Published in final edited form as:
J Acad Nutr Diet. 2013 June ; 113(6): 776–785. doi:10.1016/j.jand.2013.01.019.

Self-report of Fruit and Vegetable Intake that meets the 5 A Day Recommendation is Associated with Reduced Levels of Oxidative Stress Biomarkers and Increased Levels of Antioxidant Defense in Premenopausal Women

Stephanie M. Rink
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892

Pauline Mendola, PhD*
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892. Telephone: 301-496-5267, Fax: 301-402-2084, pauline.mendola@nih.gov

Sunni L. Mumford, PhD
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892. Telephone: 301-402-6831, Fax: 301-402-2084, mumfords@mail.nih.gov

Jill K. Poudrier
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892

Richard W. Browne, PhD
Department of Biotechnical and Clinical Laboratory Sciences, University at Buffalo, 38 Cary Hall, Buffalo, NY 14214. Telephone: 716-829-5181, Fax: 716-829-3601, rwbrowne@buffalo.edu

Jean Wactawski-Wende, PhD
Department of Social and Preventive Medicine, University at Buffalo, 270C Farber Hall, Buffalo, NY, 14214. Telephone: 716-829-5374, Fax: 716-829-2979, jww@buffalo.edu

Neil J. Perkins, PhD
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892. Telephone: 301-435-6928, Fax: 301-402-2084, perkinsn@mail.nih.gov

---

*For correspondence and reprints: Pauline Mendola, PhD Investigator Epidemiology Branch Division of Epidemiology, Statistics and Prevention Research *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH 6100 Executive Blvd, Room 7B03F Rockville, MD 20852 Phone: 301-496-5267 pauline.mendola@nih.gov.

Current affiliation: Department of Nutrition, College of Health and Human Development, The Pennsylvania State University. 110 Chandlee Laboratory, University Park, PA, 16802. Telephone: (814)863-9692, Fax: (814)863-6103, smr381@psu.edu.

Current affiliation: Department of Global and Community Health, College of Health and Human Sciences, George Mason University, Fairfax, VA, 22030. Telephone: 703-298-4411, jillyan06@gmail.com.

The authors' responsibilities were as follows- JWW and EFS designed and conducted research; SMR, PM, SLM, and JKP analyzed data; SMR and PM wrote the manuscript and had primary responsibility for final content; PM, SLM, EFS, RWB, NJP and JWW critically revised manuscript for important intellectual content.

[2] None of the authors have a personal or financial conflict of interest.

Enrique F. Schisterman, PhD
Eunice Kennedy Shriver National Institute of Child Health and Human Development, Division of Epidemiology, Statistics and Prevention Research, Bethesda, MD, 20892. Telephone: 301-435-6893, Fax: 301-402-2084, schistee@mail.nih.gov

Abstract

Background—Oxidative stress has been associated with a variety of chronic diseases and reproductive disorders. Fruits and vegetables may contribute to antioxidant vitamin and micronutrient levels and reduce oxidative stress.

Objective—To investigate the effect of meeting the 5 A Day recommendation for fruit and vegetable consumption on biomarkers of oxidative damage and antioxidant defense.

Design—In this longitudinal study, healthy premenopausal women (n=258) were followed for ≤2 menstrual cycles with ≤16 oxidative stress measures timed to cycle phase.

Main outcome measures—Plasma concentrations of F_2-isoprostane, 9-hydroxyoctadecadieneoic acid (9-HODE), and 13-hydroxyoctadecadieneoic acid (13-HODE), erythrocyte activity of superoxide dismutase (SOD), glutathione reductase (GSHR), and glutathione peroxidase (GPx), as well as blood micronutrient concentrations were measured. Dietary intake was assessed by Food Frequency Questionnaires (FFQ, 1/cycle) and 24-hour recalls (≤4/cycle).

Statistical analyses performed—Fruit and vegetable servings were dichotomized based on the 5 A Day recommendation. Linear mixed models with repeated measures were used to analyze lipid peroxidation markers, antioxidant vitamins, and antioxidant enzymes by cycle phase and in association with usual fruit and vegetable intake.

Results—For both 24-hour recall (timed to cycle phase) and cycle-specific FFQ, meeting the 5 A Day recommendation was associated with decreased F_2-isoprostanes (24-hour recall $\beta = -0.10$ (95% CI: −0.12, −0.07); FFQ $\beta = -0.14$ (95% CI: −0.18, −0.11)). GSHR was lower in association with typical 5A Day consumption by FFQ but not in the phase-specific analysis. Higher levels of ascorbic acid, lutein, β-carotene and β-cryptoxanthin were observed with both 5 A Day measures.

Conclusions—Meeting the 5 A Day recommendation was associated with lower oxidative stress and improved antioxidant status in analyses of typical diet (FFQ) and in menstrual cycle phase-specific analyses using 24-hour recalls. Green salads were commonly eaten and increasing intake of salads may be a useful strategy to impact oxidation in reproductive aged women.

Keywords

Fruit and vegetable intake; oxidative stress; premenopausal women; antioxidant

Introduction

Free radicals and reactive oxygen species are produced as by-products of normal cellular oxidative metabolism. Oxidative stress refers to a condition where overproduction of these byproducts causes damage to components of the body, particularly DNA, lipids, and proteins[1]. Oxidative stress can be balanced through multiple antioxidant mechanisms, which can stabilize or quench reactive oxygen species. Oxidative stress has been implicated in infertility[2], impaired follicular growth[3], endometriosis[4], spontaneous abortion[5, 6], and increased risk of delivering a low birth weight infant[7] as well as several chronic degenerative diseases[8–10]. Greater understanding of the potential mediators of oxidative stress, given the role it may play in reproductive health, is an important issue for reproductive-age women. Dietary strategies have been advocated to reduce the risks associated with oxidative stress, as a myriad of antioxidants can be obtained from the diet.

Fruits and vegetables are rich sources of antioxidants, including vitamins C and E, carotenoids and flavonoids, as well as numerous other phytochemicals[11]. Some intervention trials have shown decreased levels of oxidative stress markers, such as F_2-isoprostanes, with a fruit and vegetable-rich diet[12–17]. However, other studies of fruit and vegetable intake did not find an association with measures of oxidative stress, perhaps due to small numbers (< 15 adults) in the intervention groups[18, 19] or because the intervention was based on a supplement extracted from foods[19]. Additionally, supplementation with purified antioxidants has not proven to be protective against or chronic disease, and has even resulted in increased risk of disease in some rare cases[20–24]. With such conflicting results, more evidence is clearly needed to understand the impact of fruit and vegetable intake on oxidative stress levels.

