9-1-2015

Assessment of nutritional status in pregnant women on opioid maintenance therapy

Shikhar Shrestha

Shikhar Shrestha

Candidate

Pharmaceutical Science

Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Ludmila N. Bakhireva, MD, PhD, MPH, Chairperson

Dennis W. Raisch, PhD, MS, RPH

Peter Pribis, MD, DrPH, RD

ASSESSMENT OF NUTRITIONAL STATUS IN PREGNANT WOMEN ON OPIOID MAINTENANCE THERAPY

by

SHIKHAR SHRESTHA

B. PHARM., 2011
KATHMANDU UNIVERSITY

THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science
Pharmaceutical Sciences

The University of New Mexico

Albuquerque, New Mexico

July, 2015

DEDICATION

I dedicate this thesis to my parents, Suresh Kumar Shrestha and Shakuntala Shrestha for their unconditional love and support.

ACKNOWLEDGMENT

This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in preparation and completion of this study.

First and foremost, I would like to express my sincere gratitude to my advisor and thesis committee chair, Dr. Ludmila Bakhireva for her steadfast guidance and help. Dr. Bakhireva has been a constant source of encouragement and support not only during the course of this thesis project but through all the years of graduate school. She has always made time to answer all my question and overcome difficulties. She has been my inspiration as I hurdle all the obstacles in the completion of this research work. The plethora of experience I received under the guidance of Dr. Bakhireva is, in my belief, unparalleled to any form of classroom teaching. Dr. Bakhireva has been a very instrumental figure towards the development of my academic and professional goals.

I would also like to express my deepest thanks to my committee members Dr. Dennis Raisch and Dr. Peter Pribis for their constant guidance and encouragement throughout my thesis and at various stages of graduate school. I was fortunate enough to work with them and would like to thank them for their insightful comments.

I would like to thank fellow researchers Hilda Gutierrez, Laura Garrison, Sonnie Williams, and Steven Bishop without whom this research would not have been successful. I would also like to thank my girlfriend Emily Mathis for her support during the study. I owe my achievement to you all!

ASSESSMENT OF NUTRITIONAL STATUS IN PREGNANT WOMEN ON OPIOD MAINTENANCE THERAPY

By

Shikhar Shrestha

B. Pharm., Kathmandu University, 2011

ABSTRACT

Objectives: Patients who use substances or those who are on opioid maintenance therapy could be at risk of inadequate nutrition. These inadequacies could translate to adverse outcomes during pregnancy. The objective of this study was to determine differences in dietary macro and micronutrient intake in pregnant women on OMT compared to healthy controls.

Methods: Participants from a parent prospective cohort study "ENRICH" were classified into two groups: OMT users and healthy controls. Inclusion into the nutritional analysis was based on eligibility criteria of completion of food frequency questionnaire administered during hospital stay after delivery, absence of heavy drinking and adequate energy intake. Crude differences in energy, macro (carbohydrate, protein and total fat) and micronutrient (vitamin A, B1, B2, B6, B12, C, D, E, beta-carotenes, folate, iron and choline) intake between the study groups were compared using student's t-test which was

repeated after adjustment by total energy intake. To control for multiple comparisons MANOVA was used. Multivariate regression was used to control for confounders.

Results: A total of 54 subjects (34 OMT and 20 controls) were included in the nutritional analyses. No significant effect of OMT status on energy intake was observed. It was observed that OMT group had lower energy adjusted protein intake (p=0.03). Analysis of the dietary micronutrient intake showed that the subjects on OMT had significantly lower Vitamin E (-0.9a-TE/1000Kcal/day, 95%CI:-1.8, 0.1, p=0.03) and folate (-45.9 DFE/1000Kcal/day, 95%CI:-87.1,-4.6, p=0.03) intake compared to controls after controlling for marital status, insurance type, age and BMI. There was a significant effect of ethnicity on energy-adjusted carbohydrate intake (p=0.02) and employment (p<0.01) on energy-adjusted protein intake after controlling covariates. It was observed that diet alone was not able to meet the requirements of several micronutrients in both the OMT and control group.

Conclusion: It was observed that pregnant women on OMT had lower intake of several micronutrients compared to healthy controls which could lead to adverse pregnancy outcomes. The results of this study reinforces the requirement of micronutrient supplementation during pregnancy. Future studies should focus on investigating the effect of these differences in pregnancy outcomes and implement policies to promote healthy diet.

Contents

List of Tables

List of Figures

CHAPTER ONE: INTRODUCTION

Background

There is an epidemic of illicit drug use in the United States. In 2013, a national survey reported that there were 22.4 million adults in the U.S. who used illicit drugs[1]. There has been a steady increase in non-medical use of opioids over the last few decades which has become a significant concern for public health. The same survey in 2013 showed that the prevalence of opioid use among pregnant women aged 15 to 44 was approximately 5.4%. Some authorities consider that these estimates are modest due to underreporting of substance abuse in pregnancy. Still, the prevalence of illicit drug use poses a significant risk to the health of the mother and the growing fetus.

The management of opioid addiction falls onto physiological and social interventions [2]. Opioid maintenance therapy is the management of opioid dependence using pharmacological treatment to stabilize the physiologic dependence on harmful drugs[3]. The use of methadone and buprenorphine among other drugs have been shown to be effective for the treatment of opioid addiction [4,5]. Studies have revealed that treatment with opioids agonists (methadone) /mixed agonist-antagonist opioid receptor modulator (buprenorphine) along with social intervention increase the likelihood of treatment success and minimization of relapse[4]. The treatment, however, is not without its side-effects. In pregnant women treatment with methadone has been extensively studied [6-8]. Its use is associated with neonatal abstinence syndrome[9]. A few studies also report health complications such as low birth-weight to be associated with opioid maintenance therapy (OMT) [10,11]. These effects are moderate compared to the impact of use of other opioids and illicit drugs [11,12]. Hence, methadone and buprenorphine have been recommended as an effective therapy for the management of opioid dependence.

There are several explanations of the effects of opioid abuse and OMT on pregnancy outcomes. There are actual physiological mechanisms of opioid that affect the neurotransmitters [13] that regulate neurologic and physiologic development in the fetus. Other reason could be a possible effect on absorption of nutrients due to opioids [14]. Studies also show that socio-demographic reasons could lead to adverse pregnancy outcomes in opioid-addicted pregnant women [15]. Concomitant use of alcohol and tobacco among other substances has also been widely reported to be associated with adverse pregnancy outcomes such as low birth weight and fetal alcohol syndrome [16-18]. The complex nature of health behaviors related to opioid use, socio-demographic condition of the opioid using population, underlying diseases and, the nutrition affects the fetus and the mother in several ways. Singular intervention through OMT is almost never sufficient to mitigate the effects of opioid abuse and promote remission. Interventions such as social support, guidance, prenatal health checkups and adequate nutrition along with OMT are expected to promote good health in the fetus and the mother.

Nutrition during pregnancy and nutrition status of pregnant women on opioid maintenance therapy

Pregnancy is an important time for the development of the fetus. Adequate energy intake during the pregnancy along with micronutrients has been shown to decrease the probability of low birth weight (LBW) infant [19-21]. LBW has been shown to be associated with infant mortality, respiratory disorders and developmental metabolic problems in future [22,23]. The importance of good diet in pregnancy-related outcomes have been shown by several studies using dietary patterns and nutrient group analysis. Studies that analyze the association between dietary patterns around pregnancy and pregnancy outcomes reveal that food groups that had high energy, low nutrient density, high saturated and

trans-fats were associated with lower birth sizes. The converse, that is, nutrient dense food with fruits, vegetables, and whole grains were associated with positive pregnancy outcomes [24-28]. However, there are inconsistencies with the associations of pregnancy outcome with dietary patterns, as some studies have shown an association of pregnancy outcomes with socio-demographic characters [29-31].

Deficiencies of vitamins have been associated with adverse pregnancy outcomes regarding growth of the fetus, skeletal development, and future childhood development [32-34]. Apart from the studies that primarily focus on folic acid with iron supplementation and vitamin D on birth outcomes, there are a few studies that highlight the importance of B vitamins. A review by Allen (2005) states that deficiency of thiamin, riboflavin, folate or vitamin B12 is related to elevated levels of homocysteine in blood, which in turn was related to adverse pregnancy outcomes [34-36]. There are several dietary intake recommendations that provide specific information on the intake of vitamins and minerals which are considered essential for the development of the fetus during pregnancy [37-42]. Micronutrients such as folic acid, iron, zinc, magnesium, calcium, and several vitamins are necessary for proper growth and development of the fetus [43,44]. Supplementation of micronutrients is considered important and widely recommended. However, some authors also state that requirements of some of the vitamins and minerals are usually met in healthy pregnancies through regular diet and changes is physiological mechanisms [37]. However, it is essential to focus on having a healthy diet during pregnancy. Particular emphasis has been made on supplementation of folic acid for the prevention of neural tube defects (NTDs) [33,43]. Thus, concerning the importance of micronutrients in diet, our focus is on vitamin A, carotenoids, thiamin (B1), riboflavin

(B2), vitamin C, vitamin D, vitamin E, folate, and iron intake in pregnancy. The following table shows deficiency of several vitamins and their effect on maternal and fetal health.

Table 1: Micronutrient deficiency and associated maternal-fetal health outcomes

Micronutrient deficiency	**Maternal health outcome**	**Fetal health outcome**
Vitamin A	Maternal anemia[45]	Preterm delivery[45]
Vitamin B12 and Folate	Anemia, weight loss	Neural tube defects, small for gestational age birth, pre-eclampsia[33,36,43]
Vitamin D	Bone loss, subclinical myopathy, risk of pre-eclampsia[46]	Lower bone mass, risk of future osteoporosis[47]
Vitamin E	-	Miscarriage[48]
Iron	Iron deficiency anemia[32]	Pre-term delivery [32], iron deficiency anemia if delivery is preterm
Choline	Liver and neurological diseases [49]	Risk of neural tube defects[50]

Significance

Heroin and other opioid users are associated with neglect for their physical nutritional and social care[51]. It is often coupled with risk of criminal activities and prevalence of infectious diseases in the patients. Prevalence of opioid use in pregnant women and the

association of adverse outcomes related to opioid abuse in pregnancy demonstrates the need of effective interventions to minimize adverse pregnancy outcomes in those populations[1]. OMT has been established as an effective therapy for the treatment and maintenance of opioid addiction in pregnant women [6]. Compared to non-OMT related methods of addiction therapy, methadone maintenance therapy has been shown to be significantly better in retaining patients in treatment and suppressing heroin use [52]. OMT is also associated with better adherence to prenatal care, decreased risk of being incarcerated and decreased risk of acquiring blood borne infections associated with illicit drug use[52]. It should be considered that OMT, as all opioids, have some adverse effects on maternal and child health such as low birth weight and neonatal abstinence syndrome.[9,11,53]. It is important to consider these adverse outcomes associated with opioid use in pregnancy. Interventions consisting of regular prenatal care, social support and other medical treatments should be provided to such population.

Figure 1 highlights the association between maternal factors, intervention and pregnancy outcomes. We can observe that there are several factors that come into play that lead to a healthy pregnancy outcome or an adverse even. Factors such as pregnant mother's age, her immediate socio-demographic surroundings and her health and medical conditions could play a significant role in outcome of that pregnancy. To promote a healthy pregnancy interventions such as regular prenatal care, healthy diet, multivitamin supplementation, social support, management of opioid abuse could be necessary,

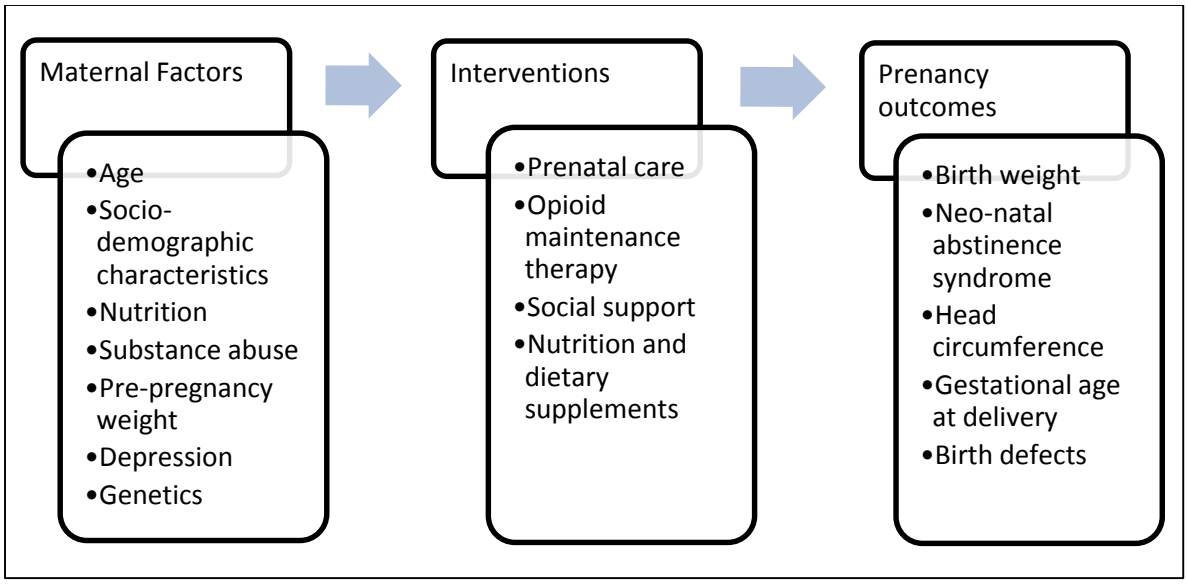

Figure 1: Relationship between maternal factors, interventions and pregnancy outcomes

Pregnant women have greater than average requirement of calories and nutrition for the development of the fetus and to develop stores of lactation[54]. Adequate nutrition, balanced in calories and micronutrients, which is based on recommended dietary allowances has been shown to be associated with positive outcomes in pregnancy. Studies consistently show the benefits of adequate weight gain and supplementation of diet with multivitamins and folic acid[55,56]. Opioid dependent pregnant women are already under the stress of the drugs they are taking. Thus, it is imperative that proper nutrition be a part of OMT in pregnant women.

There have been a few studies about nutrition in subjects undergoing OMT in non-pregnant population. These studies showed that subjects in the opioid maintenance program had higher carbohydrate intake and poor micronutrient status[57,58]. Our search of the literature resulted in just a single study on nutrition status performed on opioid

dependent pregnant women. The pilot study on pregnant women on OMT (n=22) and non-OMT pregnant women (n=119) showed that pregnant women on OMT had significantly higher energy intake which after adjusting for confounders was approximately 34% greater than the energy intake of controls[59]. The study also analyzed nutritional biomarkers and found that after adjustment serum homocysteine was higher and serum carotenoids were lower in OMT group as compared to controls.

The results of the studies on non-pregnant opioid dependent population and the pilot study on pregnant women on OMT suggest that pregnant women on OMT may be at high risk of poor nutrition. However, the pilot study on pregnant women on OMT had a small population size and did not evaluate the outcomes related to pregnancy. Thus the purpose of our project is to examine the differences in nutritional status of pregnant women on OMT with non-opioid using population. Future direction of our research could be to examine the association of these different pregnancy outcomes such as pre-term delivery and birth weight.

The result from our study is expected to confirm that there are nutritional differences in pregnant women on OMT as compared to controls. We expect the result to be in concordance with the studies in non-pregnant opioid using population. Such result would mean that the OMT population in our study would have higher energy intake and poor micronutrient status. This would place these women at a higher risk of adverse pregnancy outcomes. The result from our study is expected to be used to formulate interventions regarding dietary intake in pregnant women on OMT. In conjugation with OMT and prenatal care, proper nutrition can help minimize adverse pregnancy outcomes and promote proper growth and development of the fetus.

Study hypothesis and specific aims

Specific Aim I: To determine the differences in energy intake and the sources of energy (carbohydrates, fats, proteins) in pregnant women on OMT compared to non-opioid using pregnant women.

Research Hypothesis I: We hypothesize that there will be differences in energy intake in pregnant women on OMT compared to non-opioid using pregnant women.

Specific Aim II: To determine the differences in dietary vitamin A, B1, B2, B6, B12, C, D, E, and beta-carotenes intake in pregnant women on OMT compared to non-opioid using pregnant women.

Research Hypothesis II: We hypothesize there will be difference in dietary intake of vitamins in pregnant women on OMT compared to non-opioid using pregnant women.

Specific Aim III: To determine the differences in dietary iron, folate and choline intake in pregnant women on OMT compared to non-opioid using pregnant women.

Research Hypothesis III: We hypothesize that there will be differences in dietary folate, iron and choline intake in pregnant women on OMT compared to non-opioid using pregnant women.

Sub-aim for Specific Aims I, II and II

To determine the effect of socio-demographic characteristics on energy and micronutrient intake.

Specific Aim IV: To assess if the participants in our study meet the estimated average requirements based on their diet and micronutrient supplements.

CHAPTER TWO: LITERATURE REVIEW

Epidemiology of drug abuse in United States

The 2013 report on substance use from the National Survey on Drug Use and Health stated that an estimated 22.4 million adults older than 18 years used illicit drugs in the United States [1]. Illicit drugs included marijuana, cocaine, inhalants, hallucinogens, heroin or prescription drugs (non-medical usage). This estimate shows that approximately 9% of the American population used illicit drugs of which a significant portion were marijuana users (7.6%) and prescription drug abusers (2.5%). There has been a steady rise in drug abuse over the past five decades with a significant rise in abuse of therapeutic opioids. Hydrocodone topped all prescriptions with 136.7 million prescriptions in 2011.

The exponential increase in the abuse of illicit drugs constitutes an important public health problem in the US. Approximately 80% of the 43,982 overdose related deaths in the US in 2013 were unintentional [60]. Also, the economic impact of drug abuse is significant with an estimated cost of $55.7 billion in 2007. Lost productivity accounted for approximately 46% of the above cost and 45% was related to healthcare cost [61].

Illicit drugs use in pregnant women

Prevalence of substance abuse is difficult to establish particularly due to inaccuracies of self-report. Thus, the data regarding drug abuse in pregnant women varies according to the population sampled, the method of test used, screening period and sampling (community population or targeted population). Social stigma, fear and embarrassment could prevent women from disclosing drug abuse. The fear of legal and social action could also prevent pregnant women from disclosing drug use thus delaying care. These various factors combined with the inaccuracies of self-report makes it difficult to get

proper estimates of drug abuse during pregnancy. We can consider current estimates to be low because of the above reasons which lead to under-reporting.

