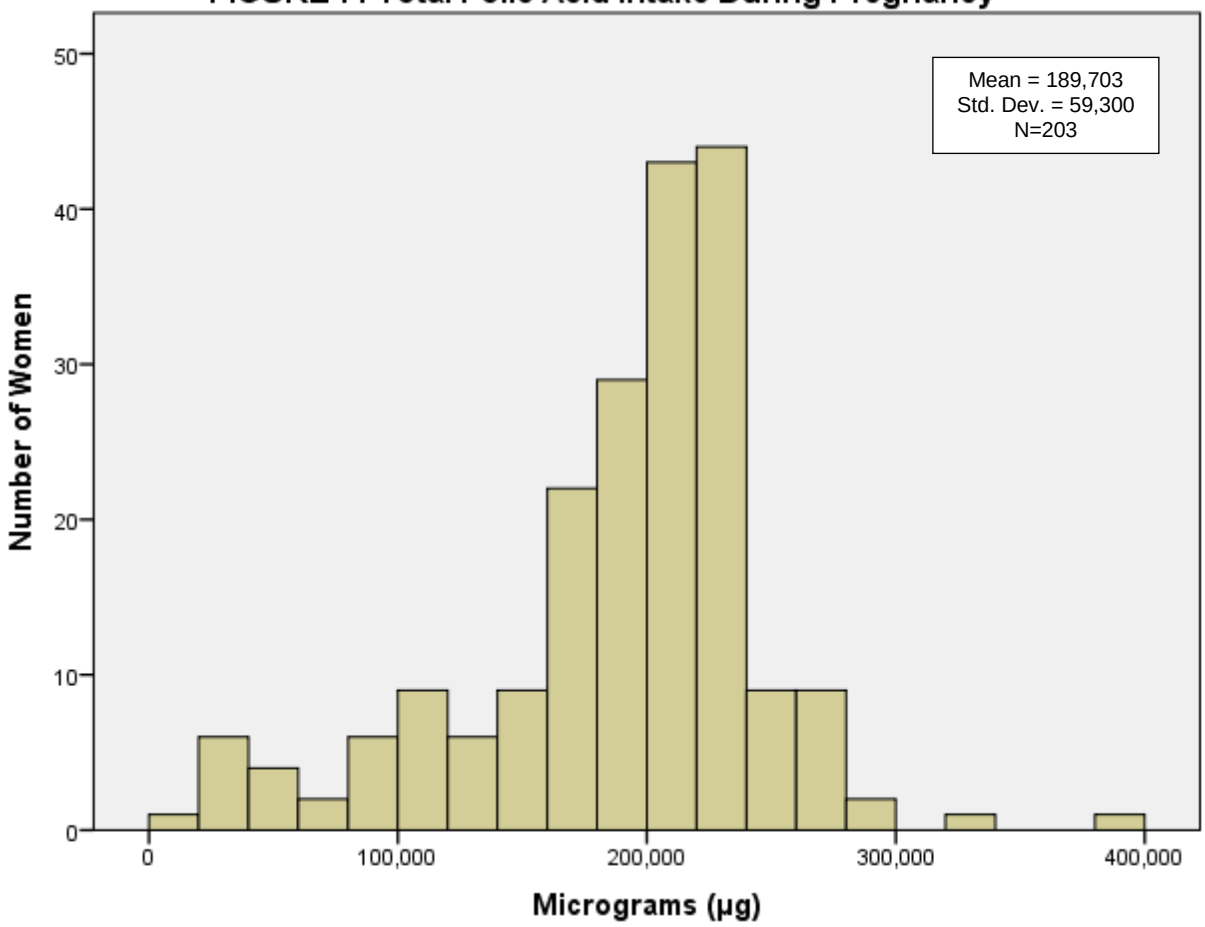


FIGURE 8: Folic Acid Intake Per Pregnancy Day

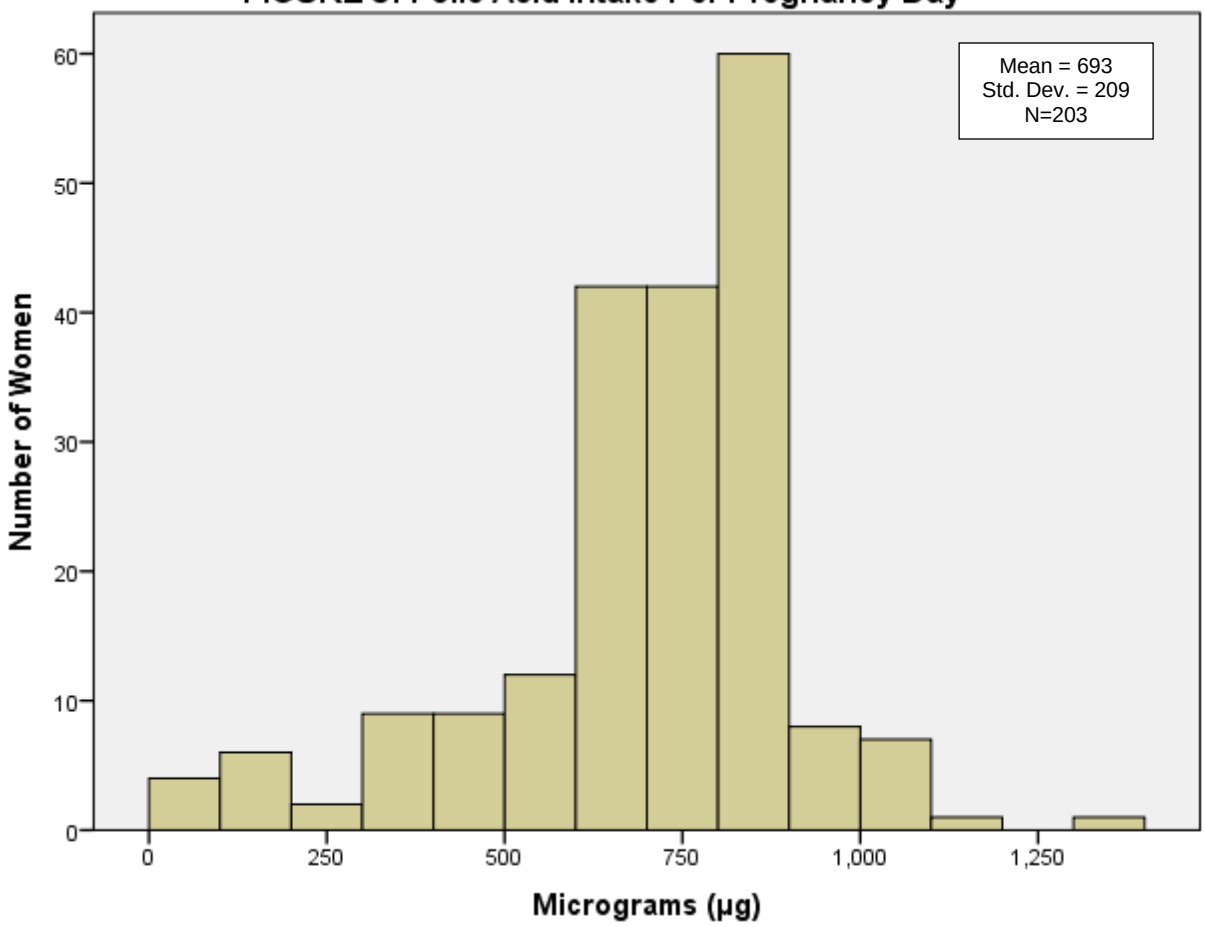
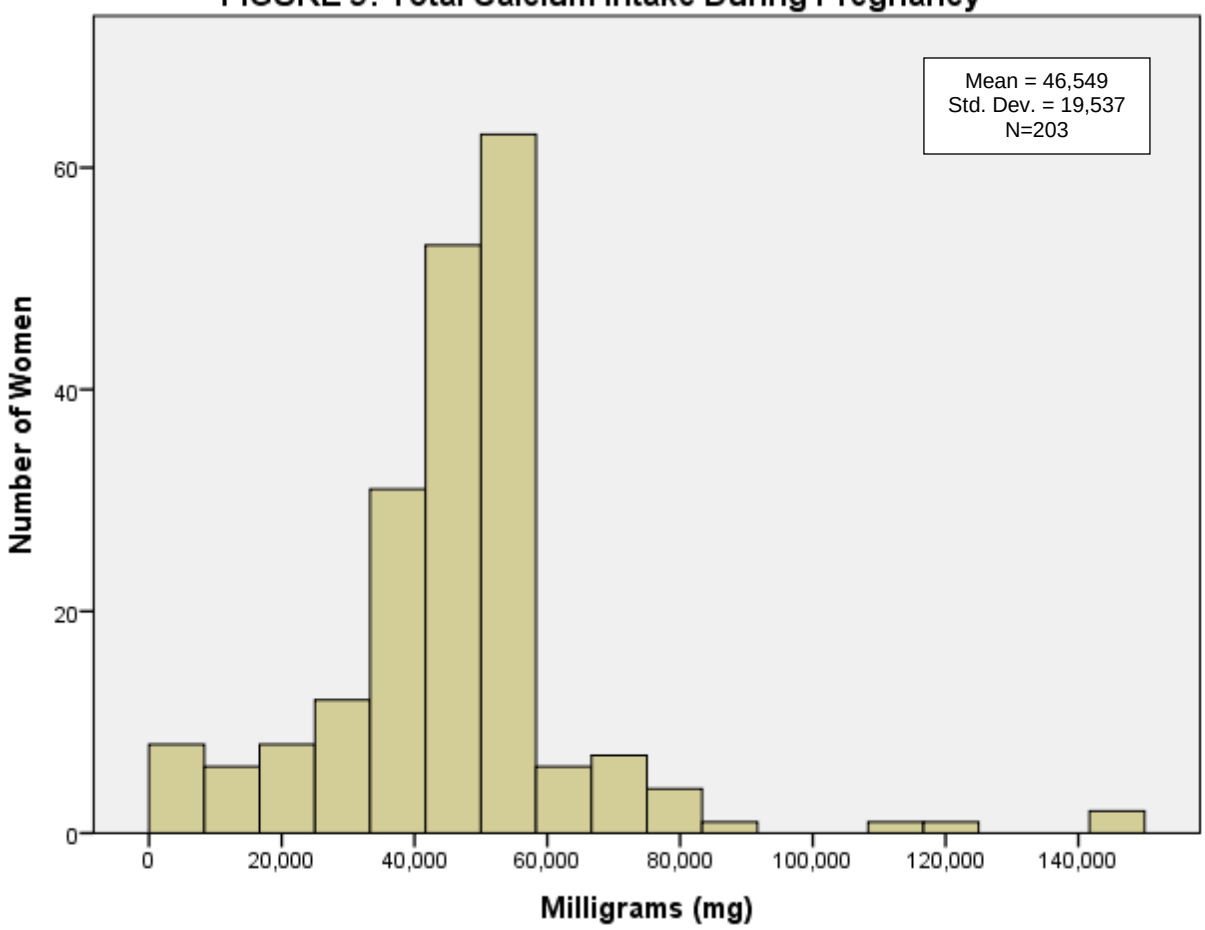


