DETERMINATION OF TOTAL OXIDANT STATUS BY DIETARY ASSESSMENT AND ASSOCIATION WITH BLOOD AND URINE BIOMARKERS

by

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STATEMENT OF THESIS APPROVAL

The following faculty members served as the supervisory committee chair and members for the thesis of Tamara Lee Artz.

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ABSTRACT

Oxidant status may influence conception after in vitro fertilization, maternal health during pregnancy, and fetal outcomes including birthweight. However, few reports exist of oxidant status in women of childbearing potential. Oxidant status may be influenced by the intake of antioxidant vitamins, minerals, and phytochemicals. The purpose of this study was to examine the intake of antioxidant vitamins and minerals, measure biomarkers of oxidative stress, and evaluate the association between them. We conducted a cross-sectional study of dietary and supplement intake and measurements for biomarkers of oxidative status (malondialdehyde (MDA), glutathione (GSH), and 8-isoprostane) in women of childbearing potential. Intake was measured using a food frequency questionnaire and an overall index of dietary quality was generated (the Healthy Eating Index-2005). Additionally, a new, integrated index of oxidant stress from dietary variables, the diet oxy-score, was calculated with intake for specific antioxidant vitamins and minerals. The total oxidant status from the biomarkers was created by integrating the measured values of MDA, GSH, GSH/GSSG ratio, and 8-isoprostane into one index. Oxidative status measured with biomarkers was correlated in a biologically plausible direction with the dietary index of oxidative status, the Healthy Eating Index-2005, zinc, manganese, vitamin E, β-carotene, iron, and selenium. The observed correlations suggest that appropriate diet and supplementary zinc, manganese, vitamin E,

β-carotene, iron, and selenium intake may be an effective strategy for augmenting oxidant status in women of childbearing potential.

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INTRODUCTION

Oxidative stress is associated with an increased production of oxidizing species or a significant decrease in the capability of antioxidant defenses. This occurs when there is an imbalance between the body's antioxidant defenses and the production of reactive oxidative species (ROS). The latter lead to damage of tissues and components of the cell including: proteins, lipids, and DNA (1). The concentrations of ROS that are generated during cellular metabolism are maintained by endogenous antioxidant enzymes and free-radical scavengers.

Oxidative stress is reported to increase from the first to the third trimester among pregnant women implying that some increase of oxidative stress during pregnancy is adaptive and "normal" (2). However, maternal pro- and antioxidant intake and oxidative status during critical stages of development, alone or in combination with genetic and environmental exposures have also been implicated in adverse pregnancy outcomes including birth defects, miscarriage, preeclampsia, and even mortality in preterm infants (3). To date we know of no work that addresses optimization of oxidative status via dietary intake in women contemplating pregnancy.

Neurodevelopmental disorders have increased significantly in recent years. The rates of ADD (including ADHD) are estimated to range from 3% to 12% (4, 5). The historical rate of autism in the United States was 5/10,000. Recent CDC studies have estimated that the current rate may be as high as 6.7/1000, a rate more than 10 times the

historical estimates (6). The increase in prevalence of neurodevelopmental disorders may be a result of changes in diagnosis, genetics and environmental studies. Or perhaps, it is a combination of these factors. There is a growing sense that environmental exposures, potentially including dietary intake, during the perinatal period are the proximate cause of the increase in neurodevelopmental disorders.

We hypothesized that there would be an association between dietary intake and blood and urine biomarkers of oxidative stress. In order to test this hypothesis this study determined dietary oxidant status in nonpregnant women and women in the first trimester of pregnancy using the National Children's Study Food Frequency Questionnaire (NCS FFQ) and dietary supplement questionnaire as a method of estimating the recalled intake of foods, energy and of individual nutrients over a 3 month period. The NCS FFQ was used to calculate an index of overall dietary quality, the healthy eating index (HEI-2005) (7). Maternal oxidant status was measured for the same subjects with blood and urine biomarkers of oxidative stress. Finally, the associations of dietary intake (e.g., HEI-2005, individual pro- and antioxidant nutrients) and biomarkers of oxidative status were examined in order to understand whether dietary intake could be utilized as a specific, accurate and noninvasive method for measuring oxidative stress.

METHODS

Study Design

We conducted an observational, uncontrolled cohort study of women of childbearing age (WCBA) to determine oxidative status by evaluating dietary intake from a food frequency questionnaire and blood and urine biomarkers. The associations between specific nutrients, the HEI-2005 score (7), individual blood and urine biomarkers, as well as a lab oxy-score were evaluated.