The National Survey on Drug Use and Health is an annual survey which provides information on use of illicit drugs, alcohol and tobacco on state and national level [1]. Current report (combined 2012-2013 data) states that illegal drug use in pregnant women aged 15 to 44 was approximately 5.4 % which is lower than the rate in non-pregnant women (11.4%). There has not been a significant increase in illicit drug use over the past couple of years; 2007 - 2009 (5.1%) and 2009-2010 (4.4%). The prevalence of illicit drug use is lower in the third trimester (2.4%) versus the first (9.0%) and the second (4.8%) trimester. The distribution of the prevalence of drug abuse over the age of pregnant women showed that the highest prevalence was for the age group 15-17 years (14.6%) followed by the age group 18 to 25 years (8.6%) and the age group 26-44 years (3.2%).

Methadone and Buprenorphine for treatment of opioid abuse in pregnant women

Opioid dependence has been defined as a "physiological disease characterized by a permanent metabolic deficiency" by Dole and Nyswander [3]. According to their treatment principle of managing opioid dependence by "a sufficient amount of drug to stabilize metabolic deficiency", they introduced treatment by oral administration of methadone. Agonist medications such as methadone act through the same receptors as the addictive substance but have different rates of action. These agonists thus reduce the harmful behavior caused by addictive opioids all the while preventing withdrawal symptoms. It is essential to consider the effects of OMT in the presence alternatives such as drug free treatment, placebo medication or detoxification. A Cochrane review on methadone maintenance therapy summarized that MMT was better able to retain patients in the

program compared to other alternatives and to suppress heroin use [52]. It also stated that the patients were less likely to be involved in criminal activities. The usefulness of OMT or MMT is not just limited to stabilizing the metabolic deficiency and curbing illegal drug use. Preventing drug abuse is followed up by better control over their lifestyle, involvement in better social activities, decrease in criminal activities and promotion of good health. The services that are provided with OMT such as social services, counseling and medical services combined with the treatment is catered towards improving outcomes for the patients.

Studies that evaluate the effects of OMT suggest that the treatment reduces illicit drug use, improves prenatal care thus leading to improved pregnancy outcomes [62]. Although methadone has the ability to cause neo-natal abstinence syndrome, maintenance treatment provides steady drug concentration in blood preventing repeated withdrawal effects on the fetus [63]. Buprenorphine has also been shown to be effective in pregnant women. Studies report lower placental transfer of buprenorphine as compared to methadone which reduces the exposure of fetus to the opioid, thus minimizing the chance of developing neonatal abstinence syndrome [8]. A recently published Cochrane review article assessed the effectiveness of OMT with or without other 'social interventions' compared to no intervention in pregnant women in randomized controlled trials. They found four randomized controlled trials with a total of 271 subjects [53,64-66]. The authors of the review presented a generalized conclusion stating that they did not observe significant difference between methadone, buprenorphine or slow release morphine to conclude if any of the treatment was superior [4]. Their study indicated that methadone had a higher patient retention rate but buprenorphine lead to a lower neo-natal abstinence syndrome rate.

Implications of opioid abuse in pregnancy

Apart from the harmful behavioral and physiological effects of opioids on the mother, there are many short and long term effects of opioids on the infants [67]. Studies show that substance of abuse resemble naturally occurring neurotransmitters, thus their long term use precipitates neurobehavioral imbalance. A technical report titled "Prenatal Substance Abuse: Short and Long-Term Effects on Exposed Fetus" from the American Academy of Pediatrics concisely presents the effects of prenatal substance abuse [67]. The study reports effects on birth-weight, withdrawal symptoms and neurobehavioral anomalies. The technical report illustrates that studies analyzing the long-term effects of illicit drug use on post-natal growth and development do not demonstrate specific effects. However, the combination of environmental factors along with lack of care mechanism and exposure to illicit substances have shown to negatively affect growth, behavior, executive functioning skills and predisposition to experiment with drugs.

A retrospective cohort study analyzed the risk factors of preterm birth in opioid addicted pregnant women being treated with methadone between 2000 and 2006 [68]. The results demonstrated that overall preterm birth rate was 29% among methadone users. The rate of preterm birth in women who reported methadone use only was approximately 24%. Further analysis showed that abuse of other "supplements" (cocaine, alcohol, opiates or marijuana) alongside methadone increased the risk of preterm birth with rates up to 64% in pregnant women who used 3 or more "supplements" along with methadone. A study analyzing the effect of methadone treatment, tobacco use and social deprivation on fetal growth stated that the infants born of methadone using mothers had a significantly lower mean weight and smaller head circumference[69]. The cohort was 366 single births in a

Scottish population. The results after controlling for gestational age, tobacco use, maternal age, infant sex and parity showed that the infant born of methadone treated mother weighed 259 g less on average than infants born of healthy mothers [69]. Another research that studied the relationship of maternal heroin and methadone use on infant birth weight stated that the reduction in birth weight associated with methadone was 279 g on average, with a non-significant risk of low birth weight. Also, the use of heroin while using methadone counteracted the positive gain effects of methadone [11]. There was an increase in NAS in newborns from 1.20 to 3.39 per 1000 hospital births per year between the years 2000 and 2009 [70]. The trend was congruent with the increase in opioid use in pregnant women from approximately 1.19 to 5.63% between the same time periods. The same study reported that infants with NAS had lower birth-weight as compared to controls (19.1% vs 7.0%) and had complications with their respiration (30.9% vs 8.9%).

These studies show that pregnant women who are on OMT are at high risk of adverse pregnancy outcomes. There are several possible reasons that could have mediated those events along with the opioids. Lack of prenatal care, medical conditions, poor social support, and poverty could have contributed to poor pregnancy outcomes. It is also important to note that nutrition could have played significant role in those outcomes.

Nutrition in pregnancy

Energy intake: There is an increased requirement of energy in pregnancy due to the extra energy utilized by the fetus, uterus and the added increased activity of lungs and the heart [71-73]. Several studies have measured the basal metabolic rate (BMR) and it was determined that there is an increase of 154 MJ on average throughout pregnancy [37]. Also

studies have found that total energy expenditure increased by up to 16.5% during the third trimester of pregnancy. The estimates state that to gain an average of 12 kg during pregnancy, the extra amount of energy required would be 310 kcal/day [54].

Protein: The estimate of total protein requirement during pregnancy is approximately 925 g. It is based on the estimate that the women will gain 12.5 kg during that time and deliver a baby of 3.3kg [72]. Other estimates suggest lower intake in the range of 497 to 696 g over the pregnancy period. The recommendation by Food and Agriculture Organization/World Health Organization/United Nations University suggests that there should be an increase in intake of protein by 6 g per day during pregnancy [74].

Vitamin A: There is an extra requirement of Vitamin A during pregnancy. The growth and development of the fetus and the maintenance of maternal tissues requires vitamin A [37].Studies suggest higher requirements during third trimers of the pregnancy. However, excess amount of retinol is considered teratogenic [75].

Vitamin B1 (thiamin), B2 (riboflavin), B12 and folate: Thiamin and riboflavin (Vitamin B1 and B2) are essential for metabolic activities in cellular level in the body. Due to this reason there is a higher need of thiamin and riboflavin during the last trimester of pregnancy. Vitamin B deficiency has been shown to be associated with elevated levels of homocysteine [34]. Elevated levels of homocysteine have been related with adverse pregnancy outcomes [35,36]. Folate supplementation during pregnancy is required to prevent megaloblastic anemia. Studies also show supplementation with folic acid to reduce the risk of neural tube defects [33].

Vitamin C: The growing fetus requires extra amount of vitamin C which it gains from maternal sources. It has an important role in improving the absorption of non-hematological sources of iron [76].

Vitamin D: For the absorption of calcium and its utilization, vitamin D is considered essential. It is needed for the development of skeletal structures of the fetus during the later stages of pregnancy. Studies have shown that deficiency in vitamin D is associated with lower bone mass in a child and poses a risk of osteoporosis in the future [47]. However most of the vitamin D status in adult women is maintained through exposure to sunlight rather than diet, extra supplementation is not usually recommended unless the population is at a specific risk of vitamin D deficiency.

Vitamin E: In pregnancy studies show that there is steady increase in the levels of plasma α-tocopherol and the results indicate that vitamin E plays vital role in platelet regulation and preventing aggregation of platelets thereby regulation placental blood circulation [77]. The role of this micronutrient was also examined in pregnant women with preeclampsia. The study found that plasma concentrations of α-tocopherol and beta-carotenes were highly reduced in patients with severe preeclampsia. However, supplementation with vitamin E did not reduce the risk of pre-eclampsia [78].

Iron: There is an increased requirement of iron during pregnancy to meet the growing needs of fetus [79]. The fetus absorbs most of the required iron during the third trimester of pregnancy. There is a risk of iron deficiency anemia in infants if it the baby is born prematurely [80]. The Dietary Reference Value panel UK states that although there is higher utilization of iron during pregnancy, the requirements were met through increased

absorption, utilizing the stores in the mother and savings from stoppage of menstruation [37]. The subjects who are at risk of iron deficiency would need supplemental iron in their diet. Such groups include women from low socio-economic groups, teenagers and who have had successive births [37].

Choline: Choline and its metabolites are primarily used in the physiologic purposes of signaling roles for cell membranes, cholinergic neurotransmission and in neurotransmitter synthesis pathways [81]. There are many health effects of choline and studies have shown that its deficiency may play a role in liver and neurologic diseases [82]. Choline is of greater interest because its deficiency has been seen in athletes, heavy drinkers and pregnant women[82]. A study had shown that high dietary intake of choline around the time of conception was related to lower incidences of NTDs [50]. Pre-clinical studies on mice have also shown that choline supplementation could minimize the health effects of prenatal alcohol exposure [83]. It is highly likely that choline could play similar role in humans which makes the evaluation of this micronutrient necessary.

Dietary Reference Intakes (DRIs):

The Institute of Medicine of the National Academies provides nutrition recommendation intended for use by general public and health professionals. The DRI includes several different types of reference values of which Estimated Average Requirements is defined and listed [38-42]. Estimated Average Requirements (EAR) is the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group.

Table 2: Estimated average requirements during pregnancy

Age group (years)	Vit A* (µg/day)	Vit B1 (mg/day)	Vit B2 (mg/day)	Vit B6 (mg/day)	Vit B12 (µg /day)	Vit D (µg/day)	Vit C (mg/day)	Vit E‡ (mg/day)	Folate‖ (µg/day)	Iron (mg/day)	Choline (mg/day)	Carbohydrate (g/day)	Protein (g/kg/day)
19-50	550	1.2	1.2	1.6	2.2	10	70	12	520	22	450	135	0.88

* Measured as micrograms (µg) of Retinol activity equivalents (RAE) per day

‡ Measured as milligrams (mg) of alpha tocopherol equivalents (a-TE) per day.

‖ Measured as micrograms (µg) of dietary folate equivalents (DFE) per day.

Nutrition in opioid dependent population

A literature review was performed to identify the studies that evaluated the nutritional status of subjects undergoing OMT. The purpose of this review was to evaluate the studies that examined the nutritional behaviors of patients on OMT measured through specific nutrition survey or questionnaire We used search terms that included "buprenorphine maintenance OR methadone treatment or methadone maintenance OR methadone maintenance treatment OR opioid maintenance treatment, AND energy intake OR nutrient intake OR diet OR nutritional status or nutrition" to identify articles in PubMed.

The inclusion criteria for the study were: a) study involving nutrition or dietary patterns in subjects undergoing OMT, b) study conducted in humans, and c) study published in English. The exclusion criteria were: a) studies that only had hematological analysis of subjects, b) studies that focused only on a specific serum protein or metabolite, c) studies that focused on generalized nutrition (e.g. "Determine your nutritional health" survey), d) literature review, and e) commentary or editorials.

We initially found 127 articles using our search terms. There were 76 studies that remained after the exclusion of non-human studies. We reviewed the abstract of the remaining studies and examined them for relevance to our review. After examination we excluded 63 studies which were not relevant based on the contents of their study. After further examination we excluded studies that were not published in English, studies that only performed hematological analysis and studies that focused on generalized nutrition. We found a total of 4 studies which met our inclusion criteria. One of the studies examined the nutritional status of pregnant women on OMT.

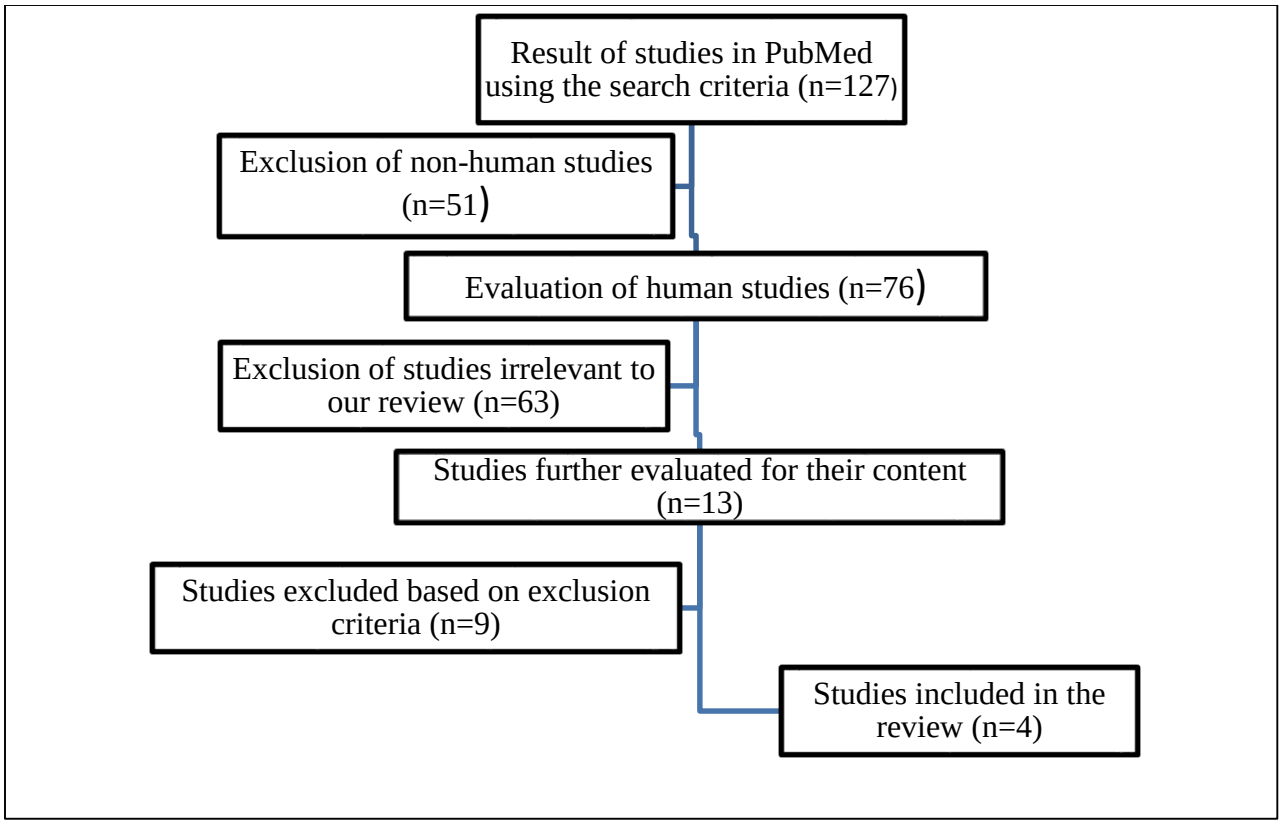

Figure 2: Literature review on nutrition in subjects on opioid maintenance therapy

Zador et al (1996) conducted a study on women (n=86) who were participants of a methadone clinic in Australia showed that they had lower daily energy intake (1547 kcal; 95% CI: 1437-1657 Kcal) as compared to a standard acquired from National Dietary Survey on Adults in 1983(1920 kcal; 95% CI: 1851-1989 kcal) [57]. The study also reported significantly lower intake of proteins (53g; 95% CI: 49-57g vs 80g; 95% CI: 76-84g) and higher intake of carbohydrates from sugar (122g; 95% CI: 112-132g vs 101g; 95% CI: 95-107g) in methadone users. The subjects also had significantly lower intake of magnesium, iron and zinc (based on percentage of recommended daily intake).

Another study conducted in Poland in 2005 evaluated nutritional status of men (n=23) and women (n=7) who attended a methadone maintenance treatment clinic for four years [58]. Their dietary intake was examined before the start of the program and at the end of 4 years into the program. Dietary intake was measured using 24 hour dietary recall. Compared to the recommended values in the study, the results showed at first test women had lower than safe levels of fibers, calcium, iron, magnesium, zinc, vitamin A and thiamin. Similarly in men, the intake levels of energy, fibers, magnesium, vitamin B1, B2, C, niacin, calcium and magnesium were lower than the recommended safe levels. The continuation of the same study reported that the participants' diet primarily consisting of high calories and low nutrients had positive changes over the period of the course of methadone maintenance therapy.

Similarly there was another study with fourteen participants (9 females and 5 males) who were in a methadone maintenance program along with 14 controls (10 females and 4 males) whose diet was assessed by using a modified questionnaire developed by Peryam and Pilgrim [84]. The basal metabolic index of the subjects in the methadone maintenance program was significantly higher than controls (28.85 ± 2.14 kg/m^2 vs 22.84 ± 0.85 kg/m^2). Their analysis showed that the patients in methadone maintenance program reported a higher degree of affinity towards consumption of desserts, chocolates and candy.

Tomedi et al(2012) conducted a study examining the nutritional status of pregnant women on OMT [59]. The subjects were pregnant women on methadone maintenance therapy (n=22) in a hospital setting. Controls, also pregnant women (n=119) were recruited from another ongoing study. The nutritional status was measured using a semi-

quantitative modified Block 98 food frequency questionnaire at 30 weeks of gestation. The study also examined nutritional biomarkers in blood.

Study of the demographics of the study population revealed that the pregnant women who were on OMT were older (27.1 vs 31.3, $p<0.01$), unmarried (59.1% vs 26.9%, $p<0.01$), unemployed (81.8% vs 43.7%, $p<0.01$), smoker (72.7% vs 12.6%, $p<0.01$), and more depressed (based on Edinburgh postnatal depression scale, $p<0.01$) compared to controls. Mean pre-pregnancy basal metabolic index of pregnant women on OMT was lower (22.9 vs 26.4, $p=0.02$). After adjustment for confounders the nutritional analysis showed that the pregnant women on OMT had higher intake of energy (3033, 95% CI: 2595-3571 kcal vs 2172, 95% CI: 2041-2303kcal). The biomarker analysis showed that after adjustment, homocysteine levels were lower and carotenoids levels were significantly lower in the methadone exposed group. Another important observation in this study was that levels of daily folate intake were lower than recommended levels in both exposed and the control groups. Elevated levels of serum homocysteine could mean that the pregnant women in OMT have thiamin, riboflavin, folate or vitamin B12 deficiencies[34]. Equally important fact is that elevated homocysteine level is associated with adverse pregnancy outcomes [35,36] which makes the finding of this study concerning.

