Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults

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ABSTRACT

JOANNE L. WATTERS: Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults
(Under the direction of Jessie A. Satia, PhD, MPH)

Cancer is the leading cause of death for those under 85 years of age in the United States. All-cause cancer rates are higher for African Americans than other racial or ethnic groups; however, the reasons for this disproportionately high cancer burden are not well understood. Diets high in fruits and vegetables have been associated with lower risk of many cancers. One mechanism by which diet may reduce cancer risk is through consumption of antioxidant nutrients, which decrease the adverse effects of reactive oxygen species (ROS) on normal physiological functions. High ROS levels can lead to oxidative stress, in which the imbalance of radical-generating agent concentrations exceeds the body's defense mechanisms. Under conditions of elevated oxidative stress (e.g., low antioxidant intakes) defenses may be overwhelmed and excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, and ultimately carcinogenesis.

Using data from a generally healthy sample of African American and White adult participants in the DIet, Supplements, and Health (DISH) study (n=164, 51% African American), we examined potential racial differences in antioxidant (vitamin C, vitamin E, and carotenoids) intakes/blood concentrations and oxidative DNA damage; associations between plasma antioxidant concentrations and oxidative DNA damage; and demographic, behavioral, and psychosocial correlates of individual antioxidant concentrations and

oxidative DNA damage. In addition, we determined psychosocial correlates of fruit and vegetable (antioxidant rich foods) intakes in African Americans in a cross-sectional study of African Americans ages 18 to 70 (n=658). This research fills important gaps in knowledge by contributing information about potential racial differences in 1) antioxidant intakes and blood concentrations, 2) oxidative stress levels, 3) associations between antioxidant concentrations and oxidative stress, 4) demographic, behavioral, and psychosocial factors that influence blood concentrations of antioxidants and oxidative DNA damage levels and also those of antioxidant-rich foods. The identification of modifiable factors (e.g., diet), mechanisms of carcinogenesis (e.g., oxidative DNA damage), and/or mediating factors that contribute to these factors (e.g., psychosocial factors) are critical for the design and implementation of cancer prevention and control programs to reduce the disparate cancer burden among African Americans.

In loving memory of the strong women who came before,

Josephine Pearl Neher Watters

Vera Rogers Maxwell

and in honor of the ones they inspired, who in turn, continue to inspire me.

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I. Introduction

A. Background

Cancer is the leading cause of death for those under 85 years of age in the United States[1]. All-cause cancer incidence and mortality rates are higher for African Americans than other racial or ethnic groups[2]. It is likely that a combination of many lifestyle, demographic, environmental, and genetic factors contribute to these disparate health risks; however, the reasons for the high cancer burden among African Americans are not well understood. Diets high in fruits and vegetables have been associated with lower risk of many cancer sites, including lung, colon, esophagus, stomach, and breast[3-5]. Although the protective relationship of fruits and vegetables is well documented, it is still unclear which elements within fruits and vegetable are responsible for the beneficial effect. One mechanism by which it is hypothesized that diet reduces cancer risk is through consumption of antioxidant nutrients, which are substances found within many foods, such as fruits and vegetables that decrease the adverse effects of reactive oxygen species (ROS) on normal physiological functions[6]. Oxidation of lipids, proteins, and nucleic acids (i.e., oxidative stress) may be causally related to the incidence of many chronic diseases, including cancer. If so, then antioxidants should mitigate the occurrence of these conditions. However, there remain significant gaps in knowledge. For example, not much is known about associations between antioxidant nutrients and oxidative stress in healthy (i.e., cancer-free) persons, factors that contribute to the blood levels of antioxidant nutrient and oxidative stress, and how demographic and psychosocial factors influence the consumption of antioxidant-rich foods, such as fruits and vegetables. Regrettably, there is even less information about

potential racial differences among these relationships of antioxidants, oxidative stress, and cancer risk.

This work provides information about potential racial differences in antioxidant (vitamin C, vitamin E, and carotenoids) intakes/blood concentrations and oxidative DNA damage, as well as the association between plasma antioxidant concentrations and oxidative DNA damage among healthy African American and White adults. Demographic, behavioral, and psychosocial correlates of individual antioxidant concentrations and oxidative DNA damage were also examined in a sample of healthy Whites and African Americans, using data from the the DIet, Supplements, and Health (DISH) study (n=164). In addition, we determined psychosocial correlates of fruit and vegetable (antioxidant rich foods) intakes in African Americans, in a cross-sectional study of African Americans ages 18 to 70 (n=658). This research fills important gaps in knowledge by contributing information about potential racial differences in 1) antioxidant intakes and blood concentrations, 2) oxidative stress levels, 3) associations between antioxidant concentrations and oxidative stress, 4) demographic, behavioral, and psychosocial factors that influence blood concentrations of antioxidants and oxidative DNA damage levels and also those of antioxidant-rich foods, and thus, may provide mechanistic support for the higher cancer burden in African Americans than Whites.

B. Research Aims

The overall goal of this work was to improve our understanding of antioxidant intake and oxidative stress levels among African Americans in North Carolina, which may

contribute to higher cancer rates for African Americans. As Whites are the most frequently studied racial/ethnic group and have lower cancer rates than African Americans, we chose to compare these two races. To do this, we examined whether racial differences existed in the levels of specific antioxidants (i.e., carotenoids, vitamin C, and vitamin E) and oxidative DNA damage levels in a sample of healthy African American and White adults. We then determined the demographic, behavioral, and psychosocial correlates of plasma antioxidant concentrations and oxidative DNA damage among this sample of African Americans and Whites and whether the correlates differed by race. Finally, we examined psychosocial factors associated with the dietary intake of fruits and vegetables (i.e., antioxidant-rich foods) in a sample of African Americans.

The **specific aims** of this work are to:

1. Determine whether antioxidant nutrient status, as measured by dietary estimates and blood levels of antioxidant nutrients, differs by race in a sample of healthy adults. The antioxidant nutrients to be evaluated include carotenoids (α-carotene, β-carotene, lycopene, lutein+zeaxanthin, β-cryptoxanthin), vitamin C, and vitamin E.
2. Determine whether oxidative DNA damage (measured as the mean comet tail moment) in healthy adults differs by race.
3. Examine associations of plasma antioxidant concentrations with oxidative DNA damage in lymphocytes, and determine whether the associations differ by race.
4. Identify demographic, behavioral, and psychosocial correlates of plasma antioxidant concentrations and oxidative DNA damage and whether these correlates differ by race.
5. Identify psychosocial correlates of the intake of fruits and vegetables, i.e., antioxidant-rich foods, among African Americans.

II. Literature Review

A. Antioxidant Nutrients and Cancer Risk

Diet and nutrition-related factors play an important role in many chronic diseases, including cardiovascular disease, diabetes, and many cancers[3,7-9]. It is estimated that at least one-third of all cancers are related to diet-related factors[10]. One way diet is thought to reduce the risk of cancer is via dietary intake of antioxidant nutrients. Antioxidants are substances within many foods that decrease the adverse effects of reactive oxygen species (ROS), reactive nitrogen species (RNS), or both on normal physiological functions in humans[6]. Antioxidants are hypothesized to decrease cancer risk by preventing tissue damage[11,12]. There are many dietary components with demonstrated antioxidant activity; however, this study focuses on carotenoids (total carotenoids, α-carotene, β-carotene, lycopene, lutein+zeaxanthin, and β-cryptoxanthin), vitamin C (ascorbic acid), and vitamin E (tocopherols) because these antioxidants have putative antioxidant function and can be assessed via the diet assessment tools selected for this study (biomarkers, diet recalls, and food frequency questionnaire)[13].

The following is a brief description of the antioxidants on which this work focuses: carotenoids, vitamin C, and vitamin E.

Carotenoids: Carotenoids, naturally occurring precursors to vitamin A, are fat-soluble red, yellow, and orange pigments produced by plants. Humans cannot produce carotenoids endogenously and thus, rely on dietary intake of fruits and vegetables for carotenoids. Carotenoids are believed to confer protection against oxidant-mediated diseases, e.g., cancers[14] and may also have effects on cell growth regulation and differentiation, modulation of gene expression, and enhancement of immune response[4,15,16]. Carotenoids are concentrated in fruits and vegetables (e.g., β-carotene in carrots, lutein in sweet corn, and lycopene in tomatoes).