The 2010 Dietary Guidelines for Americans specify a need to increase intake of fruits and vegetables to provide important nutrients, decrease chronic disease risk, and provide a low calorie food choice to maintain a healthy weight[25]. The USDA 5 A Day campaign recommends 2.5 cups (five serving equivalents) of any combination of fruits and vegetables per day for a typical American adult diet; however, the average American meets only 59% and 42% of the goal for vegetable and fruit intake, respectively[26, 27]. Previous studies of fruit and vegetable intake or antioxidant supplementation on oxidative stress markers have been mostly limited to diseased populations, or populations with particular risk for oxidative stress (i.e. smokers). Previous work from the BioCycle study found that adherence to a Mediterranean diet was associated with decreased lipid peroxidation[28] and that oxidative stress varies across the menstrual cycle[29]. The 5 A Day recommendation may be a simple, easy-to-remember guide to help improve diet and decrease oxidative stress but no prior studies have examined typical daily consumption of the recommended servings of fruits and vegetables in healthy, young women in relation to oxidative stress while taking menstrual cycle variability into account. This study aims to fill these important data gaps by investigating the association between usual fruit and vegetable intake and biomarkers for oxidative stress and antioxidant status in healthy, premenopausal women.

Methods

Study Design

The BioCycle Study is a prospective cohort study of oxidative stress and hormone variation conducted between 2005 and 2007 in 259 healthy, regularly menstruating women aged 18–44y. Participants were recruited using a variety of community-based approaches (clinics, newspaper advertisements, fliers, etc.) from across western New York and were followed for 1 ($n = 9$) or 2 ($n = 250$) menstrual cycles. Most women (71%) were employed and 58% were full-time students during their participation. Exclusion criteria included current use of oral contraceptives or for the past 3 months, regular intake of vitamin and mineral supplements or certain prescription medications; pregnant or breastfeeding in the past 6 months; and diagnosis of chronic medical conditions, including metabolic disorders and gastrointestinal diseases associated with malabsorption. At the initial telephone screening, women with a self-reported height and weight resulting in a body mass index (BMI, kg/m^2) <18 or >35 and those with current or planned dietary restrictions for weight loss or medical reasons were excluded. One participant who reported daily multivitamin use in her study diary was excluded, leaving 258 women in this analysis. Details of this study have been previously described[30]. The University at Buffalo Health Sciences Institutional Review Board (IRB) approved the study, and served as the IRB designated by the National Institutes of Health for this study under a reliance agreement. All participants provided written informed consent.

Participants were followed for up to two menstrual cycles, with up to eight clinic visits per cycle, timed to cycle phase using fertility monitors to correspond to menses, mid-follicular phase, late-follicular phase, luteinizing hormone (LH)/follicle-stimulating hormone (FSH) surge, predicted ovulation, and early luteal, mid-luteal, and late luteal phases[30, 31]. These visits correspond to approximately days 2, 7, 12, 13, 14, 18, 22, and 27 of a standardized 28 day cycle. Collection and handling protocols were designed to minimize variability in preanalytic factors, as previously described[32]. The study population was highly compliant, with 94% of women completing ≥7 clinic visits/cycle and 100% completing at least five visits/cycle, with fewer visits typically due to shorter cycles.

Dietary Assessment

Nutrient data was collected using a food frequency questionnaire (FFQ) developed and validated by the Nutrition Assessment Shared Resource (NASR) of the Fred Hutchinson Cancer Research Center (FHCRC). The semi-quantitative FFQ was administered three times, once at baseline to determine usual intake over the past 6 months and once at the end of each of two cycles to determine usual intake in the month of the previous cycle. The FFQ was administered at the appointment occurring in the late luteal phase of the menstrual cycle and was reviewed by staff to ensure completion of the questionnaire. At least one cycle-specific FFQ was available for 97% of participants.

Additionally, 24-hour dietary recalls were conducted up to four times per cycle (menses, mid-follicular phase, ovulation, and mid-luteal phase), on days corresponding with blood sample collection, for a total of up to eight recalls over two cycles. All participants completed ≥2 dietary recalls per cycle, and the majority (87%) completed 4 recalls per cycle. For both the FFQ and 24-hour recalls, average daily fruit and vegetable servings were calculated using the 5 A Day method, summing servings across fruit and vegetable items[33, 34]. A vegetable serving was defined as 1 cup (250 mL) of raw leafy vegetables, ½ cup (125 mL) of other cooked or raw vegetables, or ½ cup (125 mL) of vegetable juice. A serving of fruit was defined as 1 medium fruit, ½ cup (125 mL) of chopped, cooked, or canned fruit, ¼ cup (62.5 mL) of dried fruit, or ¾ cup (187.5 mL) of 100% fruit juice.

All dietary data were analyzed using the Nutrition Data System for Research software version 2005, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN.

Biological Specimens

Collection of fasting blood samples was scheduled to occur between 0700 and 0830 hours at each cycle visit. All lipid peroxidation measurements were performed using EDTA anticoagulated blood plasma. Free F_2-isoprostanes were the primary marker of lipid peroxidation, as it is considered to be the gold standard[35]. The performance characteristics of the analytical laboratory methods have been previously described in detail[36, 37]. F_2-isoprostanes were measured in plasma with a gas chromatography-mass spectrometry-based method by the Molecular Epidemiology and Biomarker Research Laboratory (University of Minnesota, Minneapolis, MN) (9.4% Coefficient of Variation [CV]). 9-hydroxyoctadecadieneoic acid (9-HODE, 9.0% CV) and 13-hydroxyoctadecadienoic acid (13-HODE, 9.2% CV) were determined by HLPC with diode array detection at 234 nanometers (nm)[38]. Fat-soluble antioxidant vitamins and micronutrients were measured in duplicate at the University at Buffalo (Buffalo, NY). Serum retinol (6.2% CV), carotenoids (β-carotene, β-cryptoxanthin, lycopene and lutein; 5.3%, 6.0%, 7.6%, and 6.6% CVs, respectively), and tocopherols (α- and γ-tocopherol; 5.0% and 4.6% CVs, respectively) were measured simultaneously by HPLC[39]. The dinitrophenylhydrazine method was used to determine total ascorbic acid (9.6% CV) in heparin plasma which was stabilized immediately at collection in 6% meta-phosphoric acid[40].

