HHS Public Access

Author manuscript

Paediatr Perinat Epidemiol. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Paediatr Perinat Epidemiol. 2018 January ; 32(1): 90–99. doi:10.1111/ppe.12403.

Antioxidant Consumption is Associated with Decreased Odds of Congenital Limb Deficiencies

Nelson D. Pace[a], **Tania A. Desrosiers**[a], **Suzan L. Carmichael**[b], **Gary M. Shaw**[b], **Andrew F. Olshan**[a], **Anna Maria Siega-Riz**[a,c,d], and **the National Birth Defects Prevention Study**

[a]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill

[b]Department of Pediatrics, Division of Neonatology and Developmental Medicine, Stanford University School of Medicine

[c]Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill

[d]Department of Public Health Sciences, University of Virginia School of Medicine

Abstract

Background—Approximately 1 in 2000 infants is born with a limb deficiency in the US. Research has shown that women's periconceptional diet and use of vitamin supplements can affect risk of birth defects. We investigated whether maternal consumption of nutritional antioxidants was associated with occurrence of transverse (TLD) and longitudinal limb deficiencies (LLD).

Methods—We analysed case-control data from mothers and their singleton infants with TLD (n=566), LLD (n=339), or no malformation (controls; n=9384) in the National Birth Defects Prevention Study (1997–2009). Using a modified food frequency, we estimated usual pre-pregnancy antioxidant consumption by total fruit and vegetable consumption (in grams) grouped into tertiles, and cumulative antioxidant score (ranging from 1–10) based on consumption of three antioxidants: beta-carotene, lycopene, and lutein. We estimated odds ratios (OR) adjusted for maternal age, race/ethnicity, education, smoking, alcohol use, body mass index, and total energy.

Results—Compared to women in the lowest tertile of fruit and vegetable consumption, women in the highest tertile were less likely to have infants with TLD (OR 0.74, 95% CI 0.57, 0.96) or LLD (OR 0.82, 95% CI 0.59, 1.13). Compared to the lowest antioxidant consumption score of 1, those with the highest score of 10 had ORs of 0.68 (95% CI 0.48, 0.95) for TLD and 0.77 (95% CI 0.50, 1.17) for LLD.

Conclusions—Dietary intake of antioxidants was associated with reduced odds of limb deficiencies. These findings add further evidence for women's periconceptional diet reducing occurrence of some birth defects.

Address for correspondence: Nelson Pace, Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC, nelson@unc.edu.
MR. NELSON DUANE PACE (Orcid ID : 0000-0002-6925-1444)
DR. ANNA MARIA SIEGA-RIZ (Orcid ID : 0000-0002-1303-4248)

Keywords

pregnancy; nutrition; birth defects; congenital anomalies; limb deficiency; diet

Introduction

Congenital limb deficiencies, defined as either partially formed or completely absent limb(s), are present in approximately 1 in 2000 infants at birth.[1,2] Limb deficiencies primarily originate during embryonic development early in the first trimester of pregnancy; at approximately 26 days of gestation, limb formation begins and continues through week eight.[3] The majority of limb deficiencies can be categorised as transverse limb deficiency (TLD) or longitudinal limb deficiency (LLD). TLD refers to a limb that has developed normally up to a particular point but beyond which no skeletal elements exist (e.g. arm with normal development from the shoulder up to the elbow with the arm ending in a bud at the elbow). LLD refers to a reduction or absence of bone in or along the length of the limb. In LLD there may be normal skeletal elements beyond the affected area (e.g. leg missing fibula). Single-gene mutations, chromosomal anomalies, and exposure to teratogenic drugs account for far fewer than half of all limb deficiencies.[4] The mechanism by which the majority of other limb deficiencies result remains poorly understood.

Though maternal diet is known to play a key role in fetal development,[5] the impact of overall maternal diet on the risk of congenital limb deficiencies specifically has not been investigated. However, a number of studies have examined whether folic acid, multivitamin supplementation, or individual nutrients may reduce the risk of limb deficiencies and some, but not all, studies have found a pattern of lower risk.[2,6,7]

In addition to overall healthy diet and specific nutrients like folic acid, antioxidants may be an important dietary component to consider in preventing congenital anomalies. Antioxidants act to reduce oxidative stress by inhibiting the formation of reactive oxygen species (ROS) or reducing the number of free radicals formed, thereby possibly preventing some congenital anomalies caused by this mechanism as it relates to both cell proliferation and apoptosis during embryogenesis.[8–10]

The relation between dietary antioxidant intake and limb deficiencies has not been investigated. The objective of this study was to investigate whether maternal antioxidant consumption is associated with occurrence of TLD or LLD.