Vitamin C: Vitamin C (or ascorbic acid) is a water-soluble vitamin primarily found in a wide variety of fruits and vegetables, such as citrus and leafy greens. Vitamin C's ability to scavenge free radicals has been postulated to decrease cancer risk[15].

Vitamin E: Vitamin E, a fat-soluble vitamin, consists of four different tocopherols and four different tocotrienols. Alpha-tocopherol, the most abundant form, is present in plant and seed oils, nuts, margarine, seeds, and cereal grains. Vitamin E may prevent carcinogenesis through its antioxidant properties[17], by inhibiting formation of carcinogens such as nitrosamines, or by increasing antibody production and enhancing cell-mediated immunity[15,18].

Numerous epidemiologic studies have examined the role of antioxidants, within the diet and/or from supplements, in cancer prevention. However, studies that have examined relationships between individual antioxidant nutrients cancer risk have been less consistent. Results from most observational studies provide support for a protective association between

high dietary intakes and/or blood levels of antioxidant vitamins, especially β-carotene and vitamin C, with cancer risk[3,11,19]. However, randomized trials, especially those with supplements, have generally not supported the hypothesis that individual antioxidants decrease risk for cancer, and two notable studies, two notable randomized trials, ATBC and CARET, have shown increased risk with high-dose supplementation in high-risk populations, such as smokers and asbestos workers[11,15,20,21]. There are several possible explanations for these discrepant findings: 1) observational studies are generally unable to control for confounding by unknown or unmeasured dietary and lifestyle factors, 2) the protective role of antioxidants may result from a combination of many different nutrients present in fruits and vegetables, rather than a single nutrient or combination of two nutrients that most randomized trials have tested; 3) inadequate duration of follow-up in most randomized trials; and 4) heterogeneity of the populations studied[11]. There are also biochemical mechanisms that may explain the association with increased risk of lung cancer in smokers, specifically: 1) competition between fat-soluble micronutrients in the presence of high doses of beta-carotene, and 2) pro-oxidant effects of beta-carotene under free radical-rich conditions with the lungs of smokers[22,23].

This work reports potential racial difference in antioxidant intake, plasma antioxidant concentrations, and demographic, behavioral, and psychosocial factors related to the intake of antioxidant-rich foods and plasma antioxidant concentrations. In addition, we report the associations of antioxidants and oxidative DNA damage, a potential maker of cancer risk, in a sample of healthy African Americans and Whites.

B. Associations of Antioxidant Levels with Oxidative DNA Damage

Oxidative stress is an important common factor in the etiology of many cancers. The term oxidative stress is commonly used to describe the imbalance that occurs when reactive oxygen species (ROS) or radical-generating agent concentrations exceed the body's defense mechanisms (e.g., antioxidant enzymes or plasma antioxidants)[24]. Oxidative stress is caused by exogenous factors, such as smoking, as well as endogenous processes, during normal cell metabolism. Humans have well-developed defense systems that generally maintain homeostasis by disposal of these oxidative products (e.g., catalase, superoxide dismutase, glutathione peroxidase) or by DNA excision repair (e.g., XRCC1, CRCC3, XRCC5). However, defenses may be overwhelmed under conditions of increased oxidative stress (e.g., smoking or low antioxidant intake). Although ROS are essential in some protective cell functions, excess oxidative stress can lead to oxidative damage of lipids, proteins, and DNA, and thus, increased risk of many diseases because free radicals can also attack DNA causing significant base damage, strand breaks, altered gene expression, and ultimately mutagenesis[22,25-28]. DNA is the most biologically relevant target of oxidative stress, since continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the breast, colon/rectum, and prostate[25,28-30].

Data from epidemiologic studies, including intervention trials, suggest that dietary factors may modify levels of endogenous DNA oxidation, and that antioxidant-rich diets decrease oxidative DNA damage and may prevent development of cancer[31-36]; one study

showed no effect[37]. In a randomized crossover study of healthy nonsmoking males ages 27 to 40, Pool-Zobel et al. found that supplementing the diet with tomato, carrot, or spinach products resulted in significantly decreased levels of endogenous strand breaks in lymphocyte DNA[31]. However, studies that have examined relationships between individual antioxidant nutrients and DNA damage have been less consistent. Results from most observational studies provide support for a protective association between high dietary intakes and/or blood levels of antioxidant vitamins, especially β-carotene and vitamin C, with oxidative DNA damage[38,39]. Several interventions with supplemental doses of antioxidants resulted in a significant decrease in endogenous DNA damage[40,41]. For example, in a randomized double-blind placebo-controlled intervention, Zhao et al. showed significant decreases in endogenous DNA damage after 57 days of taking supplements of lutein, β-carotene, lycopene, and a combination of all three in a sample of postmenopausal women[41].

To date, these studies have been conducted in relatively homogenous samples. One study compared oxidative DNA damage levels across five European countries; however, the study population was comprised almost entirely of White participants[42]. There remains a significant gap in knowledge about whether these associations differ by race. This work fills this gap by comparing the associations of antioxidant nutrients and oxidative DNA damage by race in a sample of healthy African American and Whites adults.

C. Oxidative DNA Damage and Cancer Risk

Another rationale for examining associations of oxidative DNA damage with antioxidant nutrient status is that indicators of oxidative DNA damage could potentially be

used as 'biomarkers' of cancer risk, i.e., oxidative DNA damage levels could be used to identify persons at high risk for cancer[43,44]. Also an examination of how DNA damage markers are affected by or associated with different dietary factors (e.g., antioxidants) could inform on optimal intakes required to suppress pro-oxidant effects or enable the antioxidant capacity of various nutrients in different populations. However, it is important to note that there are a number of reasons why oxidative DNA damage may not be a valid biomarker of cancer progression: 1) oxidative DNA damage is not always related to higher risk for cancer; 2) DNA damage in lymphocytes may not represent damage at the target tissue level; 3) oxidative DNA damage may be induced by carcinogenesis; and 4) higher rates of oxidative damage may actually reflect lower rates of repair[45]. Ascertaining whether oxidative DNA is a risk factor for or a result of carcinogenesis or would be best examined in a prospective cohort investigation[46]. Nonetheless, the available body of evidence strongly suggests that high antioxidant nutrient intakes may protect against both endogenous DNA oxidation and *in vitro* oxidative attack.

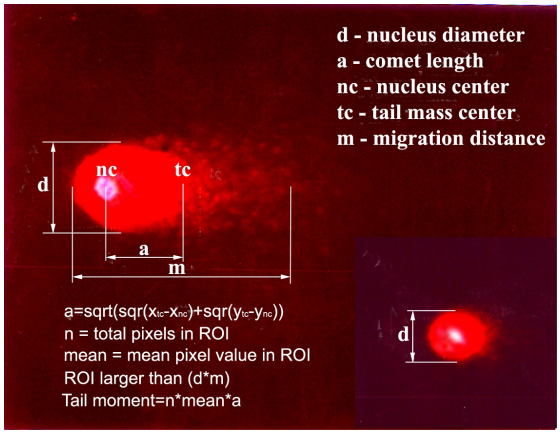
D. Measuring Oxidative DNA Damage

There are two main types of DNA damage assessment methods: direct measurements of DNA fragmentation (e.g., alkaline comet assay) and indirect measurements based on biomarkers of DNA damage (e.g., 7-hydroxy-8-oxo-2'-deoxyguanosine (8-oxo-dG))[47]. Both the comet assay and 8-oxo-dG are widely used in studies. The comet assay, also called single-cell gel electrophoresis, measures DNA strand breaks within individual cells[30,48]. Breaks in DNA allow supercoiled loops of DNA to relax and if damaged, appear like a comet with a tail under the conditions of the assay. The comet assay is relatively easy to perform,

sensitive, reasonably priced, and thus, well-suited for large population-based studies[48-51]. Recent modifications to the comet assay permit the detection of oxidized DNA bases by including a DNA digestion step using DNA glycosylase enzymes, such as formamidopyrimidine DNA glycosylase (FPG), which markedly increases specificity[52].