Antioxidant enzymes were measured using kinetic enzyme assays adapted to the Cobas Fara II autoanalyzer (Roche Diagnostic Systems, Inc., Basel, Switzerland)[41]. Erythrocyte superoxide dismutase (SOD) activity was determined by the inhibition of the oxidation of cytochrome C by xanthine/xanthine oxidase (4.6% CV). One unit of SOD activity was defined as the amount of enzyme needed for 50% inhibition of the reaction. Erythrocyte glutathione peroxidase (GPx; 5.0% CV) and erythrocyte glutathione reductase (GSHR; 3.7% CV) were performed using OxiTek reagent kits from ZeptoMetrix (Buffalo, NY). Erythrocyte enzyme activities were normalized per gram of hemoglobin (Hb)[37].

Covariate Assessment

At baseline, height (m) and weight (kg) were measured using standard protocols and used to calculate BMI. Participants completed questionnaires regarding reproductive health history, lifestyle, family, and physical activity. Standard International Physical Activity Questionnaire cut points[42] were used to create high, moderate, and low physical activity categories. In daily diaries, women recorded intake of medications, supplements, and/or vitamins.

Statistical Analysis

Descriptive statistics—Descriptive statistics for continuous and categorical covariates were compared between women meeting and not meeting the 5 A Day recommendation based on the average consumption from all 24-hour recalls (≤8 per woman) using ANOVA or Fisher's exact test, as appropriate. Total energy intake was averaged across all 24-hour recalls in the descriptive tables. Mean levels of lipid peroxidation, antioxidant enzymes, and antioxidant vitamins by menstrual cycle phase were compared between women meeting and not meeting the 5 A Day recommendation based on the average consumption per cycle (≤4 recalls per cycle) using repeated measures ANOVA. Descriptive results for FFQ measures were similar and are not presented.

Multivariable models—Oxidative stress and antioxidant measures (up to 8 measures per woman for each cycle) were analyzed in association with fruit and vegetable servings reported in the same cycle using linear mixed models that accounted for repeated measures and the correlation between and within participants. All analyses compared meeting the 5 A Day recommendation to not meeting it. For analyses based on the FFQ, the fruit and vegetable intake for each cycle was used to categorize women for that cycle. For analyses based on 24-hour recalls: dietary recall during menses was paired with oxidative stress measures during menses; dietary recall during the mid-follicular phase with mid-follicular oxidative stress measures, dietary recall on the day of predicted ovulation with the oxidative stress measures during the three peri-ovulatory period visits (late-follicular, LH/FSH surge, predicted ovulation) and dietary recall on the mid-luteal visit with early, mid, and late luteal phase oxidative stress measures. Women could change categories by cycle (FFQ and 24-hour recall analyses) and by menstrual cycle phase (24-hour recall analyses). Lipid peroxidation markers, antioxidant enzymes, and antioxidant vitamins were log transformed to improve fit in multivariable models. Models based on 24-hour recall are menstrual-cycle phase specific while those based on FFQ relate the typical diet for each cycle to multiple measures of oxidative stress in that cycle.

Covariates considered for inclusion in multivariate models were determined a priori after a review of prior literature and included: age (continuous), energy intake (continuous), clinically-measured BMI (continuous), race (Caucasian, African-American, Asian, other), smoking status (never, current/past), physical activity (low/moderate, high), marital status (married, not married), parity (0, ≥1), income (<$19,999; $20,000–39,999; $40,000–74,999; ≥$75,000), education (completed high school, did not complete high school), and past oral contraceptive use (yes, no). Variables were included in the final adjusted model if they also changed the exposure coefficient by >15% and were significant at p=0.10 in either the FFQ or 24-hour cycle specific model. The final adjusted model controlled for age, energy intake, race, income, marital status, parity, and former oral contraceptive use. SAS version 9.2 (SAS Institute, Cary, NC) was used for all statistical analyses. All testing was based on *a priori* hypotheses and no adjustments were made for multiple comparisons.

Results

Demographics

Overall, this cohort consisted of young women (mean age: 27.3 years, range 18–44) who were of healthy weight (mean BMI: 24.1 kg/m^2) and were non-smokers (Table 1). Most had completed high school (87.2%), were not married (74.8%), and had no children (74.2%). The women in this cohort had low overall fruit and vegetable intake (median=2.71 servings/d). Women who met the 5 A Day recommendation (17.8% of all subjects) tended to be older, were more likely to be Caucasian and have higher income. They were also more likely to be married, have children, and to be prior oral contraceptive users. Meeting the 5 A Day recommendation was associated with greater energy intake, but no differences were observed with respect to BMI, physical activity, education, and smoking.

5 A Day fruit And Vegetable Intake in Relation to Concentrations of Lipid Peroxidation and Antioxidants

F_2-isoprostanes were observed to be significantly lower across the menstrual cycle among women who consume 5 A Day (p<0.05), but no differences were observed for 9-HODE or 13-HODE (Table 2). SOD was also lower among those meeting the recommendation (p<0.005) with no significant differences in GPx or GSHR levels. With regard to anti-oxidant vitamins, most were significantly higher when 5 A Day was met including α-tocopherol, ascorbic acid, retinal, lutein, β-carotene and β-cryptoxanthin. An inverse association was observed for γ-tocopherol and lycopene was not related to 5 A Day consumption.

In multivariable analyses (Table 3), results were generally consistent for both cycle phase-specific models based on 24-hour recalls and the typical diet analysis based on cycle-specific FFQ. All effect estimates for lipid peroxidation markers were in the expected direction, with lower levels when 5 A Day was met, but only F_2-isoprostanes were significantly lower after adjustment. This difference may reflect the fact that total HODEs and free F2 isoprostanes represent lipid peroxides derived from different parent lipid molecules (linoleic and arachidonic acids, respectively) as well as different lipid peroxidation processes which may vary in response to the same dietary influences. We also anticipated lower antioxidant enzyme status with 5 A Day and that pattern of results was somewhat stronger for the typical diet analysis based on FFQ where GSHR was significantly lower. Ascorbic acid, lutein, β-carotene and β-cryptoxanthin were all significantly increased in both cycle phase-specific (24-hour recall) and cycle-specific (FFQ) models when 5 A Day was met. A-tocopherol was significantly increased in the phase-specific model only and no differences were observed for γ-tocopherol, retinol or lycopene.