Methods

We conducted an analysis using data from the National Birth Defects Prevention Study (NBDPS), a multi-state, population-based, case-control study of more than 30 major structural birth defects. Details of the NBDPS design and data collection protocol are published.[11] Briefly, population-based surveillance programs in each participating state (Arkansas, California, Georgia, Iowa, Massachusetts, North Carolina, New Jersey, New York, Texas, and Utah) were used to identify eligible cases of birth defects as well as live-born infants without a birth defect (controls). Cases were live births, stillbirths, or induced

abortions; controls were live births only, sampled using birth certificates or hospital records to represent the base population from which the cases arose. In this analysis, we included cases and controls with an estimated date of delivery between October 1997 and December 2009.

Maternal interviews were conducted via telephone using a standardised computer-assisted interview available in either English or Spanish. The interview collected socio-demographic, clinical, and dietary information among other things by maternal recall. Interviews were administered after 6 weeks or more had passed since the infant's estimated date of delivery (EDD) and no later than 2 years after the EDD. Participation was similar among cases (67%) and controls (65%) in NBDPS.[11] The median time since EDD to interview was 10 months for TLD, 11 months for LLD, and 8 months for controls.

Case Classification

Cases of limb deficiencies identified by each center were reviewed for eligibility by clinical geneticists at each center, and then their mothers were recruited to participate in the NBDPS interview. A secondary review of all cases across all centers was undertaken to systematically classify cases of TLD and LLD; each case was further specified as having an isolated defect (1 major limb deficiency), multiple (≥2 major yet unrelated birth defects of which at least one was a limb deficiency), or complex (≥2 major birth defects which are suspected to be related of which at least one was a limb deficiency).[12] Further, cases with chromosomal abnormalities or with recognised or strongly suspected single-gene disorders or syndromes were excluded. Eligible cases had the following British Pediatric Association diagnostic codes: TLD (755.200–209, 755.240–249, 755.300–309, 755.340–349) and LLD (755.250–279, 755.350–379) (see Table S1 for a complete list of eligible limb deficiencies). In fifteen cases, TLD and LLD were present concurrently and as such these cases were included in the analyses for TLD and again for LLD.

Exposure Assessment

As part of the maternal interview, a modified 63-item Willet food frequency questionnaire (FFQ) was administered to collect information on maternal diet in the year before pregnancy.[13] Response options included 16 levels of frequency ranging from "never" to "6 or more [servings] per day". Additional questions assessed consumption of cereals, soda, coffee, and tea. Quantity of each food consumed per day was calculated as average grams/day based on standard portion sizes. Nutrient intakes were calculated using the US Department of Agriculture (USDA) nutrient database, version 25.[14] Calculated values for specific nutrient intake were based on foods consumed and did not consider intake of the nutrient from either vitamin supplements or food supplements (e.g. meal replacement shake, protein bar).

Maternal antioxidant consumption was assessed using two independent methods, which examine antioxidant intake at the level of major food groups (Method 1) as well as individual nutrients (Method 2).

Method 1, Total Fruits and Vegetables—Fruit and vegetables are the major source of antioxidants in our daily diet.[15] We calculated total fruit and vegetables consumption by summing estimated grams per day consumed for the 21 fruit and vegetable FFQ items. We created tertiles of consumption of total fruits and vegetables (high/med/low) based on the distribution among eligible controls. In analyses, the lowest tertile was the reference category.

Method 2, Cumulative Antioxidant Score—To estimate an antioxidant score, we selected three key antioxidants, beta-carotene, lycopene, and lutein, based on their prominent consumption in an average diet and potential for accurate estimation. We calculated an antioxidant score based on consumption of these three nutrients with the following steps: (1) We summed estimated nutrient consumption per day across all food items for each of the three antioxidants; (2) Based on the distribution of the three antioxidants in controls, we created cut-points for quartiles of consumption. Each participant received a score based on their quartile of consumption for each antioxidant (1^{st} – 4^{th} quartile (lowest to highest) are scored 0, 1, 2, 3 respectively); (3) The score for each antioxidant (minimum score of 0, maximum score of 3) is summed across the three nutrients, resulting in a cumulative antioxidant score. This score (+1 to facilitate statistical modeling) was then assigned to each study participant (cumulative minimum score of 1, cumulative maximum score of 10). In analyses, we treated cumulative antioxidant score as a continuous variable.