Summary of the review on nutrition in opioid dependent population

The studies regarding nutrition in opioid dependent population showed mixed results regarding energy intake and basal metabolic index. However, they shared a common theme. Most of their energy was derived primarily from carbohydrates consisting of non-complex sugars and their diet had lower amount of micronutrients as compared to controls or lower according to recommended daily intake requirements. These

observation shows that there are inadequacies in diet of patients on OMT. Since the energy and micronutrient requirements of pregnant population are considered to be greater than the general population it can be implied that pregnant women on OMT could have poor nutrition status which can put them at a risk of adverse pregnancy outcomes.

The study conducted by Tomedi et al (2012) was a pilot study and the small sample size could have resulted in non-significant results in the intake of micronutrients after adjustment. Hence, further studies are required in this population to confirm the poor nutritional status of pregnant women. Also, the effect of nutrition status on pregnancy has not been evaluated specifically on pregnant women on OMT. Our study has greater relevance in the light of lack of information regarding the nutritional deficiencies in pregnant women on OMT. The results of this study can be used to plan interventions to promote proper nutrition in pregnant women on OMT leading to improved pregnancy outcomes.

Measurement of nutrition

Methods to estimate dietary intake can be divided into two broad categories: 1) the measurement of actual concentration of nutritional biomarkers in biological specimen along with anthropometric measurements, 2) the measurement of what the people are eating. These two approaches appear distant to each other but share the common outcome of measuring the nutritional status of a person. The first method focuses primarily on the final output, which is the end product of nutrition after the food is metabolized, absorbed and then distributed into the body producing the result in terms of plasma level concentrations, hematocrit levels, weight gain, muscle mass and fat mass. The second

method focuses on what the subject is consuming, how much of each food group is contained in his her diet.

There are various advantages and disadvantages of the two methods. The first method where plasma micronutrient levels and hematocrit level are measured are better suited to describe the nutrition at the period surrounding examination. It would be difficult to predict the overall dietary patterns or what the subjects are eating based just on the plasma levels of the micronutrients. Also the cost of conducting such analysis would be significantly greater. The second method focuses directly on what the patients are eating. Several self-reported methods are available to estimate patients' dietary intake, including the24 hour recall, food records and food frequency questionnaire (FFQ), each with its own advantages and limitations [85-87]. The final output of these self-reported measures are what the person is eating in general. These methods provide excellent information, depending upon the instrument used, on what the person eats in his day to day life. This allows the researchers to evaluate if that diet has any role in the outcome that they are evaluating. However, these methods fail to take into account if there are any problems with the absorption of the food or if the food is not being metabolized properly. Recall biases could also affect the result of such analysis. The primary goal of this research involves the measurement of dietary intake in pregnant women. Our prime goal is to evaluate if there are any differences in nutritional intake in pregnant women on OMT versus the controls. The observance of any disparity can help us identify those deficiency and formulate intervention. In the following sections Block brief food frequency questionnaire has been described which was used to collect nutritional information from the pregnant mothers in our study.

Block Brief Food Frequency Questionnaire

The interest of this research in the nutrition of pregnant women revolves around their usual diet. As discussed previously, adequate nutrition is a vital part in prenatal health care. There is rich information on the effects on inadequate or excess energy intake and effects of micronutrient supplementation on pregnancy outcomes and its long term effects. Nutritional analysis of pregnant women can provide clear information on their dietary intake and allow us to develop interventions if significant deficiencies that may be harmful for the growth of the fetus are observed.

The accuracy in measurement of nutrient intake and the context of the research are important factors in determining the technique to be used for evaluation of nutritional status. Various methods have been developed to measure dietary intake. The 24 hour recall method is a simple method, which can be administered by interviewer with minimal training. It asks the respondents to recall intake in the last 24 hours. However, there is significant deviation in day to day intake of food which can lead to imprecise estimates of nutrient intake in individuals [85,86]. The history method uses food model and consists of an extensive interview to study an individual's diet [87]. Diary method applies the concept of using a diary for a specific period of time to record food intake. Method such as seven day record has been used in studies. It is a type of diary method and it is oriented towards minimizing errors of recall. The analysis of nutritional biomarkers is a precise way of establishing the nutrients present in an individual's body. It can provide a very accurate measurement of the nutritional status of an individual. The drawbacks are the expenses and methodological difficulties regarding collection and analysis of blood

sample. Also such biomarkers are specific to a certain period of time and do not account for the fluctuation of such biomarkers in blood.

FFQ is used to measure usual and customary intake of food. The results are obtained by recording the frequency and portion size of a list of food items. Various FFQ have different food lists and work on different time frames of recall. This versatility allows the FFQ to control for variation in food intake due to seasonal variations. The FFQ used in our study was the Block Brief 2000 FFQ (NutritionQuest©). It consists of 72 food items. The average administration time is around 15-20 minutes. Due to the reduced list of food items, this FFQ could underestimate the estimates of energy and macronutrient intake. It is possible however to rank individuals along the distribution of their intake thus cancelling out the effect of underestimated intake estimates.

Validity and use of Block Brief 2000 FFQ

Development of the Block Brief 2000 FFQ was based on the same method as the full Block 98 FFQ. However, the data set used for the development of Block brief 2000 was NHANES III while Block 98 FFQ had used NHANES II. The list of food items were separate for White, Hispanics and African American so that food items specific to a single group was not omitted. Another change was the method of asking portion sizes for non-unitary foods using portion size photos to aid the estimation. Block 98 FFQ has been widely used and has been validated by several studies [88,89].

The validation of the Block Brief 2000 FFQ has not be specifically performed. However, a study was performed which evaluated a reduced questionnaire (food items =60) which was based on Block 98 questionnaire [90]. The study evaluated Block98 and reduced questionnaire against a “three four-day dietary intake” records in the Women’s Health

Trial pilot. The study reported that in the usual diet group the correlation between Block98 and multiple dietary records was 0.57, and the correlated between reduced questionnaire and multiple dietary records was 0.56. Similarly, in the reduced fat intake group, the correlation between Block98 and dietary records was 0.62, and that between reduced questionnaire and dietary records was 0.65. The result suggested that the reduced questionnaire would essentially be as effective as Block98 in measuring the dietary intake. The Block Brief 2000 is a reduced version of Block98, however, compared to the reduced questionnaire, it contains additional food items. Thus, the construction of Block Brief 2000 as an intermediate between the Block98 and reduced questionnaire from the Block et al study suggests similar validity. Block Brief 2000 has been in use in contemporary research and is an effective instrument in estimating dietary intake [91-93]

Factors that affect diet and nutrition: An ecological model

There are a multitude of factors that affect food preferences, dietary patterns and nutritional status of an individual. There are individual factors such as personal preference, taste, affordability followed up by the social and familial effect on diet such as effect on ethnicity, race, and social-perception on food choice. On a macro level, food pricing, advertisement, and government food programs could affect food choices. An ecological model representing the factors that could explain food choices which ultimately affects nutritional status is represented in figure 3.

The first level that immediately affects nutrition as shown in the ecological model is food choice. Taste is an important factor that drives our nutritional behavior[94,95]. Our basic sensations of taste which is affected primarily by our genetics and physiology is modifiable by other external factors such as age, race, culture, and location[96,97] . Another

factor that could affect food consumption is the time required to prepare food and access. The amount of work and conditions surrounding it affects meal choices as long commute from home, evening shifts and associated time challenge is related with purchasing takeout meals [98].

On the second level the interpersonal factors that consist our immediate surrounding are responsible in determining what food we eat. Four environmental factors have been identified by a study by Wansink that affect the amount of food eaten. It includes the surrounding (pleasantness); access and convenience; company (friends or family who encourage eating); and distractions such as television which can cause a person to lose track of how much he/she is eating [99].

Finally on the third and fourth levels are the factors that are distant towards actual food choices but affect the purchase and consumption of food on a bigger levels. These levels, the community level and the policy level have an overarching effect over overall food choices in social or national context. Other factors such as marketing of food (functional organic foods), awareness of nutrient content of food groups, cost of food, social perception of a product safety and prices affect how much a food is consumed [100-102]. There are larger effects of socio-cultural norms on dietary patterns [103] along with perceptions, attitudes and beliefs about nutrition [95]. Although the trends are changing due to social change, media and availability of information, it is important to consider how perceptions affect choices of food [95].

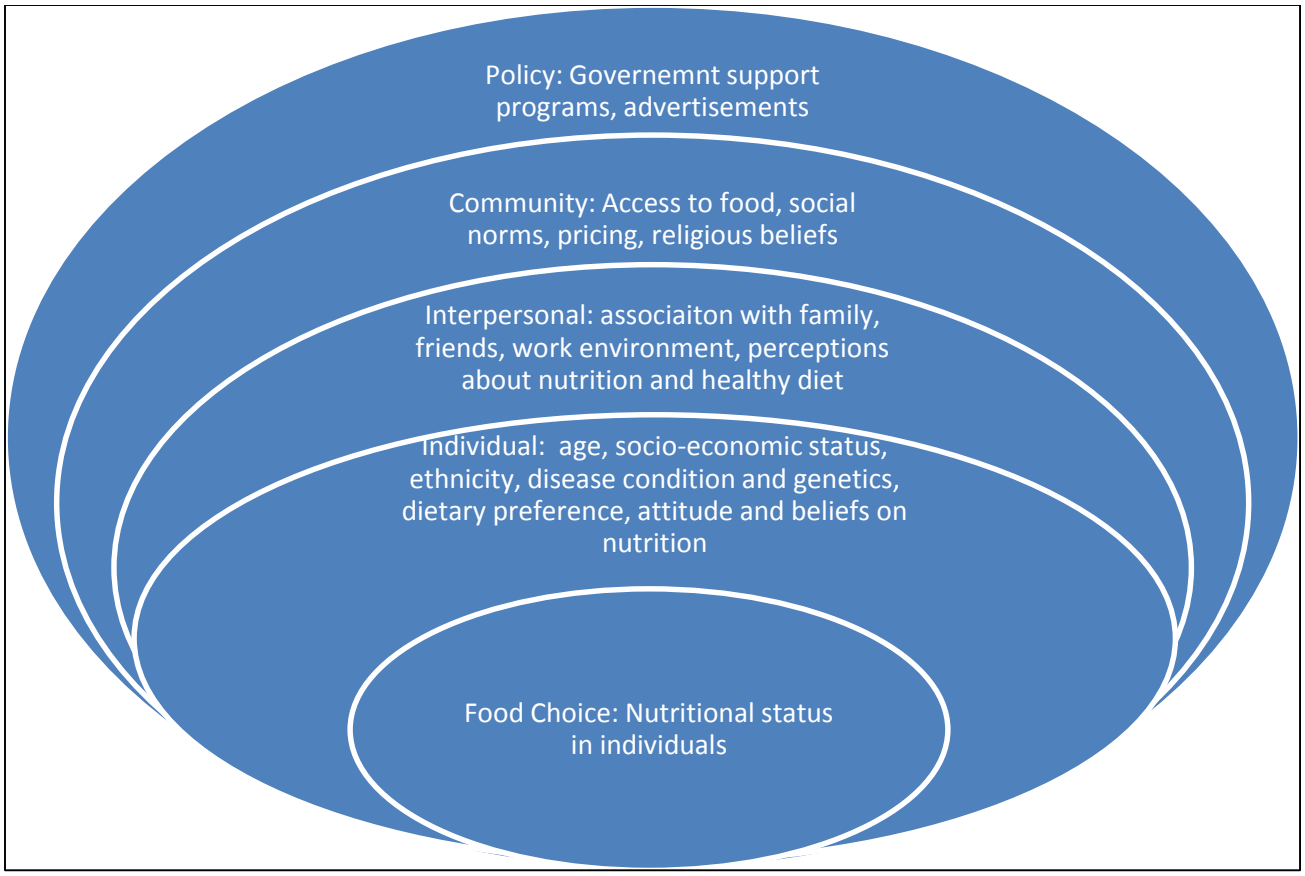

Figure 3: Ecological model: Factors that affect food choice and nutritional status in individuals

CHAPTER THREE: METHODS

Data source

The data for our study was derived from an ongoing study in University of New Mexico Health Sciences Centre. The "Ethanol Neurodevelopment Infant and Child Health" (ENRICH) study, aims to refine analytical procedures to detect the effects of prenatal alcohol exposure earlier in a child's development. The procedures consists of several questionnaires and a battery of biomarkers test to ascertain alcohol, tobacco and substance exposure. Magnetoencephalography (MEG) and electroencephalography (EEG) are used to analyze neurophysiological indices to detect indicators of fetal alcohol exposure. Such early detection could pave a way to earlier health and behavioral intervention in exposed population thereby decreasing the chances of development of disabilities in children with fetal alcohol spectrum disorders. This work has been supported by the R01 AA021771 research grant from NIAAA/NIH.

ENRICH is a prospective cohort study. It follows a cohort of pregnant women recruited during their prenatal visits, and their newborn over a course of 20 months after delivery. The patients are recruited from University of New Mexico Milagro clinic and UNM General Obstetrics clinic. There are two major study groups in the ENRICH study. First group are the healthy controls: pregnant women who have no exposure to OMT or have ever used illicit drugs in their lifetime. They are also required to have minimal exposure to alcohol or tobacco. The second group consists of pregnant women who are on OMT. The subjects in this group have other exposures such as alcohol, tobacco and other substances. The inclusion criteria for the parent study were: 1) age $\geq$ 18 years; 2) Singleton pregnancy which was confirmed by an ultrasound; 3) residence in Albuquerque metropolitan area (plan to stay here for the duration of study, approximately 2 years); 4)

capable of providing written consent; 5) No use of cocaine, crack-cocaine, ecstasy or methamphetamine for exposed groups; lifelong abstainer of illicit drugs for controls 6) no major structural anomalies identified prenatally

Assessments at baseline (Study visit 1)

At baseline (V1), demographic information such as age, marital status, ethnicity (Hispanic or not), race, education level, employment status, health insurance status, country of birth and primary language use in home was recorded. Medical and reproductive health information such as self-reported pre-pregnancy weight and height, diagnosed medical conditions, information on their last menstrual period, current gestational age, gravidity, parity, complications in current pregnancy and information on any dietary supplements the subjects are taking was collected. Maternal alcohol consumption is also measured based on time line follow back calendar[104](TLFB) around LMP and at visit 1, along with Alcohol Use Disorders Identification Test (AUDIT)[105] and TWEAK scores[106]. Lastly information on tobacco and substance use was recorded. Biological specimens (serum, whole blood, urine) were also collected at the first interview. They were used to ascertain alcohol, tobacco and substance use. The alcohol biomarkers that were examined were serum gamma-glutamyl transpeptidase (GGT), disialo carbohydrate deficient transferrin (%dCDT), and phosphatidylethanol (PEth) in the mother's whole blood. The study used the following cut-offs to classify the subjects as positive to alcohol exposure: GGT>40 U/L[107], %dCDT>2.0[108-110] and PEth $\geq$ 8ng/ml[111,112].

Assessments during the hospital stay after delivery (Study visit 2)

The second interview (V2) was conducted during the patient's stay in the hospital for delivery. In the second interview, information regarding use of supplemental vitamins, tobacco, alcohol and substance use was recorded. Information on the subject's perceived stress was also included. In the second interview, an interviewer administered a food frequency questionnaire (FFQ) which was used to evaluate the dietary intake of the participants in the study. The study used Block 2000-Brief FFQ to estimate the daily intake of nutrients in the participants. Biological specimens were also collected to ascertain if the subject had used alcohol or other substances. The alcohol biomarkers examined at the second interview were: GGT, %dCDT, PEth in maternal blood and PEth in infant dry blood spot (DBS). The cutoffs used for GGT, %dCDT and PEth in maternal blood were as described earlier. A cut off value of DBS-PETH >20ng/mL[113] ,urine ethyl glucoronate (uEtG) ≥ 25 ng/mL and urine ethyl sulphate (uEtS) ≥ 7 ng/mL[111] was used to classify the subjects as positive to alcohol exposure.

Selection of participants for the nutritional analysis from the parent cohort:

All of the participants in the nutritional assessment study were selected from the ENRICH parent study. The following inclusion criteria were applied: 1) Completion of both visit 1 and visit 2 interviews; 2) completion of the Block Brief 2000 FFQ at visit 2. The following exclusion criteria were applied:

For exclusion of heavy to moderate alcohol users we excluded the subjects who

1) Were positive for any two alcohol biomarkers (analyzed at visit1 or visit2)

   or

2) Had consumed more than 1.5 units of absolute alcohol per day on average around their last menstrual period calculated using timeline follow back calendar

For exclusion of unrealistic energy intake values for pregnant women, subjects who were outliers with respect to energy intake (<1,000 Kcal/day or > 6,000 Kcal per day) were excluded.

At the time of analysis, 61 subjects in the ENRICH parent study had completed the visit 2 interview and had completed the food frequency questionnaire. After we applied the exclusion criteria, 7 subjects were excluded; 4 subjects were excluded because they were either positive for 2 or more alcohol biomarkers or had intake of over 1.5 units of absolute alcohol per day and 3 subjects were excluded because their energy intake was either above 6000 Kcal per day or lower than 1000 Kcal per day. In total 54 subjects were qualified for the nutritional analysis.

The subjects undergoing OMT as identified by their recruitment from Milagro clinic which offers treatment to pregnant women with substance use disorders were classified as OMT group. Their exposure to OMT was further confirmed by their self-report and medical records. Alcohol, tobacco and substance use in these population was also confirmed using self-report and analysis of biological specimens. The healthy controls were those subjects who had no exposure to OMT. They also did not have any or minimal exposure to alcohol identified by self-report and confirmed by analysis of their biological specimens for alcohol biomarkers. In total 34 subjects were qualified to be included in the OMT group and 20 into the healthy control group for final analysis.