Discussion

Among healthy premenopausal women, we observed that meeting the 5 A Day recommendation was associated with lower concentrations of lipid peroxidation, as measured by F_2-isoprostanes, with lower concentrations of erythrocyte antioxidant enzymes, specifically SOD (in unadjusted models),and GSHR (after adjustment), and higher concentrations of most plasma/serum antioxidant vitamins. These findings are particularly relevant for reproductive-age women, as oxidative stress has been implicated in infertility and poor birth outcomes in addition to a host of chronic diseases[2–7, 9, 10].

No prior studies have focused on young adult women or have looked at the efficacy of the 5 A Day recommendation in terms of oxidative stress, but other investigations have reported an inverse relationship between fruit and vegetable intake and biochemical oxidative stress measures[12, 43, 44]. A cross-sectional analysis of male and female adolescents (mean age: 15 y, n=285) found a substantial inverse association between total fruit and vegetable intake and F_2-isoprostanes, primarily explained by vitamin C and folate intake (41). Studies in older adults report similar findings[9, 44]. A randomized intervention showed that increased Brassica vegetable intake, but not a micronutrient supplement, decreased F_2-isoprostane levels in men and postmenopausal women (mean age: 57 y, n=20), providing evidence that the vegetables impacted oxidative stress where supplementation did not[9]. The Study of Women's Health Across the Nation showed that in nonsmoking women (ages 42–52 y) greater daily vegetable intake was associated with a lower concentration of urinary F_2-isoprostanes[44] Our findings support the notion that increased fruit and vegetable consumption is associated with lower F_2-isoprostane levels and we observed a similar effect when 5 A Day was met within menstrual cycle phase as with typical diet.

Fruits and vegetables are thought to ameliorate oxidative stress partially due to their high antioxidant content. As anticipated, we observed increased levels of α-tocopherol, lutein, β-carotene, β-cryptoxanthin, and ascorbic acid when the 5 A Day recommendation was met. In general, we observed lower levels of antioxidant enzyme activity among women who met the 5 A Day recommendation, particularly for typical diet, consistent with the explanation that more activity is required in the presence of higher oxidative stress[45, 46]. Erythrocyte activity of SOD, GPx, and GSHR were generally inversely associated with servings of fruits and vegetables but only GSHR was significant in the adjusted model.

This study, while it expands on previous research, has several limitations. The BioCycle cohort is has strict inclusion criteria which results in a healthy, normal weight, sample of women with regular menstrual cycles and no recent pregnancy or hormonal contraception. These inclusion criteria strengthen the internal validity of the study by limiting known sources of variability in the complex biologic measures under study, but do limit generalizability. However, young adult women are not well studied in previous investigations of dietary intake and oxidative stress and our analysis fills an important knowledge gap – whether or not the straightforward message to eat 5 A Day is associated with changes in biomarkers of oxidative stress and antioxidant defense in healthy young women. A particular strength of the BioCycle Study is the multiple measures of lipid peroxidation, antioxidant enzymes and vitamins which were measured up to 8 times over the course of each menstrual cycle. Our measures of fruit and vegetable consumption are all based on self-reported intake. We used FFQs to capture typical consumption for each cycle recognizing that this method might be subject to recall bias but comparison to 24-hour recalls in the same study period and cycle phase-specific analyses yielded similar findings. We also conducted analyses using fruit and vegetable servings as a continuous measure and categorized by servings up to 5 A Day, all with generally similar findings. Finally, oxidative stress measures are known to vary across the menstrual cycle, specifically increased F_2-

isoprostanes associated with estrogen levels[29], and these analyses take this important source of variability into account. Our analyses considered both typical consumption and cycle phase-specific associations in evaluating the 5 A Day recommendation.

Of note is that 5 A Day and the Dietary Guidelines for Americans, 2010, do not exclude starchy vegetables like potatoes from their recommendations to increase fruit and vegetable consumption. We recognize that white potatoes products can be an indicator of an unhealthy diet[47] but also note that potatoes are a good source of vitamin C[48]. Since potatoes are commonly eaten and they are part of the 5 A Day recommendation, we include them in the calculation of servings for our analyses. As such, our findings reinforce the simple message that increasing fruit and vegetable consumption and meeting 5 A Day is associated with lower oxidative stress.

A potentially successful avenue for increasing fruit and vegetable consumption is to encourage more consumption of foods already being eaten. While ketchup and fried potatoes were eaten by 70–85% of women in our study, they were the most commonly eaten servings among the lowest fruit and vegetable consumers, possibly explaining the lack of association for lycopene since ketchup is a major source of lycopene[49]. Green salads were the 3rd most common serving for the lowest consumers but they were the most common serving for all other women. Given the acceptability of green salads to this population, they may represent a key avenue for increasing fruit and vegetable consumption. Strategies that encourage adding volume and/or additional vegetables and fruits to green salads and mixed dishes is a potentially easy and acceptable way to change the eating habits of young adult women to include more servings of fruits and vegetables.

In summary, we found that self-report of meeting the 5 A Day recommendation was associated with lower biomarkers of oxidative stress and improved antioxidant defense. For isoprostanes, benefits were consistently observed for both a menstrual cycle phase-specific analysis and an analysis of typical diet by cycle. Women who consume very low levels of fruits and vegetables may be encouraged to eat more fruits and vegetables in green salads and mixed dishes as a strategy to increase their average daily servings and potentially impact their oxidative stress measures.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

Abbreviations used

9-HODE	9-hydroxyoctadecadienoic acid
13-HODE	13-hydroxyoctadecadieneoic acid
DNPH	dinitrophenylhydrazine
SOD	superoxide dismutase
GPx	glutathione peroxidase
GSHR	glutathione reductase

References

1. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr. 2005; 135:969–72. [PubMed: 15867266]