Statistical Analysis

To estimate the association between antioxidant consumption and limb deficiencies, we fit separate unconditional logistic regression models for TLD and LLD to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each method: with antioxidant consumption categorised in tertiles of total grams of fruits and vegetables (Method 1), and then for cumulative antioxidant score (Method 2). The following variables, selected a priori based on subject matter expertise and use of causal directed acyclic graphs (Figure S1),[16] were included in adjusted models to control for confounding: maternal age (continuous, squared), maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), maternal education (≤high school or >high school), pre-pregnancy body mass index (BMI) (four categories: underweight, normal, overweight, obese),[17] smoking (any or none while pregnant), and alcohol use (any or none 1 month prior to pregnancy through the first trimester). We also adjusted for total energy intake.[18] Additionally, we explored possible effect measure modification and confounding by folic acid-containing supplement use (any or none) anytime from 1 month prior to pregnancy through the second month of pregnancy. Covariate information was obtained during the interview. Effect measure modification was evaluated for statistical significance of the interaction terms and through visual inspection of a graph plotting antioxidant consumption and the odds ratio of limb deficiency by folic acid use.

To assess model fit by the form of the exposure variable, we allowed for flexibility in modeling the cumulative antioxidant score (i.e. quadratic, cubic, and categorical treatment of the cumulative antioxidant score). The same was done for total fruit and vegetable

consumption (i.e. continuous, quadratic, quartiles of consumption). We also included other antioxidants (Vitamin C and Vitamin E), a pro-oxidant (iron), and folic acid supplementation when assessing model fit to see if these additional nutrients could further explain the exposure-outcome relation. If strong attenuation of exposure-outcome relation occurred when adding these nutrients to the model, collinearity would be present.

Our analysis included 890 cases (81% of available cases) and 9,384 controls (91%) after applying the following exclusion criteria: (1) limb deficiency other than transverse or longitudinal (n=56); (2) multiple/unknown number of babies (n=394); (3) pre-pregnancy Type I or Type II diabetes (n=91); (4) folate antagonist medication use (n=101); (5) first-degree family history of limb deficiencies (n=33); (6) extreme or out-of-range caloric intake (<1st or >99th percentile, based on controls) (n=378); (7) more than one missing food item on the FFQ (n=222). Among the 566 cases of TLD and 339 cases of LLD included in the analysis, approximately 73% were cases of isolated limb deficiencies, and the majority were livebirths (95%).

Results

In our first approach to capturing antioxidant consumption, we determined tertiles (high, medium, low) of fruit and vegetable consumption. The lowest tertile consumed less than 342 grams per day of fruits and vegetables, while those in the highest tertile consumed more than 567 grams per day (see Table 1: Method 1). The shape of the distributions of total gram for fruit and vegetable consumption and the three antioxidants were similar among cases and controls (further details in Table S2). Our second approach created quartiles of estimated nutrient intake for the three key antioxidants with scores based on the quartiles with cumulative antioxidant score ranging from 1 to 10. The different nutrients had slightly different distributions, and all were reported in micrograms (further details in Table 1: Method 2).

Control and case mothers had similar distributions of age, BMI, use of folic acid supplementation, and caloric intake (Table 2). Hispanics were more represented among cases while Non-Hispanic white mothers were more represented among controls. Control mothers had slightly higher education attainment: 58.6% attended college compared to 55.2% among case mothers. Also, 18.0% of control mothers smoked sometime during pregnancy compared to 20.1% of case mothers. Control mothers had slightly elevated alcohol consumption (36.6%) compared to case mothers (33.6%). There was some variation by center in the distribution of cases and controls. Live births, stillbirths (fetal deaths ≥20 weeks' gestation), and infants delivered as result of an induced abortion made up 95.3%, 1.9%, and 2.8% of limb deficiency cases, respectively.

Results for Method 1

Maternal consumption in the highest tertile of fruit and vegetable consumption was associated with an approximately 27% (95% confidence Interval (CI): 5, 44) reduced odds of having children born with TLDs compared to mothers who were in the lowest tertile of fruit and vegetable consumption over the study period after adjustment for potential confounding. The highest tertile compared to the lowest tertile was associated with an

approximately 17% (95% CI −14, 40) reduced odds of having a child born with LLD. The observed association was stronger in magnitude for the highest tertile than for the middle tertile, indicating a possible dose-response, but confidence intervals overlapped considerably (see Table 3). No meaningful difference in the association was observed when isolated and non-isolated defects were considered separately for either TLD or LLD.

Results for Method 2

Using the cumulative antioxidant score, we analysed the relation between antioxidant consumption in the year before pregnancy and the risk of limb deficiencies. We assumed a linear relation of cumulative antioxidant score with odds of limb deficiency. To test this assumption, we tried more flexible forms of the exposure variable (e.g. quadratic, higher order polynomials, categories) all of which resulted in no measureable improvement of model fit. Thus, Figure 1 presents the graphical depiction of the linear relation between the odds of TLD or LLD and antioxidant consumption as measures by cumulative antioxidant score. Increased consumption of antioxidants is associated with reduced odds of both types of limb deficiencies.