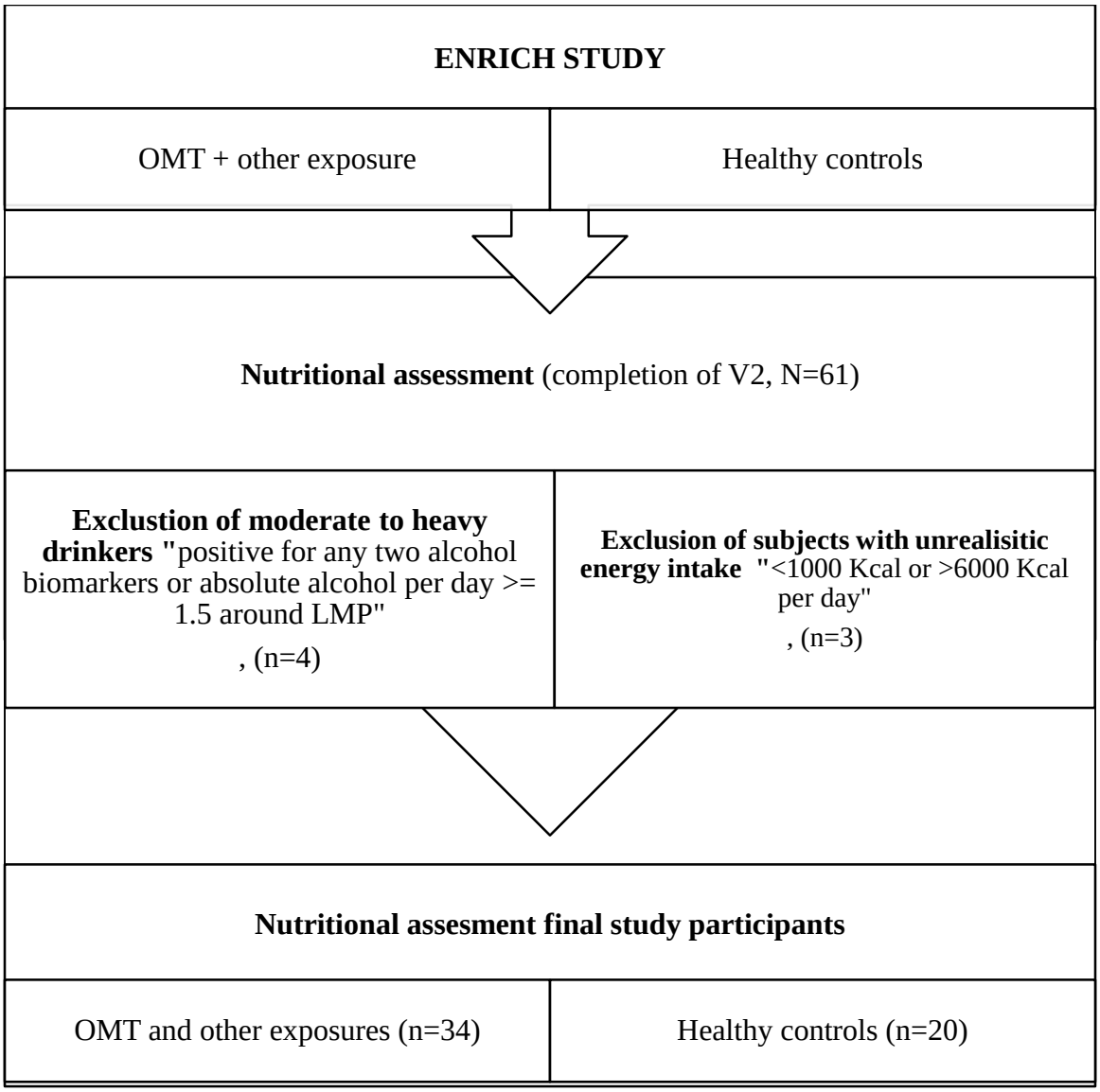

Figure 4: Study participant selection for nutritional analysis

Block Brief Food Frequency Questionnaire

The Block Brief Food Frequency Questionnaire was used to evaluate the nutritional status in pregnant women during their hospital stay after their delivery. The FFQ was administered by the researchers interviewing the patients for V2. As mentioned previously in the literature review section, the FFQ measures usual and customary intake in the study participants. Information on different types of food groups, their frequency of consumption and the portion size are all collected during the interview using the FFQ instrument. The information thus collected goes through analysis at NutritionQuest© and results which are in the terms of energy, micro and macronutrients consumed per day are generated. This information was collected as a part of our study and was used in the nutritional analysis.

Apart from the measurement of food, the FFQ also collects information on intake of alcohol and vitamins. The consumption of alcohol related beverage plays a significant role in the nutritional analysis. A review by Yeomans et al, 2010 effectively summarizes the effect of alcohol consumption on its effect on appetite and the balance in energy intake[114]. The study summarizes that short term alcohol intake could possibly promote food intake and that alcohol consumption is additive to energy obtained from other sources. Although the study suggest that mild to moderate alcohol intake might not be associated with weight gain, nonetheless there are effects on appetite with the consumption of alcohol. The food frequency questionnaire lists 3 items in the beverage section: 1) beer, 2) wine or wine coolers 3) liquor or mixed drinks along with the frequency of intake in the past year and portion sizes to measure alcohol intake.

Description of covariates

Inclusion of the covariates was based on the ecological model on nutrition. As described earlier, individual preferences, economic status, societal and cultural norms, attitudes and beliefs on diet along with governmental policies could have effect on dietary habits. Under the restraints of the study design, the following covariates will be used to adjust for the differences in nutritional intake in our study.

Age: Age was measured at the first interview and is expressed as years.

Marital Status: Marital status was ascertained in the first interview. Marital status was categorized as 1) single, never married; 2) married, living with spouse; 3) not married, living with partner; 4) separated from spouse; 5) divorced; 6) widowed. In our analysis used a binary variable for marital status 1=married or not married, living with partner and 0=single, not married or separated or divorced.

Ethnicity: The question regarding ethnicity which recorded if the subject was Hispanic, Latino or of Spanish descent was asked in first interview. A binary variably (1=yes, 0= no) was used to record the answer.

Race: The information on race was recorded during the first interview. Race was categorized as: 1) White; 2) Black or African-American; 3) American Indian or Alaskan Native; 4) Asian or Asian American or Pacific Islander; 5) Other group; 6) prefer not to answer. For our calculation a binary variable for race was used (1= White. 0=others).

Education: Education level was recorded during the first interview. It was categorized as: 1) less than high school graduate; 2) high school graduate or GED; 3) some college or vocational school; 4) college degree; 5) Masters, Doctorate or professional degree. A

binary variable for education for the calculations was used. Education level was dichotomized as high school education or lower and some college education or higher.

Employment status: Employment status was recorded as a binary variable at the first interview.

Insurance status: Insurance status was used as a binary variable in our analysis. First group was subjects who had Medicare as their insurance and the other group had any other insurance except Medicare.

Presence of any medical condition: During the first interview the subjects were asked if they had any medical condition that required treatment. Information on any medical conditions described was recorded. This information was first self-reported and then confirmed using electronic medical records.

Parity: Parity is the number of pregnancies carried to viable gestational age and delivery. It was measured during the first interview.

Gravidity: Gravidity refers to the number of times the women has been pregnant. It was also recorded at the first interview.

Tobacco use: Tobacco use was recorded during the first interview. The information recorded was current smoking, if yes then the amount of cigarette smoked daily. If the subject was not a current smoker and if the subject was a user previously, then the amount that the subject used to smoke prior to quitting was acquired. For our analysis, the smoking status of the subject at visit 1 and overall smoking status (i.e. did the patient ever smoke more than 100 cigarettes in lifetime) was used.

Substance use: Other illicit substance and their frequency of use during pregnancy was recorded during the first interview and at the second interview. The study collected the information on substances such as marijuana, cocaine, methamphetamine, benzodiazepines, barbiturates, opioid analgesics, methadone and buprenorphine. Self-reported frequency of use was collected from the subjects. Toxicology screens for substances were conducted to confirm the self-report.

Data analysis plan

The description of the baseline demographics of the study population (measure at first interview) was described using means (for continuous variables) and percentages (categorical variables). The difference in the general characteristics of the groups was evaluated using students t-test for the continuous variables and the fisher exact tests for categorical variables.

For specific aim I, to determine the difference in energy intake in pregnant women on OMT compared to non-opioid using pregnant women, we performed a student's t-test. The dependent variable was total energy intake measured in kilo-calories per day. The independent variable was OMT status. We also performed the same test on log transformed energy intake. This method was used to account for the non-normal distribution of energy intake. We also examined the effect of other demographics factors such as race, ethnicity, marital status, employment, education level and health insurance type on energy-adjusted macronutrient intake[115]. However, to minimize the effect of multiple testing we used multivariate analysis of variance (MANOVA) instead of individual tests on the dependent variables. MANOVA allows us to perform a joint test for any significant effect among a set of variables at a fixed alpha level. The dependent

variables were energy-adjusted carbohydrate, protein and total fat intake. Another analysis was a multivariate analysis of covariance (MANCOVA) model that took into account the age, pre-pregnancy BMI, ethnicity, education level, marital status, employment status of subjects along with the variable OMT status. Inclusion of variables in the MANCOVA model was ascertained by the MANOVA analysis. Independent variables with a significance level of $p<0.2$ were selected into the MANCOVA analysis. The rationale for this analysis was to control for the confounders that could have affected energy-adjusted macronutrient intake. This was followed up by a multivariate regression for each individual energy-adjusted macronutrient. We also calculated the percentage of energy coming from different sources such as carbohydrates, fats and proteins. This analysis evaluated the primary macronutrient groups that formed the sources of energy in OMT and non-opioid using pregnant women.

For specific aim II, to determine the difference in intake of vitamins A, B1, B2, B6, B12, C, D, E, and beta-carotenes in pregnant women on OMT compared to controls, we performed a student's t-test on unadjusted micronutrient intake. In the next step, the dependent variables (vitamins and carotenoids) were adjusted on the basis of total caloric intake. The initial adjustment by total calorie intake takes into account the ratio of micronutrients to energy [115]. To account for multiple testing, multiple analysis of variance on the micronutrients as a group was performed. The dependent variables were energy-adjusted vitamin A, thiamin, riboflavin, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, folate, iron and choline. To adjust for other confounders MANOVA test was performed by individual dependent variables such as race, ethnicity, education level, marital status, employment status and type of health insurance. Finally a multiple analysis

of covariance was performed to control for age, pre-pregnancy weight, ethnicity, race, education level, marital status and employment status. Inclusion of a covariate in the MANCOVA was dependent upon the significance of the MANOVA test. Independent variables with $p<0.02$ were included in the final model. It was followed by a multivariate regression for those micronutrients that were observed to have significant difference in the energy-adjusted analysis.

For specific aim III, to determine the difference in intake of folates and iron, we performed student's t test. Then, the dependent variables were adjusted on the basis of total caloric intake. We performed the t-test to calculate the difference in intake after adjustments. The two variables (folate and iron) were included in the MANOVA model along with all other micronutrients to evaluate the composite effect of OMT exposure and other covariates. A multiple analysis of covariance was performed to control for age, pre-pregnancy weight, ethnicity, race, education level, marital status and employment status based. Inclusion in the MANCOVA was dependent upon the significance of the MANOVA test. Independent variables with $p<0.02$ were included in the final model. Finally, a multivariate regression was performed to control for age, pre-pregnancy weight, ethnicity, race, education level, marital status and employment status.

For specific aim IV, to assess if the study participants meet the EAR based on their exposure, we calculated the proportion of subjects who met the EAR for the macro and micronutrients through their diet alone. Secondly, we calculated the proportion of study subjects in each group who met the EAR after addition of micronutrient supplements.

For data analysis, we modified the covariates into simpler groups to account for the small sample size in our study. Marital status was converted to a binary variable married/not married but living with spouse and single/separated/divorced/widowed. Race was converted into a binary variable White and others. Education was converted into a binary variable high-school or lower and some college or higher. All analyses were conducted using Stata (version 13, Stata Corp, College Station, Texas, USA).

Multivariate analysis of variance (MANOVA)

MANOVA is generalized analysis of variance (ANOVA) when there are two or more dependent variables. It is a statistical test that compares the multivariate means of several groups[116]. When there are several dependent variables and we try to ascertain the effect of an independent variable, multiple testing might lead to a type I error due to inflation of α. To control for the type I error, MANOVA allows us to perform a joint test for any significant effect among a set of variables at a fixed alpha level. After a significant effect of an independent variable is found among a set of variables, ANOVA or regression analysis can be performed to ascertain the effect on individual variables. Thus, we can state that MANOVA generally describes a pattern of difference in means in a group of dependent variable which is due to an independent variable. A MANOVA test generally is dependent upon a set of conditions that determine if the distribution of data with respect to each other are normal and correlated. We analyzed the correlation between the dependent variables to assess the effectiveness of MANOVA for our analysis. The inclusion of multiple variables included in our study would make it difficult to prove multivariate normality, hence multivariate normality of the dependent variables was not be tested. Similarly, multivariate analysis of covariance (MANCOVA) is an extension of

analysis of covariance (ANCOVA) where there are multiple dependent variables and continuous independent variables need to be controlled.

Power calculations

We based the sample size calculation on specific aim I, to determine the differences in energy intake in pregnant women on OMT and non-opioid using pregnant women. In that analysis the dependent variable was total energy intake and independent variable was OMT status. We used sample size calculating software (PASS 13, Kaysville, Utah) for the power analysis.

The alpha value which is the probability of rejecting the null hypothesis when the null is true was set at 0.05. We performed the power analysis to calculate the sample size required in OMT exposed and the control group to achieve power in the range of 0.8 to 0.9. The sample size for group 1 (OMT group) was varied from 10 to 50 at increments of 10 and power for each combinations were calculated.

We used the estimates for difference in energy intake from a previous study [59].The mean energy intake in each group along with standard deviations was extracted for the calculations. We used these estimates to calculate the sample size in each group. We also performed power calculations based on the estimates of Vitamin A and Iron intake from the same study. The power calculation was performed to account for the different analysis we were performing on the data and the difference in energy intake versus intake of micronutrients in a person's diet. The basis of multiple power analysis was based on the hypothesis that caloric intake would not directly correlate with micronutrient intake in our study population. Another driving factor was the difference in the levels of micronutrient intake in the previous study [59]. They had observed that the difference in

average energy intake was significantly greater but the difference in micronutrient although present was non-significant. Hence, use of the same power analysis and sample size calculation could possibly lead to our study to have less power in detecting a difference in micronutrient intake. The result from the preliminary power analysis is shown in table 3, 4 and 5.

We used the estimates of variance in energy and micronutrient intake from a previous study and used projected sample size for our study to calculate minimum detectable difference for our study. The results are shown in table 6.

Table 3: Power calculation based on the estimates of energy intake (specific aim I)

Target power	Actual power	N1	N2	N	μ1	μ2	μ1 - μ2	σ1	σ2	Alpha
0.8	1.0	10	2	12	3033.0	2172.0	861.0	223.8	66.8	0.05
0.9	1.0	10	2	12	3033.0	2172.0	861.0	223.8	66.8	0.05

Table 4: Result of power calculation based on the estimates of Vitamin A intake (specific aim II)

Target Power	Actual Power	N1	N2	N	μ1	μ2	μ1 - μ2	σ1	σ2	Alpha
0.80	0.88510	20	4	24	3326.0	3818.0	-492.0	498.0	183.7	0.050
0.90	0.92061	20	5	25	3326.0	3818.0	-492.0	498.0	183.7	0.050

Table 5: Power calculation based on the estimates of Iron intake (specific aim III)

Target Power	Actual Power	N1	N2	N	μ1	μ2	μ1 - μ2	σ1	σ2	Alpha
0.80	0.80259	50	94	144	6.8	7.3	-0.5	0.6	1.5	0.050
0.90	0.90072	50	136	186	6.8	7.3	-0.5	0.6	1.5	0.050

Target Power was varied between 0.8 and 0.9..

Actual power was the power obtained according to the estimated differences and sample size.

N1(exposed group) and N2 (controls) are the number of items sampled from each population.

N is the total sample size (N1 + N2)

μ1 and μ2 are the estimated mean (values from previous studies were used)

μ1 - μ2 is the difference between the estimates of the means

σ1 and σ2 are the estimated standard deviation for exposed and controls respectively.

Alpha, the probability of rejecting the null hypothesis when it is true was set at 0.05.

The means and standard deviations for the power calculation were derived a previous pilot study[59].

Table 6: Calculation of minimum detectable difference based on estimated of sample size

Actual power	N1	N2	N	μ1	μ2	μ1 - μ2	σ1	σ2	Alpha
Based on estimated of caloric intake (Specific Aim I)									
0.573	40	25	65	2200	2100	100	180	180	0.05
0.89	40	25	65	2250	2100	150	180	180	0.05
0.99	40	25	65	2350	2100	200	180	180	0.05
Based on estimates of Vitamin A intake (Specific Aim II)									
0.48	40	25	65	3600	3800	-200	400	400	0.05
0.82	40	25	65	3500	3800	-300	400	400	0.05
0.97	40	25	65	3400	3800	-400	400	400	0.05
Based on estimates of Iron intake (Specific Aim III)									
0.56	40	25	65	6.3	7.3	-0.6	1	1	0.05
0.87	40	25	65	6.5	7.3	-0.8	1	1	0.05
0.98	40	25	65	6.8	7.3	-1.1	1	1	0.05

Actual power was the power obtained according to the estimated differences and sample size.

N1(exposed group) and N2 (controls) are the number of items sampled from each population.

N is the total sample size (N1 + N2)

μ1 and μ2 are the estimated mean (values from previous studies were used)

μ1 - μ2 is the difference between the estimates of the means

σ1 and σ2 are the estimated standard deviation for exposed and controls respectively.

The means and standard deviations for the power calculation were derived a previous pilot study [59].

Summary of power calculations

The results from the power analysis showed that very small sample size in each group were enough to achieve a significant power (100%) to detect the estimated difference in energy intake between the groups. This was attributed to the significant difference in the energy intake in between OMT and the control group in the previous study. Similarly the power calculation based on the estimates of Vitamin A intake showed that 90% power could be achieved with a sample size of 24 (20 in OMT group and 4 in controls) to detect an estimated mean difference of 492 IU/day. The third power calculation showed that it would be difficult to detect a significant difference in daily intake of iron between the groups. To have a 70% power to detect an estimated difference of 0.5 g/day in iron intake, the sample size needed would be 121 (50 in the OMT group and 71 in the control group).

Based on the estimates of our target sample size (n=40 in the exposed group and n=25 in controls) we can observe a difference of 150 Kcal in energy intake with 90% power, a difference of 300 IU in vitamin A intake with 82% power, and a difference of 0.8 mg in iron intake between the groups with 82% power.

Human research review committee (HRRC) approval

The data has been abstracted from an ongoing NIH funded study which has HRRC approval. The Principal Investigator of the study has agreed to provide all the data required for the study. Available data is de-identified; hence this study did not require a further HRRC approval.

CHAPTER FOUR: RESULTS

This chapter presents the results of our analysis pertaining to the aims of the study. Description of the study population is presented first, which is followed up by the analysis of difference in energy and nutrient intake. The analysis is presented as unadjusted analysis based on exposure status followed up by unadjusted analysis based on covariates and finally adjusted analysis (multivariate linear regression).