2. Rayman MP. The importance of selenium to human health. Lancet. 2000; 356:233–241. [PubMed: 10963212]
3. Murray AA, Molinek MD, Baker SJ, Kojima FN, Smith MF, Hillier SG, Spears N. Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles in vitro. Reproduction. 2001; 121:89–96. [PubMed: 11226031]
4. Murphy AA, Santanam N, Parthasarathy S. Endometriosis: a disease of oxidative stress? Semin Reprod Endocr. 1998; 16:263–273.
5. Samborskaia E, Ferdman T. Mechanism of abortion induced by ascorbic acid. Biulletin Eksperimentalnoi Biologii I Meditsiny. 1966; 62:96–98.
6. Jenkins C, Wilson R, Roberts J, Miller H, McKillop JH, Walker JJ. Antioxidants: their role in pregnancy and miscarriage. Antioxid Redox Sign. 2000; 2:623–628.
7. Kim YJ, Hong YC, Lee KH, Park HJ, Park EA, Moon HS, Ha EH. Oxidative stress in pregnant women and birth weight reduction. Reproductive Toxicology. 2005; 19:487–492. [PubMed: 15749262]
8. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr. 1993; 57:715S, 724S. discussion 724S–725S. [PubMed: 8475889]
9. Epplein M, Franke AA, Cooney RV, Morris JS, Wilkens LR, Goodman MT, Murphy SP, Henderson BE, Kolonel LN, Le Marchand L. Association of plasma micronutrient levels and urinary isoprostane with risk of lung cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev. 2009; 18:1962–70. [PubMed: 19531680]
10. Polidori MC, Pratico D, Parente B, Mariani E, Cecchetti R, Yao Y, Sies H, Cao P, Mecocci P, Stahl W. Elevated lipid peroxidation biomarkers and low antioxidant status in atherosclerotic patients with increased carotid or iliofemoral intima media thickness. J Investig Med. 2007; 55:163–7.
11. Brevik A, Gaivao I, Medin T, Jorgenesen A, Piasek A, Elilasson J, Karlsen A, Blomhoff R, Veggan T, Duttaroy AK, et al. Supplementation of a western diet with golden kiwifruits (Actinidia chinensis var.'Hort 16A':) effects on biomarkers of oxidation damage and antioxidant protection. Nutr J. 2011; 10:54. [PubMed: 21586177]
12. Fowke JH, Morrow JD, Motley S, Bostick RM, Ness RM. Brassica vegetable consumption reduces urinary F2-isoprostane levels independent of micronutrient intake. Carcinogenesis. 2006; 27:2096–102. [PubMed: 16704986]
13. Visioli F, Riso P, Grande S, Galli C, Porrini M. Protective activity of tomato products on in vivo markers of lipid oxidation. Eur J Nutr. 2003; 42:201–6. [PubMed: 12923651]
14. Thompson HJ, Heimendinger J, Sedlacek S, Haegele A, Diker A, O'Neill C, Meinecke B, Wolfe P, Zhu Z, Jiang W. 8-Isoprostane F2alpha excretion is reduced in women by increased vegetable and fruit intake. Am J Clin Nutr. 2005; 82:768–76. [PubMed: 16210705]
15. Thompson HJ, Heimendinger J, Haegele A, Sedlacek SM, Gillette C, O'Neill C, Wolfe P, Conry C. Effect of increased vegetable and fruit consumption on markers of oxidative cellular damage. Carcinogenesis. 1999; 20:2261–6. [PubMed: 10590217]
16. Sanchez-Moreno C, Cano MP, de Ancos B, Plaza L, Olmedilla B, Granado F, Martin A. Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F2-isoprostanes, prostaglandin E2 and monocyte chemotactic protein-1 in healthy humans. J Nutr Biochem. 2006; 17:183–9. [PubMed: 16169205]
17. Miller ER 3rd, Erlinger TP, Sacks FM, Svetkey LB, Charleston J, Lin PH, Appel LJ. A dietary pattern that lowers oxidative stress increases antibodies to oxidized LDL: results from a randomized controlled feeding study. Atherosclerosis. 2005; 183:175–82. [PubMed: 16216596]
18. Nelson JL, Bernstein PS, Schmidt MC, Von Tress MS, Askew EW. Dietary modification and moderate antioxidant supplementation differentially affect serum carotenoids, antioxidant levels and markers of oxidative stress in older humans. J Nutr. 2003; 133:3117–23. [PubMed: 14519794]
19. Dragsted LO, Pedersen A, Hermetter A, Basu S, Hansen M, Haren GR, Kall M, Breinholt V, Castenmiller JJ, Stagsted J, et al. The 6-a-day study: effects of fruit and vegetables on markers of oxidative stress and antioxidative defense in healthy nonsmokers. Am J Clin Nutr. 2004; 79:1060–72. [PubMed: 15159237]