Mothers with the highest antioxidant score of 10 had an approximately 33% (95% CI: 6–47%) reduced odds of having a pregnancy affected by TLD compared to mothers with a lowest score of 1 (Table 4). Mothers with the highest score of 10 had an approximately 27% (95% CI: −13–52%) reduced odds of having a pregnancy affected by LLD compared to mothers with a lowest score of 1. Mothers with a score of 10 (6.7% of cases and 7.2% of controls) were in the highest quartiles consumption for beta-carotene, lycopene, and lutein/zeaxanthin consumption. Mothers with a score of 1 (9.6% of cases and 7.5% of controls) were in the lowest quartile of consumption for all 3 antioxidants. A similar pattern of decreased odds of TLD and LLD with increased antioxidant consumption is seen, for non-isolated, and isolated deficiency categories, although precision was reduced with these smaller sample sizes (Table 4).

Periconceptional folic acid supplementation (FA) did not appear to significantly modify the effect of high antioxidant consumption in these data (Likelihood Ratio Test: $p>0.05$). Adjusting for potential confounding by FA did not alter the exposure-outcome relation previously indicated (Table S3). Neither adjustment for FA as a potential confounder nor treatment of FA as a potential effect modifier changed the observed results for fruit and vegetable consumption or cumulative antioxidant score.

Comment

Main findings

Higher intake of antioxidants in the maternal diet – assessed by two methods – was associated with a reduced odds of congenital limb deficiencies. This association tended to be stronger for transverse than longitudinal limb deficiencies.

When considering antioxidants, there are a wide variety of nutrients that have antioxidant properties. Some antioxidants have been consistently associated with better health outcomes (i.e. cancer prevention, cardiovascular health) while others have not.[19] The largest source of

antioxidants in the average American adult diet is coffee and tea. A prior analysis of NBDPS data found no convincing evidence of coffee and tea being related to risk of selected birth defects (not including limb deficiency) when considering their contribution to maternal intake of caffeine.[20] This study did not to concentrate on these forms of antioxidants (i.e. flavonoids and other polyphenols) as they have not been associated with risk of birth defects. [20] Further, mothers tend to decrease their consumption of these beverages, during pregnancy. [21] Instead it focused on food groups that contribute to antioxidant intake (fruits and vegetables) and three specific antioxidants (beta-carotene, lycopene, and lutein).

The mechanism by which antioxidants might decrease the risk for limb deficiency and particularly for TLD is unknown. One pathway by which antioxidants might decrease risk is the reduction of ROS. ROS overproduction often leads to a deleterious process, called oxidative stress, which can cause damage to cell structures (i.e. lipids and membranes, proteins, and DNA)[8]. With limb deficiencies in particular, oxidative stress can lead to damaged cells causing a reduction in cell proliferation and interfering with cell signaling pathways. Consequently abnormal limb formation may occur in the developing embryo and fetus such as a shortened limb (e.g. leg ending at the knee). ROS have been associated with many negative health outcomes such as teratogenesis[10] (including limb deficiency),[22] carcinogenesis[23], cardiovascular disease, and neurogenerative diseases.[8,24]

Interpretation of the findings

In comparing high to low tertiles of fruit and vegetable consumption, women in the highest tertile were less likely to have an infant with TLD. From a public health messaging perspective, the difference in grams between the lowest and highest tertile of consumption is similar to 2 to 3 servings (0.7 to 1.0 cup equivalents). The U.S. Food Patterns corresponding to the 2010 U.S. Dietary Guidelines[31] recommends consuming daily 2 cups of fruits and 2.5 cups of vegetables (based on a 2,000 calorie diet). A mother who consumed the daily recommended intake of fruits and vegetables[31] would be around the cut-point for the high and medium tertile. These results support existing fruit and vegetable consumption recommendations.[31] The relation observed suggests, should a causal relation exist, an increase of approximately 2.5 additional servings per day among low fruit and vegetable consuming moms could have a population-level impact on the prevalence of congenital limb deficiencies.

Limitations of the data

This study had some limitations. First, the FFQ used to determine antioxidant exposure asked about average food intake during the year before pregnancy, and while most diets remain very similar during pregnancy,[25–27] it is not known if this is the case in this study sample. That said, it is unlikely for usual diet to change substantially in the first few weeks of pregnancy, before pregnancy recognition and before nausea and vomiting which may then lead to some changes in diet. Further the diet prior to pregnancy itself is important to consider as foods eaten in the months prior to conception would impact the nutritional stores used during pregnancy. Second, the self-reported nature of the questionnaire may lead to potential bias of effect estimation by two means (1) non-differential misclassification of diet due to the difficulty in accurately recalling a distant past behavior (e.g. >1.5 years

previously) and (2) differential misclassification of diet by case versus control status. The direction of the latter form of misclassification cannot be determined with the data available and could, in this study, either attenuate (e.g. a mother with an infant born with a limb deficiency might be hesitant to recall a less than health diet) or intensify (e.g. said mother might be more critical of her own diet and thereby recall a poorer diet compared to a control mother) the magnitude of the estimates. Was the differential misclassification to bias estimates away from the null, it is unlikely the misclassification would explain the observed associations and trends in their entirety. Lastly, while a significant association is detected at the level of food groups and cumulative antioxidant score, one cannot separate out the effect of a dietary pattern that is high in antioxidants from the antioxidants themselves.