FIGURE 9: Total Calcium Intake During Pregnancy



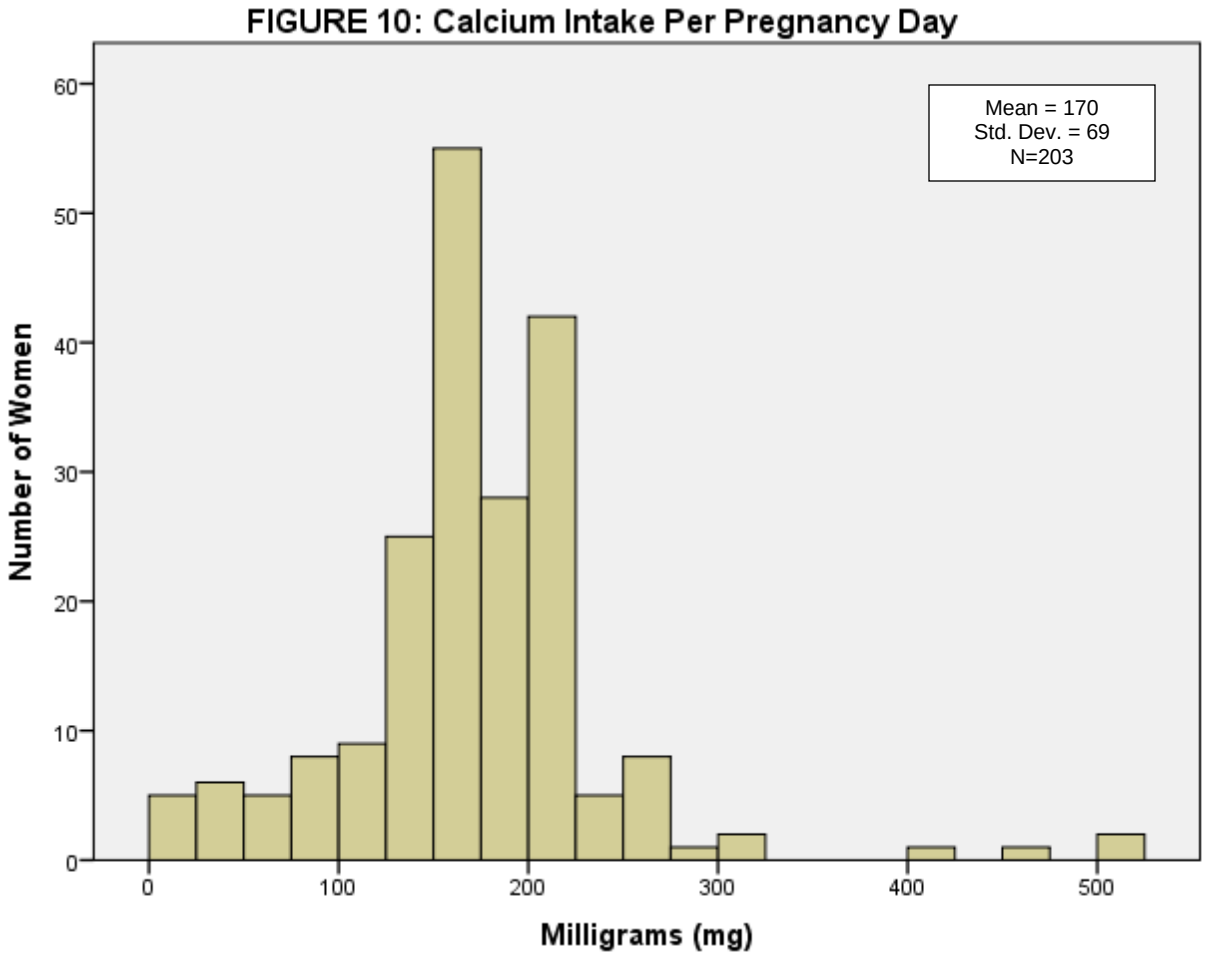


FIGURE 10: Calcium Intake Per Pregnancy Day
Mean = 170
Std. Dev. = 69
N=203
Number of Women
0
10
20
30
40
50
60
0
100
200
300
400
500
Milligrams (mg)