Study Procedures

A convenience sample of 100 WCBA volunteers was recruited by posted flyers and through the University of Utah Hospital's Obstetrics and Gynecology Research Network and the University and Medical Center Clinical Care Units. This represents two methods of recruitment, clinic-based and community based. Both methods focused on enrolling women of diverse racial and socioeconomic backgrounds. Demographics (name, age, race, contact information) as well as gravida, parity, height, weight, and date of last menstrual period were recorded and an anonymous study ID was assigned. Data from all sources were linked by the study ID.

Potential participants were screened for eligibility by a research assistant. After subject consent was obtained, the subject either a) had blood and urine samples collected on that day if the subject was found to have fasted at the time of enrollment or b) had

blood and urine samples taken on a conveniently scheduled date to allow fasting. All consented women were asked to complete the NCS FFQ, the 3-day food checklist, the dietary supplement questionnaire, and the General Information Questionnaire at the time of enrollment. Subjects received $15 in the form of a gift card upon completion of the blood and urine samples and $10 when the NCS FFQ and other forms were returned, to partially compensate them for their time.

Participant Selection and Criteria

The criteria for the inclusion and exclusion of participants are summarized in Table 1. Briefly, eligible participants were women of childbearing potential that were either not pregnant or in the first trimester of pregnancy. Women who presented with diabetes mellitus, metabolic disorder cystic fibrosis, malabsorption syndrome, chronic diarrhea, and inborn errors of metabolism were excluded. Two women did not provide blood and urine samples and two subjects had implausibly low reported dietary intake (<800 kcal per day). These four subjects were excluded from the statistical analysis.

Table 1. Participant Inclusion and Exclusion Criteria

Inclusion criteria:	Exclusion criteria:
Women	< 18 years of age or > 49 years
Ages between 18 and 49	Sterile or Menopausal
Of childbearing potential	More than 13 weeks Pregnancy
Not pregnant or less than 14 weeks pregnant	Cystic Fibrosis
English speaking and comprehension	Diabetes (type 1 or 2 or gestational diabetes)
	Any maternal inborn error of metabolism
	HIV positive
	Known multiple pregnancy (twins or higher order)

Blood and Urine Collection

Eleven ml of blood were drawn (a 4 ml red top and a 7 ml green top tube). Blood from the 4 ml red top tube was wrapped in aluminum foil to protect from light when centrifuged. The 500 µl aliquots were kept in dark containers and frozen at -80 degrees Celsius within 1-2 hrs. Blood from the 7 ml green top was inverted several times. Two 100 µl aliquots were placed into an eppendorf tube and 10 µl of M2VP was added. Two 50 µl aliquots were placed into tubes and all four aliquots were frozen at -80 degrees C within 1-2 hours. The remaining heparinized sample was centrifuged and plasma was stored in 2, 250 µl screw-top tubes. Samples were frozen at -80 degrees C within 1-2 hours of blood draw. A minimum of 4 ml of urine was collected and aliquoted into three 1.2 ml samples and frozen at -80 degrees C within 1-2 hours of collection.

Malondialdehyde (MDA) Assay

Malondialdehyde (MDA) was measured using the MDA-586 assay (Oxis Research, Portland, OR). This assay utilizes a method that produces a carbocyanine dye (max absorbance at 586 nm) from the reaction of MDA with a chromogenic agent, N-methyl-2-phenylindole (NMPI). The concentration of MDA in each sample was run in duplicate and was determined using the calibration curve obtained from the MDA standard provided in the kit.

Glutathione (GSSG) and Reduced Glutathione (GSH) Assay

Concentrations of GSSG and GSH were determined in whole blood using the GSH/GSSG-412 kit (Oxis International, Foster City, CA) in duplicate. This assay uses Ellman's reagent, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), which reacts with GSH to form a spectrophotometrically detectable product at 412 nm. The reaction rate is

proportional to the GSH and GSSG concentrations and is equal to the slope of the linear regression equation supplied by the manufacturer. A calibration curve was constructed by plotting the net rate (difference between the rate at each concentration of GSH and the blank rate) versus the concentration of GSH. The concentrations of GSH, GSSG, and the ratio were calculated by the using the linear regression equation of the net rate calibration curve.