That said, findings remained consistent in several sensitivity analyses in models that included other antioxidants (Vitamin C and Vitamin E), a pro-oxidant (iron), and allowed for flexibility of exposure modelling, demonstrating stability of the exposure-outcome relation (data not shown). Further, the reduced risk of observed with antioxidants was not diminished when FA supplementation was included in models as a covariate. In post-hoc analyses that included FA supplementation as a covariate there was no change in the magnitude of the association, but the precision of the estimates improved suggesting that FA supplementation may only being associated with the outcome not antioxidant intake.[28]

Supplemental forms of beta-carotene, lycopene, and lutein were not included in nutrient consumption estimates. While beta-carotene is present in many multivitamins, lycopene and lutein are not typically found in multivitamins and are not common supplements. If supplementation had an effect on the odds of limb deficiency, it is anticipated that this would have diluted the odds ratio estimates.

Strengths of the study

There are several strengths of this study. Extensive surveillance paired with a thorough questionnaire in the NBDPS allowed for maternal diet to be examined in relation to the risk of a rare birth defect, congenital limb deficiencies. Second, the representative nature of the study due to population-based sampling and 10 states contributing data increases confidence in generalising results for multiple regions of the United States. Indeed, Cogswell et al. found that NBDPS controls were representative of their base populations in many ways.[29] Further, the geographic, temporal, and demographic diversity of the NBDPS sample captures a large degree of variability in diet, allowing for assessment of a wider range of antioxidant exposure. Third, the limb deficiency case definition was based on strict inclusion/exclusion criteria and systematic classification. Individual case review by a single clinical geneticist limits the potential for outcome misclassification. Fourth, the etiologies of transverse limb deficiencies (TLD) and longitudinal limb deficiencies (LLD) are suspected to be different,[6,30] and the NBDPS provided sufficient sample size to examine each phenotype individually.

Conclusions

Findings from this study indicate that higher maternal intake of fruits and vegetables and higher intake of antioxidants in the diet in the year before pregnancy are associated with a reduced odds of congenital limb deficiencies, particularly TLD, in offspring. These findings

add further evidence to the importance of the quality of a women's periconceptional diet in reducing the occurrence of some birth defects. The literature on maternal nutrition and birth defects would benefit from a detailed examination of the role of antioxidants in relation to other birth defects including rarer forms of limb deficiencies (i.e. intercalary deficiencies) not examined here. Further investigation of causal mechanisms by which antioxidants may act as protective agents in birth defect etiologies is needed. Replication of this research in other studies to examine consistency of results is recommended.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the contributions of Richard Olney (Division Chief, Genetic Disease Screening Program, California Department of Public Health), who classified all limb deficiency cases for the NBDPS and provided expert consultation on this project. We thank the NC Center for Birth Defects Research and Prevention (NCCBDRP) for preparing data for these analyses and all NBDPS centers for collecting this data as well as the mothers who participated to make this research possible. In particular, we acknowledge the incredible work of Kathy Wisniewski in preparing the analytic dataset and replicating results. We also thank Sydney Thai for his helpful feedback on conceptual framework of the nutrient analysis. We also acknowledge the work of the Nutrition Epidemiology Core of the University of North Carolina Clinical Nutrition Research Center for help with the nutrient database (Grant #DK56350). Coding of drug information in the NBDPS used the Slone Drug Dictionary under license from the Slone Epidemiology Center of Boston University.

We thank the California Department of Public Health Maternal Child and Adolescent Health Division for providing data, and the California Center for Birth Defects Research and Prevention (CA CBDRP) for coding all the self-reported data on folic acid supplement intake. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the California Department of Public Health.

Funding

This work was supported through cooperative agreements under PA 96043, PA 02081 and FOA DD09-001 from the Centers for Disease Control and Prevention to the Centers for Birth Defects Research and Prevention participating in the NBDPS including cooperative agreement U50CCU422096 to the NCCBDRP. While conducting this research, Mr. Pace was supported by the NIH/NICHD T32HD052468 Training Program in Reproductive, Perinatal, and Pediatric Epidemiology, and the Royster Society of Fellows, University of North Carolina at Chapel Hill – The Graduate School.