20. Rytter E, Vessby B, Asgard R, Ersson C, Moussavian S, Sjodin A, Abramsson-Zetterberg L, Moller L, Basu S. Supplementation with a combination of antioxidants does not affect glycaemic control, oxidative stress or inflammation in type 2 diabetes subjects. Free Radic Res. 2010; 44:1445–53. [PubMed: 20942575]
21. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet. 2002; 360:23–33. [PubMed: 12114037]
22. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med. 1994; 330:1029–35. [PubMed: 8127329]
23. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med. 1996; 334:1150–5. [PubMed: 8602180]
24. Greenwald P, Anderson D, Nelson SA, Taylor PR. Clinical trials of vitamin and mineral supplements for cancer prevention. Am J Clin Nutr. 2007; 85:314S–7S. [PubMed: 17209217]
25. U.S. Department of Agriculture. U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition. U.S. Government Printing Office; Washington, DC: Dec. 2010
26. U.S. Department of Agriculture. Agricultural Research Service. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. What We Eat In America, NHANES 2001–2004, 1 day mean intakes for adult males and females, adjusted to 2,000 calories and aver-aged.
27. Heimendinger J, Van Duyn MA, Chapelsky D, Foerster S, Stables G. The National 5 A Day for Better Health Program: A Large-Scale Nutrition Intervention. J Public Health Man. 1996; 2(2):27–35.
28. Gaskins AJ, Rovner AJ, Mumford SL, Yeung E, Browne RW, Trevisan M, Perkins NJ, Wactawski-Wende J, Schisterman EF. Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women. Am J Clin Nutr. 2010; 92:1461–7. [PubMed: 20943796]
29. Schisterman E, Gaskins AJ, Mumford SL, Browne RW, Yeung E, Trevisan M, Hediger M, Zhang C, Perkins N, Hovey K, Wactawski-Wende J. Influence of endogenous reproductive hormones on F_2-isoprostane levels in premenopausal women: The BioCycle Study. Am J Epidemiol. 2010; 172:430–439. [PubMed: 20679069]
30. Wactawski-Wende J, Schisterman EF, Hovey KM, Howards PP, Browne RW, Hediger M, Liu A, Trevisan M. BioCycle study: design of the longitudinal study of oxidative stress and hormone variation during the menstrual cycle. Paediatr Perinat Epidemiol. 2009; 23:171–84. [PubMed: 19159403]
31. Howards PP, Schisterman EF, Wactawski-Wende J, Reschke JE, Frazer AA, Hovey KM. Timing clinic visits to phases of the menstrual cycle by using a fertility monitor: the BioCycle Study. Am J Epidemiol. 2009; 169:105–12. [PubMed: 18974081]
32. Browne RW, Bloom MS, Schisterman EF, Wactawski-Wende J, Hovey K, Trevisan M, Gross M. Analytical and biological variation of F2-isoprostanes during the menstrual cycle. Clin Chem. 2009; 55:1245–7. [PubMed: 19359531]
33. Schakel SF. Procedures for estimating nutrient values for food composition databases. J Food Comp and Anal. 1997; 10:102–114.
34. Thompson FE, Subar AF, Smith AF, Midthune D, Radimer KL, Kahle LL, Kipnis V. Fruit and vegetable assessment: performance of 2 new short instruments and a food frequency questionnaire. J Am Diet Assoc. 2002; 102:1764–72. [PubMed: 12487538]
35. Milne GL, Sanchez SC, Musiek ES, Morrow JD. Quantification of F2-isoprostanes as a biomarker of oxidative stress. Nat Protoc. 2007; 2:221–6. [PubMed: 17401357]
36. Browne RW, Bloom MS, Schisterman EF, Wactawski-Wende J, Hovey KM, Trevisan M, Gross M. Analytical and biological variation of F2-isoprostanes during the menstrual cycle. Clinical Chemistry. 2009; 55(6):1245–47. [PubMed: 19359531]

37. Browne RW, Bloom MS, Schisterman EF, Hovey K, Trevisan M, Wu C, Liu A, Wactawski-Wende J. Analytical and biological variation of biomarkers of oxidative stress during the menstrual cycle. Biomarkers. 2008; 13(2):160–83. [PubMed: 18270869]

38. Browne RW, Armstrong D. Simultaneous determination of polyunsaturated fatty acids and corresponding monohydroperoxy and monohydroxy peroxidation products by HPLC. Methods Mol Biol. 2002; 186:13–20. [PubMed: 12013761]

39. Browne RW, Armstrong D. Simultaneous determination of serum retinol, tocopherols, and carotenoids by HPLC. Methods Mol Biol. 1998; 108:269–75. [PubMed: 9921536]

40. Chalmers AH, McWhinney BC. Two spectrophotometric methods compared for measuring low concentrations of ascorbate in plasma and urine. Clin Chem. 1986; 32:1412–3. [PubMed: 3719958]

41. Pippenger CE, Browne RW, Armstrong D. Regulatory antioxidant enzymes. Methods Mol Biol. 1998; 108:299–313. [PubMed: 9921539]

42. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003; 35:1381–95. [PubMed: 12900694]

43. Holt EM, Steffen LM, Moran A, Basu S, Steinberger J, Ross JA, Hong CP, Sinaiko AR. Fruit and Vegetable Consumption and Its Relation to Markers of Inflammation and Oxidative Stress in Adolescents. Journal of the American Dietetic Association. 2009; 109:414–21. [PubMed: 19248856]

44. Tomey M, Sowers M, Li X, McConnell DS, Crawford S, Gold EB, Lasley B, Randolph JF Jr. Dietary Fat Subgroups, Zinc, and Vegetable Components are Related to Urine F2a-Isoprostane Concentration, a Measure of Oxidative Stress, in Midlife Women. The Journal of Nutrition. 2007; 137:2412–9. [PubMed: 17951478]

45. Salminen A, Vihko V. Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. Acta Physiol Scand. 1983; 117:109–13. [PubMed: 6858698]

46. Kalra J, Rajput AH, Mantha SV, Prasad K. Serum antioxidant enzyme activity in Parkinson's disease. Mol Cell Biochem. 1992; 110:165–8. [PubMed: 1584206]

47. Heroux M, Janssen I, Lam M, Lee D, Hebert JR, Sui X, Blair SN. Dietary patterns and the risk of mortality: impact of cardiorespiratory fitness. Int J Epidemiol. 2010; 39:197–209. [PubMed: 19380370]

48. Kondo Y, Higashi C, Iwama M, Ishihara K, Handa S, Mugita H, Maruyama N, Koga H, Ishigami A. Bioavailability of vitamin C from mashed potatoes and potato chips after oral administration in healthy Japanese men. Br J Nutr. 2012; 107:885–892. [PubMed: 21917196]

49. Rao AV, Shen H. Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress. Nutr Res. 2002; 22:1125–31.

Table 1

Demographic characteristics and total energy at baseline for women who met the 5 A Day recommendation for fruit and vegetable consumption and those who did not in the BioCycle study.