FIGURE 11: Total Iron Intake During Pregnancy

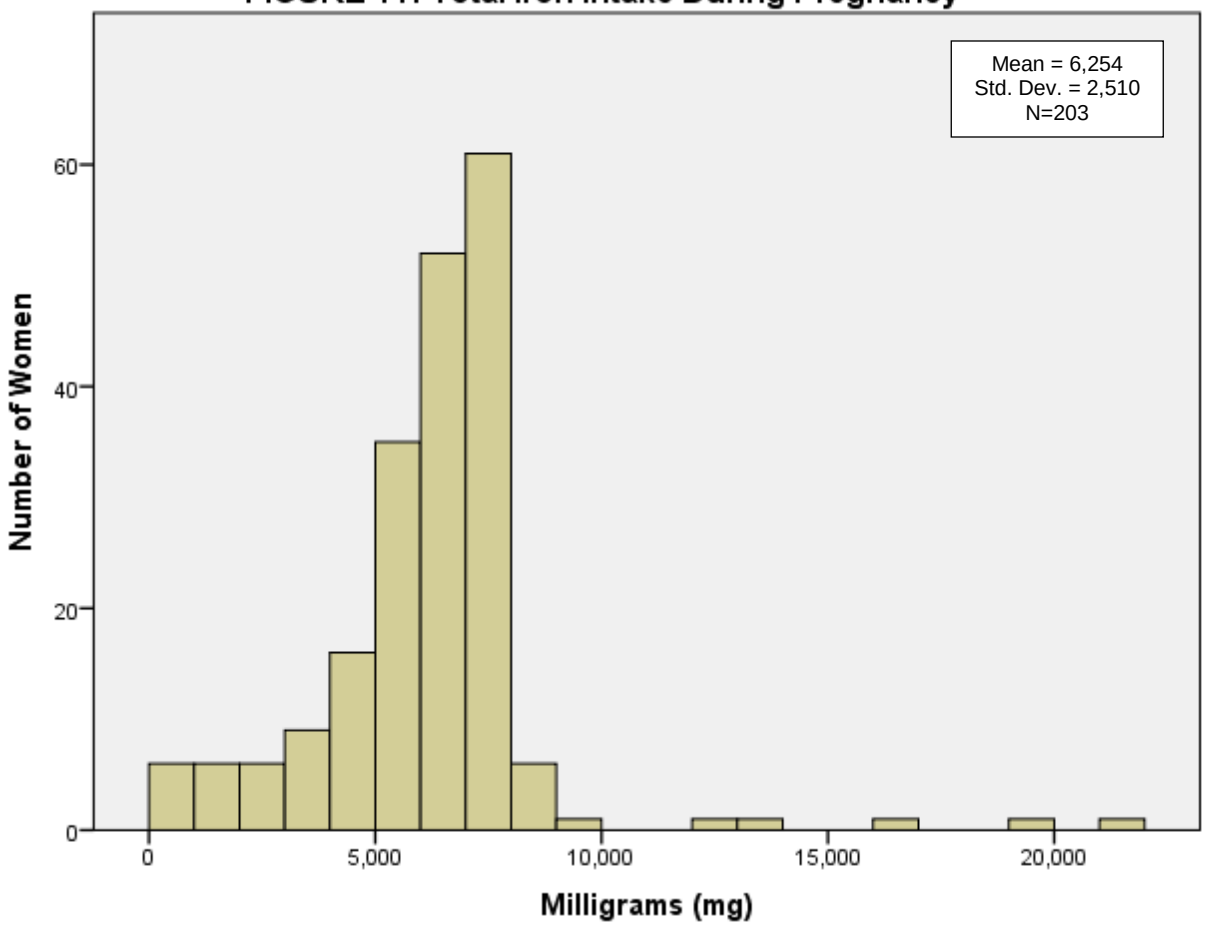
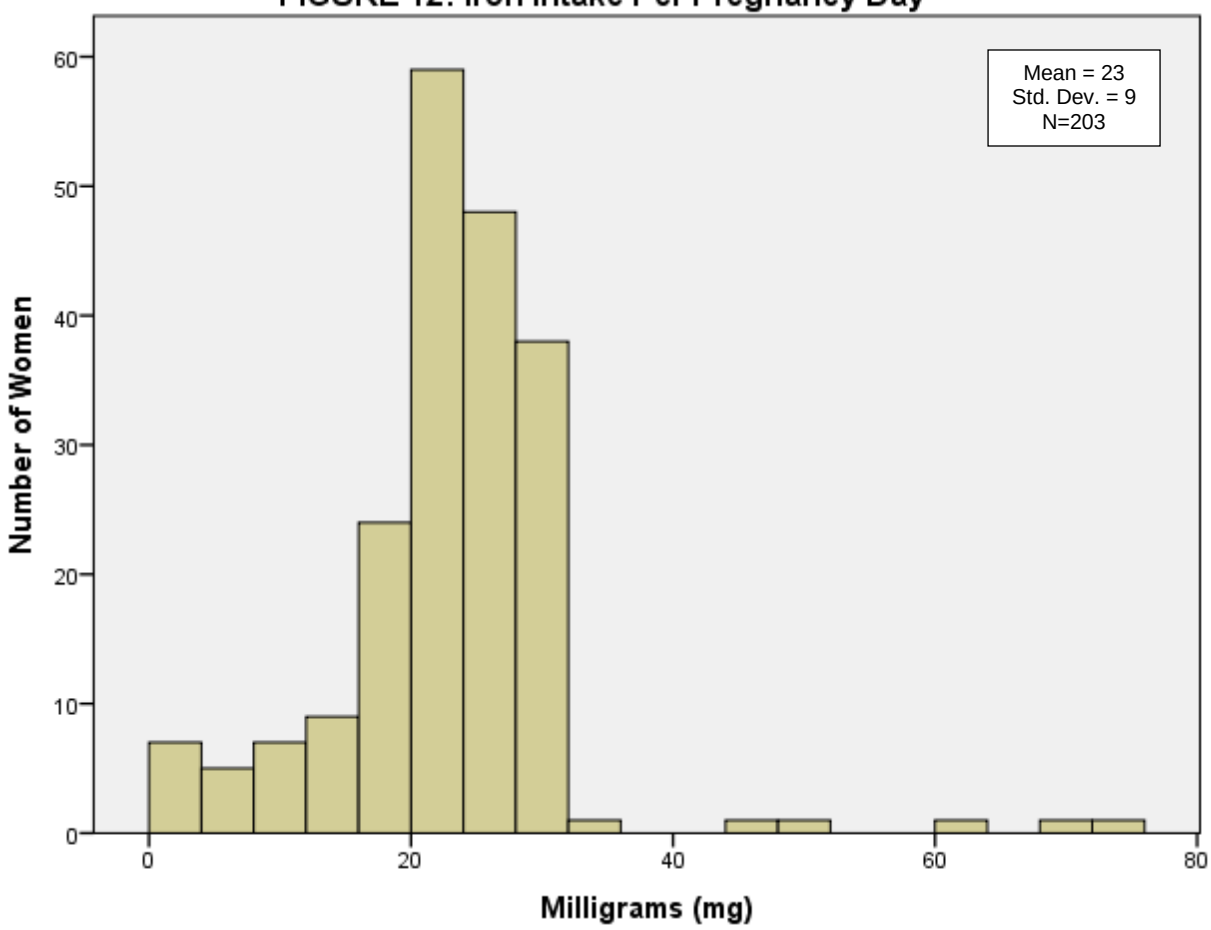


FIGURE 12: Iron Intake Per Pregnancy Day



The mean intake of vitamin C was 94 mg per day, while the RDA is 85 mg per day. The maximum intake was 207 mg per day.

The RDA for vitamin D is set at 600 IU per day, while the mean intake was 333 IU per day. However, maximum intake from our subjects was 917 IU per day, which is far from the UL of 4,000 IU per day.

Vitamin E mean intake from supplements was 23 IU per day, meeting 100% of the RDA. Maximum intake was 100 IU per day.

The mean intake of folic acid was 693 µg per day, exceeding the RDA of 600 µg. Maximum intake was 1,328 µg per day. Nine subjects (4%) supplemented with additional folic acid as a single nutrient during pregnancy. The UL for folic acid is set at 1 mg per day.

Mean intake of calcium was 170 mg per day with the RDA set at 1,000 mg per day. The highest reported intake from supplements was 518 mg per day.

The RDA for iron is set at 27 mg per day. Mean iron intake was 23 mg per day with the maximum intake was 74 mg per day.

Median intakes were calculated for the three emerging nutrients: iodine, choline and DHA. Median intake for all three nutrients was zero. Iodine was found in 43% of prenatal supplements (16/37), with amounts ranging from 0-290 µg. The RDA is set at 220 µg. Choline was found in 19% (7/37) of prenatal supplements, with amounts ranging from 0-60 mg. The RDA for choline is set at 450 mg per day. DHA was found in 30% of the supplements (11/37), ranging from 0-300 mg. No RDA has been established for DHA.

Other single nutrient supplements consumed during pregnancy included additional DHA and folic acid in addition to that provided by the prenatal or multivitamin. Women who consumed DHA supplements consumed an average of 230 mg per day (n=20). Additional folic acid was taken during pregnancy (n=9), but specific nutrient amounts could not be determined.

Supplement intake prior to pregnancy

Women who took supplements prior to pregnancy compared to those that did not had different income by zip code of residence at the time of enrollment, maternal education, maternal age, maternal race and smoking status. Women taking a supplement prior to pregnancy were more likely to be living in a higher average income zip code (F=46.920 p<0.001). Women with a higher average education were also more likely to take supplements prior to pregnancy (F=99.233 P<0.001). Age of the woman at enrollment was found to be significant (F=31.540 P<0.001). The older women were more likely to take a supplement prior to pregnancy. Supplement use prior to pregnancy was higher in white, including Hispanic, women as shown in Table 3 (χ^2(2)=33.064 P<0.001). Smoking pack years was also found to be lower in women taking pre-pregnancy supplements (F=8.832 P<0.003) compared to women who did not take supplements. Reproductive history, specifically the number of previous living children, was uncorrelated to pre-pregnancy supplement use but trended toward supplement use before pregnancy if a woman had previous children (F=3.315 P=0.070). Table 4 includes variables that were significantly related to pre-pregnancy supplement use.

TABLE 3: Supplement Use Prior to Pregnancy with Maternal Race

n=231

	Yes (n=116)*	No (n=115)*
White	98	57
African American	16	56
Other	2	2

*P<0.001 using Chi-Square Test

TABLE 4: Factors Affecting Supplement Use Prior to Pregnancy

n=231

	Yes (n=116)	No (n=115)	*P*-value
Income by Zip Code ($)	53,874	39,105	<0.001
Maternal Education (years)	16	13	<0.001
Maternal Age at Enrollment	28	24	<0.001
Smoking Prior to Pregnancy (pack years)	1	2	0.003

*P values were calculated using a Pearson Chi Square Test
Note: Income information not available for 4 women;
1 woman did not report smoking history

We separated supplements taken prior to pregnancy into a number of categories. A total of 181 supplements were taken by 116 women. Some women were taking more than one supplement prior to pregnancy (Table 5).