The unwound, relaxed DNA is able to migrate out of the cell during electrophoresis and can be visualized by SYBR Green staining. DNA loops containing breaks extend under electrophoresis to form comet tails. Cells that have accumulated DNA damage appear as fluorescent comets with tails of DNA fragments, whereas normal, undamaged DNA does not migrate far from the cell origin. Comet tail length (the distance of DNA migration from the nuclear core) was visualized by using a fluorescence microscope (typically, 100 cells/sample) and SCION IMAGE software[53]. The comet tail moment (defined as the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail) was calculated by using the NIHIMAGEANALYSISMACRO language software. The higher the comet tail moment value, the greater the amount of cellular DNA strand breaks.

Figure 1. Calculation of comet tail moment



Comparing results of oxidative damage across studies can be problematic. Although the comet assay is widely used, oxidative DNA damage may be assessed qualitatively by visual scoring, a subjective method whereby comets are classified into categories of damage by eye, or quantified using computer-based image analysis, which can be expressed as the tail length, relative tail intensity (% of DNA in tail), or the comet tail moment[54]. There is potential for inter-study variation with visual scoring as readers differ across studies; however, visual scoring was shown to correspond well to the percentage of DNA in tail within a study by Collins et al.[54]. Objective measures are generally preferred when feasible by time and cost. Both the percentage of DNA in tail and comet tail moment have been described as optimal measures[54,55]. The percentage of DNA in tail is linearly related to break frequency and is scale-independent[48], whereas the comet tail moment was shown to be the most sensitive approach for low levels of damage[55]. The comet assay with FPG has been heralded as the most convenient and reliable method currently available for assessing oxidative stress in general[56]. The limitations of the comet assay include considerable intra- and inter-individual variation, which may be affected by various demographic and lifestyle factors including age, gender, smoking, physical activity, environmental pollutants, and diet[50]. We have collected information on each of these variables, which will be used to control for potential confounding.

E. Racial Differences in Antioxidant Nutrient and Oxidative Stress Levels

African Americans have the highest cancer burden of any racial or ethnic group in the US[2]. In 2001, the age-adjusted national mortality rate for all cancers was 243.8 per 100,000

persons for African Americans, as compared to 193.3 per 100,000 persons for Whites[57]. Similar trends exist in North Carolina (NC). For example, prostate cancer incidence and mortality rates for African Americans in NC are among the highest in the US and the world, and considerably higher than among Whites in NC[58]. From 1993-1997, African Americans in NC had higher mortality rates for cancers of the breast, colon/rectum, liver, lung, pancreas, prostate, stomach, cervix, and all cancers combined than Whites in NC[57]. There is convincing evidence that diets high in fruits and vegetables (i.e., foods high in antioxidants) are inversely associated with the incidence of many of these cancers with disparately high numbers of African Americans, including colorectal, esophageal, pancreas, lung, mouth, pharynx, breast (probable evidence), and bladder[3]. Given the disparate cancer burden among African-Americans in North Carolina, it is especially important to identify potentially modifiable factors, such as diet, that may be associated with cancer risk in this population.

Both national and NC-specific survey data show substantial differences in antioxidant-related dietary habits between African Americans and Whites. Using data from the Third National Health and Nutrition Examination Survey (NHANES III), Ford reported that African Americans had statistically significantly lower intakes of vitamin E than Whites and also had the lowest concentrations of serum α-tocopherol[59]. Based on 2002 Behavioral Risk Factor Surveillance Survey (BRFSS) results for NC[60], only 19% of African American respondents consumed the recommended 5 fruits and vegetables daily (26% of Whites) and only 38% of African Americans reported current use of multivitamins, compared to 51% of Whites. These national surveys are in agreement with values seen in epidemiologic studies. For example, in a recent case-control study conducted in central NC that found serum levels

of α-carotene, lycopene, and vitamin E were significantly lower for African Americans than Whites[61]. Also, in a study of seventh-day Adventists, African Americans had lower blood levels and dietary intakes of vitamins C and E, but higher total carotene levels than Whites[62]. Based on these data, it appears that African Americans, including those in North Carolina, have dietary patterns that may put them at higher risk for oxidative stress.

Given the relationship between oxidative stress and carcinogenesis, it is biologically plausible that African Americans have higher rates of oxidative stress than Whites, irrespective of dietary intake. Two laboratory studies provided evidence of elevated oxidative stress in African Americans; endothelial cells taken from African Americans were less capable of preventing damage from oxidative stress than endothelial cells from Whites[63] and African Americans responded to induced hyperlipidemia with higher levels of oxidative stress than Whites[64]. However, two epidemiological studies have found the opposite. In a randomized controlled study of vitamins C and E supplements, oxidative DNA damage (assessed by urinary 7-hydroxy-8-oxo-2'-deoxyguanosine) was lower in African American than Whites participants at baseline[65]. Huang et al. concluded these differences were not explained by diet or lifestyle factors[65]. Similarly, Toraason et al. reported statistically significant lower oxidative DNA damage levels in African Americans than Whites in a study of female dry cleaners[66]. Further research is necessary to put these potentially conflicting results in context. This work reports oxidative DNA damage levels stratified by race, which can serve as a comparison to the work by Huang et al. and Toraason et al. In addition, we collect considerable data on dietary, demographic, and behavioral factors that may help explain any potential differences found.

F. Measuring Antioxidant Nutrients

Diet is generally measured using self-report dietary assessment instruments (e.g., food records, dietary recalls, and food frequency questionnaires (FFQ)) or biochemical measures (e.g., markers in serum, plasma, urine, or toenails). Biomarker measures are usually preferred because they obviate many of the limitations of self-reported instruments; however, biological markers are problematic for some nutrients[67]. For example, vitamin C is tightly regulated in the body and thus, self-report measures may be the preferred method[13]. Therefore, the optimal approach for quantification of antioxidant nutrient status involves collecting both self-report and biomarker information to provide complementary information[13]. The work presented here combines two measures of self-reported intake, 24-hour recalls and FFQ, with plasma biomarker concentrations, in an attempt to capture antioxidant status as accurately as possible.

1. Self-Reported Intake: Two commonly used self-report methods are 24-hour recalls and FFQs. 24-hour recalls they do not require literacy, have a relatively low respondent burden, and are less likely than food records to affect participants' eating patterns, since information is collected after consumption[67]. 24-hour recalls have the advantages of being based on actual intake, are open-ended, allow high specificity of detail in food description, and can accommodate a wide range of foods or food combinations. The greatest limitation of 24-hour recalls is that they rely on memory and they also require trained interviewers[67]. 24-hour recalls may fail to capture *usual* diet, as one or even a few days may not reflect true variability in dietary intake. Increasing the number of recalls performed and including both weekends and weekdays may increase accuracy[67]. FFQs are

the most commonly used method of assessing diet in epidemiological studies, as they are relatively inexpensive and provide reasonable estimates of *usual* intake over a designated time (e.g., one month). Foods included on questionnaires should be consumed relatively often, have substantial concentration of nutrient of interest, and intake should vary across people[67].

2. Biomarkers: Biological markers of dietary exposure are considered objective and therefore, often the preferred method of assessment. However, there are several limitations that must be considered. First, many antioxidant nutrients are under homeostatic regulation (e.g., vitamin C), which affects the amount of circulating levels in the body[67]. Second, levels of a nutrient in blood or tissues can be affected by genetic influences, lifestyle factors such as smoking and physical activity, and/or the intake of other nutrients[13,67]. Third, potential errors can occur if there are inappropriate specimen collection, handling, storage, and quality control techniques[13,67,68]. Fourth, blood-based biomarkers, by definition, represent concentrations of circulating amounts integrated over time, as compared to absolute intakes or recovery markers (e.g., urinary nitrogen) whose nutrient units and time periods are clearly defined[13,67,68]. Fifth, many biomarkers do not reflect the exposure period of interest in diet and cancer studies (i.e., years) because most indicators are sensitive to relatively short-term intakes (e.g., hours or months)[13,67,68]. Sixth, even with an ideal biomarker, repeated measures are desirable to account for individual changes and secular trends in nutrient intake[13,67]. Finally, many biomarkers are prohibitively expensive for use in large-scale and/or population-based studies[13,67].