Description of the study population

At the time of analysis the ENRICH study had 61 participants who had completed Visit 2 along with complete information on nutritional analysis. Fifty four subjects out of the 61 who had complete information qualified for our study. The demographic characteristics of the study population are presented in Table 7. The mean age of the study population was 27.0 ± 5.2 years. Majority of the study participants were under the age of 30. Of the total study population, 64.8% were Hispanics. Ninety percent of the population identified themselves racially as White. The marital status of the participants varied with 44.4% married or living with partner and 55.6% either single, separated or divorced. Approximately 61% of the study population had education level of high-school or lower, and 38.9% had some education after high-school. The employment rate of the study sample was low at approximately 31%. Approximately 79% of the study sample had Medicaid as their health insurance.

The physical health, reproductive characteristics, alcohol, and tobacco use the study sample has been described in Table 8. The average pre-pregnancy weight of the study participants was 148.5 ± 32.3 lbs. The BMI on average was 26.0 ± 5.9. At baseline 60% of the subjects had a BMI of 24.9 or lower. Approximately 20% of the subjects had a

BMI in between 25.0 and 25.9. About 19% of the subjects had a BMI of 30 or above. At the time of recruitment (V1) their mean gestational age was 23.2 weeks. On average the study participants had around 3 pregnancies. However, for 27% of the participants it was their first pregnancy. A significant proportion of the study subjects (63%) reported that the pregnancy was unplanned. Approximately 50% of the participants revealed that they had some kind of medical condition that required treatment. Most prevalent conditions were hepatitis (25.9%), anxiety (16.7%) and depression (16.7%).

Population characteristics based on exposure to Opioid Maintenance Therapy

Table 7 and 8 shows the population characteristics based on exposure to OMT. There was no significant difference in the average age in the two groups (p=0.16), however, the subjects in the control group were slightly younger (25.7 years in controls vs 27.8 years in OMT exposed). Seventy percent of the subjects in the OMT group identified themselves as Hispanics compared to 55% in the control group (p=0.37). There was a significant difference in the marital status in the subjects in OMT versus control group (p=0.02). Higher proportion of the subjects in the OMT exposed group were single/separated/divorced (67%) but a larger proportion of the subjects in the controls were married or cohabitating (65%). There was a significant difference in education level between OMT exposed and controls (p=0.02). In the OMT exposed group 73.5% of subjects had an education status of high-school or lower compared to 40% in the controls. Controls had a higher proportion of subjects who had some college degree or higher compared to the OMT exposed group (60% vs 26.5%). Employment rate was 45% in the control group but 23.5% in the OMT group. In the OMT exposed group 91.2% of the subjects had Medicaid compared to 60.0% in the control group (p=0.01).

There was no significant difference in pre-pregnancy weight (p=0.95), BMI (p=0.85) or gestational age at first interview (p=0.10) in between the two study groups. However, 45% of the subjects in control group reported that they did not plan to get pregnant with the baby compared to 73.5% in the OMT group (p=0.04). On average subjects in the OMT group had been pregnant 3.4 times compared to 1.9 times in the control group (p<0.01). We observed a significant difference in presence of a medical condition in subjects between the two groups (p=0.01). Sixty four percent of the subjects in the OMT exposed group reported that they had a medical condition compared to 25% in the control group. The most commonly prevalent medical condition in the OMT group was hepatitis (41%), anxiety (23.5%), depression (20.6%), and asthma or allergies (11.8 %) whereas the most common conditions in the control group were asthma or allergies (15 %) and depression (10%).

We did not observe specific differences in self-reported multivitamin or iron use during the period surrounding pregnancy based on the OMT exposure. Twenty two percent of the subjects reported using multivitamin more than 4 times a week in the month before their last menstrual period. Although a higher proportion of the controls (35% vs 14.7%) were using multivitamins during the month before their last menstrual period compared to OMT group, the difference was not statistically significant (p=0.10). At the time surrounding the first interview i.e. after the subjects had discovered that they were pregnant, the prevalence of regular multivitamin users (more than 4 times a week) had increased to 90% in both the study groups. This value increased to 93% in the OMT group and 100% in the controls by the time of the second interview. A relatively lower proportion of the study subjects reported using multivitamin containing iron compared to

regular multivitamins. At visit 1, 25% of the healthy controls and 18% of OMT users reported that they used iron supplements.

Table 7: Demographics characterisitcs of the study sample

	Total sample (N=54)	**OMT Exposed (n=34)**	**Controls (n=20)**	**p-value**
Age (years)	27.0 ± 5.2	27.8 ± 5.2	25.7 ± 5.1	0.16*
	N (%)	n (%)	n (%)	
Hispanic	35 (64.8)	24 (70.6)	11 (55.0)	0.37ǂ
Marital Status				0.02ǂ
Single/ Separated/ Divorced	30 (55.6)	23 (67.6)	7 (35.0)	
Married/ Unmarried (cohabitating)	24 (44.4)	11 (32.3)	13 (65.0)	
Race				0.14ǂ
White	49 (90.7)	29 (85.3)	20 (100.0)	
Others	5 (9.3)	5 (14.7)	0 (0.0)	
Education level				0.02ǂ
High-school or less	33 (61.1)	25 (73.5)	8 (40.0)	
Some college or higher	21 (38.9)	9 (26.5)	12 (60.0)	
Employed	17 (31.5)	8 (23.5)	9 (45.0)	0.13ǂ
Health insurance				0.01ǂ
Medicaid	43 (79.6)	31 (91.2)	12 (60.0)	
Other	11 (20.4)	3 (8.8)	8 (40.0)	

*student's t-test, unequal variances

ǂfisher exact test, r x c contingency table

Table 8: Physical charactersitics, reproductive characteristics, alcohol and tobacco use in the study population

	Total Sample (N=54)	OMT Exposed (n=34)	Controls (n=20)	p-value
Pre-pregnancy weight (lb.)	148.5 ± 32.3	148.2 ± 29.5	148.8 ± 37.4	0.95*
BMI (kg/m^2)	26.0 ± 5.9	25.9 ± 5.6	26.2 ± 6.6	0.85*
Total number of pregnancies	2.8 ± 1.8	3.4 ± 2.0	1.9 ± 0.9	<0.01*
Gestational age at first interview (weeks)	23.2 ± 7.1	21.9 ± 6.7	25.3 ± 7.5	0.10*
	N (%)	n (%)	n (%)	
Weight status (BMI)				1.00ǂ
<=24.9	32 (60.4)	20 (60.6)	12 (60.0)	
>=25.0 to <=29.9	11 (20.7)	7 (21.2)	4 (20.0)	
>=30.0	10 (18.9)	6 (18.8)	4 (20.0)	
Presence of any medical condition	27 (50.0)	22 (64.7)	5 (25.0)	0.01ǂ
Hypertension	1 (1.8)	1 (2.9)	0 (0.0)	
Seizure disorder	2 (3.7)	2 (5.8)	0 (0.0)	
Thyroid disorder	3 (5.5)	2 (5.8)		
Asthma or allergies	7 (12.9)	4 (11.8)	3 (15.0)	
Hepatitis	14 (25.9)	14 (41.2)	0 (0.0)	
Depression	9 (16.7)	7 (20.6)	2 (10.0)	
Anxiety	9 (16.7)	8 (23.5)	1 (5.0)	
Migraine headaches	4 (7.4)	4 (11.8)	0 (0.0)	
Others	5 (9.3)	4 (11.8)	1 (5.0)	
Unplanned pregnancy	34 (62.9)	25 (73.5)	9 (45.0)	0.04ǂ
Primigravida	15 (27.8)	7 (20.6)	8 (40.0)	0.06ǂ
Multivitamin Use				
During LMP(>4 times a week)	12 (22.2)	5 (14.7)	7 (35.0)	0.10ǂ
At V1	49 (90.7)	29 (85.3)	20 (100.0)	0.14ǂ
Frequency (n=49)				1.00ǂ
At least 4 times a week	44 (89.8)	26 (89.7)	18 (90.0)	
At V2	51 (94.4)	32 (94.1)	19 (95.0)	1.00ǂ
Frequency (n=51)				1.00ǂ
At least 4 times a week	48 (96.0)	29 (93.5)	20 (100.0)	
Multivitamin (Iron) use	11 (20.7)	6 (18.2)	5 (25.0)	0.72ǂ

*student's t-test, unequal variances

ǂfisher exact test, r x c contingency tables

Substance and alcohol use in the OMT group

Table 9 shows the prevalence of substance use in the subjects in the OMT group at first interview (V1) and at the follow up interview (V2). The information on prevalence of substance and alcohol use for the healthy control is not presented because the subjects in healthy control group were designed to have no exposure to substances such as opioids and tobacco. Another characteristic of the healthy control group was absence or minimal alcohol exposure during the period surrounding the last menstrual period and overall pregnancy. The obvious lack of such exposures in the healthy control group gave us sufficient leverage to exclude them from the substance, alcohol and tobacco exposure table.

Overall there was a decreasing trend of substance abuse from V1 to V2. The most commonly used substance was marijuana (after excluding methadone and buprenorphine). At V1 29% of the subjects had reported some use of marijuana but at V2 it had decreased to 14.7%. Other commonly used substances were pain relievers, heroin, benzodiazepine, methamphetamine and barbiturates. All of these substances also showed a decreased trend in use from V1 to V2. There were 15 (44%) methadone users at V1 who used it daily. There were 19 (56%) buprenorphine users at V1. One of the subjects had shifted from using methadone to using buprenorphine at V2.

Table 10 shows the frequency of alcohol use and tobacco users in the OMT group. The results in this table have been calculated after the exclusion of subjects based on the alcohol intake criteria. Alcohol use in the OMT group reveals a decreasing trend from calendar 1 to calendar 2. Calendar 1 is the 29 day period surrounding the last menstrual period and calendar 2 is the 29 day period surrounding the first interview. The total

drinking day on average during the calendar 1 was 2.8 days with an average amount of alcohol consumed per day amounting to 1.0 units of absolute alcohol per drinking day. These values decreased to an average 0.2 drinking days in calendar 2 with average amount of alcohol consumed in that period totaling 0.04 units of absolute alcohol per drinking day. Table 10 also shows the prevalence of tobacco use in the pregnant women on OMT. Ninety one percent of the subjects reported that they had smoked but only 50% were regularly smoking at visit1. At visit 1 the proportion of current smokers had decreased to approximately 41%.

Table 9: Frequency of self reported substance use in the OMT group at enrollement and at visit 2 (n=34)

Substance abuse in OMT group	Almost everyday	Once a week	Once every 2-3 weeks	Once a month	Occasionally (less than monthly)	Any use
Marijuana						
Visit 1	3 (8.8)	2 (5.9)	0 (0.0)	1 (2.9)	4 (11.8)	10 (29.4)
Visit 2	1 (2.9)	2 (5.9)	0 (0.0)	1 (2.9)	1 (2.9)	5 (14.7)
Heroin						
Visit 1	4 (11.8)	1 (2.9)	0 (0.0)	1 (2.9)	1 (2.9)	7 (20.6)
Visit 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Methamphetamine						
Visit 1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (8.8)	3 (8.8)
Visit 2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Methadone						
Visit 1	15 (44.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (44.1)
Visit 2	14 (41.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (41.2)
Buprenorphine						
Visit 1	17 (50.0)	1 (2.9)	0 (0.0)	1 (2.9)	0 (0.0)	19 (55.9)
Visit 2	20 (58.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	20 (58.8)
Opioid analgesics						
Visit 1	7 (20.6)	1 (2.9)	0 (0.0)	1 (2.9)	1 (2.9)	10 (29.4)
Visit 2	1 (2.9)	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.9)	3 (8.8)
Benzodiazepines						
Visit 1	3 (8.8)	1 (2.9)	0 (0.0)	0 (0.0)	2 (5.9)	6 (17.6)
Visit 2	0 (0.0)	1 (2.9)	0 (0.0)	0 (0.0)	1 (2.9)	2 (5.9)
Barbiturates						
Visit 1	0 (0.0)	1 (2.9)	0 (0.0)	0 (0.0)	1 (2.9)	2 (5.8)
Visit 2	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)

Table 10: Alcohol and tobacco use in the OMT group

	TLFB Calendar1*		**TLFB Calendar2***	
	Mean	SD	Mean	SD
Alcohol use				
Total drinking days	2.8	4.3	0.2	0.8
Total drinks (Absolute alcohol)	13.5	22.6	0.3	1.3
Average amount of alcohol per drinking day	1.0	1.6	0.04	0.2
Tobacco use	**Visit 1**		**Visit 2**	
	n (%)		**n (%)**	
Current smoker	17 (50.0)		14 (41.2)	
Ever smoker	31 (91.2)		-	

*TLFB Calendar 1: 29 day period surrounding last menstrual period, TLFB calendar 2: 29 day period surrounding visit 1 interview.

Analysis of energy intake and sources of energy

Descriptive statistics and the results of a t-test to assess the difference in energy intake per day and sources of energy in OMT subjects and controls is presented in Table 11. The average total energy intake in the study sample was 2234.8 ± 992.2 Kcal/day. Average carbohydrate intake per day was 284.8 ± 123.7g, average protein intake was 84.9 ± 38.4g and average fat intake was 100.2 ± 47.2g in the study population. The primary source of energy in the study population was carbohydrate 48.7%. The crude analysis of difference in energy intake between the groups showed that subjects in OMT group had higher intake in calories compared to controls (2469.6 ± 1091.7g vs 2105.6 ± 766.9, p=0.15), but the difference was not statistically significant. There was no significant difference in consumption of carbohydrates (304.9 ± 130.0g vs 250.5 ± 106.6g, p=0.1), protein (86.2 ± 43.4g vs 82.5 ± 29.1g, p=0.71) and total fat (105.3 ± 52.9g vs 91.6 ± 35.2g, p=0.25) in OMT group compared to subjects in controls. We observed that significantly higher percentage of energy was derived from protein in controls compared to the OMT subjects (16 ± 3.6% in controls vs 14 ± 2.5% in OMT, p=0.03)

After log transformation of energy intake, no significant difference in energy intake was observed (p=0.24). The difference in energy-adjusted macronutrient (protein, fat and carbohydrates) intake in OMT group vs control group was analyzed and it was found that the adjusted total protein intake was significantly higher in the control group (35.0±1.1 vs 40.1±9.1 gm per 1000 Kcal per day, p=0.03). We did not observe any difference in energy-adjusted total carbohydrate intake (p=0.45) and energy-adjusted total fat intake (p=0.14).The results are shown in Table 12.

The results of the MANOVA which tested the effect of OMT exposure on composite energy-adjusted carbohydrate, protein and total fat are shown in Table 13. The null hypothesis that the vectors of means are equal for the two groups was rejected ($p=0.02$) which showed that there is an effect of OMT status on energy-adjusted macronutrient intake.

Table 11: Description of energy intake and sources of energy by study group

	Total study sample (N=54)		**OMT Exposed (n=34)**		**Controls (n=20)**		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	p-value*
Total Energy intake (Kcal/day)	2334.8	992.2	2469.6	1091.7	2105.6	766.9	0.15
Total Protein intake (g/day)	84.9	38.4	86.2	43.4	82.5	29.1	0.71
Total Fat intake (g/day)	100.2	47.2	105.3	52.9	91.6	35.2	0.25
Total Carbohydrate intake (g/day)	284.8	123.7	304.9	130.0	250.5	106.6	0.10
Percentage of energy from:							
Fat	38.5	5.31	38.1	5.2	39.2	5.6	0.45
Protein	14.8	3.1	14.0	2.5	16.0	3.6	0.03
Carbohydrate	48.7	6.9	49.6	7.0	47.1	6.7	0.19
Sweets	13.1	10.8	13.8	9.9	11.9	12.5	0.55
Alcohol	0.7	2.3	1.0	2.8	0.05	0.1	0.05

*students t-test, unequal variance

Table 12: Analysis of log-transformed energy intake and energy-adjusted micronutrient intake by study group

	Total study sample (N=54)		**OMT Exposed (n=34)**		**Controls (n=20)**		
	Mean	SD	Mean	SD	Mean	SD	p-value**
Log-transformed Energy Intake	7.7	0.4	7.7	0.4	7.6	0.3	0.24
Adjusted total protein intake*	36.9	7.7	35.0	1.1	40.1	9.1	0.03
Adjusted total fat intake *	42.8	5.9	42.3	5.8	43.6	6.2	0.45
Adjusted total carbohydrate* intake	121.7	17.4	124.0	17.5	117.7	16.7	0.14

*Adjustment based on total energy intake, adjusted macronutrient = (macronutrient intake/ total energy intake) * 1000, units: g per 1000 Kcal.

**students t-test, unequal variance

Analysis of the effect of socio-demographic characteristics on energy and macronutrient intake

The result of the Hotelling's test (equivalent of MANOVA when the independent variables is binary) examining the effect of OMT exposure, ethnicity (Hispanic or not), race (White or others), employment, marital status (married/cohabitating vs single, divorced, separated), education level (high-school or less vs some college or more) and insurance status (Medicare or others) are shown in Table 13. We observed that ethnicity (p=0.03), race (0.04) and marital status (0.05) had a significant effect on the composite energy-adjusted macronutrient intake.

Table 13: Hotelling's T^2 (Test of effect of OMT exposure and other covariates on macronutrient intake)

Predictors	**p-value**
OMT exposure	0.02
Ethnicity	0.03
Race	0.04
Employment	0.07
Marital status	0.05
Education level	0.06
Insurance	0.44

The dependent variables in the MANCOVA were energy-adjusted carbohydrate, fat and protein intake.

Multivariate analysis for energy and macronutrient intake

The results of the MANVCOVA to examine the effect of OMT on macronutrient intake (energy-adjusted carbohydrate, fat and protein intake) after controlling for ethnicity, employment status, marital status, education level, age and BMI (categorical) are shown in Table 14. Race was not used in the analysis because none of the subjects in control had identified themselves as others. The effects of OMT exposure on the composite macronutrient intake was not observed when controlled for other covariates ($p=0.27$). However, we observed a significant effect of ethnicity on composite energy-adjusted macronutrient intake ($p=0.02$). We also observed a borderline significant effect of employment status on composite energy-adjusted macronutrient intake ($p=0.07$). This could possibly mean that being Hispanic would be attributable to having a different composite mean on the dependent variables compared to being non-Hispanic.

The results for the multivariate regression analysis based on the MANCOVA model are shown in Table 15. No significant effect of OMT exposure on any of the three outcomes of interest (energy-adjusted carbohydrate, proteins or fats) was observed. However, ethnicity had a significant impact on energy-adjusted carbohydrate intake; Hispanic subjects on average consumed 11.9g/1000 Kcal more than non-Hispanics after controlling for other factors ($p=0.02$). Similarly, employment was found to have significant impact on energy-adjusted protein intake. After controlling for all the other variables, subjects who were employed consumed 5.8g/1000 Kcal more proteins than their unemployed counterparts ($p<0.01$).