8-Isoprostane Assay

Urinary 8-isoprostane, also know as $iPF_{2\alpha}$-III, levels were analyzed in duplicate by an enzyme-immunoassay method using a commercially available kit (Cayman Chemical Co., Ann Arbor, MI, USA). A urinary creatinine assay was run using a colorimetric assay (Cayman Chemical Co., Ann Arbor, MI, USA) and each 8-isoprostane sample was normalized (ng/mmol).

Modified Lab Oxy-Score

A modified lab oxy-score (ML oxy-score) was created based on the method of Veglia et al. (8). Briefly, log-transformed biomarker values were standardized by subtracting the sample mean value from the individuals' value and dividing by the sample standard deviation. Since neither α-tocopherol nor an assay of Individual Antioxidant Capacity (IAC) was measured in plasma, the protection score excluded these values and contained only the standardized GSH. A damage score combining standardized MDA, 8-isoprostane and the GSSG/GSH ratio was also created. The ML oxy-score was calculated by subtracting the protection score from the damage score.

Therefore, a positive score indicates a higher indication of damage where a negative score suggests greater protection.

National Children's Study Food Frequency Questionnaire (NCS FFQ) and Dietary Supplement Questionnaire

The NCS FFQ (P1-T3, OMB Number 0925-0593) used in this study was adapted from the National Cancer Institute Dietary History Questionnaire (Version 1.0. National Institutes of Health, Applied Research Program, National Cancer Institute. 2007). This NCS FFQ was used to calculate the dietary intake of foods and nutrients over a three month period. Data from the questionnaires were scanned by Optimum Solutions Corp, (Lynbrook, NY). The dietary supplement questionnaire determined what non-prescription (over-the-counter) and prescription vitamins, minerals, and other dietary supplements the subject used over the previous 3 months. Missing data from all forms were minimized by contacting participants shortly after receipt of questionnaires to assess the cause for missing responses. Nutrient analysis was completed using Diet*Calc Version1.5 and was downloaded and processed with the responses from the NCS FFQ. Both food and nutrient data were generated. The primary nutrient database is from the United States Department of Agriculture.

Nutrients of Interest

The nutrients of interest for this project include total energy intake, zinc, manganese, selenium, β-carotene, vitamin E, vitamin C, and iron. Vitamin C, vitamin E, and β-carotene act as antioxidants (9), while zinc, manganese, selenium, and iron are constituents of antioxidant enzymes (10-12). Total nutrient intake was calculated by

summing the amount from supplements and diet measured from the NCS FFQ and supplement questionnaire.

Healthy Eating Index-2005

An index of overall dietary quality was calculated by creating subscores for 12 dietary components (HEI 1 through 12 listed in Table 2) obtained from the NCS FFQ and summing them up to reach a total, the HEI-2005 (7). Each of these components is assigned a minimum, median, or maximum number of points based on participant's conformance to federal dietary guidance (13).

Diet Oxy-Score

For this study, a diet oxy score was created using adequacy of intake based on the Recommended Dietary Intakes (DRI) (14) for the following variables: vitamin C, vitamin E, β-carotene, iron and Selenium. Some variables may act as antioxidants or pro-oxidants depending on the level of intake. Therefore, dichotomization of individual variables was used for vitamin C and iron because these variables favor anti-oxidant activity at lower intakes, while acting as pro-oxidants at higher intakes (15). A plus 1 was assigned for calculated intake exceeding the DRI: vitamin C > 60 mg and < 1000 mg, iron > 8.1 mg and <180 mg, vitamin E ≥ 15 mg, β-carotene ≥ 500 μg, and Selenium ≥ 25 μg. A minus 1 was assigned if the calculated intake was: vitamin C ≥ 1000 mg, iron ≥ 180 mg, and vitamin E ≤ 15 mg. The dietary oxy-score was computed by summing the values for vitamin C, vitamin E, β-carotene, iron and Selenium.