Biomarkers of carotenoids, α-tocopherol, and vitamin C have all been shown to increase in response to higher dietary intakes; however, associations between intake and biomarker indicators for these nutrients are modest, ranging from 0.1-0.6[67]. Plasma levels of fat-soluble vitamins are related to the concentration of cholesterol levels as fat-soluble vitamins are transported by lipoproteins[67]. If biomarker values are not adjusted for plasma cholesterol levels, one risks misclassifying the bioavailable amounts of vitamin E and carotenoids. Plasma carotenoids are generally excellent biomarkers because of they are exclusive dietary sources (i.e., not produced endogenously in humans), detected easily, and fat-soluble[13]. Vitamin C can be detected in plasma for 35 to 40 days, compared to 100 to 120 days leukocytes[67,69]. Although leukocyte levels may reflect more long-term intake, saturation occurs at only 100 mg per day and thus, intake over 100 mg per day will not be reflected[69]. Therefore, plasma levels are usually used to measure vitamin C status in epidemiologic studies. There are limitations to using plasma vitamin C concentrations, including 1) plasma samples need to be acid stabilized (e.g., with trichloroacetic or metaphosphoric acid) to prevent degradation of ascorbic acid, 2) levels fluctuate considerably in response to dietary intake, so fasting blood samples are essential, and 3) due to tight regulation, plasma ascorbic acid levels may be accurate for those with extremely high intakes (e.g., supplement users)[13]. As noted earlier, the amount of vitamin E obtained from foods is relatively small compared to doses that can be obtained from supplements. Correlations between self-reported vitamin E dietary intake and serum or plasma α-tocopherol (adjusted for total cholesterol) are usually less than 0.35[67,70], while correlations with supplemental vitamin E are usually greater than 0.60[71,72].

3. Dietary Supplements: Vitamin and mineral supplements are an important source of micronutrient intakes in the US[71,73-76]. Doses of supplemental antioxidant nutrients vary greatly and many supplements are available in doses much larger than can typically be obtained from the diet. For example, on average 8-10 mg of vitamin E comes from food compared to doses that can be obtained from dietary supplements (e.g., 180 mg from single supplements)[71,74,75]. Thus, vitamin and mineral supplements represent a significant component of micronutrient exposure and should be added to intakes obtained from foods to determine total micronutrient intake in epidemiologic studies. Most epidemiologic studies typically use personal interviews or self-administered questionnaires to obtain information on supplement intake[73,77]. However, these methods may not adequately capture the wide variety of supplements available on the market after the passage of the Dietary Supplements and Health Education Act (DSHEA) in 1994 that deregulated the supplement industry[76]. One approach to assessing nutrient intakes from dietary supplements that has shown reasonable validity is the supplement inventory method in which study staff directly enter data about multiple vitamins/minerals and single supplement(s), including the dose, frequency, and duration of use[72,74,78]. In a recent validation study, assessment of supplemental nutrient intakes using this inventory approach yielded higher correlations with biomarkers than a detailed self-administered questionnaire[72].

This work utilized two self-reported methods of dietary intake, i.e., 24-hr dietary recalls and FFQ, and also plasma biomarker concentrations to measure vitamin C, vitamin E, and carotenoids (α-carotene, β-carotene, lycopene, lutein+zeaxanthin, β-cryptoxanthin). Collecting complementary dietary measures is the optimal approach as the self-reported

methods are not limited by potential inter-individual differences in metabolism and absorption and biomarkers are not subject to many of the biases, e.g., recall bias, of the self-reported methods[13]. To capture potential variations in diet, two of the diet recalls were conducted on weekdays and two on weekends. In addition, we used a FFQ specifically designed for this study that queried diet *over the past month* and included antioxidant-rich foods. Carotenoids and vitamin C are contained mostly in fruits and vegetables and vitamin E is mostly found in oils, cereals, and nuts. Since these antioxidants are concentrated in a moderate number of foods, the FFQ should adequately measure intake. Finally, to assess dietary supplement use, we used an open-ended interview, where all labels were transcribed by a trained nutritionist, which has been to be more accurate than a detailed self-administered questionnaire[72].

G. Psychosocial Factors and Fruit and Vegetable Intake

According to the 2002 Behavioral Risk Factor Surveillance Survey (BRFSS), less than 25% of the US population consumed at least 5 fruit and vegetable servings per day, which is far lower than national guidelines[79,80]. Programs designed to increase fruit and vegetable intake are most effective when based in theory and rooted in an understanding of how these factors affects the people it serves[81]. Interventions to increase fruit and vegetable consumption have typically examined sociodemographic characteristics, such as age, gender, education, and socioeconomic status, and a handful have considered psychosocial factors as potentially mediating variables[81-83]. However, psychosocial factors may be important predictors or correlates of dietary behavior, particularly fruit and vegetable consumption. For example, results from NCI's 5 A Day program showed that psychosocial factors were more

important determinants of fruit and vegetable intake than demographic factors alone[84]. Identifying salient psychosocial factors is important for several reasons: 1) they can provide a foundation for behavior change strategies, 2) mediating factors provide insight into underlying motivations for behaviors, and 3) measuring mediating factors allows for evaluation of change. Three successful dietary interventions aimed at African American churches incorporated both demographic and psychosocial factors and had relatively large increases of 0.7 to 1.4 fruit and vegetable servings per day[85]. Still, few studies have examined the possible influence of psychosocial factors on fruit and vegetable intake, and there is even less such data for African Americans.

Theory-based research promotes an understanding of behavior change mechanisms, the underlying reasons why the mechanism worked or failed, and identification of relevant mediators that an intervention should target. One method for examining psychosocial factors is the PRECEDE (Predisposing, Reinforcing, and Enabling Constructs in Educational Diagnosis and Evaluation) planning framework, which is used to understand motivations for healthy dietary behaviors and mediating factors in dietary interventions, categorizes psychosocial factors into 3 main categories: predisposing, reinforcing, and enabling factors[86]. Predisposing factors are antecedents that influence the likelihood of how one will behave and include the individuals' knowledge, attitudes, beliefs, existing skills, personal preferences, and self-efficacy (i.e., the extent one believes he/she can successfully perform a given behavior)[86]. Reinforcing factors are incentives following a behavior that may affect the likelihood that this behavior will be repeated over time, such as social support, peer

influence, significant others, and rewards[86]. Enabling factors help facilitate a behavior and may include programs, services, and resources necessary for a behavior to occur[86].

There are other health behavior theories, in additional to the PRECEDE framework, that may be used to examine the psychosocial factors associated with fruit and vegetable intake. For example, a particularly useful theory may be social cognitive theory since it incorporates principles on predicting health habits, guiding behavior change, and also includes outcomes expectancies and self-efficacy[34]. Self-efficacy, the extent one believes s/he can successfully perform a given behavior, has consistently been shown to influence healthy dietary behavior[82,84,87,88]. We chose to base this work on the PRECEDE framework because it has been used successfully in previous research of fruit and vegetable intakes[81,87,89,90] and also because it is particularly well-suited for studies of minority populations[91]. Specifically, it assumes that factors that affect behavior vary across populations, and is therefore an excellent model to use in cross-cultural research.

H. Summary and Significance

Given the high rates of cancer in African Americans, it is especially important to identify potentially modifiable factors, such as diet, that may be associated with cancer risk in this population. This work is among the first to provide information on associations of antioxidant nutrients with oxidative stress in healthy African American and White adults. There remains a gap for research examining potential mechanisms of carcinogenesis in African Americans. Considering the associations of antioxidant nutrients and oxidative DNA damage with cancer, identifying factors that may influence these levels is important for

several reasons. First, it will provide information for those involved in study design of future research to ensure key factors are considered and adequately measured. Second, identification of potential confounders will be useful in data analyses. Third, information on the demographic, behavioral, and psychosocial factors of fruits and vegetables may have important implications for cancer prevention initiatives. Examining racial differences in antioxidant nutrients, foods rich in antioxidants, and oxidative DNA damage in African Americans and Whites may provide important mechanistic support for addressing health disparities in cancer.