TABLE 5:
Supplement Intake Prior to Pregnancy
n=116

Multivitamins	**# of Women**
MVI	42
Children's MVI	3
PNV	61
PNV Rx	8
Lipids	
Omega 3 - Long Chain and Essential Fatty Acid	13
Independent Vitamins	
Carotene	1
Folic Acid	5
Vitamin C	8
Vitamin D	3
Vitamin E	4
Independent Minerals	
Iron	5
Calcium	9
Potassium	1
Zinc	1
Amino Acids/Protein	
L-Lysine	1
Spirulina	1
Herbals	
St. John's Wort	1
Alfalfa	1
Bromelain	1
Herb	2
Lemon Balm	1
Olive Leaf Extract	1
Fenugreek	1
Microorganism	
Probiotic	1
Functional Food Extracts	
Grapefruit Seed Extract	1
Cayenne	1
Elderberry	1
Cranberry Extract	3

Five women were taking additional folic acid beyond that provided in a standard multivitamin before pregnancy and three continued throughout pregnancy. On average, the folate supplement was taken 68 days prior to conception by these 5 women. Data was not available for the amount of nutrient the supplement provided.

Post-natal supplementation

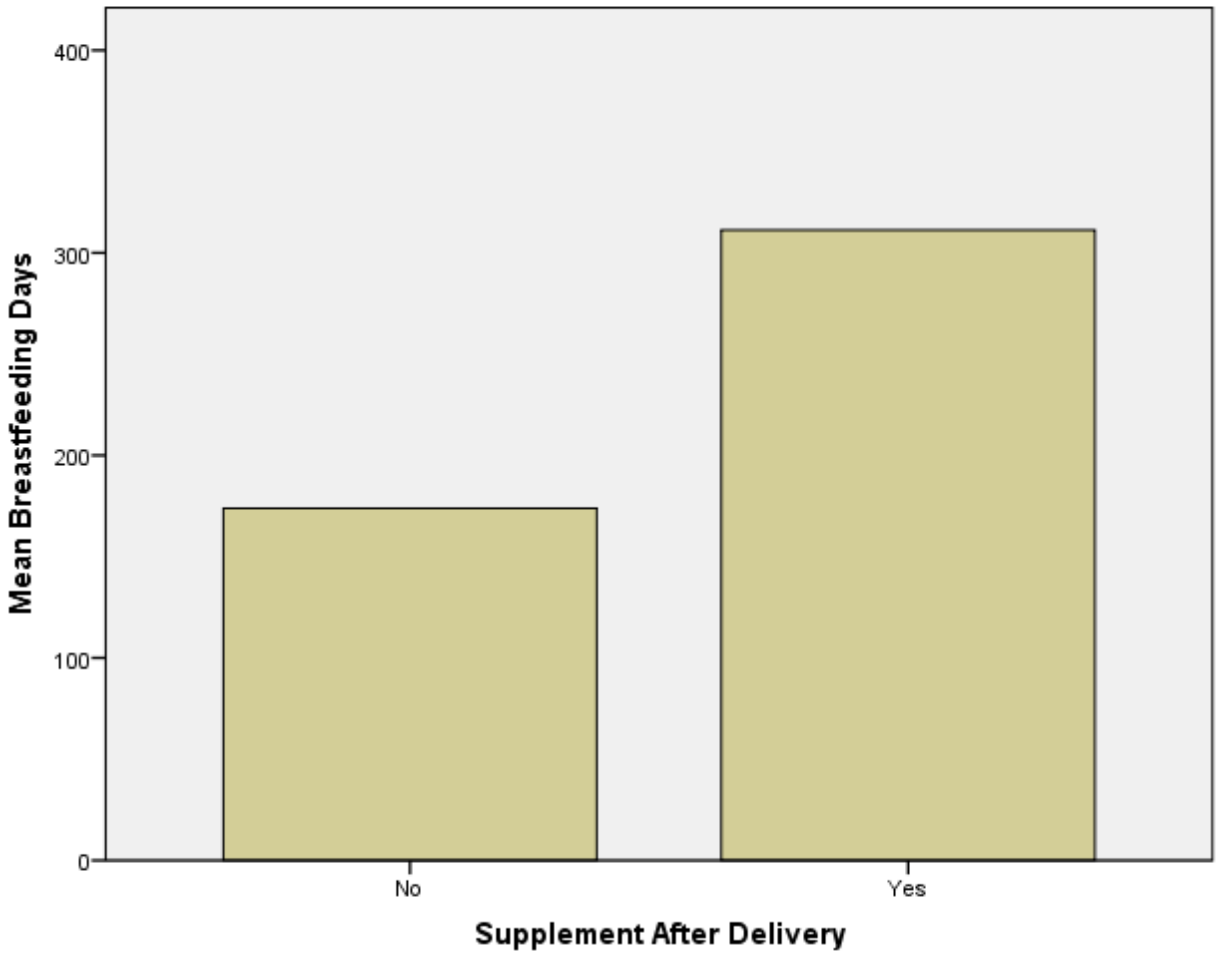
Post-natal supplementation was defined as supplement use that continued ≥30 days after delivery. A total of 104 supplements were consumed post-natally by 82 women (TABLE 6). Similar to pre-pregnancy supplementation, some women were taking more than one supplement.

TABLE 6: Post-Natal supplement intake
n=82

Supplement Name	# of Women	% of Women
Calcium	2	2
Flaxseed Oil	1	1
Iron	11	13
MVI	1	1
Omega	6	7
PNV- OTC	54	66
PNV Rx	27	33
Vitamin C	2	2

Women were more likely to take supplements post-natally if they were breastfeeding ($F=16.546$ $P<0.001$). Post-natal supplementation was associated with longer breastfeeding duration (Figure 19). Women still consuming supplements post-natally had a mean breastfeeding length of 311 days, while the women who stopped supplements prior to 30 days postpartum had a mean breastfeeding length of 174 days.

Figure 13: Breast Feeding Days with Post-Natal Supplementation



Prenatal supplement formulations

Supplement formulations varied from brand to brand and over-the-counter (OTC) to prescription (Rx). Table 7 outlines the differences in formulations. Sixteen different Rx supplements taken by subjects in the study were used in the analysis and 21 OTC supplements were used. Three nutrients were found to have statistical significance between Rx supplements and OTC supplements. Rx supplements provided significantly more folic acid (F=52.973 *P*<0.001) than OTC supplements and significantly more DHA

(F=8.852 *P*=0.005). Iron was also found to be significantly higher in prescription prenatals (F=4.309 *P*=0.045).

TABLE 7: Comparison of Prenatal Nutrient Contents

	Rx (n=16)	OTC (n=21)	P value*
Vitamin C (mg)	123	110	0.205
Vitamin D (IU)	351	390	0.174
Vitamin E (IU)	26	37	0.081
Folic Acid (µg)	1000	800	<0.001
Calcium (mg)	179	331	0.073
Iron (mg)	55	26	0.045
Iodine (µg)	66	66	0.998
Choline (mg)	7	1	0.176
DHA (mg)	132	29	0.005

**P* values were calculated using a one-way ANOVA.

Chapter 5

DISCUSSION

Nutrient intake during pregnancy from prenatal vitamins

Vitamin C intake from prenatal supplements was found to exceed the RDA for pregnancy by 11%. Since vitamin C is a water-soluble nutrient, there is not much concern for toxicity. A tolerable upper intake level (UL) has been set at 2 grams resulting in gastrointestinal problems such as nausea and diarrhea (24). None of the subjects came close to consuming this amount per pregnancy day. In other words, there is no concern with the exceeding the RDA by 11%. Gennaro et al (1) found that 79.3% of their subjects were not meeting the RDA from diet alone indicating that vitamin C intake from supplements is important.

Mean vitamin D intake from supplements met 55% of the RDA for pregnancy. Maximum intake was far from the UL in the subjects. An excess of supplementation from vitamin D could lead to potential toxicity evidenced by nausea, headache, weight loss and in extreme cases, mineralization of the heart, blood vessels and cutaneous tissue (24). Gennaro et al. (1) found that 91.4% of their subjects were not meeting the RDA from diet. In combination of diet and supplements, there is a potential deficit in vitamin D intake.

Mean vitamin E intake from prenatal supplements met 100% of the RDA. Gennaro et al. (1) found that 98.3% of their subjects were not meeting the RDA through diet. This leaves little concern with supplements providing 100% of the RDA because many women are not likely to get adequate intake from diet alone. When diet and supplementation are combined, it can be inferred that many women are likely to exceed the RDA. Vitamin E has been proposed to be one of the least toxic vitamins, so this is

not of concern (24). High intakes of vitamin E have been associated with gastrointestinal distress, increased bleeding and possible increased risk of respiratory infections (24).

Supplemental folic acid intake exceeded the RDA by 15%. The UL is set at 1mg due to insomnia, malaise, irritability, and gastrointestinal distress. Also, excessive folate intake can mask potential vitamin B12 deficiency. Zeisel (25) found that during development, excessive intake of dietary methyl groups, such as folate, can alter DNA. Altered DNA then results in epigenetic changes to gene expression that can be seen throughout a lifetime. In pregnant rodent models, high folic acid intake was found to alter the coat color of the offspring or result in tail kinking. In humans, it is believed that this can be translated to an increased risk of cancer of the offspring. Rett Syndrome has also been suggested to result from defective epigenetic regulation. From dietary intake, Gennaro et al. (1), found 89.7% of their subjects were not meeting the folate RDA. While excessive folate can result in potential complications, women are not getting adequate intake from diet, so supplementation is necessary, but not in excessive amounts. While folate fortification in foods has occurred since 1998, Gennaro et al, (1) shows that food sources alone are not meeting the needs of pregnant women.