References

1. Rynn L, Cragan J, Correa A. Update on overall prevalence of major birth defects—Atlanta, Georgia, 1978–2005. Morbidity and Mortality Weekly Report. 2008; 57:1–5. [PubMed: 18185492]
2. Robitaille J, Carmichael SL, Shaw GM, Olney RS. Maternal nutrient intake and risks for transverse and longitudinal limb deficiencies: data from the National Birth Defects Prevention Study, 1997–2003. Birth Defects Research Part A: Clinical and Molecular Teratology. 2009; 85:773–779. [PubMed: 19350655]
3. Cuccurullo, SJ. Physical medicine and rehabilitation board review. Demos Medical Publishing; 2014.
4. McGuirk CK, Westgate MN, Holmes LB. Limb deficiencies in newborn infants. Pediatrics. 2001; 108:E64. [PubMed: 11581472]
5. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. The Journal of nutrition. 2004; 134:2169–2172. [PubMed: 15333699]
6. Yang Q, Khoury MJ, Olney RS, Mulinare J. Does periconceptional multivitamin use reduce the risk for limb deficiency in offspring? Epidemiology. 1997:157–161. [PubMed: 9229207]

7. Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. American Journal of Medical Genetics. 1995; 59:536–545. [PubMed: 8585581]
8. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 2007; 39:44–84. [PubMed: 16978905]
9. Wan J, Winn LM. In utero–initiated cancer: The role of reactive oxygen species. Birth Defects Research Part C: Embryo Today: Reviews. 2006; 78:326–332.
10. Wells PG, Bhuller Y, Chen CS, Jeng W, Kasapinovic S, Kennedy JC, et al. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicology and applied pharmacology. 2005; 207:354–366. [PubMed: 16081118]
11. Reefhuis J, Gilboa SM, Anderka M, Browne ML, Feldkamp ML, Hobbs CA, et al. The national birth defects prevention study: a review of the methods. Birth Defects Research Part A: Clinical and Molecular Teratology. 2015; 103:656–669. [PubMed: 26033852]
12. Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA. Guidelines for case classification for the national birth defects prevention study. Birth Defects Research Part A: Clinical and Molecular Teratology. 2003; 67:193–201. [PubMed: 12797461]
13. Willett WC, Reynolds RD, Cottrell-Hoehner S, Sampson L, Browne ML. Validation of a semi-quantitative food frequency questionnaire: comparison with a 1-year diet record. Journal of the American Dietetic Association. 1987; 87:43–47. [PubMed: 3794132]
14. U.S. Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 25. 2012.
15. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agricultural and Food Chemistry. 2004; 52:4026–4037. [PubMed: 15186133]
16. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999:37–48. [PubMed: 9888278]
17. National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. Obesity research. 1998; 6(Suppl 2): 51S–209S. [PubMed: 9813653]
18. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. The American Journal of Clinical Nutrition. 1997; 65:1220S–1228S. discussion 1229S–1231S. [PubMed: 9094926]
19. Schmidt HH, Stocker R, Vollbracht C, Paulsen G, Riley D, Daiber A, et al. Antioxidants in translational medicine. Antioxidants & redox signaling. 2015; 23:1130–1143. [PubMed: 26154592]
20. Browne ML, Hoyt AT, Feldkamp ML, Rasmussen SA, Marshall EG, Druschel CM, et al. Maternal caffeine intake and risk of selected birth defects in the National Birth Defects Prevention Study. Birth Defects Research Part A: Clinical and Molecular Teratology. 2011; 91:93–101. [PubMed: 21254365]
21. Peck JD, Leviton A, Cowan LD. A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: a 2000–2009 update. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2010; 48:2549–2576. [PubMed: 20558227]
22. Lee CJ, Goncalves LL, Wells PG. Embryopathic effects of thalidomide and its hydrolysis products in rabbit embryo culture: evidence for a prostaglandin H synthase (PHS)-dependent, reactive oxygen species (ROS)-mediated mechanism. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2011; 25:2468–2483. [PubMed: 21502285]
23. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. Apmis. 2007; 115:81–103. [PubMed: 17295675]
24. Devore EE, Feskens E, Ikram MA, den Heijer T, Vernooij M, van der Lijn F, et al. Total antioxidant capacity of the diet and major neurologic outcomes in older adults. Neurology. 2013; 80:904–910. [PubMed: 23427318]