III. Methods

A. The DIet, Supplements, and Health Study (DISH)

1. Study Overview From March 2005 to January 2006, 168 healthy African American and White participants were recruited from the Research Triangle Area of North Carolina for a study examining antioxidant intakes and oxidative DNA damage. Participants completed four 24 hour dietary recall interviews by phone and a demographic and health questionnaire at home. Participants had height, weight, and waist circumference measured, provided urine and blood samples, and participated in a dietary supplement inventory at UNC's General Clinical Research Center (GCRC). Blood samples were analyzed for levels of antioxidant nutrients, cholesterol, oxidative damage, hemoglobin A1C, and cotinine, a metabolite of nicotine. Plasma levels of total carotenoids, α-carotene, β-carotene, lycopene, lutein+zeaxanthin, β-cryptoxanthin, vitamin A (retinols), vitamin C (ascorbic acid), vitamin E (α-tocopherol), and cholesterol were determined. Lymphocytes were assessed for oxidative DNA damage using the comet assay. Red blood cells were used for hemoglobin A1C and cotinine was tested in serum. Upon completion, each participant received $100 compensation for his/her time.

2. DISH Participant Recruitment and Eligibility Participants were recruited via flyers displayed in public venues, such as local churches, community centers, gyms, and on campus buildings throughout the Research Triangle Area (i.e., Chatham, Durham, Franklin, Johnston, Orange, and Wake counties) and an informational email distributed to all faculty, students, and staff members at UNC. Interested persons called the advertised toll free phone

number and the study coordinator determined eligibility according to a prepared script. Study participants were required to be generally healthy, free of cancer and other chronic diseases, fluent in written and spoken English, and have transportation to the GCRC. Persons who were likely to have high levels of oxidative stress, such as current smokers and those with diseases related to oxidative stress (e.g., diabetes, heart disease, or Alzheimer's disease) were ineligible. Since obesity is positively associated with increased oxidative stress, participants with a self-reported body mass index (BMI) of 30 or greater were ineligible. Persons with anorexia or bulimia nervosa, those who have not maintained a stable weight (within 15 pounds) in the last year, those who are unable to fast for 6 hours, and pregnant women were also ineligible.

Of the 191 respondents deemed eligible during the screening interview, 168 (88.0%) participants enrolled in the study and 164 (85.9%) participants successfully completed the study. Of these 164, 83 (51.6%) were African American and 81 (49.4%) were white.

3. Diet Assessment Tools

3a. 24-hour Dietary Recall Interviews After the signed consent form was received, the CNRC's Nutritional Epidemiology Core conducted four 24-hour recalls by phone over approximately the subsequent four weeks. Two recalls were conducted for weekend days (i.e., Saturday or Sunday) and 2 were conducted for weekdays. Repeated attempts to reach the participant were made until each of the four recalls was completed.

All recalls were conducted via telephone by trained nutritionists, using a computerized multiple pass approach with the Nutrition Data System (NDS) software (version 5.0.35, University of Minnesota, Minneapolis), and a standard introduction script. The foods, beverages, preparation methods, amounts, and recipes reported by the participant were entered by one of the Core's trained nutritionist into the NDS-R software package to obtain an estimate of nutrient intake. The trained nutritionist asked the participant what s/he has eaten during the previous day and prompted the participant for additional information when necessary. The NDS-R database contains over 18,000 foods, 8,000 brand name products, and many ethnic foods.

3b. Demographic and Health Questionnaire The Demographic and Health Questionnaire contains 37 questions pertaining to general health and diet in 12 pages. We conducted a small pilot study (n=10) in a convenience sample with representative demographic characteristics (i.e., equally divided by race and gender) to test the questionnaire for feedback as to the design, content, and ease of completion. Based on feedback from the focus group, the questionnaire could be completed in approximately 15 to 30 minutes. The questionnaire contains sections on general health, physical activity, attitudes and beliefs regarding diet, medical history, smoking and alcohol use, demographics, dietary supplement use, and also includes the newly developed antioxidant nutrient questionnaire. All data was manually key-entered and a randomly selected 10% were re-entered to assess accuracy. The Fred Hutchinson Cancer Research Center Nutrition Assessment Shared Resource (FHCRC- NASR) analyzed all nutrient intake records using Nutrition Data System (NDS). NDS, developed by the University of Minnesota's Nutrition

Coordinating Center, combines USDA's Nutrient Database for Standard Reference, information from scientific literature, and food manufacturers, to maintain the most accurate and comprehensive nutrition calculation software available in the US.

3c. Antioxidant Nutrient Questionnaire We have developed an antioxidant nutrient questionnaire for use in this study, which will be included within the demographic and health questionnaire booklet. Although food frequency questionnaires (FFQ) have several limitations, the FFQ is a practical and relatively inexpensive tool for estimating usual intake, even for large populations[67]. Our FFQ is a semi-quantitative food frequency questionnaire (FFQ) designed to capture *usual* dietary and supplemental intake of carotenoids, vitamin C, and vitamin E. The questionnaire includes more than 80 foods that either are natural sources of carotenoids, vitamin C, and vitamin E (e.g., fruits and vegetables) or fortified sources (e.g., cold cereals). Participants were asked to report how often s/he ate each listed food *in the past month* and selected from the following choices: never or less than once per month, once per month, 2-3 per month, 1-2 per week, 3-4 per week, 1 per day, or 2+ per day. Participants also recorded whether s/he usually consumed a small, medium, or large amount (medium serving size is shown as a reference).

Dietary supplement information was collected separately from the food portion in a different format. Participants were asked whether they had taken a multivitamin *in the past month* and if so, selected from a list of common multivitamins or wrote in their brand if it was not listed. Participants were then asked if they take a single nutrient supplement of beta-carotene, vitamin A, vitamin C, or vitamin E and if so, frequency (number of days per week)

and supplement dose (amount of nutrient per day). Daily intake was calculated as "frequency (days per week) x dose per day / 7 (days)" as reported in the questionnaire[72]. Capturing supplement intake of these antioxidants is crucial as supplements can contribute a large percentage of the total intake. This is especially true for vitamin E, as typical dietary intake (8-10 mg) is much smaller than typical doses in dietary supplements (e.g., 180 mg from single supplements)[71].

3d. Dietary Supplement Inventory Participants were instructed to bring the bottles for all vitamin, mineral, and herbal supplement(s) taken (even once) in the past month to the GCRC visit. The study coordinator collected the information from the participant's vitamin, mineral, and herbal supplement bottles in an open-ended format. For each supplement, the interviewer recorded the supplement name, brand name, amount of *each* "nutrient" in supplement per pill, whether it is a single- or multi- nutrient supplement, total number of supplements taken, how many pills taken each time, number of years taken since 1995, when usually taken (morning, afternoon, evening), and when the supplement was last taken. This method has been shown to be more valid than self-administered questionnaires[72,74,78]. Average daily nutrient intake was calculated as "frequency (days per week) x number of pills taken each time x dose per pill / 7" from the information collected during the interview[72]. We then summed intakes of each individual nutrient from all multivitamins and single supplements reported to determine a total average daily intakes for each nutrient. Beta-carotene, retinol, and vitamin E were converted into activity units as follows: 1 IU of vitamin A = 0.3 μg of retinol and 0.6 μg of beta-carotene; and 1 IU of vitamin E = 0.45 mg of alpha-tocopherol.[92]

4. GCRC Visit GCRC staff measured participants' height and weight and collected a urine and blood samples. Finally, the participant met with the study coordinator and completed the dietary supplement inventory, had his/her waist circumference measured, and answered questions about the use of NSAIDS and lipid-lowering drugs, current occupation, usual outdoor exposure, and last menstrual cycle (women only).