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Table 2. Healthy Eating Index-2005 Subscore Components

HEI subscore	Description	Max points	Standard for maximum score	Standard for minimum score of zero
HEI 1	Fruit including juice	5	≥0.8 cup equiv. Per 1,000 kcal	No fruit
HEI 2	Non-juice fruit	5	≥0.4 cup equiv. Per 1,000 kcal	No whole fruit
HEI 3	Total vegetables	5	≥1.1 cup equiv. Per 1,000 kcal	No vegetables
HEI 4	Dark green or orange vegetables or legumes	5	≥0.4 cup equiv. Per 1,000 kcal	Dark green or orange vegetables or legumes
HEI 5	Total grains	5	≥3.0 oz equiv. Per 1,000 kcal	No grains
HEI 6	Whole grains	5	≥1.5 oz equiv. Per 1,000 kcal	No whole grains
HEI 7	Milk/dairy eq.	10	≥1.3 cup equiv. Per 1,000 kcal	No milk or dairy eq.
HEI 8	Meat & beans	10	≥2.5 oz equiv. Per 1,000 kcal	No meat or beans
HEI 9	Oils	10	≥12 grams per 1,000 kcal	No oil
HEI 10	Sodium	10	≤0.7 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
HEI 11	Saturated fat	10	≤7% of energy	≥15% of energy
HEI 12	Solid fat, alcohol, added sugar	20	≤20% of energy	≥50% of energy
HEI-2005	Sum of all categories	100		

Statistical Methods, Data Analysis and Interpretation

Descriptive methods (e.g., box plots, kernel density curves) were used to provide preliminary univariate summaries of the dietary food, nutrient, and laboratory variables. Nutrient and laboratory variables that exhibited substantial skewness were log transformed prior to subsequent analyses. Pairwise relationships between laboratory measures (GSH, GSSG, GSH/GSSG ratio, MDA, 8-isoprostane, and ML oxy-score) and selected dietary measures were analyzed and were summarized using Pearson correlations and regression coefficients. When the p-value was <0.05, we concluded that the relationship was statistically significant. Statistics were run using SAS version 9.2. The study protocol was approved by the University of Utah's Institutional Review Board.

RESULTS

The sample mean and standard deviation for the subject characteristics of the 96 women that were included in this study are summarized in Table 3. The women averaged 31 years (range 18 - 49) with up to 4 live births (Table 3). The race and ethnicity of this convenience sample was reflective of the population of Utah with over 95% of women selecting white or Hispanic as the race they most closely identify.

Sixty-one women reported using any vitamin or mineral supplement, 23 used a multivitamin and 11 reported taking a prenatal vitamin. The majority of participants consumed the nutrients of interest (total from diet and supplements) at or above the Dietary Reference Intake (DRI) levels (14) (Table 4).

The HEI-2005 scores ranged from 42.9 to 82.2 and averaged 67.1 (Table 5). The vast majority of participant's diets scored in the fair range (n=91) (7). Few had diets that met the criteria for good quality (n=4) or poor (n=5). Participants lost the most points on their intake of sodium (HEI10), whole grains (HEI6), and the HEI12, which summarizes the percentage of solid fats, alcohol and sugar in the subject's diets.

Table 6 displays the data from the blood and urine biomarkers of oxidative stress and the laboratory oxy-score.

Table 3. Participant Characteristics

	Mean ±SD
Age (years)	31.0 ±8.3
Height (cm)	166.5 ±7.8
Weight (kg)	71.2 ±20.2
Gravida	1.5 ±1.7
Parity	1 ±1.1
Miscarriages	1.5 ±1.2
BMI (kg/m^2)*	25.5 ±6.1
Weight status	
Underweight*	7**
Normal*	57
Overweight*	20
Obese*	16

*BMI= Body mass index, BMI <18.5 is underweight, BMI of 18.5 to 24.9 is optimal weight, BMI > 24.9 and ≤30 is overweight, BMI >30 is obese. ** Number of participants

Table 4. Mean Intake for Nutrients of Interest, Adequacy of Intake, and the Diet Oxy-Score

	Mean ±SD	Number of Participant's whose intake ≥ DRI
Energy intake (kcal)	2028 ±914	-
Vitamin C (mg) *	259.2 ±272.3	95
Vitamin E (IU)	35.3 ±34.3	69
β-carotene (µg)	6697 ±8077	-
Selenium (µg)	119.0 ±62.6	94
Iron (mg)	30.4 ±49.9	91
Zinc (mg)	17.8 ±9.2	92
Manganese (mg)	3.7 ±2.3	82
Dietary oxy-score	2.4 ±1.3	-

*Total nutrient intake calculated as the sum of the nutrient from the NCS FFQ and reported supplement use

Table 5. Mean and Standard Deviation for the Healthy Eating Index-2005 and Healthy Eating Index Components