25. Cuco G, Fernandez-Ballart J, Sala J, Viladrich C, Iranzo R, Vila J, et al. Dietary patterns and associated lifestyles in preconception, pregnancy and postpartum. European journal of clinical nutrition. 2006; 60:364–371. [PubMed: 16340954]
26. Sotres-Alvarez D, Herring AH, Siega-Riz AM. Latent transition models to study women's changing of dietary patterns from pregnancy to 1 year postpartum. American Journal of Epidemiology. 2013; 177:852–861. [PubMed: 23538942]
27. Rifas-Shiman SL, Rich-Edwards JW, Willett WC, Kleinman KP, Oken E, Gillman MW. Changes in dietary intake from the first to the second trimester of pregnancy. Paediatric and perinatal epidemiology. 2006; 20:35–42. [PubMed: 16420339]
28. Brookhart MA, Schneeweiss S, Rothman KJ, Glynn RJ, Avorn J, Sturmer T. Variable selection for propensity score models. American Journal of Epidemiology. 2006; 163:1149–1156. [PubMed: 16624967]
29. Cogswell ME, Bitsko RH, Anderka M, Caton AR, Feldkamp ML, Hockett Sherlock SM, et al. Control selection and participation in an ongoing, population-based, case-control study of birth defects: the National Birth Defects Prevention Study. American Journal of Epidemiology. 2009; 170:975–985. [PubMed: 19736223]
30. Holmes LB. Teratogen-induced limb defects. American Journal of Medical Genetics. 2002; 112:297–303. [PubMed: 12357474]
31. US Department of Agriculture, US Department of Health and Human Services. Dietary guidelines for Americans, 2010. 2010.

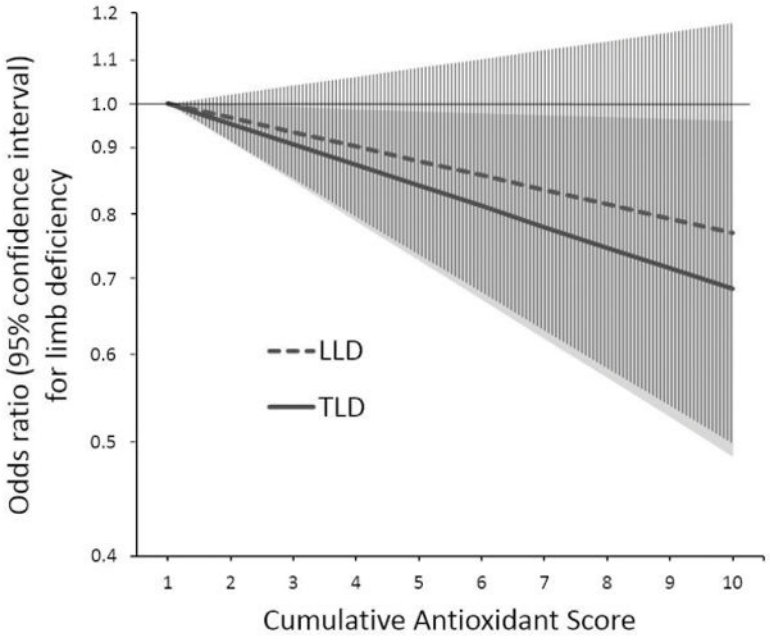

Figure 1.

Table 1

Description of the two methods used to measure antioxidant consumption, National Birth Defects Prevention Study, 1997–2009.

	Method 1		Method 2	
Characteristic	**Total Fruits and Vegetables**		**Cumulative Antioxidant Score**	
Description	Total consumption of fruits and vegetables is quantified based on the FFQ information.		Score (0–3) assigned based on quartile for each estimated nutrient intake of three key antioxidants. The 3 scores are added plus 1 additional point to calculate a cumulative score.	
Units (min, max)	Grams	(0.0, 5611)	Points[a]	(1, 10)
Quantiles[b]	Fruit and Vegetable consumption (g)		Nutrient intake (μg) quartiles needed for a score of (0,1,2,3)	
	High Tertile	>566.7	β-carotene	(≤1161, >1161 and ≤2167, >2167 and ≤3756, >3756)
	Middle Tertile	>341.6 and 566.7≤	Lycopene	(≤547, >547 and ≤1994, >1994 and ≤4152, >4152)
	Low Tertile	≤341.6	Lutein	(≤894, >894 and ≤1441, >1441 and ≤2409, >2409)

[a]Total score to represent the cumulative antioxidant score calculated as the sum of the scores for each antioxidant plus 1

[b]Based on Food Frequency Questionnaire data among controls eligible for analysis (n=9384)

Table 2

Demographic Characteristics of Cases of Congenital Limb Deficiency and Controls in the National Birth Defects Prevention Study, 1997–2009.