5. Biological Specimens After ensuring participant was eligible to have blood samples collected (i.e., no food or drink, except plain water within 6 hours), the GCRC nursing staff drew approximately 42 mL of blood into 4 ACD (yellow top) 8.5 mL vacutainer tubes, one 3ml lavender top tube, and one 5 ml of blood in 1 red top tube 5 mL vacutainer. All tubes were wrapped in aluminum foil upon completion of blood draw, as the nutrients being analyzed (carotenoids) are light sensitive. All samples were processed *within 2 hours of collection.* Levels of cholesterol, carotenoids, vitamin A, and vitamin C were measured in plasma. The aliquot of plasma designated for ascorbic acid assessment was preserved with a 6% weight/volume metaphosphoric acid (MPA) solution added in a 1:4 ratio plasma to MPA to stabilize vitamin C. Serum levels of cotinine, a nicotine metabolite, were measured using Cotinine Direct ELISA Kit (BioQuant, Inc., San Diego, CA) and hemoglobin A1C was measured via turbidimetric immunoinhibition using a hemolized whole blood sample. Lymphocytes were used to measure oxidative DNA damage. Lineberger Comprehensive Cancer Center's Tissue Culture Facility (TCF) isolated peripheral blood lymphocytes (PBL) from whole blood collected in the ACD tubes. Lymphocytes were washed in PBS, counted

using a hemacytometer, and cryopreserved in 1 ml RPMI-1640 + 15% BSA+ 10% DMSO. All samples were stored at –80ºC until assays were performed.

In addition, two 10 mL aliquots of urine were collected (one preserved with 20mg ascorbic acid and the other unpreserved) and stored at –80ºC for future research. Participants also provided a sample of toenails, clipped at home, for future analyses of antioxidant minerals. Toenails are excellent sources of long-term (26 to 52 weeks) exposure to selenium and zinc[67]. Toenail samples were stored in sealed paper coin envelopes in a dry, cool place until needed for analysis.

5a. Plasma Nutrient Analyses Craft Technologies, Inc. evaluated the plasma concentrations of carotenoids (total carotenoids, α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin), retinol, tocopherols (α-tocopherol, γ-tocopherol, and δ-tocopherol), and ascorbic acid. Total plasma cholesterol was also analyzed to adjust the values of the nutrients that are associated with plasma lipoproteins (i.e., carotenoids and tocopherols)[93]. Quality control samples and 10% duplicates were included in each batch. Craft Technologies, Inc. participates in the National Institute of Standards and Technology (NIST) Micronutrients Measurement Quality Assurance Program.

Plasma Carotenoids and Tocopherols Method: Serum concentrations of vitamin A (retinol, retinyl palmitate, retinyl stearate), vitamin E (alpha-, delta-, and gamma-tocopherols), and nine carotenoids (lutein, zeaxanthin, alpha-cryptoxanthin, beta-cryptoxanthin, trans lycopene, cis lycopene, alpha-carotene, trans beta-carotene, cis beta-carotene) were measured using

high performance liquid chromatography (HPLC) with multiwavelength photodiode-array absorbance detection[93]. A small volume (150 μL) of serum/plasma was mixed with an equal volume of buffer, then mixed with 2 volumes of ethanol containing the internal standard (tocol). The analytes were extracted from the aqueous phase into hexane. The combined hexane extracts were then dried under vacuum. The extract was redissolved in ethyl acetate and diluted in mobile phase. An aliquot was injected onto a C18 reversed phase column and eluted isocratically. The analytes all possess absorbance and/or fluorescence proportional to their concentration in solution; therefore these properties are used for quantitative analysis. The mode of detection was chosen to provide the highest sensitivity and selectivity. Carotenoids were measured by absorbance at 450 nm. Retinol, retinyl esters, phytoene and phytofluene were measured by UV absorbance near their absorption maxima of 325 nm, 280 nm and 340 nm. Tocopherols have absorption maxima between 292 and 300 nm. Chromatograms were recorded using a computer data system. Analytes were quantified by external standard quantitation using neat standards to calculate response factors based on the peak area of the analyte. The quantities of analytes were corrected for recovery post-run based upon tocol as an internal standard.

Plasma Cholesterol Method: Plasma cholesterol concentrations were measured by enzymatic/colorimetric analyses ("Trinder" procedure), using adaptations of commercially available kits. For total cholesterol, cholesterol esterase cleaves cholesterol esters into free cholesterol; then cholesteryl oxidase produces hydrogen peroxide which is converted into a quinoneimine dye by peroxidase enzyme. The absorbance of the quinoneimine dye product

at 520 nm was proportional to the amount of total cholesterol in the sample. The working range for this assay was 0.1 mmol/l (4 mg/dl) to 12.0 mmol/l (464 mg/dl).

Plasma Vitamin C Method: Ascorbic acid was quantified using high performance liquid chromatography (HPLC). After thawing the samples and mixing, 100 mL aliquots of plasma are transferred to microcentrifuge tubes and 300 µL of DPP/TCEP buffer (0.435g dipotassium phosphate + 0.0312g TCEP (Tris(2-carboxyethyl)phosphine HCl) in 25mL H2O) was added, mixed and incubated at room temperature for 30 minutes. Meta-phosphoric acid (100 µL of 40% (w/w) solution) was added and samples were vortex-mixed and centrifuged at 10,000 rpm for 5 minutes. The supernatant was transferred to an HPLC autosampler vial. Duplicate 20-µL aliquots were injected. The HPLC system consisted of a computer data system, solvent degasser, an autosampler maintaining samples at 4C, a Synergi Hydro-RP column (4 mm, 4.6 x 250 mm), a Security Guard C18 guard column (Phenomenex, CA), a programmable UV detector set at 245 nm and a two channel coulometric detector. The first cell of the detector was set at 350 mV and 5mA full scale with a 1 sec filter time to measure the ascorbic acid. The second cell was set at 500 mV and used to oxidize extraneous components in the sample. The separation was performed isocratically using a mobile phase of 25 mM potassium phosphate monobasic containing 1% methanol and 0.1 mM EDTA, pH 2.7 at a flow rate of 0.8 mL/min. Ascorbic acid elutes at ~5 minutes with a total run time of 12 minutes.

Calibration was by peak area using external standard method. Calibrants of 0.1, 0.5, and 1.0 mg/mL were prepared by diluting the stock ascorbic acid solution (2 mg/mL) with

5% meta-phosphoric acid. A set of calibrants was injected at the beginning and end of each set of samples. The NIST SRM 1846, Infant Formula, was analyzed with each set of samples along with three levels of serum controls from the Center for Disease Control.

5b. Oxidative DNA Damage Measurement via the Comet Assay The single cell gel electrophoresis or comet assay is a widely used method for measuring DNA strand breaks at the level of a single cell, in which lymphocytes are digested with lesion-specific repair endonucleases[45,52]. We used a slightly modified version of the comet assay, in which formamidopyrimidine DNA glycosylase (FPG) (provided by Dr. A.R. Collins, Aberdeen, Scotland, UK) was added to convert oxidized purines including formamidopyrimidines and 8-oxoGua into strand breaks[30,94]. Lymphocytes were sandwiched between 0.5% agarose and 0.5% low-melting-point (37 °C) agarose (Fisher, Fair Lawn, NJ). The resulting slides were placed into cold, freshly made lysis solution [10 mmol Tris/L (pH 10), 2.5 mol NaCl/L, 100 mmol EDTA/L, 1% sodium sarcosinate, 10% DMSO, and 1% Triton X-100] at 4 °C for _1 h and then treated for 20 min in electrophoresis buffer [300 mmol NaOH/L, 1 mmol EDTA/L (pH 13)][53]. After electrophoresis was performed at 25Vand 300mAfor 20 min, slides were incubated 3 times for 5 min in neutralization buffer [0.4 mol Tris/L (pH 7.5)] with FPG, washed with methanol, and stained with SYBR Green. Comet tail length (the distance of DNA migration from the body of the nuclear core) was visualized by using a fluorescence microscope (typically, 100 cells/sample) and SCION IMAGE software[53]. The comet tail moment (defined as the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail) was calculated by using the

NIHIMAGEANALYSISMACRO language software

(http://dir.nhlbi.nih.gov/labs/ldn/macroanalysis.asp).