	HEI-2005 Components	Maximum points	Mean ±SD
HEI-2005	HEI -2005 Total Score	100	67.1 ±8.0
HEI 1	Fruit including juice	5	4.2 ±1.1
HEI 2	Nonjuice fruit	5	3.2 ±1.3
HEI 3	Total vegetables	5	4.3 ±1.0
HEI 4	Dark green or orange vegetables or legumes	5	3.7 ±1.5
HEI 5	Total grains	5	4.1 ±0.2
HEI 6	Whole grains	5	1.1 ±0.9
HEI 7	Milk/dairy eq.	10	6.0 ±2.6
HEI 8	Meat & beans	10	9.8 ±0.8
HEI 9	Oils	10	2.2 ±1.7
HEI 10	Sodium	10	2.7 ±2.0
HEI 11	Saturated fat	10	6.2 ±2.3
HEI 12	Solid fat, alcohol, added sugar	20	12.9 ±4.5

Table 6. Blood and Urine Biomarkers of Oxidative Stress

	Mean ±SD
Total glutathione (μ mol)	118.0 ±485.4
GSH (μ mol)	670.1 ±463.0
Ratio GSH/GSSG	8.8 ±16.1
8-isoprostane (ng/mmol)	95.8 ±132.0
MDA (μ mol)	21.4 ±17.2
β -carotene (μ g)	229.8 ±197.5
Lutein (μ g)	131.8 ±61.8
Zeazanthin (μ g)	20.6 ±10.1
ML oxy-score*	-0.111 ±1.942

*The modified lab (ML) oxy-score is composed of reduced glutathione (GSH), Malondialdehyde (MDA), 8-isoprostane and the GSSG/GSH ratio

Table 7 presents the Pearson correlations between dietary variables and laboratory variables. An inverse association was observed between the diet oxy-score and the ML oxy-score where a positive correlation was observed with GSH and the ratio of GSH and GSSG. Although the HEI-2005 (7) total score was not significantly associated with the ML oxy-score, it was significantly associated with GSH, total glutathione, and the GSH/GSSG ratio. The strongest association observed was between vitamin E and all biomarkers of oxidative status except for MDA. The correlations were inline with the putative biological effect of vitamin E intake on oxidant status. The strength of the association of beta-carotene with all biomarkers except MDA and 8-isoprostane was similar. Interestingly, vitamin C was not correlated with any of the laboratory measures including the ML oxy-score.

Table 7. Association of Dietary Antioxidants and Biomarkers of Oxidative Status

	ML oxy-score	$iPF_{2\alpha}$-III	GSH	GSH/GSSG ratio	Total GSH	MDA
Diet oxy-score	**-0.34**	-0.17	**0.24**	**0.27**	**0.18**	-0.11
p-value	**0.002***	0.11	**0.02**	**0.01**	**0.01**	0.29
HEI-2005	-0.20	-0.12	**0.21**	**0.21**	**0.23**	0.001
p-value	0.07	0.23	**0.05**	**0.05**	**0.03**	0.99
HEI 1	-0.09	0.02	0.05	0.07	0.08	0.008
p-value	0.41	0.84	0.64	0.54	0.47	0.94
HEI 2	-0.03	-0.02	0.04	0.003	0.07	-0.001
p-value	0.80	0.84	0.69	0.97	0.48	0.99
HEI 3	**-0.31**	-0.001	0.14	0.05	0.18	**-0.23**
p-value	**0.005***	0.997	0.18	0.63	0.09	**0.02**
HEI 4	-0.21	-0.04	0.09	0.02	0.14	-0.08
p-value	0.06	0.71	0.38	0.84	0.19	0.46
Zinc	**-0.35**	**-0.24**	**0.23**	0.19	0.19	-0.05
p-value	**0.001***	**0.02**	**0.03**	0.07	0.08	0.62
Manganese	**-0.30**	-0.17	0.19	0.10	**0.18**	-0.03
p-value	**0.009***	0.11	0.08	0.35	**0.099***	0.77
Vitamin C	-0.17	-0.10	0.18	0.03	0.18	0.04
p-value	0.14	0.34	0.08	0.80	0.08	0.69
Vitamin E	**-0.41**	**-0.24**	**0.25**	**0.21**	**0.21**	-0.12
p-value	**0.0002***	**0.03**	**0.02**	**0.05**	**0.05**	0.28
β-carotene	**-0.23**	-0.003	**0.26**	**0.22**	**0.25**	0.15
p-value	**0.04**	0.98	**0.01**	**0.04**	**0.02**	0.14
Iron	**-0.24**	-0.12	0.13	0.01	0.12	-0.09
p-value	**0.03**	0.28	0.20	0.90	0.24	0.39
Selenium	**-0.22**	**-0.24**	**0.23**	0.14	**0.21**	0.07
p-value	**0.05**	**0.02**	**0.03**	0.19	**0.05**	0.51