Calcium intake from prenatal supplements met 17% of the RDA. When assessing dietary consumption of pregnant women, Gennaro et al. (1) found that 32.6% of the subjects were not getting adequate calcium from the diet. And our study demonstrates that pregnant women are not getting sufficient intakes from supplements. Without specific nutrient values from diet, it is difficult to determine if supplement use in combination with dietary intake would meet the RDA for pregnant women. It has been

shown that calcium intake can inhibit iron absorption (26). In pregnancy, iron needs are increased, so additional calcium supplements are not recommended beyond the average prenatal supplement. In other words, it is imperative to have adequate calcium stores prior to conception. Calcium deficiency can result in tetany for the mother and may increase the long-term risk of osteoporosis.

Mean iron intake from supplements met 85% of the RDA. Mean intake from supplements was slightly lower than the CDC recommendations of 30 mg/d (9). Gennaro et al. (1) found that 82.8% of the women were not meeting the iron needs through diet. With the specific amounts from the diet unknown, it is hard to say if supplement intake meeting 85% of the RDA is adequate.

Iodine is thought to be imperative in the production of thyroid hormones (27). During pregnancy, a 50% increase in iodine is recommended to meet the thyroid hormone need of both the mother and the fetus. Iodine deficiency can result for the mother and fetus without adequate intake. Deficiency during pregnancy can result in damage to the fetus' brain development as evidenced by cretinism and ultimately mental retardation (27). Of the supplements providing iodine, two formulations exceeded the RDA by 32%. Twelve formulations delivered 68% of the RDA by providing 150 µg and two supplements provided 25 µg, meeting 11% of the RDA.

While choline requirements are thought to be increased during pregnancy (28), 0% of the RDA was met through prenatal supplements. Choline is an emerging nutrient in pregnancy that has been found to have potential benefits in reducing neural tube defects (28). Shaw et al. (28) found decreased risk of neural tube defects with choline intake as evidenced by a strong linear association. Deficiency of choline is thought to

decrease folic acid metabolism, resulting in an increased risk of neural tube defects. Choline is not a common component of prenatal supplements (28), but as research continues to be published, it can be expected that prenatal formulations will be altered.

While DHA does not have an RDA set for pregnancy, much research is being conducted on the cognitive benefits on the fetus. While the primary study was a double-blind, placebo controlled trial using DHA, the amount of DHA from other supplements was closely monitored by study personnel. Women were not able to consume more than 300 additional mg per day outside of their study capsules. Although this was not an initial issue at the beginning of the study, as more research was published regarding the benefits of supplementation, the manufacturers of supplements began adding in DHA. Prior to this time, some women were taking more than the restricted limit. Research is still being conducted to establish an optimal level during pregnancy. Higher maternal DHA status during pregnancy has been shown enhanced infant attention in the first year of life. Notable differences in the Bayley scores of children at 18 months of age have also been recorded in several observational studies, suggesting an increase in cognitive abilities in infants whose mothers were supplemented with DHA. Visual acuity at 4 months of age has been shown to be more mature in infants with mothers with higher DHA status (29).

While this study did not look at dietary intake of the mothers, Gennaro et al. (1) examined the dietary intake of pregnant women. Caloric intake was found to be excessive, while inadequate micronutrient consumption was reported. All the micronutrients examined (iron, folate, vitamin D, vitamin C, vitamin E, and calcium) were

all inadequate from the diet, thus showing the importance of prenatal supplementation during pregnancy (1).

Factors affecting supplement intake prior to pregnancy

While nearly all of our subjects used supplements during pregnancy, we wanted to examine factors that affected supplement use prior to pregnancy. Many studies examine use during pregnancy, but since all of our subjects were using supplements we were unable to make the same comparisons.

Maternal race was found to impact supplementation prior to pregnancy. White mothers were more likely to use supplements than African American mothers or other races ($P<0.001$). Suitor et al. found this same finding in pregnant women during pregnancy (6), while our study did not find the same result. All of the subjects, no matter the race, used supplements during pregnancy.

Family income, as determined by zip code, was also found to significantly affect supplement use prior to pregnancy ($P<0.001$). This finding is not surprising considering that supplements can be expensive and access may be limited for those with a lower income.

Maternal education at enrollment was found to be positively associated with supplement use prior to pregnancy ($P<0001$). Women taking supplements prior to pregnancy had an average education of 16 years, while women that did not take supplements prior to pregnancy had an average education of 13 years. Yu et al. found a similar finding in pregnant women with 89.9% of the pregnant women with education levels ≥16 years using supplements during pregnancy (5).

Another positive association with supplement use prior to pregnancy was maternal age at enrollment (P<0.001). The younger the mother, the less likely she was to use supplements prior to pregnancy. The average age for women that did not supplement prior to pregnancy was 24, while the average supplemented woman was 28 years old. Yu et al. had a similar finding in pregnancy, the older the mother, the more likely she was to use supplements (5).

Smoking status was also negatively associated with supplement use prior to pregnancy (P=0.003). Women consuming supplements before pregnancy were less likely to smoke than women that did not use supplements. Women using supplements before pregnancy had an average pack year consumption of 1, while women not using supplements had an average pack year consumption of 2. This finding could be contributed to the fact that smokers may be less mindful about health concerns if they smoke. Jasti et al. (22) and Yu et al. (5) both found higher adherence in supplementation during pregnancy for women that were current smokers.

No association was found between supplement use prior to pregnancy and the number of previous children (P=0.070). This suggests that if mothers did not take supplements with previous children, the women believe there is no benefit to supplementation with subsequent pregnancies.

Supplements taken prior to pregnancy

One hundred eighty one supplements were taken by 116 women prior to pregnancy. Some of the supplements were the same, while there were many supplements taken by only a handful of subjects.

The multivitamin category was made up of adult multivitamins, children's multivitamins, over-the-counter prenatals and prescription prenatals. Almost all of the women (n=114) taking a supplement prior to pregnancy were consuming a multivitamin.

Long chain and essential fatty acids, such as omega-3, was the next most commonly used supplement with 13 women taking the supplement prior to pregnancy.

Folic acid is recommended to decrease the risk of neural tube defects during pregnancy. Only five women (2%) took folic acid prior to pregnancy, as recommended by the CDC (9). Average intake was 68 days prior to conception. This could suggest that these subjects were purposively attempting to conceive. In order to increase folic acid consumption in the United States, grains have been fortified since 1998. As a public health initiative, this suggests that not many women of child bearing age are consuming folic acid supplements, so the consumption through grains is imperative to decrease the risk of neural tube defects.

Calcium, as a single nutrient, was taken 8% of our subjects prior to pregnancy, lower than the 44% found in the Australian study (7). Specific vitamins used before pregnancy included carotene, vitamin C, vitamin D and vitamin E. Independent nutrients such as iron, potassium and zinc were also reported. The Australian study found that 7% of the women used independent zinc supplements during pregnancy (7), although this study did not find the same results.

Different herbals, microorganisms, amino acids, and functional food extracts were reported in limited consumption.

Post-natal supplementation

Eighty-two women reported taking a supplement at least 30 days after delivery. This study found that women were more likely to take a postnatal supplement if they were breastfeeding, suggesting that they were trying to improve the quality of their milk supply. Women were also found to have a longer mean breastfeeding length if they were consuming supplements. Again, suggesting that the quality of the milk was trying to be enhanced by supplementation. Of the supplements consumed, the most common supplement was a prenatal vitamin, with over-the-counter formulations making up about half of the prenatal vitamin consumption.

Prenatal supplement formulations

Since prenatal formulations are not standardized, there is much variation from supplement to supplement. Sixteen prescription formulations were averaged to develop the prescription formulation and 21 over-the-counter prenatals were averaged to develop the over-the-counter formulation. When comparing average prescription prenatals to over-the-counter prenatals it was surprising not to find much statistical significance in the nutrient amounts. Folic acid content was found in larger amounts in prescription supplements ($P<0.001$). Evidence regarding the benefits of supplementing folic acid in pregnancy has been around since the 1980's, yet over-the-counter formulations have not increased to the similar dosage in prescription supplements. Iron was also found to be significantly higher in prescription prenatals as ($P=0.045$). Iron recommendations vary greatly, so it is not surprising to find almost a two-fold increase in prescription prenatals content.

Limitations

Limitations of this study include the fact that all women had to have the ability to swallow capsules, making them more likely to take supplements.

A major limitation of the study is that there is no diet information provided for these women, so we do not know how much of their diet is meeting the RDA.

The majority of the study population was from KUMC and utilized the on-site pharmacy. The majority of the women took the pharmacy generic provided. Also, since most subjects were from KUMC, the number of doctors was limited. Doctors often prescribed the same prenatal multivitamins to all of their patients.