Table 14: MANCOVA (Test of effect of OMT exposure after adjustment for ethnicity employment, marital status, education level age at V1 and BMI on macronutrient intake)

Predictors	**p-value**
OMT exposure	0.27
Ethnicity	0.02
Employment	0.07
Marital status	0.33
Education level	0.24
Age at V1	0.62
BMI (categorical)	0.90

The dependent variables in the MANCOVA were energy-adjusted carbohydrate, fat and protein intake.

Table 15: Multivariate regression: Analysis of the effect of OMT exposure on energy-adjusted macronutrient intake after controlling for covariates

	Energy-adjusted carbohydrate intake (g/1000Kcal/day)		**Energy-adjusted protein intake (g/1000Kcal/day)**		**Energy-adjusted total fat intake (g/1000Kcal/day)**	
	β coefficient (95% CI)	p-value	β coefficient (95% CI)	p-value	β coefficient (95% CI)	p-value
OMT exposure	4.4 (-6.5, 15.3)	0.42	-2.6 (-7.1, 1.9)	0.26	-1.5 (-5.6, 2.5)	0.45
Ethnicity	11.9 (1.5, 22.4)	0.02	-2.1 (-6.4, 2.3)	0.34	-2.6 (-6.5, 1.3)	0.19
Employment	-6.1 (-16.5, 4.2)	0.24	5.8 (1.5, 10.2)	<0.01	0.4 (-4.2, 3.5)	0.84
Marital status	9.0 (-1.4, 19.5)	0.08	-1.1 (-5.5, 3.3)	0.61	-2.7 (-6.6, 1.2)	0.16
Education level	-2.5 (-14.4, 9.1)	0.67	3.5 (-1.3, 8.3)	0.15	0.3 (-4.0, 4.6)	0.88
BMI category						
25 - 29.9	-0.3 (-12.2, 11.6)	0.95	1.2 (-3.8, 6.2)	0.62	-0.7 (-5.1, 3.7)	0.75
30 >=	-3.5 (-15.7, 8.6)	0.56	1.85 (-3.2, 6.9)	0.73	-1.2 (-3.4, 5.7)	0.61
Age at V1	-0.1 (-1.0, 0.8)	0.79	0.2 (-0.2, 0.6)	0.24	-0.02 (-0.4, 0.3)	0.90

Analysis of micronutrient intake from diet in pregnant women by study group

Unadjusted analysis of the micronutrient intake from diet in pregnant women in our study is shown in Table 16. We observed that the intake of beta-carotene was significantly lower in OMT group compared to controls exposed group (2936.3 ± 2549.4 mcg vs 4898 ± 3825.7 mcg, p=0.05). We did not observe any significant difference in micronutrient in the OMT group versus the controls. However, we observed that there was slightly higher but statistically insignificant intake of several of several micronutrients such as thiamin (p=0.45), riboflavin (p=0.27), vitamin B6 (p=0.36), vitamin B12 (p=0.33), vitamin C (p=0.59), vitamin D (p=0.59), vitamin E (p=0.71), iron (p=0.54), folate (p=0.80), and choline (p=0.42) in the OMT group.

Analysis of energy-adjusted micronutrient intake showed opposite trends in the results compared to the unadjusted analysis (Table 17). OMT group had lower intake of Vitamin A, thiamin, riboflavin, vitamin B6, vitamin B12, beta-carotene, vitamin C, vitamin D, vitamin E, iron, folate and choline per 1000 Kcal energy compared to controls. However, these differences were not significant except for vitamin A (388.4 ± 142.5 vs 485.7 ± 188.4 RAE/1000 Kcal, p=0.05), beta-carotene (1271.3 ± 1153.9 vs 2386.3 ± 1750.8 mcg/1000 Kcal, p=0.01), vitamin E (4.5 ± 0.8 vs 5.5 ± 1.9 a-TE/1000 Kcal, p=0.03) and folate (245.5 ± 62.3 vs 290.5 ± 66.9 DFE/1000 Kcal, p=0.01). Despite these individual differences for specific micronutrients, the MANOVA model showed that OMT exposure did not have a statistically significant effect on the difference in means of composite energy-adjusted micronutrients (p=0.10)

Table 16: Micronutrient intake by study groups

Micronutrient intake from diet	Total Study sample (N=54)		OMT Exposed group (n=34)		Controls (n=20)		
	Mean	SD	Mean	SD	Mean	SD	p-value*
Vitamin A (mcg-RAE)	939.23	421.9	907.7	409.9	992.9	447.2	0.48
Thiamin (mg)	1.8	0.8	1.9	0.8	1.7	0.7	0.45
Riboflavin (mg)	2.3	0.9	2.4	1.0	2.1	0.7	0.27
Vitamin B6 (mg)	2.2	0.8	2.3	0.8	2.1	0.7	0.36
Vitamin B12 (µg)	5.4	2.7	5.7	2.8	5.0	2.5	0.33
Beta-carotene (µg)	3662.9	3195.0	2936.3	2549.4	4898.0	3825.7	0.05
Vitamin C (mg)	183.0	132.3	190.3	134.4	170.6	131.1	0.59
Vitamin D (mcg)	5.8	3.9	6.1	4.0	5.5	3.8	0.59
Vitamin E (a-TE)	11.1	4.7	10.9	4.8	11.4	4.6	0.71
Iron (mg)	16.6	7.1	17.0	7.6	15.9	6.2	0.54
Folate (DFE)	591.6	226.9	585.8	232.0	601.6	223.4	0.80
Choline (mg)	353.5	158.4	365.4	181.3	333.3	110.4	0.42

* Student's t-test

Table 17: Micronutrient in study group after adjustment for total energy intake

Energy-adjusted micronutrient* intake from diet	Total Study sample (N=54)		OMT Exposed group (n=34)		Controls (n=20)		
	Mean	SD	Mean	SD	Mean	SD	p-value ǂ
Vitamin A (mcg-RAE)	424.4	166.2	388.4	142.5	485.7	188.4	0.05
Thiamin (mg)	0.8	0.1	0.7	0.1	0.8	0.1	0.10
Riboflavin (mg)	1.0	0.3	1.0	0.2	1.1	0.3	0.66
Vitamin B6 (mg)	1.0	0.2	0.9	0.2	1.0	0.2	0.62
Vitamin B12 (µg)	2.4	1.1	2.4	0.9	2.5	1.4	0.75
Beta-carotene (µg)	1684.3	1491.1	1271.3	1153.9	2386.3	1750.8	0.01
Vitamin C (mg)	76.8	44.2	76.3	48.4	77.6	37.3	0.91
Vitamin D (mcg)	2.6	1.6	2.5	1.2	2.8	2.1	0.62
Vitamin E (a-TE)	4.9	1.4	4.5	0.8	5.5	1.9	0.03
Iron (mg)	7.3	1.9	7.1	2.0	7.7	1.6	0.25
Folate (DFE)	262.1	67.1	245.5	62.3	290.5	66.9	0.01
Choline (mg)	155.1	37.9	150.8	34.7	162.5	42.8	0.3

*Adjustment based on total energy intake, adjusted micronutrient = (micronutrient intake/ total energy intake) * 1000, units per 1000 Kcal.

ǂ Student's t-test

Analysis of the effect of socio-demographic factors on dietary micronutrient intake

A MANOVA that included all of the micronutrient variables being tested except beta-carotene (beta-carotene is used in calculation of total vitamin A intake) as the dependent variable was used to analyze the effect of ethnicity, race, marital status, education level, employment status and insurance type. We observed that insurance type ($p=0.02$) and marital status ($p=0.01$) had significant effect on the difference of means in the micronutrients being tested. Our analysis showed that ethnicity ($p=0.63$), race ($p=0.40$), employment status ($p=0.64$), and education level ($p=0.44$) did not have an effect on the composite energy-adjusted micronutrient intake. The results of the individual tests are shown in Table 18.

Table 18: Hotelling's T^2 (Test of effect of OMT exposure, ethnicity, race, employment, marital status, education level and insurance on energy-adjusted dietary micronutrient intake)

Predictors	**p-value**
OMT exposure	0.10
Ethnicity	0.63
Race	0.40
Employment	0.64
Marital status	0.01
Education level	0.44
Insurance	0.02

The dependent variables are energy-adjusted Vitamin A, B1, B2, B6, B12, C, D, E, beta-carotenes, iron, folate and choline.

Multivariate analysis for energy-adjusted dietary micronutrient intake

The results of the MANCOVA test which examined the effect of OMT on energy-adjusted composite measure of micronutrient intake after adjustment for marital status, insurance, age at V1 and BMI (categorical) is shown in Table 19. After adjustment for the covariates, OMT status was not observed to have an effect on the composite micronutrient intake (p=0.19).

The results of the multivariate regression on energy-adjusted vitamin A, vitamin E, folate and choline based on the MANCOVA model are shown in Table 20. These micronutrients were selected because significant difference was observed between the OMT and the control group in the energy-adjusted analysis. Although statistically significant difference in between the groups was not observed for choline, it was included in the multivariate regression because of its clinical importance. No significant effect of OMT exposure was seen on vitamin A intake (p=0.12). OMT subjects had a lower intake of vitamin E on average by -0.9 a-TE/1000 Kcal per day (95% CI:-1.8, -0.1, p=0.03) compared to controls after controlling for other factors. In addition, folate intake in OMT group was 45.9 DFE/1000 Kcal (95% CI: 4.6, 87.1, p=0.03) lower on average compared to healthy controls after controlling for other factors. No significant effect of OMT exposure on choline intake was observed (p=0.38).

Table 19: MANCOVA (Test of effect of OMT exposure after adjustment for marital status, insurance, age and BMI on adjusted micronutrient intake)

Predictors	**p-value**
OMT exposure	0.19
Marital status	0.07
Insurance	0.42
Age at V1	0.01
BMI (categorical)	0.38

The dependent variables are energy-adjusted Vit A, B1, B2, B6, B12, C, D, E, iron, folate and choline

Table 20: Multivariate regression: Analysis of the effect of OMT exposure on energy-adjusted micronutrient intake after controlling for covariates

	Energy-adjusted Vitamin A intake (mcg-RAE/1000Kcal/day)		**Energy-adjusted Vitamin E intake (a-TE/1000Kcal/day)**		**Energy-adjusted folate intake (DFE/1000Kcal/day)**		**Energy-adjusted total choline intake (mg/1000Kcal/day)**	
	B-coefficient (95% CI)	p-value	B-coefficient (95% CI)	p-value	B-coefficient (95% CI)	p-value	B-coefficient (95% CI)	p-value
OMT exposure	-82.0 (-189.4, 24.9)	0.12	-0.9 (-1.8, -0.1)	0.03	-45.9 (-87.1, -4.6)	0.03	-10.9 (-36.1, 14.2)	0.38
Marital status	-20.4 (-121.5, 80.6)	0.68	0.2 (-0.6, 1.0)	0.59	-8.9 (-47.8, 29.9)	0.694	-6.7 (-30.5, 17.0)	0.57
Insurance type	-79.2 (-214.3, 55.8)	0.24	-0.3 (-1.3, 0.8)	0.60	-15.5 (-67.4, 36.4)	0.42	-12.6 (-44.4, 19.2)	0.42
BMI category								
25 – 29.9	37.7 (-80.6, 156.0)	0.64	1.2 (0.2, 2.1)	0.01	43.1 (-2.4, 88.6)	0.06	-0.1 (-27.9, 27.6)	0.99
30>=	-54.2 (-174.1, 65.8)	0.36	0.3 (-0.6, 1.2)	0.49	-22.5 (-68.6, 23.6)	0.33	-15.9 (-44.1, 12.3)	0.26
Age at V1	2.33 (-6.8, 1.5)	0.6	0.04 (-0.03, 0.1)	0.26	1.4 (-2.1, 4.9)	0.42	0.9 (-1.2, 3.1)	0.37

Difference in intake of supplementary micronutrients among OMT exposed and controls

The results of the analysis comparing intake of dietary supplements (vitamin A, thiamin, riboflavin, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, iron and folate) are shown in Table 21. No significant difference in micronutrient intake was observed among OMT user and control group (all $p>0.05$). We observed borderline statistical difference in iron intake (23.3 ± 21.3 vs 36.4 ± 30.4 mcg, $p=0.09$). A MANOVA conducted to evaluate the effect on composite multivitamin supplement did not generate results because of multicollinearity between the micronutrients in the supplements which signals that these micronutrients were highly likely to have come from a single supplement.

Table 21: Differences in intake of micronutrient supplementation in pregnant women in OMT group compared to controls

Micronutrient intake from supplements	**OMT Exposed**		**Controls**		**p-value***
	Mean	SD	Mean	SD	
Vitamin A (mcg-RAE)	1304.6	484.5	1478.6	848.3	0.40
Thiamin (mg)	1.9	2.5	1.9	2.4	0.90
Riboflavin (mg)	2.1	2.5	2.1	2.4	0.90
Vitamin B6 (mg)	2.0	1.4	2.1	1.4	0.88
Vitamin B12 (µg)	5.9	3.6	6.1	3.5	0.87
Vitamin C (mg)	88.9	128.9	97.8	128.1	0.80
Vitamin D (mcg)	353.8	134.2	384.3	153.6	0.46
Vitamin E (a-TE)	22.6	24.7	25.8	31.2	0.69
Iron (mg)	23.3	21.3	36.4	30.1	0.09
Folate (DFE)	631.9	352.6	704.0	358.5	0.47

*student's t-test

Comparison of dietary intake in study subjects to recommended dietary allowances.

The results of this analysis comparing dietary intake among study participants to EAR is shown in Table 22. Carbohydrate EAR intake levels were met by 95% of the controls and 91% of the OMT exposed pregnant women. Protein intake levels were met by 75% of the healthy controls and 73% of the OMT exposed pregnant women. Analyzing the micronutrient intake levels excluding the dietary supplements we found that a significant proportion of our study subjects were deficient in vitamin E, iron, folate and choline. Vitamin E EAR levels were only met by 45% of the controls and 35% of the OMT exposed pregnant women. Similarly, iron EAR levels were only met by 15% of the controls and 23.5% of the OMT exposed group, folate intake levels were only met by 55% of the healthy controls while 56% of the OMT exposed group met the requirements and choline intake levels was met by 15% of the controls compared to 23.5% in the OMT exposed group. It should also be noted that mean values of dietary iron and choline intake were also lower than the specified EAR. After accounting for intake of multivitamins and supplements, over 90% of the pregnant women in both group met the requirements for almost all of the micronutrients that were being analyzed. There were a few subjects in the OMT groups that did not meet the EAR requirements, but this could be due to inadequate or no micronutrient supplementation in those individuals.

Table 22: Proportion of study subjects meeting the EAR with and without micronutrient supplementation

	Met EAR based on diet alone		**p-value (exact-test)**	**Met EAR based on diet and supplementations**		**p-value (exact-test)**
	OMT exposed n (%)	Controls n (%)		OMT exposed n (%)	Controls n (%)	
Macronutrients						
Carbohydrates	31 (91.2)	19 (95.0)	0.52	-	-	-
Proteins	25 (73.5)	15 (75.0)	0.58	-	-	-
Micronutrients						
Vitamin A (mcg-RAE)	28 (82.3)	16 (80.0)	0.55	34 (100)	19 (95.0)	0.37
Thiamin (mg)	28 (82.3)	19 (95.0)	0.23	33 (97.0)	20 (100.0)	1.00
Riboflavin (mg)	32 (94.1)	19 (95.0)	1.00	34 (100.0)	20 (100.0)	-
Vitamin B6 (mg)	28 (82.3)	16 (80.0)	1.00	33 (97.1)	20 (100.0)	1.00
Vitamin B12 (mcg)	33 (97.1)	19 (95.0)	1.00	34 (100.0)	20 (100.0)	-
Vitamin C (mg)	27 (79.4)	18 (90.0)	0.45	33 (97.1)	20 (100.0)	1.00
Vitamin D (mcg)	7 (20.6)	1 (5.0)	0.23	30 (88.2)	18 (90.0)	1.00
Vitamin E (a-TE)	12 (35.3)	9 (45.0)	0.56	30 (88.2)	19 (95.0)	0.64
Iron (mg)	8 (23.5)	3 (15.0)	0.51	30 (88.2)	19 (95.0)	0.64
Folate (DFE)	19 (55.8)	11 (55.0)	1.00	32 (94.1)	19 (95.0)	1.00
Choline (mg)*	8 (23.5)	3 (15.0)	0.51	-	-	-

CHAPTER 5: DISCUSSION

This section includes the discussion of the results followed up by recommendations for future research. The section begins with the discussion on the effect of OMT exposure on energy and micronutrient intake. Then, results of dietary intake based on socio-demographic factors are discussed. Finally, strengths, limitations, recommendations for future research and conclusions are presented.

Discussion of the demographic characteristics of the study participants

There were some typical characteristics of the subjects in the OMT exposed group. A significant proportion were Hispanics, unmarried, had lower education level, unemployed, had Medicare for their health insurance and significant proportion (64.7%) of the subjects had reported medical conditions such as hepatitis, anxiety and depression. The demographic characteristics of the OMT exposed study group mirrors several other studies which included patients with substance abuse [59,84]. This observation shows that the patients on OMT are significantly different than those who were included as healthy controls. It is important to consider these differences in the light of the analysis. The impact of ethnicity, education level, employment and medical condition could all possibly have a strong effect on dietary intake. The impact of these factors might contribute to poor nutritional status in people undergoing OMT along with the effect of OMT itself. We have controlled for some of these factors in our multivariate analysis, but the cumulative impact of these socio-demographic characters on dietary intake cannot be undermined.

Effect of OMT exposure on energy intake

There was no significant difference in energy intake between the OMT group and controls (p=0.15) although the average intake in the OMT group was higher by 250 Kcal per day compared to the controls. The difference in energy intake in our study subjects was lower than that reported by the previous study[59]. The unadjusted analysis showed that the bulk of the energy was derived from carbohydrates. The energy-adjusted analysis also showed that total protein intake was lower in OMT group as compared to controls (p=0.03). This means that if an average subject consumed 2000 Kcal per day, the controls intake would be 80g per day while for OMT exposed it would be 70 g per day. This difference of an average of 10g per day per 2000 Kcal diet could have a significant implications during pregnancy. This difference that we observed in energy-adjusted protein intake was observed only in the crude analysis. Although the MANOVA analysis on energy adjusted composite macronutrients showed that there was some effect of OMT, We did not observe any effects after controlling for age, BMI, ethnicity, employment, marital status and education level. We did not observe significant effect of OMT exposure on energy-adjusted protein intake after we adjusted for the same covariates even though an association was observed in the energy adjusted analysis.