Bolded values show a statistically significant correlation (p-value <0.05). Healthy eating index (HEI), HEI 1 (total fruit), HEI 2 (non-juice fruit), HEI 3 (total vegetables), HEI 4 (dark green or orange vegetables or legumes), 8-isoprostane ($iPF_{2\alpha}$-III), reduced glutathione (GSH), reduced/oxidized glutathione ratio (GSH/GSSG ratio), malondialdehyde (MDA) and Total GSH= (GSH-2GSSG)/GSSG. Vitamin E is the sum of all forms. Zinc, manganese, vitamin E, iron, selenium, $iPF_{2\alpha}$-III, GSH, GSH/GSSG ratio, total GSH and MDA were log transformed. *p-value <0.01

DISCUSSION

The present study demonstrates that overall diet quality, several individual anti-oxidant nutrients, and an integrated score of pro- and antioxidant nutrients are associated with biomarkers of oxidative status in women of child bearing potential. These data imply the opportunity to optimize oxidative status via optimal nutrient intakes using diet and supplement use in women planning pregnancy. Importantly, the associations of nutrients with biomarkers were reflective of their biological roles. For example, urinary isoprostanes are biomarkers of lipid peroxidation and were inversely associated with antioxidant nutrients (zinc, selenium and vitamin E). In contrast, the oxidized to reduced glutathione ratio (GSH/GSSG) is one of the primary determinants of cellular redox state and is decreased in individuals with higher oxidative stress (16). As expected, the ratio of GSH/GSSG was positively associated with overall diet quality, the integrated score of dietary pro- and antioxidants as well as vitamin E and β-carotene intake.

Much of the previous research addressing the relationship of dietary intake with oxidative stress has been conducted on subjects in a diseased state and/or the primary objective was to evaluate changes in the disease state. Changes in oxidative status associated with food and nutrient intake has rarely been addressed. For example, the Women's Healthy Eating and Living study (17) that focused on breast cancer risk found that subjects in the intensive dietary intervention group (three servings of fruit, five servings of vegetables, 16 ounces of vegetable juice, 30 grams of fiber, and only 15 to 20

percent of fat) had significant increase in intake of vitamins E and C and beta-carotene from baseline to 12 months. Vitamin E intake was inversely associated with 8-isoprostane. Dierckx et al. (18) found that in diabetic patients vs. control, oxidative damage (assessed with glutathione and MDA) was only related to intakes of saturated fats and cholesterol.

One previous observational study observed an association between the Mediterranean diet pattern (focusing on high consumption of fruit and vegetables, olive oil as principal source of fat, low consumption of meat and dairy products and moderate consumption of wine as measured with a dietary diary) and oxidative status measured by MDA (19). Maternal oxidative status during critical stages of development, alone or in combination with genetic and environmental exposures has been implicated in adverse pregnancy outcomes including birth defects, miscarriage, preeclampsia, and even mortality in preterm infants (3). A few studies have investigated the role of antioxidant supplementation in reducing the risk of preeclampsia (20, 21). Neither study found significant differences between the supplement group (vitamins C and E, 100mg and 400 IU respectively) and the placebo groups in the risk of preeclampsia. Based on published results, the potential to modify total intake by both diet and supplement use is appealing. To our knowledge, this is the first study to demonstrate that better overall diet quality using the HEI -2005 is associated with oxidative status in healthy women of childbearing potential.

We created a novel dietary score of pro- and antioxidant nutrients in women of childbearing potential, the diet oxy-score, and showed its direct association with an integrated biomarker of oxidative status (ML oxy-score). We created the diet oxy-score

to represent the complexity of diet as a contributor to oxidant status, because intake of a single nutrient may not accurately represent the contribution of diet to oxidative status. This score reflects both pro- and antioxidant influences of vitamin C, vitamin E, β-carotene, iron and selenium based on their recommended levels of intake and known biological roles. The diet oxy-score was positively related to biomarkers that reflect better oxidative status (total GSH and the ratio of GSH to GSSG) in healthy, normal weight women (of child bearing age).