Women who took the time to enroll in the study may be more motivated than other women, thus increasing their likelihood of taking supplements.

In order to determine frequency, the most compliant mothers returned the monthly questionnaires routinely giving us a better picture of their supplement frequency. There were no high risk families enrolled in the study.

Chapter 6

SUMMARY

Supplementation during pregnancy appears to be quite widespread, but the formulation of supplements is not as consistent. Many women are not meeting the RDAs during pregnancy through prenatal supplements. While our study did not investigate dietary intake, other studies such as Gennaro et al.(1), suggest that pregnant women are not meeting the RDAs through dietary sources either. As a public health message, it would be beneficial for formulations of prenatal supplements to be standardized to be sure that women are providing necessary nutrients to the developing fetus.

Formulations should include increased calcium content, as well as choline, iodine and DHA. All of the emerging nutrients have been found to be beneficial in pregnancy, and thus should be given to the fetus.

Future directions include assessing not only what supplements women are taking during pregnancy, but also diet information to measure what percentage of the women are meeting the RDAs for pregnancy. More comprehensive research should be conducted on the benefits and levels of the emerging nutrients. Future research could also include supplementation outcomes on birth.

References

1. Gennaro S, Biesecker B, Fantasia HC, Nguyen M, Garry D. Nutrition profiles of American women in the third trimester. MCN Am J Matern Child Nurs 2011;36(2):120-6. doi: 10.1097/NMC.0b013e3182057a13.
2. Allen L. Pregnancy and lactation. Present Knowledge in Nutrition 2006;9(2):529-43.
3. Picciano MF, McGuire MK. Use of dietary supplements by pregnant and lactating women in North America. Am J Clin Nutr 2009;89(2):663S-7S. doi: 10.3945/ajcn.2008.26811B.
4. Botto LD, Khoury MJ, Mulinare J, Erickson JD. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. Pediatrics 1996;98(5):911-7.
5. Yu SM, Keppel KG, Singh GK, Kessel W. Preconceptional and prenatal multivitamin-mineral supplement use in the 1988 National Maternal and Infant Health Survey. Am J Public Health 1996;86(2):240-2.
6. Suitor CW, Gardner JD. Supplement use among a culturally diverse group of low-income pregnant women. J Am Diet Assoc 1990;90(2):268-71.
7. Forster DA, Wills G, Denning A, Bolger M. The use of folic acid and other vitamins before and during pregnancy in a group of women in Melbourne, Australia. Midwifery 2009;25(2):134-46. doi: 10.1016/j.midw.2007.01.019.
8. Kaiser LL, Allen L. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. J Am Diet Assoc 2002;102(10):1479-90.

9. Recommendations to prevent and control iron deficiency in the United States. Centers for Disease Control and Prevention. MMWR Recomm Rep 1998;47(RR-3):1-29.

10. Stolzfus RJ, Dreyfuss ML. Guidelines for use of iron supplements to prevent and treat iron deficiency anemia. International Life Sciences Institute 1998.

11. Becker DV, Braverman LE, Delange F, et al. Iodine supplementation for pregnancy and lactation-United States and Canada: recommendations of the American Thyroid Association. Thyroid 2006;16(10):949-51. doi: 10.1089/thy.2006.16.949.

12. Scholl TO, Hediger ML, Bendich A, Schall JI, Smith WK, Krueger PM. Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. Am J Epidemiol 1997;146(2):134-41.

13. Czeizel AE. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. Am J Med Genet 1996;62(2):179-83. doi: 10.1002/(sici)1096-8628(19960315)62:2<179::aid-ajmg12>3.0.co;2-l.

14. Cogswell ME, Parvanta I, Ickes L, Yip R, Brittenham GM. Iron supplementation during pregnancy, anemia, and birth weight: a randomized controlled trial. Am J Clin Nutr 2003;78(4):773-81.

15. Mishra V, Thapa S, Retherford RD, Dai X. Effect of iron supplementation during pregnancy on birthweight: evidence from Zimbabwe. Food Nutr Bull 2005;26(4):338-47.

16. Christian P, Khatry SK, Katz J, et al. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: double blind randomised community trial. BMJ 2003;326(7389):571. doi: 10.1136/bmj.326.7389.571.

17. Shah PS, Ohlsson A. Effects of prenatal multimicronutrient supplementation on pregnancy outcomes: a meta-analysis. CMAJ 2009;180(12):E99-108. doi: 10.1503/cmaj.081777.

18. Scholl TO, Hediger ML, Schall JI, Fischer RL, Khoo CS. Folate Intake during pregnancy:its association with preterm and very preterm delivery. American Journal of Clinical Nutriton 1996(63):520-25.

19. Inskip HM, Crozier SR, Godfrey KM, Borland SE, Cooper C, Robinson SM. Women's compliance with nutrition and lifestyle recommendations before pregnancy: general population cohort study. BMJ 2009;338:b481.

20. Cogswell ME, Kettel-Khan L, Ramakrishnan U. Iron supplement use among women in the United States: science, policy and practice. J Nutr 2003;133(6):1974S-7S.

21. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. J Am Diet Assoc 2001;101(3):294-301. doi: 10.1016/s0002-8223(01)00078-5.

22. Jasti S, Siega-Riz AM, Cogswell ME, Hartzema AG, Bentley ME. Pill count adherence to prenatal multivitamin/mineral supplement use among low-income women. J Nutr 2005;135(5):1093-101.

23. Yu SM, Kogan MD, Huang Z. Vitamin-mineral supplement use among US women. Journal of American Medical Women's Association 2003(58):167-64.

24. Sarren G, Jack S, James G, eds. Advanced Nutrition and Human Metabolism. Fifth ed. Belmont: Wadsworth Cenage Learning, 2009.

25. Zeisel SH. Epigenetic mechanisms for nutrition determinants of later health outcomes. Am J Clin Nutr 2009;89(5):1488S-93S. doi: 10.3945/ajcn.2009.27113B.

26. Hallberg L. Does calcium interfere with iron absorption? Am J Clin Nutr 1998;68(1):3-4.

27. Skeaff SA. Iodine deficiency in pregnancy: the effect on neurodevelopment in the child. Nutrients 2011;3(2):265-73. doi: 10.3390/nu3020265.

28. Shaw GM, Finnell RH, Blom HJ, et al. Choline and risk of neural tube defects in a folate-fortified population. Epidemiology 2009;20(5):714-9. doi: 10.1097/EDE.0b013e3181ac9fe7.

29. Carlson SE. Docosahexaenoic acid supplementation in pregnancy and lactation. Am J Clin Nutr 2009;89(2):678S-84S. doi: 10.3945/ajcn.2008.26811E.

APPENDIX A

CONSENT FORM FOR KU DHA OUTCOMES STUDY (KUDOS)

CONSENT FORM
The Effects of DHA on Pregnancy and Infant Outcome
(Kansas University DHA Outcomes Study or KUDOS)

Sponsor: NIH (1R01 HD047315)

INTRODUCTION

As a pregnant woman who is between 8 and 20 weeks of gestation, you are being invited to enroll in a research study of a nutrient (DHA) that is a component of normal brain and important for brain development. The centers involved in the study are the University of Kansas Medical Center in Kansas City, Kansas, St. Luke's Hospital in Kansas City, Missouri, and Truman Medical Center in Kansas City, Missouri. If you decide to enroll in this study, your baby will participate in research procedures at the University of Kansas Medical Center. Dr. Susan Carlson is the main investigator for this study. A total of 350 pregnant women will be enrolled in this study between October 2005 and January 2010.

You do not have to participate in this research study. It is important that before you make a decision to participate, you read the rest of this form. You should ask as many questions as you need to understand what will happen if you participate in the study.

BACKGROUND

Docosahexaenoic acid (DHA) is a fat that is found in very large amounts in the brain. DHA is important for how my baby sees and learns. Breast milk and, since 2002, US formulas contain DHA. Many studies have shown that DHA in the diet helps the baby's vision, attention, and ability to learn. In this way, DHA is considered an important nutrient for babies after they are born.

DHA may also be important before babies are born. Four studies found that women's DHA during pregnancy was related to higher infant/child function. These studies are called observational studies, meaning that the women's normal DHA status was studied in relation to development of the baby/child. There is only one study that gave women DHA during pregnancy and measured development of their babies/children. That study showed higher IQ at 4 years of age in children whose mothers took fish oil capsules during the last 6 months of pregnancy. (Fish oil contains a lot of DHA). However, because women in the study also consumed DHA while they were breastfeeding they provided more DHA to their babies after they were born. Therefore, the study does not prove that giving DHA before babies are born will help their development. There are no studies that have varied DHA intake only during pregnancy. You and your child are being asked to participate in such an experimental study.

PURPOSE

The purpose of this study is to determine if a dietary supplement of DHA during pregnancy will help babies be born at the right time and help their development. If you decide to be in the study, you will have a 50-50 chance of receiving capsules with the supplement of DHA or ordinary food oil, which does not contain any DHA.