Although significant difference in energy intake in pregnant women on OMT was not observed, the result that the average energy intake OMT group was slightly higher than that in the controls is in agreement with previous studies [58,59,84]. However, the magnitude of difference that we observed was lower (difference of approximately 250 Kcal between groups) than that reported by Tomedi et al (2012) (difference of approximately 800Kcal between groups). This could have been possible due to a variety of reasons. Firstly, the

characteristics of the study population could have played a significant role in energy intake. The participants in the Tomedi et al (2012) study were recruited from Philadelphia which has a significantly different social and cultural aspect compared to subjects in New Mexico. Also, majority of the study participants in that study were non-Hispanic whites compared to a majority of Hispanic subjects in this study. Second, since our study consists of pregnant women who were recruited from clinics associated with UNM hospital, it is highly likely that there is some surrounding social structure around the participants that lead to overall better health and diet compared to the subjects in the previous studies. It is to be noted that the average energy intake in the controls this study and the study by Tomedi et al are almost similar at approximately 2100 Kcal per day. Statistical significance in that study could have been primarily driven by the high energy intake level of approximately 3000 Kcal per day observed in the subjects in the OMT group. Similarly, Tomedi et al (2012) also observed significant difference in energy percentage of energy derived from proteins. It is also important to note that that difference was found to be not significant after controlling for other covariates. These similarities in our results support the finding that OMT exposure is associated with difference in energy and macronutrient intake and the possible reason that statistical significance was not observed could be small sample size.

Effect of OMT exposure on micronutrient intake

The analysis of difference in mean intake of micronutrient in our study groups showed that there was lower intake of beta-carotenes in the OMT group. Although not statistically significant, it was observed that for most micronutrients the intake level was higher in the OMT group in the crude analysis. However, the difference in energy-

adjusted micronutrient intake was opposite of the difference we observed in crude micronutrient intake. This study found significant deficiencies in several important micronutrients among OMT patients. Specifically, lower intake of beta-carotenes, vitamin E, and folate was observed in OMT patients. In addition, there was a trend towards lower intake of vitamin A in OMT patients of borderline statistical significance. The difference in intake of folates in between OMT patients and controls was particularly significant. A 45 DFE/1000 Kcal difference in between OMT and controls is a difference of approximately 90 DFE per day if we assume a subject consumes 2000 Kcal a day on average. The estimated average requirement of folate during pregnancy is 520 DFE per day, hence a difference of 90 DFE is significant considering the recommendations. This difference is particularly important because studies have consistently shown that deficiency of folate is associated with neutral tube disorders [33,36,43]. The differences in vitamin A and vitamin E intake was relatively minor compared to the difference that was observed in the intake of folate. However, these micronutrients play an important role in the growth and development of fetus. Significant deficiencies in vitamin A could lead to pre-eclampsia [45]and there are reports of miscarriage due to deficiencies in vitamin E [45].

These results shows that the nutrient density in pregnant women on OMT is lower than those in controls. The implication of these findings could be significant in promoting healthier diet in pregnant women on OMT. Promoting a healthier diet in these individuals would not only involve them to cut down on their energy intake but also to improve their micronutrient intake from other sources. The findings of our study are reflected in the previous study by Tomedi et al (2012), where it was found that after adjustment for total energy intake per day, pregnant women on OMT had significantly less intake of folate,

vitamin C and vitamin E after controlling for energy intake[59]. Similar observation in this study certainly reinforces our hypothesis that subjects in the OMT group have lower intake of micronutrients in their diet.

Socio-demographic characteristics and their association with energy and micronutrient intake

The results of the multivariate regression showed that there was some effect of demographic factors on the intake of energy-adjusted carbohydrate and proteins. We observed that Hispanics were likely to have more carbohydrate intake compared to non-Hispanics by an average of 11.9 g/1000 Kcal/day. This would round up to an average of 24 g of carbohydrate more per day if we consider that the average subject consumed 2000 Kcal per day. Similarly, it was observed that the subjects who were employed had a significantly higher intake of proteins compared to the unemployed subjects by an average of 5.8 g/1000 Kcal/day. This would be an average of 10 g a day if we consider the average subject consumed 2000 Kcal per day.

There are several factors that might have caused these differences. Consider the higher carbohydrate intake in Hispanics. It could be driven by their dietary patterns and food groups that might be rich in carbohydrates [117]. The observation of the difference in carbohydrate intake could be possibly related other sociological factors associated with the ethnicity such as employment rates, education level, marital status all of which could have a possible effect on dietary patterns. Similarly, the observation that higher protein intake in the employed subjects after controlling for other covariates demonstrates that there is a strong tendency in subjects with a job to have a diet rich in protein. Usually higher income is associated with employment and studies have reported higher adherence

to dietary guidelines in subjects with higher income [118]. This could be a possible reason that we observed higher protein intake in subjects who were employed. Although there are several other factors that might result the opposite such as lesser time for food preparation and consumption of fast food that could lead to poor diet qualities. This study, however, did not observe such effect. We might also consider the fact that employment rates are associated with higher education level. The multivariate analysis did not produce a significant effect of education level on adjusted protein intake, but a trend was observed that employment and higher education level were associated with higher protein intake and lower carbohydrate intake.

In the intake of micronutrients, specific trends based on socio-demographic characteristics was not observed. The Hotelling's test showed that insurance type and marital status showed some effect on composite intake of energy-adjusted micronutrients. However, these effect were not observed in the multivariate analysis, There could be various possible explanation for the lack of difference in our observations. Firstly the FFQ ensures that ethnicity specific food groups are not missed which eliminates the risk of ethnicity being a factor in determining differences in dietary intake. Also, the population is derived from a specific study area whereby the effect of cultural variety on dietary patterns is reduced. Small sample size and a large variance in the intake of micronutrients could have been another reason that significant differences due to the socio-demographic characteristics were not observed.

Effect of alcohol consumption on energy intake

According the design of the parent study, healthy controls had to be very light alcohol users before pregnancy and abstain from alcohol use after the last menstrual period.

However, in the OMT group, subjects with exposure to alcohol were not excluded. Thus, the effect of alcohol use on nutritional status could not be controlled for in multivariate analysis given that healthy controls had zero prevalence. The study minimized this limitation by excluding subjects from the OMT groups who were heavy drinkers. Among OMT users, 35% had reported some drinking around their LMP, but only 6% of the OMT subjects reported some alcohol exposure during the 1 month period before their first interview. The amount of alcohol consumption in those subjects who reported alcohol was also significantly lower because we excluded subjects who had reported consuming more than 1.5 units of absolute alcohol per day around their LMP. As seen in Table 10 the average amount of drinking during the 30 days period before enrollment was 0.08 oz. per drinking day (equivalent to 0.04 units of absolute alcohol/drinking day). Thus, it can be argued that there would be minimal effect of alcohol on dietary patterns in our study population.

Biological mechanisms affecting dietary pattern in pregnant women on OMT

There are several speculations about the biological mechanisms that affect dietary patterns in opioid using pregnant women. American Dietetic Association states that substance abusers could have an accelerated nutritional requirement to higher levels than required on a regular basis [119]. This could suggest that the body would develop a compensatory mechanism to increase energy and nutrient intake to balance the energy deficit. This could be a possible explanation of higher energy intake in subjects on OMT. A systematic review published in 1994 associated substance use to bulimia [120], that subjects who use substance are more likely to binge eat. Studies also point towards reward mechanism, impulsivity and vulnerability to impulse related disorders in

substance using subjects [121]. Although this study is not examining eating disorders in the subjects, the modulation of the reward pathway by opioids and a compensatory response by the body to account for energy deficit in substance abusing patients could be possible explanations in the increased dietary intake in subjects on OMT.

Dietary intake of study participants in relation to estimated average requirements

Comparison of dietary intake of the pregnant women in our study to the EAR showed that the pregnant women in both groups were not meeting the requirements of some micronutrients through their diet alone. The observation that the study participants' diet was especially deficient in vitamin E, folate and iron demonstrates the need for dietary supplementation. After supplementation, pregnant women in both group had adequate intake of iron and folate. Also concerning is the significantly large proportion of the study subjects in both group not meeting the adequate intake requirements for choline. The adequate intake suggested by IOM is 450 mg/day [42], but the mean intake in both the study groups is approximately 350 mg/day with only 23% of the subjects in OMT group and 15% in the controls consuming the adequate amount. Many studies have shown the importance of choline in human diet, especially during pregnancy [50,81,82]. This deficiency of choline in such significant proportion of the population may contribute to developmental disorders in the growing fetus. Since choline is not primarily supplemented in multivitamins, other sources of choline should be sought after. The observed results calls for more attention to supplementation with choline by diet modification and intake of multivitamins which contain choline. The dietary intake of Vitamin D was remarkably low in both groups without supplementation. This deficiency in their diet is less concerning because most of the vitamin D requirements for the body

comes from exposure to sun rays [122]. Dietary supplementation of vitamin D was able to control for this deficiency in diet. It is important to acknowledge that supplementation with vitamin D could be essential because studies show that significant proportion of the US population is deficient in vitamin D[123]. Based on the results of comparing dietary intake of pregnant women in our study and the EAR, it can be stated that a significant proportion of the subjects were not taking satisfactory levels of micronutrients in their diet. This highlights the importance of micronutrient supplementation in pregnancy. The communication of this finding can enable health care providers to promote healthy diet in pregnant women.

Limitations

The results of this study should be interpreted in the light of some limitations. The study is limited by sample size. The limited sample size makes it difficult to observe smaller differences that could have been possibly present. We had calculated that a sample size of 65 with 40 subjects on the OMT group and 25 on the HC group had a 90% power to detect a difference in energy intake of 150 Kcal per day. However, our sample size was slightly smaller with only 34 subjects in the OMT group and 20 subjects in the controls. Contributing to this problem was a greater than expected variability in energy and some other nutrients within each study group.

Another limitation is the potential for confounding effect of alcohol, tobacco, other substances and maternal medical conditions. These risk factors could not be controlled for in the multivariate analyses given that the controls were selected to be life-long abstainers from drugs and, non-smokers. A sub-group analysis on these factors within the OMT group is statistically possible, but it would still be limited by the sample size.

With respect to chronic medical conditions, the study groups were very unbalanced for viral hepatitis (64% in OMT vs. 0% in health controls), as expected in opioid-dependent population. Other medical conditions were also either incomparable or had low prevalence thus restricting our analysis. The variation in the type of medical condition also was a limiting factor in determining if medical condition played any role in the subjects' nutrition.

The generalizability of the study might be limited since the cultural and ethnic heritage of the state of New Mexico is significantly different from the general US population. The interpretation of the results of this study in the context of other geographical and ethnic region could be inaccurate. Recall bias could affect the results of the dietary analysis. The FFQ questionnaires are usually designed to control for these factors but recall data than spans a year could be imprecise. FFQ presents an average value of dietary intake over the course of the year. Pregnancy is a time which is characterized by several physical, reproductive and dietary changes over a span of 9 months. This could have a significant impact on the reliability of the FFQ. Although FFQ is considered better than the 24 hour recall method, several recall interviews during the period surrounding pregnancy could have produced better precision in dietary intake of our subjects rather than a single assessment after delivery.

There are several other factors that were not addressed in our study. Biomarkers could be an important marker of absorption of nutrients. The food frequency questionnaire only provides us information on what the pregnant women were consuming, it does not provide any information on absorption and metabolism of food. If there are any metabolic

or mal-absorptive symptoms in study subjects, the biomarker analysis would play a vital role in identifying if there are any deficiencies in nutrient intake.

Another limitation of this study is that it focuses on quantitative data. The absence of data that specifically pertains to the individuals' knowledge on food groups, nutrition requirements, attitudes and behaviors regarding diet, family support and help during pregnancy could be important driving factors affecting dietary intake. Since it is an individual and his/her conceptions and cravings that drive dietary habits, information on such factors could be crucial in interpreting the results of effect of OMT exposure in pregnant women.

Strengths

The strengths of our study is the initial design. A cohort study is a powerful tool to measure multiple effects based on an initial exposure. Although the primary purpose of the study delves in early indices of prenatal alcohol exposure, the robust study design and wealth of information gives us high quality data on the subjects who are enrolled in the study. The recruitment of the study subjects from specific clinic (Milagro) that provides for pregnant women who are undergoing opioid maintenance therapy ensured that there is no misclassification of patients. The patients recruited in OMT group specifically received OMT through the clinic. This enabled precise classification of subjects into OMT exposed or controls. Further, screening using urine biomarkers of opioid or substance abuse, alcohol biomarkers in serum and blood, along with biomarkers of tobacco consumption confirmed if a patient is abusing other drugs. This ensured that the researchers are not relying on self-report alone to recruit the subjects and it is a significant strength of this study. The initial interview process during prenatal visits and

the subsequent follow up around the time of delivery provided adequate time to collect all relevant information regarding the study. This system of collection of information on different time points allowed the study to track changes of multivitamins, alcohol, tobacco and substance use over the period of pregnancy. The study also boasts of a double entry system to maximize quality assurance and minimize missing data.

Future directions and policy implications

Future studies should focus on evaluating patients' knowledge regarding diet, food habits, choices, behaviors, cultural attributes and attitudes towards eating healthy food. Our study added to the increasing evidence that there are significant and important deficiencies in dietary intake among opioid dependent pregnant women. The effect of OMT might be difficult to disentangle from the effect of socio-demographic and other environmental characteristics (unstable housing, financial insecurities, involvement of the legal system, lack of social support and peer pressure etc.) on dietary habits. These direction could be multi-directional and quite complex. Additional work is needed to further examine the effect and interplay of these factors on dietary intake.

The results that were observed gives significant information that can be applied towards improving the diet of pregnant women in general. It was observed that there was significant deficiencies regarding iron, folate and choline which need supplementation. Specific supplementations regarding choline are available which could be recommended to pregnant women. It is imperative that prenatal care providers emphasize the importance of micronutrient supplementation as the results of this study explicitly states that micronutrient supplementation are required to meet the dietary recommendation of several micronutrients.

Future direction in regards to research and analysis could also include stratification of OMT group by methadone or buprenorphine use. Also, the effect of other substances such as marijuana, heroin, benzodiazepines that were concomitantly used by the study subjects can be controlled for in the analysis of dietary patterns. Also other analysis could be performed that accounted for alcohol and tobacco use. It would also be interesting to see if any medical condition played a role in affecting dietary patterns in pregnant women. Future research could be the examination of pregnancy outcomes based on the dietary patterns in OMT group versus the controls. When pregnancy outcomes are associated with dietary patterns, we could possibly have results that further promote the fact that diet plays a significant role in healthy pregnancy, all the more in pregnancy involving use of opioid maintenance therapies. Such research would provide an immense strength to the argument that healthy diet is necessary for healthy nutrition as dietary patterns could be associated with health outcomes.

The communication of this finding can inform health care practitioners and pregnant women to choose healthier nutrient rich diet which could be beneficial during their pregnancy. Intervention that focus on dietary change should focus on several domains of behavior change. A review by Malek et al (2015) summarizes 34 studies that evaluate the factors which influence women's dietary choices during their pregnancy[124]. The review suggested that perceptions regarding benefit and risk of healthy nutrition, individual beliefs, self-efficacy knowledge, financial standing and environment of food all played a vital role in determining the food choices during pregnancy. The review also started that key factors that influenced change in diet were received from their care providers. The review also highlighted the need of other methods such as persuasive communication,

automated feedback and modification of food culture and environment that enabled healthier food preferences in pregnant women. This review highlights the fact that promotion of healthy nutrition does not fit a single avenue. There are several programs such as the Special Supplemental Nutrition Program for Woman, Infants and Children (WIC), Supplemental Nutrition Assistance Program (SNAP) which can help provide assistance to low income pregnant women to get access to healthy foods. Along with such programs addition of a dietician in substance use treatment program, social support and awareness about the importance of healthy nutrition could be paramount in promoting healthy diet in pregnant women. Through an effective collaboration between health care workers, family, supplemental government program and educational agencies, healthy diet and pregnancy can be ensured.

Conclusion

This study demonstrates that there are significant deficiencies in macro and micronutrient intake in opioid dependent pregnant women compared to controls. It was observed that pregnant women on OMT consumed lower proteins on average compared to controls. Also their caloric intake was higher than controls but the results was not statistically significant. In addition to OMT, socio demographic characteristics such as ethnicity and employment were significant predictors of energy-adjusted carbohydrate and protein intake respectively. Further, the diet in the OMT groups had lower nutrient density than controls. Multivariate analysis demonstrated that pregnant women on OMT had significantly lower intake of vitamin E and folate after controlling for marital status, insurance type, BMI and maternal age. Our results were in agreement with previous

research on several avenues such as higher energy intake and low nutrient density in the OMT group.

Comparison of dietary intake in the study subjects with estimated average requirements demonstrated that diet alone was insufficient to meet the requirements of several micronutrients. The results showed that supplementation with multivitamins was necessary to meet the requirements, especially for iron and folate. Choline, which is increasingly being studied as an essential micronutrient, was observed to be deficient in a significant proportion of the study population. Thus supplementation of choline through diet modification or dietary supplements should be recommended by healthcare workers to pregnant women to ensure that they receive adequate amounts during pregnancy.

Additional research with a larger sample size along with qualitative information regarding health behaviors surrounding diet and nutrition should be another step in future research. These analyses when coupled with pregnancy outcomes data can provide further support towards healthy nutrition during pregnancy. Promoting healthy nutrition is important because of its role in preventing premature births, low birth weights and birth defects. Diet of a pregnant mother is certain to affect the fate of the growing fetus. The effects on the child could possibly be chronic and might affect the future of the baby. Hence, it is paramount that the results of this research in nutrition in pregnant women is communicated well to pregnant mothers, their families and health care workers such that effective measures can be taken to promote a healthy diet and a healthy pregnancy.

REFERENCES

1. National Survey on Drug Use and Health. Inter-university Consortium for Political and Social Research (ICPSR) 2013.

2. Veilleux JC, Colvin PJ, Anderson J, York C, Heinz AJ. A review of opioid dependence treatment: pharmacological and psychosocial interventions to treat opioid addiction. Clin Psychol Rev 2010;30:155-66.

3. Dole VP, Nyswander M. A medical treatment for diacetylmorphine (heroin) addiction: a clinical trial with methadone hydrochloride. JAMA 1965;193:646-50.

4. Mattick RP, Breen C, Kimber J, Davoli M. Buprenorphine maintenance versus placebo or methadone maintenance for opioid dependence. The cochrane library 2014.

5. Ball JC, Ross A. The effectiveness of methadone maintenance treatment: Patients, programs, services, and outcome: Springer Science & Business Media; 2012.