The diet oxy-score differs from the HEI-2005 in that it includes only select nutrients involved in oxidant status whereas the HEI-2005 is reflective of adequate intake of all food types as well as optimal fat and sodium intake which bear no influence on oxidative status. Interestingly, the total HEI-2005 score was positively associated with GSH and the ratio of GSH to GSSG. However the subscore reflecting vegetable intake was more strongly positively associated with the ML oxy-score, perhaps reflecting the contribution of vegetable consumption to antioxidant intake (22). The overwhelming majority of participants diet was categorized as "fair" (67.1) and in range with other reports (65.7) for an adult, female population (23). Nonetheless, there was a change in oxidative status as dietary quality improves, as defined by HEI-2005 (7).

The total intake (diet and supplement) of the antioxidant nutrients of interest was adequate as compared to the DRI for the vast majority of participants (Table 4). The proportion of women consuming adequate vitamin E as compared to the DRI (14) was 69% but was lower than the other variables. The low intake of whole grains, reflected in subscore HEI 5, may explain lower vitamin E intake. Increasing whole grain consumption may contribute to optimization of oxidative status.

The total diet and supplement intake of antioxidants (vitamin E, beta carotene and selenium) were associated with several biomarkers of oxidative status, except for vitamin C. Vitamin E was associated with all biomarkers except MDA, reflecting both roles in preventing lipid peroxidation and creating a more favorable glutathione ratio and oxidative status. One of the forms of vitamin E, α-tocopherol, has been shown to completely inhibit selenium deficiency induced cell death by abolishing the usual rise in ROS that occurs prior to death (24). alpha-tocopherol is also believed to have a sparing affect on GSH (25), favoring a better redox status. Beta-carotene is thought to be a quenching agent of singlet oxygen (26) and was correlated with all biomarkers except MDA and 8-isoprostane and the ML oxy-score. Selenium is a component in the glutathione peroxidase enzyme and oxidant status is attenuated with decreased selenium intake or enhanced with adequate intake (27). It is also thought to play a role in adverse outcomes such as miscarriages, neural tube defects, diaphragmatic hernia, premature birth, low birth weight, preeclampsia, glucose intolerance and gestational diabetes (28). As expected, selenium intake was favorably correlated with all biomarkers except for MDA and GSH/GSSG ratio.

In contrast, vitamin C was not associated with any of the biomarkers of oxidative status. Vitamin C is able to recycle vitamin E, allowing it to function again as an antioxidant. The absence of correlation of vitamin C with any of the biomarkers could be explained by most of the women having adequate intakes of vitamin C. Additionally, it is possible that high intakes of vitamin C, those usually associated with use of large amounts of vitamin C from supplements, could result in a pro-oxidant state (15). In this case, the relationship between vitamin C and oxidative status would not be linear.

The strength of the associations of dietary intake of individual nutrients with their corresponding biomarkers varies. For example, studies measuring tocopherols in the blood of female subjects in order to validate intake from an NCS FFQ produced correlation coefficients that ranged from slight (r=0.11) to moderate (r=0.51) (29, 30). Similar studies measuring ascorbic acid yielded slightly stronger associations, but still low to moderate (r= 0.32 to 0.61) (31, 32). The strength of the relationships of nutrients and oxidative status biomarkers in this study also ranged from low to moderate (r= 0.21 to 0.41). Future studies may improve the strength of the relationships between dietary variables of pro- and antioxidant nutrients and biomarkers of oxidant status by controlling for other factors influencing total oxidative status or by including individuals with a wider range of dietary adequacy.

Limitations

One factor that may have an effect on oxidative stress is exercise. Acute, as well as chronic, exercise has been shown to increase the production of endogenous antioxidant enzymes such as catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase (33, 34). Adjustment of the association of dietary intake with biomarkers of oxidative stress for exercise intensity, duration and at the very least frequency may be useful in future studies.

Oxidative stress is in constant flux within the body as ROS are constantly being destroyed and quenched rapidly. This presents a challenge to researchers attempting to accurately measure oxidative stress. While every attempt was made to store and analyze samples in a timely manner, the half-lives of the species being measured (particularly

MDA and GSH) are extremely short. The instability of these species may account for or contribute to the lack of correlation of the MDA assay with the other variables.

Many studies have shown that smoking has an effect on maternal and newborn levels of oxidative stress biomarkers (35). Chelchowska et al. showed that tobacco smoke enhances lipid peroxidation (measured with MDA) and depletes antioxidant potential in the plasma of pregnant women and umbilical cord blood (36). Smoking during pregnancy may stimulate free radical damage in the mother and the growing fetus. Although smoking rate for pregnant women in Utah is low 5.4% in 2008 (37), future studies should evaluate the influence of smoking.