Maternal Characteristic	Cases No. (%)/Mean (sd) (N=890)	Controls No. (%)/Mean (sd) (N=9384)
Age at conception, yr	26.5 (6.0)	26.9 (6.0)
min, max	13, 45	13, 49
Race/ethnicity		
Non-Hispanic White	491 (55.2)	5489 (58.5)
Non-Hispanic Black	89 (10.0)	1012 (10.8)
Hispanic	257 (28.9)	2210 (23.6)
Other	53 (6.0)	667 (7.1)
Missing	0 (0.0)	6 (0.1)
Education		
≤ High school	391 (43.9)	3827 (40.8)
> High school	491 (55.2)	5499 (58.6)
Missing	8 (0.9)	53 (0.6)
Prepregnancy Body Mass Index		
Underweight (<18.5)	44 (4.9)	490 (5.2)
Normal (≥18.5, <25)	441 (49.6)	4880 (52.0)
Overweight (≥25, <30)	203 (22.8)	2060 (22.0)
Obese (≥30)	158 (17.8)	1560 (16.6)
Missing	44 (4.9)	394 (4.2)
Alcohol[a]		
Yes	299 (33.6)	3437 (36.6)
No	583 (65.5)	5885 (62.7)
Missing	8 (0.9)	62 (0.7)
Smoke[b]		
Yes	179 (20.1)	1685 (18.0)
No	704 (79.1)	7672 (81.8)
Missing	7 (0.8)	27 (0.3)
Folic Acid Supplementation[c]		
Yes	666 (74.8)	7015 (74.8)
No	224 (25.2)	2368 (25.2)
Missing	0 (0.0)	1 (0.0)
Energy, kcal	1661.2 (711.6)	1669.1 (698.5)
min, max	535.9, 4694.9	502.7, 4898.8

[a] Drinking alcohol anytime between 1 month before pregnancy through the first trimester

[b] Anytime during pregnancy

[c] Folic acid supplementation anytime from 1 month prior to pregnancy through the second month of pregnancy

Table 3

Odds ratios for the association between tertile[a] of fruit and vegetable consumption and odds of limb deficiency among offspring, National Birth Defects Prevention Study, 1997–2009.

		Selected Limb Deficiencies (n: TLD=566, LLD=339)	Non-isolated only (n: TLD=89, LLD=153)	Isolated only (n: TLD=477, LLD=186)
Model		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Unadjusted models				
TLD	High	0.8 (0.7, 1.0)	1.0 (0.6, 1.6)	0.8 (0.6, 1.0)
	Mid	0.9 (0.7, 1.1)	0.8 (0.5, 1.4)	0.9 (0.7, 1.1)
	Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
LLD	High	0.8 (0.6, 1.1)	0.8 (0.6, 1.2)	0.9 (0.6, 1.3)
	Mid	1.1 (0.8, 1.4)	0.9 (0.6, 1.3)	1.3 (0.9, 1.8)
	Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Adjusted models[b]				
TLD	High	0.7 (0.6, 0.9)	0.6 (0.3, 1.1)	0.8 (0.6, 1.0)
	Mid	0.9 (0.7, 1.1)	0.7 (0.4, 1.2)	0.9 (0.7, 1.2)
	Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
LLD	High	0.8 (0.6, 1.2)	0.9 (0.5, 1.4)	0.8 (0.5, 1.3)
	Mid	1.1 (0.8, 1.4)	0.9 (0.6, 1.4)	1.2 (0.9, 1.8)
	Low	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)

[a]Tertiles based off fruit and vegetable consumption among controls; see Table 1 for more details.

[b]Adjusted for maternal race/ethnicity, education, age, body mass index, smoking, alcohol and caloric intake.

Table 4

Odds ratios for the association between cumulative antioxidant scores[a] of 10 and 5 compared to 1 and odd of limb deficiency among offspring, National Birth Defects Prevention Study, 1997–2009.

		Selected Limb Deficiencies (n: TLD=566, LLD=339)	Non-isolated (n: TLD=89, LLD=153)	Isolated only (n: TLD=477, LLD=186)
Model		**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Unadjusted models				
TLD	10	0.7 (0.5, 0.9)	1.2 (0.6, 2.5)	0.6 (0.5, 0.9)
	5	0.9 (0.8, 1.0)	1.1 (0.8, 1.5)	0.8 (0.7, 0.9)
	1	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
LLD	10	0.7 (0.5, 1.0)	0.6 (0.3, 1.0)	0.8 (0.5, 1.3)
	5	0.9 (0.7, 1.0)	0.8 (0.6, 1.0)	0.9 (0.7, 1.1)
	1	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
Adjusted models[b]				
TLD	10	0.7 (0.5, 0.9)	0.9 (0.4, 2.0)	0.6 (0.4, 0.9)
	5	0.8 (0.7, 1.0)	0.9 (0.6, 1.4)	0.8 (0.7, 1.0)
	1	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)
LLD	10	0.7 (0.5, 1.1)	0.6 (0.3, 1.1)	0.9 (0.5, 1.6)
	5	0.9 (0.7, 1.1)	0.8 (0.6, 1.1)	0.9 (0.7, 1.2)
	1	1.0 (Reference)	1.0 (Reference)	1.0 (Reference)

[a] Higher scores indicate greater consumption of antioxidants

[b] Adusted for maternal race/ethnicity, education, age, body mass index, smoking, alcohol consumption and caloric intake.