B. Study examining methods to recruit African Americans into cancer prevention studies

1. Study Overview Data presented here were collected as part of a study examining methods and strategies to recruit African Americans into cancer prevention studies[23]. Briefly, 5,000 potential African American participants, 18-70 years, residing in 6 North Carolina counties were randomly selected from Department of Motor Vehicle rosters and assigned at random to one of five recruitment strategies, based on variations of approach letters and incentives. All prospective participants were sent an 11-page questionnaire by mail with a pre-paid return envelope, as well as instructions for completing the survey via the Internet or by telephone. An advance postcard was sent to alert potential participants to the upcoming questionnaire mailing and a reminder letter was sent 2-3 weeks later with information for obtaining a replacement questionnaire and instructions for completing the survey by telephone or the internet.

2. Study Population Eligible participants were African Americans between ages 18-70 years who resided in 6 contiguous North Carolina (NC) counties (3 urban and 3 rural). Names and addresses for the sampling frame (n=50,000) were obtained from Department of Motor Vehicle (DMV) rosters. The choice to use DMV records was motivated by results of a study of 8 rural NC counties which found that DMV rosters contained more African

Americans than did voter registration lists[95]. Also, DMV records do not contain business and non-residential addresses, which makes mailing more efficient.

3. Data Collection All prospective participants were sent an 11-page questionnaire by mail with a pre-paid return envelope, as well as instructions for completing the survey via the Internet or by telephone. An advance postcard was sent to alert potential participants to the upcoming questionnaire mailing and a reminder letter was sent 2-3 weeks later with information for obtaining a replacement questionnaire and instructions for completing the survey by telephone or the internet. The study had a 17.5% response rate (n=747): 87.7% by mail, 11.2% via the Internet, and 1.1% by telephone. Data were excluded from 89 respondents who did not meet eligibility criteria and whose questionnaires failed quality-control checks; data from the remaining 658 persons were used for the analyses presented here. The study was approved by the Institutional Review Board of the School of Public Health at the University of North Carolina - Chapel Hill.

Each of the 5,000 potential participants were randomly allocated to each of the five strategies (1,000 per group) based on variations of the approach (cover) letters and use of incentives. The five strategies are explained in detail below:

Generic approach letter: This letter stated the purpose of the study, how and why the prospective participant was selected, and cited reasons why each person's participation is vital, but did not make a direct appeal to African Americans. Participants were assured that their data would be kept confidential and used exclusively for research purposes. The Principal Investigator was presented as a cancer researcher.

Culturally sensitive approach letter: This letter was similar to the generic version, but also included the Principal Investigator's picture to identify her as African American. In addition, the letter mentioned the lack of research on issues specific to African Americans and appealed to values by noting that the respondent's participation may be of benefit to other African Americans. The purpose of this strategy was to increase respondent ethnic/cultural identification with the researcher and the study.

Culturally sensitive approach letter plus promise of incentive: This letter was identical to the culturally sensitive letter described above, but also included a promise of an incentive upon receipt of the completed survey. The incentive was a 60-minute pre-paid telephone calling card that cost $3.60.

Generic approach letter plus incentive: The fourth strategy tested the effect of including an incentive (i.e., 60-minute pre-paid telephone calling card) along with the generic approach letter.

Culturally sensitive approach letter plus incentive: To evaluate this strategy, the fifth group received the incentive along with the culturally sensitive letter.

4. Survey Instrument Using the PRECEDE framework as a guide, an 11-page questionnaire was designed to measure demographic, psychosocial, lifestyle, and behavioral factors related to cancer prevention. The broad areas addressed in the questionnaire include the following: current dietary intake and physical activity, dietary supplement use, psychosocial factors related to diet, motivation for healthful dietary change, use of nutrition labels, and attitudes and beliefs about genetic testing for colon cancer. Three sets of these

questions were used in these analyses: diet-related psychosocial factors, demographic characteristics, and fruit and vegetable intake. All data are self-reported.

4a. Diet-related psychosocial factors Questions designed to capture psychosocial factors were adapted from the Behavioral Risk Factor Surveillance System (BRFSS) and previous research using PRECEDE framework.[81,96,97] According to PRECEDE, factors affecting behavior can be broadly grouped as predisposing, reinforcing, and enabling[86,91]. Predisposing factors, such as attitudes, beliefs, and values provide the rationale or motivation for a behavior. Enabling factors are skills, resources, and barriers that facilitate or hinder change. Reinforcing factors include variables such as social support, which provide an incentive for a behavior. Because PRECEDE recognizes that factors affecting behavior are culturally determined and can vary across populations, it is an excellent model to use for crosscultural research[86].

Predisposing factors included questions regarding *knowledge* -- whether participants had heard about the U.S. Department of Agriculture's Food Guide Pyramid (yes, no, don't know/not sure) and what they believed to be the fruit and vegetable daily servings recommendations (1-2, 3-4, 5 or more, or don't know); *attitudes* -- whether they believe a relationship between diet and cancer exists and if so, whether the relationship is strong, moderate, or weak and how important is was for them to personally eat a diet high in fruits and vegetables (very important, somewhat important, or not important); *taste preferences* (whether they like the taste of most fruits and vegetables, yes, sometimes, no); and *self-efficacy*. Healthful eating self-efficacy was assessed by a Likert-scale (very confident,

somewhat confident, or not very confident) item about respondents' confidence in their ability to eat more fruits and vegetables.

Reinforcing factors addressed social support. Respondents were asked whether they felt they could count on those close to them: *to encourage them to eat healthfully; to tell them about healthier foods and how to prepare them; to prepare healthier foods with them;* and *to eat healthier foods with them***.** Possible responses were a lot, some, or not at all.

Enabling factors included four items related to perceived barriers to healthy eating and queried respondents on whether: they *can afford to purchase healthy foods and meals; it takes too much time and trouble to prepare healthy meals; it is easy for them to order healthy foods in restaurants***;** and *they need more information on how to prepare healthy foods and meals***.** Response options were yes, sometimes, or no. Scales were created for each set of factors by linearly summing responses to individual questions (least healthy responses scored the lowest and the healthiest responses scored the highest). All questions had an equal number of possible responses and a summary score for each scale was computed as the mean of the non-missing responses. The distinctions "least healthy" and "most healthy" are used only to categorize the responses to each psychosocial factor; we do not intend to make any inference to actual behavior**.**

4b. Demographic characteristics Various demographic characteristics were assessed, including age (categorized approximately into tertiles), sex, education (less than or equivalent to high school, some college, college graduate, or advanced degree), marital status

(never married, married/living with partner, or divorced/separated/widowed), and self-rated health status (excellent, very good, good, fair, or poor). Using self-reported height and weight, body mass index (BMI) was calculated as kg/m^2 and further categorized as normal (18.5 to 24.9), overweight (25.0 to 29.9), or obese ($\geq$30.0)[24]. Information was collected about other lifestyle and behavioral characteristics, such as physical activity and smoking, but was not included in these analyses.

4c. Fruit and vegetable intake Fruit and vegetable consumption during the past 3 months was assessed using the seven-item fruit and vegetable screener developed at the National Cancer Institute[25,26]. Fruit intake was the sum of "fruit juice" and "fruit, not counting juice", and vegetable intake was calculated as the sum of green or lettuce salad, potatoes (boiled, baked, or mashed), other vegetables, beans and peas, and vegetables in mixed dishes. Fruit and vegetable intake was calculated as the sum of all seven items. The standard approach for evaluation in the 5 A Day program was used to calculate fruit and vegetable servings per day[27].

C. Data Analysis

1. Overview All study information (including participant's identification, lab values, questionnaire responses, 24-hour dietary recalls) for the DISH study was stored in a password-protected access database created by the GCRC's Bioinformatics Core. Final datasets used in analyses here contained no personal identifiers. Statistical analyses were conducted using STATA (version SE 8.2, STATACorp, College Station, TX). Descriptive

statistics (means, standard deviations, percentiles, and graphical displays) were computed for all key study variables. Raw and log transformed values were examined since some variables were right-skewed. A 5% significance level was used for all statistical tests.