Conclusion

Favorable oxidative status among women prior to pregnancy is thought to be desirable to optimize maternal and infant outcomes (38). We showed that evaluation of oxidative stress parameters including nutrient intake and integrated dietary scores were modestly correlated with individual biomarkers and the ML oxy-score. Overall diet quality was positively associated with the concentration of GSH, the GSH/GSSG ratio. Consequently, dietary variables and an integrated index of oxidative stress from dietary pro- and antioxidant components (the diet oxy-score) obtained from the NCS FFQ should be further evaluated for their utility in evaluating oxidative stress in lieu of measuring oxidative stress biomarkers. Further studies should consider modification of diet (improved diet quality by adherence to the US Dietary Guidelines (13)) and antioxidant supplementation prior to and during pregnancy. Research should focus on understanding what is the optimal diet and/or supplement combination and ultimately whether optimization of maternal oxidative status via pro- and antioxidant intake can positively

influence maternal health through pregnancy and neurodevelopmental outcomes in their children.

APPENDIX A

SCREEENING FORM

Screening Form Date_____

Pregnancy Status
Not Pregnant
Pregnant < 13 wks (by LMP or Ultrasound)

Exlcusion Criteria: (do not recruit)
- ☐ Pregnant > 13 weeks
- ☐ Menopausal
- ☐ Diabetes Mellitus (GDM, Type 1 or Type 2)
- ☐ Cystic Fibrosis
- ☐ Inborn Error of Metabolism
- ☐ HIV +
- ☐ Current diagnosis of any Cancer
- ☐ Chronic Diarrhea
- ☐ Malabsorption syndrome (any)
- ☐ Gastric Bypass

Study Explained yes _____ No_____

- ☐ Not Interested
- ☐ No time
- ☐ Consented

Ethnicity (check all that apply)
- ☐ Hispanic
- ☐ Non-Hispanic White
- ☐ Black/African American
- ☐ Asian
- ☐ American Indian/Alaska Native
- ☐ Native Hawaiian/Pacific Islander

Age: _____yrs

Gravity/ Parity/LIvebirths/Stillbirths/Abortions/miscarriages
G_____ P_____ L_____A_____

APPENDIX B

DEMOGRAPHICS FORM

Demographics Form

Ethnicity (check all that apply)

- ☐ Hispanic
- ☐ Non-Hispanic White
- ☐ Black/African American
- ☐ Asian
- ☐ American Indian/Alaska Native
- ☐ Native Hawaiian/Pacific Islander

Birthdate _____

Age: _____yrs

Gravity/ Parity/Livebirths/Stillbirths/Abortions/miscarriages
G_____ P_____ L_____A_____

Height _____inches_

Weight Current _____lbs__ Pre-Pregnancy_____lbs__

Contact information
Mailing Address _____

_____

Telephone number _____

Best time to call
Weekdays

- ☐ Morning
- ☐ Afternoon
- ☐ Evening

Weekend

- ☐ Morning
- ☐ Afternoon
- ☐ Evening

Lab appointment Date _____

APPENDIX C

FOOD FREQUENCY QUESTIONNAIRE

See supplemental file

APPENDIX D

DIETARY SUPPLEMENT QUESTIONNAIRE

1. Over the past 3 months have you taken any **over-the-counter or nonprescription** vitamins, minerals, or other dietary supplements?

☐ NO (GO TO QUESTION 2)

☐ YES

1a. List all **over-the-counter or nonprescription** vitamins, minerals, or other dietary supplements.

NAME OF SUPPLEMENT	UNITS DOSE	WHEN DID YOU TAKE THEM
		Over past month Last 3 months Last 2 months
		Over past month Last 3 months Last 2 months
		Over past month Last 3 months Last 2 months

Go to next page to write in additional supplements.

2. Over the past 3 months have you taken any **prescription** vitamins, minerals or other dietary supplements?

☐ NO

☐ YES

2a. List all **prescription** vitamins, minerals, or other dietary supplements.

NAME OF SUPPLEMENT	UNITS DOSE	WHEN DID YOU TAKE THEM
		Over past month Last 3 months Last 2 months
		Over past month Last 3 months Last 2 months
		Over past month Last 3 months Last 2 months

Go to next to write in additional supplements.

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