Participant Characteristics		**Met the 5 A Day recommendation**[1]		
	Total Cohort	**Yes**	**No**	**p-value**[2]
n (% of total)	258	46 (17.8)	212 (82.2)	
Demographics				
Age, *y, mean (SE)*	27.3 ± 0.51	29.3 ± 1.2	26.8 ± 0.6	0.0596
BMI, kg/m^2, *mean (SE)*	24.1 ± 0.24	23.6 ± 0.6	24.2 ± 0.3	0.3789
Physical Activity, *n (%)*				0.208
Low	25 (9.7)	2 (4.4)	23 (10.9)	
Moderate	92 (35.7)	21 (45.7)	71 (33.5)	
High	141 (54.7)	23 (50.0)	118 (55.7)	
Race, *n (%)*				0.0134
Caucasian	153 (59.3)	36 (78.3)	117 (55.2)	
African-American	51 (19.8)	5 (10.9)	46 (21.7)	
Asian	39 (15.1)	2 (4.4)	37 (17.5)	
Other	15 (5.8)	3 (6.5)	12 (5.7)	
Income, n (%)				0.0983
<$19,999	55 (21.5)	4 (8.7)	51 (24.3)	
$20,000–39,999	61 (23.8)	14 (30.4)	47 (22.4)	
$40,000–74,999	71 (27.7)	14 (30.4)	57 (27.1)	
≥75,000	69 (27.0)	14 (30.4)	55 (26.2)	
Completed High School, *n (%)*	225 (87.2)	42 (91.3)	183 (86.3)	0.4690
Marital Status, *n (%)*				0.0011
Married	65 (25.2)	21 (45.7)	44 (20.8)	
Not Married	193 (74.8)	25 (54.4)	168 (79.3)	
Parity, *n (%)*				0.0263
0	187 (74.2)	28 (60.9)	159 (77.2)	
≥1	65 (25.8)	18 (39.1)	47 (22.8)	
Past or current smoker, *n (%)*	10 (3.9)	2 (4.4)	8 (3.8)	0.6941
Past OC use, *n (%)*	139 (54.7)	36 (78.3)	103 (49.5)	0.0005
Total energy[3], *kcal, mean (SE)*	1605.5 ± 22.0	1806.2 ± 50.4	1562.0 ± 23.5	<.0001

[1] Based on the average 24-hour recall levels for each cycle.

[2] Based on ANOVA for continuous measures and Fisher's exact test for categorical measures.

[3] Based on the average of all 24-hour recalls (≤8 measures).

Table 2

Biomarkers of oxidative stress and antioxidant defense across the menstrual cycle for women who met the 5 A Day recommendation for fruit and vegetable consumption and those who did not in the BioCycle study.

Cycle Phase		Menses	Mid-Follicular	Late-Follicular	LH/FSH surge	Predicted ovulation	Early Luteal	Mid-Luteal	Late-Luteal	
Standardized Cycle Day		**Day 2[1]**	**Day 7**	**Day 12**	**Day 13**	**Day 14**	**Day 18**	**Day 22**	**Day 27**	
	Met 5A Day[2]	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**p-value[3]**
Lipid Peroxidation										
F_2-Isoprostanes, *pg/mL*	Yes	60.27 (4.166)	49.93 (4.145)	48.71 (4.124)	48.76 (4.145)	46.68 (4.166)	48.77 (4.209)	45.78 (4.253)	48.79 (4.919)	0.0495
	No	57.03 (2.060)	53.98 (2.065)	52.38 (2.057)	53.30 (2.060)	50.71 (2.075)	51.61 (2.088)	52.52 (2.107)	52.75 (2.354)	
9-HODE, μ*mol/L*	Yes	0.22 (0.022)	0.23 (0.022)	0.19 (0.022)	0.20 (0.022)	0.18 (0.022)	0.19 (0.022)	0.23 (0.022)	0.19 (0.026)	0.5985
	No	0.21 (0.011)	0.20 (0.011)	0.21 (0.011)	0.22 (0.011)	0.21 (0.011)	0.21 (0.011)	0.21 (0.011)	0.18 (0.012)	
13-HODE, μ*mol/L*	Yes	0.26 (0.031)	0.27 (0.030)	0.24 (0.030)	0.25 (0.030)	0.21 (0.030)	0.26 (0.030)	0.27 (0.031)	0.28 (0.036)	0.9332
	No	0.27 (0.015)	0.24 (0.015)	0.25 (0.015)	0.27 (0.015)	0.26 (0.015)	0.27 (0.015)	0.27 (0.015)	0.23 (0.017)	
Antioxidant Enzymes										
SOD, *IU/g Hb*	Yes	4612.76 (93.471)	4655.10 (93.003)	4618.97 (93.003)	4674.52 (92.541)	4732.84 (93.003)	4553.60 (94.920)	4588.72 (94.920)	4542.64 (110.370)	0.0046
	No	4714.97 (46.213)	4702.57 (46.271)	4717.23 (46.213)	4764.73 (46.156)	4713.48 (46.386)	4752.86 (46.677)	4756.00 (47.337)	4741.99 (52.907)	
GPx, *IU/g Hb*	Yes	36.24 (1.438)	35.50 (1.423)	36.65 (1.438)	37.23 (1.423)	37.07 (1.430)	36.05 (1.460)	36.82 (1.460)	36.13 (1.698)	0.9464
	No	35.45 (0.712)	36.04 (0.712)	36.66 (0.711)	37.16 (0.711)	36.35 (0.714)	35.95 (0.718)	37.02 (0.728)	37.01 (0.811)	
GSHR, *IU/g Hb*	Yes	4.04 (0.132)	3.88 (0.131)	3.91 (0.131)	3.80 (0.131)	3.87 (0.131)	3.81 (0.134)	3.79 (0.134)	3.77 (0.156)	0.708
	No	3.86 (0.065)	3.84 (0.065)	3.82 (0.065)	3.85 (0.065)	3.80 (0.066)	3.79 (0.066)	3.87 (0.067)	3.92 (0.075)	
Antioxidant Vitamins										
α-Tocopherol, μ*g/mL*	Yes	8.42 (0.238)	8.75 (0.234)	8.63 (0.236)	8.73 (0.226)	8.51 (0.236)	8.58 (0.239)	8.58 (0.242)	8.53 (0.282)	<.0001
	No	8.06 (0.118)	8.33 (0.118)	8.20 (0.118)	8.21 (0.118)	8.13 (0.119)	8.09 (0.119)	8.12 (0.121)	7.9 2 (0.135)	
γ-Tocopherol, μ*g/mL*	Yes	1.75 (0.070)	1.72 (0.069)	1.70 (0.069)	1.61 (0.069)	1.68 (0.069)	1.73 (0.070)	1.68 (0.071)	1.64 (0.082)	<.0001
	No	1.80 (0.035)	1.85 (0.035)	1.84 (0.035)	1.85 (0.035)	1.80 (0.035)	1.85 (0.035)	1.81 (0.036)	1.80 (0.040)	
Ascorbic acid, *mg/dL*	Yes	1.87 (0.055)	1.9 4 (0.054)	1.95 (0.054)	1.92 (0.054)	1.94 (0.054)	1.96 (0.056)	1.96 (0.056)	2.00 (0.065)	<.0001
	No	1.69 (0.027)	1.74 (0.027)	1.71 (0.027)	1.74 (0.027)	1.74 (0.027)	1.75 (0.027)	1.76 (0.028)	1.70 (0.031)	
Retinol, μ*g/mL*	Yes	0.37 (0.009)	0.40 (0.008)	0.40 (0.008)	0.39 (0.008)	0.40 (0.008)	0.39 (0.009)	039 (0.009)	0.38 (0.010)	<.0001
	No	0.36 (0.004)	0.38 (0.004)	0.37 (0.004)	0.38 (0.004)	0.38 (0.004)	0.37 (0.004)	0.37 (0.004)	0.36 (0.005)	