6. Minozzi S, Amato L, Bellisario C, Ferri M, Davoli M. Maintenance agonist treatments for opiate-dependent pregnant women. The Cochrane Library 2013.

7. Ashley OS, Marsden ME, Brady TM. Effectiveness of substance abuse treatment programming for women: A review. Am J Drug Alcohol Abuse 2003;29:19-53.

8. Rayburn WF, Bogenschutz MP. Pharmacotherapy for pregnant women with addictions. Am J Obstet Gynecol 2004;191:1885-97.

9. Cleary BJ, Donnelly J, Strawbridge J, et al. Methadone dose and neonatal abstinence syndrome—systematic review and meta-analysis. Addiction 2010;105:2071-84.

10. Schindler SD, Eder H, Ortner R, Rohrmeister K, Langer M, Fischer G. Neonatal outcome following buprenorphine maintenance during conception and throughout pregnancy. Addiction 2003;98:103-10.

11. Hulse G, Milne E, English D, Holman C. The relationship between maternal use of heroin and methadone and infant birth weight. Addiction 1997;92:1571-9.

12. Kandall SR, Albin S, Lowinson J, Berle B, Eidelman AI, Gartner LM. Differential effects of maternal heroin and methadone use on birthweight. Pediatrics 1976;58:681-5.

13. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988;242:715-23.

14. Sternini C, Patierno S, Selmer IS, Kirchgessner A. The opioid system in the gastrointestinal tract. Neurogastroenterol Motil 2004;16:3-16.

15. Schempf AH, Strobino DM. Illicit drug use and adverse birth outcomes: is it drugs or context? J Urban Health 2008;85:858-73.

16. Horta BL, Victora CG, Menezes AM, Halpern R, Barros FC. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. Paediatr Perinat Epidemiol 1997;11:140-51.

17. Murphy CC, Schei B, Myhr TL, Du Mont J. Abuse: a risk factor for low birth weight? A systematic review and meta-analysis. Can Med Assoc J 2001;164:1567-72.

18. Clarren SK, Smith DW. The fetal alcohol syndrome. N Engl J Med 1978.

19. Godfrey K, Robinson S, Barker D, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. BMJ 1996;312:410.

20. Godfrey KM, Barker DJ. Maternal nutrition in relation to fetal and placental growth. Eur J Obstet Gynecol Reprod Biol 1995;61:15-22.

21. Ramakrishnan U. Nutrition and low birth weight: from research to practice. Am J Clin Nutr 2004;79:17-21.

22. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. JAMA 2008;300:2886-97.

23. White SL, Perkovic V, Cass A, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Am J Kidney Dis 2009;54:248-61.

24. Knudsen V, Orozova-Bekkevold I, Mikkelsen T, Wolff S, Olsen S. Major dietary patterns in pregnancy and fetal growth. Eur J Clin Nutr 2008;62:463-70.

25. Grieger JA, Grzeskowiak LE, Clifton VL. Preconception dietary patterns in human pregnancies are associated with preterm delivery. J Nutr 2014;144:1075-80.

26. Englund-Ögge L, Brantsæter AL, Haugen M, et al. Association between intake of artificially sweetened and sugar-sweetened beverages and preterm delivery: a large prospective cohort study. Am J Clin Nutr 2012;96:552-9.

27. Okubo H, Miyake Y, Sasaki S, Tanaka K, Murakami K, Hirota Y. Maternal dietary patterns in pregnancy and fetal growth in Japan: the Osaka Maternal and Child Health Study. Br J Nutr 2012;107:1526-33.

28. Ferland S, O'Brien HT. Maternal dietary intake and pregnancy outcome. J Reprod Med 2003;48:86-94.

29. Colón-Ramos U, Racette SB, Ganiban J, et al. Association between dietary patterns during pregnancy and birth size measures in a diverse population in Southern US. Nutrients 2015;7:1318-32.

30. Northstone K, Emmett P, Rogers I. Dietary patterns in pregnancy and associations with socio-demographic and lifestyle factors. Eur J Clin Nutr 2008;62:471-9.

31. Thompson J, Wall C, Becroft DM, Robinson E, Wild CJ, Mitchell EA. Maternal dietary patterns in pregnancy and the association with small-for-gestational-age infants. Br J Nutr 2010;103:1665-73.

32. Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000;71:1280s-4s.

33. Group MVSR. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. The lancet 1991;338:131-7.

34. Allen LH. Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 2005;81:1206S-12S.

35. Vollset SE, Refsum H, Irgens LM, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr 2000;71:962-8.

36. Bergen N, Jaddoe V, Timmermans S, et al. Homocysteine and folate concentrations in early pregnancy and the risk of adverse pregnancy outcomes: the Generation R Study. BJOG 2012;119:739-51.

37. Williamson C. Nutrition in pregnancy. Nutr Bull 2006;31:28-59.

38. 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;101:294-301.

39. Monsen ER. Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. J Am Diet Assoc 2000;100:637-40.

40. Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621-30.

41. Del Valle HB, Yaktine AL, Taylor CL, Ross AC. Dietary reference intakes for calcium and vitamin D: National Academies Press; 2011.

42. Finglas PM. Dietary Reference intakes for thiamin, riboflavin, niacin, vitamin B 6, folate, vitamin B 12, pantothenic acid, biotin and choline. Elsevier; 2000.

43. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832-5.

44. Ramakrishnan U, Grant F, Goldenberg T, Zongrone A, Martorell R. Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: a systematic review. Paediatr Perinat Epidemiol 2012;26:285-301.

45. Radhika M, Bhaskaram P, Balakrishna N, Ramalakshmi B, Devi S, Kumar BS. Effects of vitamin A deficiency during pregnancy on maternal and child health. BJOG 2002;109:689-93.

46. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. Am J Obstet Gynecol 2010;202:429. e1-. e9.

47. Javaid M, Crozier S, Harvey N, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. The Lancet 2006;367:36-43.

48. Shamim AA, Schulze K, Merrill RD, et al. First-trimester plasma tocopherols are associated with risk of miscarriage in rural Bangladesh. Am J Clin Nutr 2015;101:294-301.

49. Fischer LM, Ann daCosta K, Kwock L, et al. Sex and menopausal status influence human dietary requirements for the nutrient choline. Am J Clin Nutr 2007;85:1275-85.

50. Shaw GM, Carmichael SL, Yang W, Selvin S, Schaffer DM. Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. Am J Epidemiol 2004;160:102-9.

51. Fischer G. Treatment of opioid dependence in pregnant women. Addiction 2000;95:1141.

52. Mattick RP, Breen C, Kimber J, Davoli M. Methadone maintenance therapy versus no opioid replacement therapy for opioid dependence. Cochrane Database Syst Rev 2009;3.

53. Jones HE, Kaltenbach K, Heil SH, et al. Neonatal abstinence syndrome after methadone or buprenorphine exposure. N Engl J Med 2010;363:2320-31.

54. Butte NF, King JC. Energy requirements during pregnancy and lactation. Public Health Nutr 2005;8:1010-27.

55. Siega-Riz AM, Viswanathan M, Moos M-K, et al. A systematic review of outcomes of maternal weight gain according to the Institute of Medicine recommendations: birthweight, fetal growth, and postpartum weight retention. Am J Obstet Gynecol 2009;201:339. e1-. e14.

56. Ramakrishnan U, Grant FK, Goldenberg T, Bui V, Imdad A, Bhutta ZA. Effect of multiple micronutrient supplementation on pregnancy and infant outcomes: a systematic review. Paediatr Perinat Epidemiol 2012;26:153-67.

57. Zador D, Wall PL, Webster I. High sugar intake in a group of women on methadone maintenance in south western Sydney, Australia. Addiction 1996;91:1053-61.

58. Kolarzyk E, Chrostek MJ, Pach D, Janik A, Kwiatkowski J, Szurkowska M. Assessment of daily nutrition ratios of opiate-dependent persons before and after 4 years of methadone maintenance treatment. Przegl Lek 2004;62:368-72.

59. Tomedi LE, Bogen DL, Hanusa BH, Wisner KL, Bodnar LM. A pilot study of the nutritional status of opiate-using pregnant women on methadone maintenance therapy. Subst Use Misuse 2012;47:286-95.

60. Centers for Disease Control and Prevention. National Vital Statistics System mortality data (2015).

61. Birnbaum HG, White AG, Schiller M, Waldman T, Cleveland JM, Roland CL. Societal costs of prescription opioid abuse, dependence, and misuse in the United States. Pain Med 2011;12:657-67.

62. Fajemirokun-Odudeyi O, Sinha C, Tutty S, et al. Pregnancy outcome in women who use opiates. Eur J Obstet Gynecol Reprod Biol 2006;126:170-5.

63. Jarvis MA, Schnoll SH. Methadone treatment during pregnancy. J Psychoactive Drugs 1994;26:155-61.

64. Fischer G, Ortner R, Rohrmeister K, et al. Methadone versus buprenorphine in pregnant addicts: a double-blind, double-dummy comparison study. Addiction 2006;101:275-81.

65. Fischer G, Jagsch R, Eder H, et al. Comparison of methadone and slow-release morphine maintenance in pregnant addicts. Addiction 1999;94:231-9.

66. Jones HE, Johnson RE, Jasinski DR, et al. Buprenorphine versus methadone in the treatment of pregnant opioid-dependent patients: effects on the neonatal abstinence syndrome. Drug Alcohol Depend 2005;79:1-10.

67. Behnke M, Smith VC, Levy S, et al. Prenatal substance abuse: short-and long-term effects on the exposed fetus. Pediatrics 2013;131:e1009-e24.

68. Almario CV, Seligman NS, Dysart KC, Berghella V, Baxter JK. Risk factors for preterm birth among opiate-addicted gravid women in a methadone treatment program. Am J Obstet Gynecol 2009;201:326. e1-. e6.

69. Mactier H, Shipton D, Dryden C, Tappin DM. Reduced fetal growth in methadone-maintained pregnancies is not fully explained by smoking or socio-economic deprivation. Addiction 2014;109:482-8.

70. Dryden C, Young D, Hepburn M, Mactier H. Maternal methadone use in pregnancy: factors associated with the development of neonatal abstinence syndrome and implications for healthcare resources. BJOG 2009;116:665-71.

71. Butte NF, Wong WW, Treuth MS, Ellis KJ, Smith EOB. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr 2004;79:1078-87.

72. Hytten F, Chamberlain G. Clinical physiology in obstetrics: Blackwell Scientific Publications; 1980.

73. Goldberg G. Nutrition in pregnancy and lactation. Nutrition Through the Life Cycle 2002:63-90.

74. Joint F, Organization WH. Energy and protein requirements: report of a Joint FA. 1985.

75. Rothman KJ, Moore LL, Singer MR, Nguyen U-SD, Mannino S, Milunsky A. Teratogenicity of high vitamin A intake. N Engl J Med 1995;333:1369-73.

76. Hallberg L, Brune M, Rossander L. Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am J Clin Nutr 1989;49:140-4.

77. Akada S, Iioka H, Moriyama I, Hisanaga H, Morimoto K, Ichijo M. The role of vitamin E during pregnancy--anti-platelet aggregation activity of alpha-tocopherol. Nihon Sanka Fujinka Gakkai zasshi 1991;43:523-8.

78. Poston L, Briley A, Seed P, Kelly F, Shennan A, Consortium ViP-eT. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. The Lancet 2006;367:1145-54.

79. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000;72:257s-64s.

80. Thomas B. Manual of dietetic practice: Blackwell Science; 2001.

81. Glier MB, Green TJ, Devlin AM. Methyl nutrients, DNA methylation, and cardiovascular disease. Mol Nutr Food Res 2014;58:172-82.

82. Zeisel SH, Da Costa K-A. Choline: an essential nutrient for public health. Nutr Rev 2009;67:615-23.

83. Thomas JD, Abou EJ, Dominguez HD. Prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats. Neurotoxicol Teratol 2009;31:303-11.

84. Nolan LJ, Scagnelli LM. Preference for sweet foods and higher body mass index in patients being treated in long-term methadone maintenance. Subst Use Misuse 2007;42:1555-66.

85. Balogh M, Kahn HA, Medalie JH. Random repeat 24-hour dietary recalls. Am J Clin Nutr 1971;24:304-10.

86. Hankin JH, Reynolds WE, Margen S. A short dietary method for epidemiologic studies II. Variability of measured nutrient intakes. Am J Clin Nutr 1967;20:935-45.

87. Burke BS, Stuart HC. A method of diet analysis: Application in research and pediatric practice. J Pediatr 1938;12:493-503.

88. Subar AF, Thompson FE, Kipnis V, et al. Comparative Validation of the Block, Willett, and National Cancer Institute Food Frequency Questionnaires The Eating at America's Table Study. Am J Epidemiol 2001;154:1089-99.

89. Boucher B, Cotterchio M, Kreiger N, Nadalin V, Block T, Block G. Validity and reliability of the Block98 food-frequency questionnaire in a sample of Canadian women. Public Health Nutr 2006;9:84-93.

90. Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. Epidemiology 1990:58-64.

91. Brehm B, Gates D, Singler M, Poeppelman A, Succop P, D'Alessio D. Prevalence of obesity and cardiovascular risk factors among manufacturing company employees in Kentucky. AAOHN J 2007;55:397-406.

92. Chester D, Himburg S, Weatherspoon L. Spirituality of African-American women: correlations to health-promoting behaviors. Journal of National Black Nurses' Association: JNBNA 2006;17:1-8.

93. Harley K, Eskenazi B, Block G. The association of time in the US and diet during pregnancy in low-income women of Mexican descent. Paediatr Perinat Epidemiol 2005;19:125-34.

94. Drewnowski A. Taste preferences and food intake. Annu Rev Nutr 1997;17:237-53.

95. Freeland-Graves JH, Nitzke S. Position of the academy of nutrition and dietetics: total diet approach to healthy eating. J Acad Nutr Diet 2013;113:307-17.

96. Abumrad NA. CD36 may determine our desire for dietary fats. J Clin Invest 2005;115:2965.

97. Mennella JA, Pepino MY, Reed DR. Genetic and environmental determinants of bitter perception and sweet preferences. Pediatrics 2005;115:e216-e22.

98. Devine CM, Farrell TJ, Blake CE, Jastran M, Wethington E, Bisogni CA. Work conditions and the food choice coping strategies of employed parents. J Nutr Educ Behav 2009;41:365-70.

99. Wansink B. Environmental factors that increase the food intake and consumption volume of unknowing consumers*. Annu Rev Nutr 2004;24:455-79.

100. Hasler CM, Brown AC. Position of the American Dietetic Association: functional foods. J Am Diet Assoc 2009;109:735-46.

101. Carlson A, Frazão E. Are healthy foods really more expensive? It depends on how you measure the price. It Depends on How You Measure the Price (May 1, 2012) USDA-ERS Economic Information Bulletin 2012.

102. Lipsky LM, Iannotti RJ. Associations of television viewing with eating behaviors in the 2009 Health Behaviour in School-aged Children Study. Arch Pediatr Adolesc Med 2012;166:465-72.

103. Johnson-Askew WL, Gordon L, Sockalingam S. Practice paper of the American Dietetic Association: Addressing racial and ethnic health disparities. J Am Diet Assoc 2011;111:446-56.

104. Sobell LC, Sobell MB. Timeline follow-back. Measuring alcohol consumption: Springer; 1992:41-72.

105. Saunders JB, Aasland OG, Babor TF, De la Fuente JR, Grant M. Development of the alcohol use disorders identification test (AUDIT). WHO collaborative project on early detection of persons with harmful alcohol consumption-II. Addiction 1993;88:791-.

106. Russell M, Martier S, Sokol R, Mudar P, Bottoms S, Jacobson S. Screening for pregnancy risk-drinking: TWEAKing the tests. Alcohol Clin Exp Res 1991;15:368.

107. Hietala J, Puukka K, Koivisto H, Anttila P, NIEMELÄ O. Serum gamma-glutamyl transferase in alcoholics, moderate drinkers and abstainers: effect on gt reference intervals at population level. Alcohol Alcohol 2005;40:511-4.

108. Bakhireva LN, Cano S, Rayburn WF, et al. Advanced gestational age increases serum carbohydrate-deficient transferrin levels in abstinent pregnant women. Alcohol Alcohol 2012;47:683-7.

109. Bianchi V, Ivaldi A, Raspagni A, Arfini C, Vidali M. Pregnancy and variations of carbohydrate-deficient transferrin levels measured by the candidate reference HPLC method. Alcohol Alcohol 2011;46:123-7.

110. Kenan N, Larsson A, Axelsson O, Helander A. Changes in transferrin glycosylation during pregnancy may lead to false-positive carbohydrate-deficient transferrin (CDT) results in testing for riskful alcohol consumption. Clin Chim Acta 2011;412:129-33.

111. Bakhireva LN, Leeman L, Savich RD, et al. The validity of phosphatidylethanol in dried blood spots of newborns for the identification of prenatal alcohol exposure. Alcohol Clin Exp Res 2014;38:1078-85.

112. Viel G, Boscolo-Berto R, Cecchetto G, Fais P, Nalesso A, Ferrara SD. Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. Int J Mol Sci 2012;13:14788-812.

113. Bakhireva LN, Savich RD, Raisch DW, et al. The feasibility and cost of neonatal screening for prenatal alcohol exposure by measuring phosphatidylethanol in dried blood spots. Alcohol Clin Exp Res 2013;37:1008-15.

114. Yeomans MR. Alcohol, appetite and energy balance: is alcohol intake a risk factor for obesity? Physiol Behav 2010;100:82-9.

115. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S-8S.

116. Warne RT. A primer on multivariate analysis of variance (MANOVA) for behavioral scientists. Practical Assessment, Research & Evaluation 2014;19:1-10.

117. Aldrich L, Variyam JN. Acculturation erodes the diet quality of US Hispanics. FOOD REVIEW-WASHINGTON DC- 2000;23:51-5.

118. Kirkpatrick SI, Dodd KW, Reedy J, Krebs-Smith SM. Income and race/ethnicity are associated with adherence to food-based dietary guidance among US adults and children. J Acad Nutr Diet 2012;112:624-35. e6.

119. Ozier AD, Henry BW. Position of the American Dietetic Association: nutrition intervention in the treatment of eating disorders. J Am Diet Assoc 2011;111:1236-41.

120. Holderness CC, Brooks-Gunn J, Warren MP. Co-morbidity of eating disorders and substance abuse review of the literature. Int J Eat Disord 1994;16:1-34.

121. Dawe S, Loxton NJ. The role of impulsivity in the development of substance use and eating disorders. Neurosci Biobehav Rev 2004;28:343-51.

122. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 1995;61:638S-45S.

123. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.

124. Malek L, Umberger W, Zhou SJ, Makrides M. Understanding Drivers of Dietary Behavior before and during Pregnancy in Industrialized Countries. 2015.