PROCEDURES

If you choose to enroll yourself and your infant in this study, the investigators will record some information from your medical record about your pregnancy and medical history. They will also ask you a few questions about foods that you usually eat. You will have a blood sample collected from a vein in your arm. One-half teaspoon of blood will be drawn. The blood will be used to measure DHA in your blood as well as other nutrients. You will be asked to provide a current address and phone number where you can be contacted. .

During pregnancy: You will be randomly assigned (like flipping a coin) to capsules with DHA-oil or ordinary food oil (which does not contain any DHA). The DHA-oil is the same oil that is used in US infant formulas and has been fed safely to millions of infants.

You will be given enough capsules each month to take 3 capsules each day and you agree to try to consume all 3 capsules. If you consume all 3 capsules, you will consume 600 mg of DHA. The capsules are relatively small and you should find them easier to swallow than many nutrient supplements. They are orange-flavored, so if you burp (common in pregnancy and in the first week of taking any nutrient supplement), the taste should not be unpleasant. You do not need to take the capsules at any specific time as they are a nutrient and not a drug. However, you should decide upon a regular time to take them so that taking the capsules will become a habit and you won't forget. For example, you might wish to take them just before you go to bed or when you have your first beverage of the day.

Neither you nor the investigators will know which capsules you have been assigned to. On the day you enroll for the study, we will send you home with your first bottle of capsules. About 30 days later (early enough so that you do not run out of capsule), you will receive another bottle of capsules in the mail. AT THAT TIME, YOU AGREE TO PLACE THE FIRST BOTTLE WITH ANY REMAINING CAPSULES IN THE ENVELOPE AND DROP IT INTO THE MAIL.

This process will be repeated each month until your baby is born and you will continue to take 3 capsules per day until your baby is born. Each time you receive a new bottle, you will mail back the bottle that you have been using and that day will open and begin using the new bottle.

The investigators will contact you by phone at least once per month. They will ask about capsule intake and they will ask how you are doing. Maintaining contact with our study personnel on a monthly basis is very important.

IF YOUR PHONE NUMBER OR ADDRESS CHANGES AT ANY TIME DURING THE STUDY, YOU WILL LET THE INVESTIGATORS KNOW BY CALLING 913-588-3781 AND LEAVING A MESSAGE.

Delivery: After you are admitted to the hospital to deliver, you should telephone study personnel or ask the person at admitting to telephone them. You will be given a cell phone number today to call. Once you deliver your baby, the investigators will visit you in the hospital to collect data about your delivery and your baby's health. A sample of your baby's cord blood will be collected after delivery by nurses at the hospital and given to the investigators. A nurse will also draw a small blood sample (one-half teaspoon) from you while you are in the hospital. The blood samples will be used to measure DHA and other nutrients. The investigators will visit you, and give you an appointment for your baby's first follow-up visit at KUMC.

Visit 1 (6 weeks of age): The investigators will measure how your baby sees using a test that involves placing 3 electrodes directly on your baby's head. The process involves cleaning the area then placing a small amount of paste similar to toothpaste on the head. The electrodes are placed on top of the paste. The electrodes will be used to record your baby's brain waves while he/she is looking at pictures. Your child's weight, height and head circumference will be measured again and you will be asked questions about what your baby eats. If you are breastfeeding your baby, you will be asked to provide a teaspoon of breast milk to the investigator. The sample will be frozen and analyzed for fats that are found in the capsules. The visit should last about 40 minutes. You should arrive on time and allow that amount of time for the visit.

Visit 2 (4 months of age): The investigators will measure how your baby sees using the same test as before and another vision test. Your baby will wear a pair of plastic glasses during the second test. In another test, your child will be given an object to look at several times. The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. Your child will be video recorded during the test. Your baby's heart rate will be measured during the test. Your baby's height, weight and head circumference will be measured and you will be asked about what food your baby eats. Your baby will have a blood sample collected by either heel stick or drawn from a vein. If it is necessary to use a heel stick, the investigator may use a cream or spray that will numb the area before obtaining the sample. One-half teaspoon of blood will be drawn. The blood will be used to measure DHA and other nutrients. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit will take 60-90 minutes.

Visit 3 (6 months of age): The investigators will measure how your baby sees using the test that requires him/her to wear a pair of plastic glasses. In another test, he/she will be given an object to look at several times (just like at 4 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. Your child will be video recorded during the test. Your baby's heart rate will be measured during the test. Your baby's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit should take 40 -60 minutes.

Visit 4 (9 months of age): Your baby will have both tests that measure how he/she sees. In another test, your child will be given an object to look at several times (just like at 4 and 6 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object and your baby's heart rate will be measured during the test. Your child will be video recorded during the test. Your baby's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. The visit should take about 40-60 minutes.

Visit 5 (10 months of age): During this visit your baby will be placed on your lap in front of a small table. A test will be completed with a small toy, foam block and 2 cloths that will be placed in front of your child. You will also take a short language test. The small toy will be given to your child to keep. In another test, your baby will be asked to take turns with the researcher building fun toys. After your baby has played for a moment with the pieces, the researcher will show him or her how to build the toy. Then, your baby will be given a turn to put the toy together. Your baby's turn will happen either immediately or after 10-minutes of play with other things. Your child will be video recorded during the tests. You should let the investigator know if your baby has been sick or not acting well since his/her last visit. You will be asked questions about what your baby eats. The entire 10-month visit should last 65 - 70 minutes.

Visit 6 (12 months of age): The investigators will measure how your baby sees using both vision tests. Your child will be video recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. Your child's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You should let the investigator know if your child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that your child be rested before the testing at this visit. If for some reason your baby cannot finish the tests that day – this may happen if he/she is unusually fussy or tired – you will be asked to return to finish the remaining tests within 7 days.

Visit 7 (18 months of age): The investigators will measure how your child sees using the test that he/she had while wearing plastic glasses. Your child will be video recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. Your child will also be given a standardized test to measure mental and physical development. Your child's height, weight and head circumference will be measured. You will be asked questions about what your baby eats. You will be asked questions about the words your child uses and understands. You should let the investigator know if your child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that your child be rested before the testing at this visit. If for some reason your child cannot finish the tests that day – this may happen if he/she is unusually fussy or tired – you will be asked to return to finish the remaining tests within 7 days.

RISKS
Some redness, soreness, or bruising may occur at the site of blood sampling. There is also a very slight risk of infection.

You may experience burping from the capsules and find this unpleasant

There are no known risks of consuming the amount of DHA you will be provided if you receive the DHA. Even if you forget to take your capsules for one or two days, there is no known risk of deciding to "catch up" on the third day. The amount is smaller than pregnant women in many countries eat every day. Nevertheless, you could develop a problem that has not been observed before.

NEW FINDINGS STATEMENT
You will be informed if any significant new findings develop during the course of the study that may affect your willingness to participate or to allow your child to participate in this study.

BENEFITS
You and your child may or may not benefit from participating in this study**.** If you receive the supplement, it may help your baby to be born at the right time and your baby's/child's development**.** If you will not get the supplement, your baby and you will not be getting any of those benefits. It is also possible that all infants/children will get some benefit from being followed closely with developmental testing. It is hoped that additional information gained in this research study may be useful in understanding if DHA can help your baby be born at the right time and help your baby's vision, attention, and learning as he or she grows. You will receive a video recording of your infant doing the 4, 6, and 9 month looking test when the 12 month visit is complete.

ALTERNATIVES
You do not have to participate in this study to be able to take DHA supplements while you are pregnant. You may purchase capsules containing DHA at local stores without a prescription (for example, Osco, Costco, Wal-Mart). There are also several brands of prenatal supplements with DHA available by prescription or over the counter. The prenatal capsules typically contain 200 mg of DHA each and are marketed to take one capsule/day as a DHA supplement.

COSTS
Capsules containing either DHA or food oil will be provided to you at no cost while you are participating in this study. You will not incur any costs because of your or your child's participation

PAYMENT TO SUBJECTS
If study investigators are able to communicate with you each month you will be given 2 bonus gift cards to either Wal-Mart or Target of $25 each. The first gift card will be given to you half way through your treatment phase if communication is maintained at least one time each month during the first half of your treatment. The second gift card will be

given at delivery if communication maintained at least one time each month during the second half of your treatment.

Additionally, if the study investigators are called after you are admitted for delivery you will be given your choice of a bonus gift card worth $50 from either Wal-Mart or Target. You may make the call yourself or have someone else call for you. Study personnel will give you the gift card when they come to the hospital after your baby is born.

Once your baby is born, you will receive a check for $50 after your baby completes each of the following visits: 6 weeks, 4 months, 6 months, 9 months, and 10 months. You will receive a check for $100 after your child completes each of the following visits: 12 and 18 months. The reimbursements are to cover the costs of transportation and to partially compensate you for your time required to participate in the study**.**

Your name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

IN THE EVENT OF INJURY

In the event you experience any serious health problem (hospitalization, life-threatening illness, or death) for any reason during your pregnancy, you should immediately seek treatment or help in the way you normally would as if you were not in a study. You should let Susan Carlson, Ph.D. know about any of these problems as soon as possible by calling her office (913-588-5359) or the study office (913-588-3781). A message may be left at both numbers. Dr. Carlson may also be reached at home (816-960-1805).