Cycle Phase		Menses	Mid-Follicular	Late-Follicular	LH/FSH surge	Predicted ovulation	Early Luteal	Mid-Luteal	Late-Luteal	
Standardized Cycle Day		**Day 2[1]**	**Day 7**	**Day 12**	**Day 13**	**Day 14**	**Day 18**	**Day 22**	**Day 27**	
	Met 5A Day[2]	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**Mean (se)**	**p-value[3]**
Lutein, μ*g/mL*	Yes	0.14 (0.005)	0.15 (0.005)	0.15 (0.005)	0.15 (0.005)	0.15 (0.005)	0.14 (0.005)	0.14 (0.005)	0.14 (0.006)	<.0001
	No	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	0.12 (0.003)	
Lycopene, μ*g/mL*	Yes	0.44 (0.019)	0.46 (0.019)	0.47 (0.019)	0.46 (0.019)	0.46 (0.019)	0.46 (0.019)	0.48 (0.019)	0.49 (0.023)	0.6803
	No	0.46 (0.009)	0.47 (0.009)	0.47 (0.009)	0.47 (0.009)	0.47 (0.009)	0.47 (0.009)	0.48 (0.010)	0.47 (0.011)	
β-Carotene, μ*g/mL*	Yes	0.25 (0.014)	0.27 (0.013)	0.26 (0.013)	0.26 (0.013)	0.25 (0.013)	0.26 (0.014)	0.26 (0.014)	0.27 (0.016)	<.0001
	No	0.17 (0.007)	0.18 (0.007)	0.18 (0.007)	0.18 (0.007)	0.18 (0.007)	0.19 (0.007)	0.18 (0.007)	0.18 (0.008)	
β-Cryptoxanthin, μ*g/mL*	Yes	0.11 (0.006)	0.12 (0.006)	0.12 (0.006)	0.12 (0.006)	0.12 (0.006)	0.12 (0.006)	0.12 (0.006)	0.12 (0.007)	<.0001
	No	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	0.09 (0.003)	

Table Notes: N of women=258; n of cycles = 507.

Number of cycles meeting 5 A Day ranges: day 2 (96–99); day 7 (99–101); day 12 (98–101); day 13 (98–101); day 14 (97–100); day 18 (94–97); day 22 (93–96); day 27 (68–71).

Number of cycles not meeting 5 A Day ranges: day 2 (379–405); day 7 (380–404); day 12 (379–406); day 13 (379–406); day 14 (372–402); day 18 (373–397); day 22 (362–387); day 27 (289–311).

[1] Measures were standardized to cycle phase using a fertility monitor.

[2] Based on the average 24-hour recall levels for each cycle.

[3] Based on repeated measures ANOVA.

Table 3

Change in biomarkers of oxidative stress and antioxidant defense associated with meeting the 5 A Day recommendation for fruit and vegetable consumption across the menstrual cycle in the BioCycle study.

Oxidative Stress Markers	Met the 5 A Day Recommendation		
	No (reference category)	**Yes 24-hour recall timed to menstrual cycle phase[1]**	**Yes FFQ typical diet by menstrual cycle[2]**
Lipid Peroxidation			
F_2-Isoprostanes, *pg/mL*	1.00	−0.10 (−0.12, −0.07)	−0.14 (−0.18, −0.11)
9-HODE, μ*mol/L*	1.00	−0.02 (−0.08, 0.03)	−0.04 (−0.11, 0.04)
13-HODE, μ*mol/L*	1.00	−0.03 (−0.09, 0.04)	−0.08 (−0.16, 0.002)
Antioxidant Enzymes[2]			
SOD, *IU/g Hb*	1.00	−0.01 (−0.02, 0.01)	−0.004 (−0.02, 0.02)
GVx, *IU/g Hb*	1.00	0.02 (−0.01, 0.05)	−0.02 (−0.05, 0.02)
GSHR, *IU/g Hb*	1.00	0.01 (−0.003, 0.05)	−0.05 (−0.09, −0.02)
Antioxidant Vitamins			
α-Tocopherol *(μg/mL)*	1.00	0.02 (0.01, 0.04)	0.007 (−0.02, 0.03)
γ-Tocopherol *(μg/mL)*	1.00	−0.03 (−0.06, 0.001)	−0.04 (−0.08, 0.005)
Ascorbic acid *(mg/dL)*	1.00	0.10 (0.08, 0.12)	0.10 (0.07, 0.12)
Retinol *(μg/mL)*	1.00	0.001 (−0.01, 0.02)	0.01 (−0.007, 0.03)
Lutein *(μg/mL)*	1.00	0.14 (0.11, 0.18)	0.33 (0.28, 0.37)
Lycopene *(μg/mL)*	1.00	0.02 (−0.02, 0.05)	−0.04 (−0.08, 0.01)
β-Carotene *(μg/mL)*	1.00	0.30 (0.25, 0.35)	0.38 (0.32, 0.44)
β-Cryptoxanthin *(μg/mL)*	1.00	0.28 (0.24, 0.32)	0.35 (0.30, 0.40)

Table Note: Models are adjusted for age, race, income, marital status, parity, past oral contraceptive use and energy intake.

[1] Linear mixed models assessed up to 8 oxidative stress measures in association with up to 4 24-hour recall by menstrual cycle phase (dietary recall during menses was paired with oxidative stress measures during menses; dietary recall during the mid-follicular phase with mid-follicular oxidative stress measures, dietary recall on the day of predicted ovulation with the oxidative stress measures during the three peri-ovulatory period visits (late-follicular, LH/FSH surge, predicted ovulation) and dietary recall on the mid-luteal visit with early, mid, and late luteal phase oxidative stress measures) accounting for repeated measures.

[2] Linear mixed models assessed the association with FFQ for each menstrual cycle with up to 8 oxidative stress measures per cycle accounting for repeated measures.