INSTITUTIONAL DISCLAIMER STATEMENT

If you believe you have been injured as a result of participating in research at Kansas University Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Compensation to persons who are injured as a result of participating in research at KUMC may be available, under certain conditions, as determined by state law or the Kansas Tort Claims Act.

Truman Medical Center (TMC) will provide medical attention to you if you suffer any injury or harm as a direct result of participating in this research project. TMC, your study doctor, and the sponsor of this study will decide, at their discretion, who should pay for the medical care. TMC will provide treatment to you in the event of any medical emergency while present at TMC, whatever the cause. Moreover, you will have the benefit of the coverage of any existing healthy insurance you own. Participation in this research study does not take the place of routine physical examinations or clinic visits to your person physician. If you believe you have been injured as a result of participating in this study you are encouraged to contact the study investigator, Dr. Susan Carlson, at her work number, 913-588-5359.

The University of Missouri-Kansas City appreciates the participation of people who help

it carry out its function of developing knowledge through research. Although it is not the University's policy to compensate or provide medical treatment for persons who participate in studies, if you think you have been injured as a result of participating in this study, please call the investigator, Dr. Susan Carlson at 913-588-5359 (work) or Sheila Anderman, IRB administrator of UMKC's Adult Health Sciences Institutional Review Board at 816-235-6150

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Videotapes of your baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students and to make a videotape copy for you. The videotapes will be secured under lock and key like all other information that could be linked directly to your child. The videotape of your child will not be shown without specific permission from you and even then would not identify your child by name. Efforts will be made to keep you and your child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies you and/or your baby will be removed.

The privacy of you and your child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If you choose to participate in this study, you will be asked to give permission for researchers to use and disclose your and your baby's health information that is relevant to the study.

To perform this study, researchers will collect health information about me and my child from his/her and my medical records and from the study activities that are listed in the Procedures section of this consent form. My and my baby's study-related health information will be used at KU Medical Center by Dr. Carlson, members of the research team, Truman Medical Center, St. Luke's Hospital and the KU Hospital Medical Record Department. The KUMC Research Institute as well as officials at Truman Medical Center and St. Luke's Hospital that oversee research, including the KUMC Human Subjects Committee, the IRB that governs Truman Medical Center and St. Luke's Hospital and other committees and offices that review and monitor research studies, may also see my and my baby's study-related health information.

Dr. Carlson and her team may share information about me and my baby with representatives of Martek Biosciences, the monitoring company who verifies study data, the laboratory that processes study lab samples, other business partners who help with the study, the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues arise). Martek Biosciences (Columbia, MD) donated the capsules for this study that is otherwise supported by the National Institute of Child Health and Human Development.

Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

If I enroll in the study, the investigators cannot tell me what capsule I was assigned to until the study ends. This may be after I have stopped taking the capsules.

QUESTIONS

I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my or my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

My and my child's participation in this study is voluntary and the choice to not participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study, by the sponsor of the study, or the FDA. My child's participation can be discontinued by the investigator or by the sponsor if it is felt to be in my child's best interest or if I do not follow the study requirements. If I choose to withdraw before my child is 18 months of age, I may be asked to answer questions about the study on the telephone.

If I want to cancel permission to use my or my child's health information, I should send a written request to Dr. Carlson. The mailing address is Susan Carlson, Ph.D., Dept. of Dietetics and Nutrition, MS 4013, 4019 Delp, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160. If I cancel permission to use my child's health information, the research team will stop collecting any additional information about me and my child.

Should the study be terminated prior to the completion of my pregnancy, neither the investigator nor the University of Kansas Medical Center will be under any obligation to provide me with DHA capsules used in the study.

CONSENT

Dr. Carlson or her associates have given me information about this research study.
They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my and my child's health information to be used and disclosed for the purposes of this research study. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to my and my child's participation in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

_____
Type/Print Name of Principal Investigator

_____ _____
Signature of Principle Investigator Date

May the investigators contact you after the study is over to ask if you interested in continuing your child's participation? If you agree to be contacted, the investigators would explain any new study to you later and you would have the chance to decide if you wanted to participate at that time (please circle your response).

Yes

No

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

_____
Type/Print Name of Principal Investigator

_____ _____
Signature of Principle Investigator Date

APPENDIX B

CASE REPORT FORM

SUPPLEMENT USE AT ENROLLMENT

SUPPLEMENT INTAKE DATA

PATIENT NUMBER	PATIENT INITIALS	DATE

Ethnicity **(Check one box)**	o **African-American** o **Asian-American** o **Hispanic** o **European-American (other than Hispanic):** o **Other_____**
Initial Hgb (at 1st OB appt):_____ **2nd Hgb (at 28 week lab):_____**	**Date:_____** **Date:_____**
Was an additional iron supplement prescribed *during* pregnancy?	o **yes** o **no** **Date:_____**
Separate iron supplements taken *during* pregnancy	o **yes** o **no** **If yes, frequency: _____**
Prenatal Vitamins taken *during* pregnancy *if just prescribed, complete at subsequent visit or by phone interview	o **yes** o **no** **If yes, name of supplement: _____** **Approximate date started: _____** **Gestational Age at start of intake:_____** **Intake frequency: _____**
Was an additional DHA supplement taken *during* pregnancy?	o **yes** o **no** **If yes, name of supplement: _____** **Dose:_____ Start Date:_____**
Vitamin supplements *prior to* pregnancy	o **yes** o **no** **If yes, what: _____**
Other nutritional supplements *prior to* pregnancy	o **yes** o **no** **If yes, what: _____**

Date enrolled: _____/_____/_____
mmm/dd/yyyy

Age Group – (check one)

16 – 25.99 Group I _____ **26 – 35.99 Group II _____**

Date of Initial Capsule Dispensing: ____/_____/_____
mmm/dd/yyyy

APPENDIX C

SUPPLEMENT USE AT 6 WEEK POST-NATAL VISIT

INVESTIGATOR	PROTOCOL	RANDOM CODE	FORM ID
CARLSON	HSC #10186		**D**

NUTRITIONAL SUPPLEMENT USE

BEFORE PREGNANCY

☐ **Multivitamin/Prenatal**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Iron**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Calcium**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Other**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

DURING PREGNANCY

☐ **Multivitamin/Prenatal**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Iron**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **DHA** (other than study capsules)

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Other**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

INVESTIGATOR	PROTOCOL	RANDOM CODE	FORM ID
CARLSON	HSC #10186		**D**

NUTRITIONAL SUPPLEMENT USE, CONTINUED

AFTER PREGNANCY

☐ **Multivitamin/Prenatal**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Iron**

Name

_____

Dosage _____

Amount taken _____

Date/Year Started

_____

Stop Date, if applicable

_____

☐ **DHA**

Name _____

Dosage _____

Amount taken _____

Date/Year Started _____

Stop Date, if applicable _____

☐ **Other**

Name

_____

Dosage _____

Amount taken _____

Date/Year Started

_____

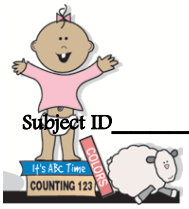
Stop Date, if applicable

_____

APPENDIX D

MAILED MONTHLY SUPPLEMENT USE QUESTIONNAIRE

Prenatal Supplement Questionnaire



Subject ID_____

Dear Study Participant,

Part of our study requires that we keep track of *all* vitamins and/or supplements that you may take during pregnancy. We do not want to influence the type of supplements you take nor tell you how often you should take non-study supplements. If you have questions about your vitamin/supplement intake you should ask your doctor. Instead, we would like an idea of your daily supplement routine.

It is especially important that we know the **exact kind** of supplement(s) you take. You may have a prescription from your doctor or a bottle you purchased from the store. Regardless of where it came from, we ask that you look at the bottle and write down **exactly** what the label says. Also, if you are not taking any vitamins or supplements at this time because of nausea, vomiting, or any other reason, that's fine too – we'd just like to be able to make note of it.

Listed below are several situations. Please pick the statement(s) that best describes your situation for **the past month** and fill in the blanks. Also, because you will be returning your study bottle there is no need to write down information about your study capsules unless there is something unique you'd like to add. After you have filled in the appropriate blanks, please return this letter with your bottle. If you have any questions, call my office (913.588.3781). I'm always willing to help!

1) I *am not* taking a prenatal vitamin at this time because:

   _____

2) I *am* taking a prenatal vitamin. The name of the vitamin is: _____.
   I got my vitamin from: _____. In the past week I've taken my vitamin _____ time(s).

3) I *am* taking other supplements. The name of the supplement(s) is/are:
   _____. In the past week I've taken this supplement(s) _____ time(s).

PS—I will be sending this same questionnaire every month with your new bottle so we can keep track of any changes that occur.