2. Statistical Methods for Aims 1-3

Aim 1. Determine whether antioxidant nutrient status, as measured by dietary estimates and blood levels of antioxidant nutrients, differs by race in a sample of healthy adults.

Aim 2. Determine whether oxidative stress status in healthy adults differs by race.

Aim 3. Examine associations of antioxidant nutrients with oxidative stress and determine whether the associations differ by race.

Data analyses were performed using Stata (version SE 8.2, STATACorp, College Station, TX). Descriptive statistics (means and percentages for continuous and categorical variables, respectively) were calculated for all variables. Missing data were excluded from analyses; on average less than one percent of data were missing. For each demographic characteristic, chi-square tests were used to test for equality by race. Antioxidant nutrient intakes were assessed in four main ways: as 1) biomarker (plasma) levels 2) average daily dietary intakes from the FFQ in the past month; 3) mean intakes across the 4 dietary recalls; and 4) the average daily intake from supplements as reported in the supplement inventory. Levels of oxidative DNA damage assessed by the comet assay were quantified by the comet tail moment (the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail). Log transformations were applied to the dietary and oxidative DNA damage estimates to meet the normality distribution assumptions, as all distributions were skewed to the right.

For continuous responses (i.e., antioxidant nutrient status and oxidative DNA damage), differences in responses between African Americans and Whites were compared using analysis of variance, while controlling for participant characteristics and other potentially important confounders. Age, gender, income, education, and alcohol use are associated with antioxidant (or fruit and vegetable) intake in studies of both African Americans and Whites [67,82,98-101] and were included in the adjusted models. BMI has been associated with antioxidant intake in largely White populations[100], however, our study population is restricted to those with a self-reported BMI under 30 to limit confounding and thus, should not be as great a factor in the analyses. We examined both crude and adjusted estimates to determine gross and net effects. In addition, plasma cholesterol was included when evaluating fat-soluble plasma antioxidant levels, as it affects bioavailability[67]. All analyses were performed in the combined sample and also stratified by race to examine effect modification by race. Intra- and inter-individual variation for both methods of assessing oxidative DNA damage may be affected by factors including age, gender, smoking, physical activity, environmental pollutants, and diet[50]. We have direct measures of each of these factors, except for "environmental pollutants." Extreme obesity is also thought to increase oxidative stress. However, as we have restricted the study population to non-obese individuals, BMI should not strongly affect our estimates of oxidative stress.

Multiple linear regression analyses[102] were performed to assess associations between the dietary estimates and blood levels of the antioxidant nutrients and oxidative DNA damage (measured as comet tail moment), controlling for the effects of race and other potential covariates (e.g., age, sex, BMI, income, physical activity, cotinine, and alcohol

consumption). In different regression models, the association between the plasma level of each antioxidant nutrient and oxidative stress was examined stratified by race. Tertiles of the dietary estimates were also computed and compared to oxidative DNA damage using multiple regression analyses and *p* for linear trend was computed. To approximate total antioxidant concentration, z-scores were calculated for each antioxidant biomarker value and averaged. Hypothesis tests and 95% confidence intervals were used to make inferences about the regression coefficients.

3. Statistical Methods for Aim 4

Aim 4. Identify correlates of antioxidant nutrients and oxidative stress and whether these correlates differ by race.

Data analyses were performed using Stata (version SE 8.2, STATACorp, College Station, TX). Descriptive statistics were calculated for all variables. Missing data were excluded from analyses; on average less than one percent of data were missing. For each study population characteristic, chi-square tests were used to test for equality by race. Antioxidant nutrient levels were assessed as biomarker (plasma) concentrations. Oxidative DNA damage was quantified by the comet tail moment. Log transformations were applied to the dietary and oxidative DNA damage distributions to meet the normality distribution assumptions, as they were right-skewed. Mean levels of antioxidant nutrients and oxidative DNA damage were reported separately by race for each demographic, psychosocial, and behavioral factor. Potential differences between African Americans and Whites were evaluated using analysis of variance for dichotomous variables and *p* for linear trend and spearman's correlations were calculated for categorical variables. Plasma cholesterol was

included in all analyses evaluating fat-soluble plasma antioxidant levels, as it affects bioavailability[67]. Forward stepwise regression analyses, with a retention criteria of 0.05 and plasma cholesterol forced into all models of fat soluble nutrients, were computed separately for each race to determine associations between the demographic, behavioral, and diet-related psychosocial correlates and plasma antioxidant concentrations and between the demographic and behavioral correlates and oxidative DNA damage. Statistical tests were two-sided and p values ≤ 0.05 were considered statistically significant.

4. Statistical Methods for Aim 5

Aim 5. Identify psychosocial correlates of fruit and vegetable intakes among African Americans.

For each demographic characteristic, one-way ANOVA models were used to assess whether there were statistically significant differences between the mean values of each psychosocial (i.e., predisposing, reinforcing, and enabling) scale and mean fruit and vegetable consumption (servings per day). To examine associations between the psychosocial scales (categorized into approximate tertiles) and fruit and vegetable intake, we used multiple linear regression models to calculate unadjusted and adjusted (for age, sex, education, and BMI) means for fruit, vegetable, and total fruit and vegetable intake (servings per day) as well as overall p values. We also compared associations of each psychosocial factor (categorized by least healthy to most healthy response) with fruit and vegetable intake by using multiple linear regression models to generate mean values for fruit and vegetable intake, unadjusted and adjusted for age, sex, education, BMI, and the other predisposing, reinforcing, and enabling factors. The fruit and vegetable variables used for aim 4 were not

transformed because the data were not markedly skewed. Statistical tests were two-sided and p values ≤ 0.05 were considered statistically significant.

IV. Associations of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults

A. Introduction

Diet and nutrition-related factors play an important role in carcinogenesis[3,7-9] and are estimated to account for at least one-third of all cancers[10]. One mechanism by which it is hypothesized that diet reduces cancer risk is through consumption of antioxidant nutrients, which are substances found within many foods, such as fruits and vegetables that decrease the adverse effects of reactive oxygen species (ROS) on normal physiological functions[6]. High ROS levels can lead to oxidative stress, in which the imbalance of radical-generating agent concentrations exceeds the body's defense mechanisms[28,103]. Humans have well-developed defense systems that generally maintain homeostasis by disposal of these oxidative products; however, under conditions of elevated oxidative stress (e.g., low antioxidant intakes) defenses may be overwhelmed. Oxidative stress is caused by exogenous factors, e.g., smoking, as well as endogenous processes during normal cell metabolism. Excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, altered gene expression, and ultimately mutagenesis[22,25-28]. Continuous oxidative damage to DNA is believed to be a significant contributor to the age-related development of the major cancers, such as those of the breast, colon/rectum, and prostate[25,28-30].

Numerous studies have examined associations of antioxidant intakes (from diet and/or supplements) with oxidative DNA damage and cancer risk. Most intervention trials that focused on intakes of fruits and/or vegetables have shown significant reductions in

oxidative DNA damage levels[31-36]; one study showed no effect[37]. In a randomized crossover study of healthy nonsmoking males ages 27 to 40, Pool-Zobel et al. found that supplementing the diet with tomato, carrot, or spinach products resulted in significantly decreased levels of endogenous strand breaks in lymphocyte DNA[31]. However, studies that have examined relationships between individual antioxidant nutrients and DNA damage or cancer risk have been less consistent. Results from most observational studies provide support for a protective association between high dietary intakes and/or blood levels of antioxidant vitamins, especially β-carotene and vitamin C, with cancer risk[3,11,19] and oxidative DNA damage[38,39]. Several interventions with supplemental doses of antioxidants resulted in a significant decrease in endogenous DNA damage[40,41]. For example, in a randomized double-blind placebo-controlled intervention, Zhao et al. showed significant decreases in endogenous DNA damage after 57 days of taking supplements of lutein, β-carotene, lycopene, and a combination of all three in a sample of postmenopausal women[41]. Conversely, two notable randomized trials, ATBC and CARET, reported elevated risk of lung cancer with high-dose supplementation in high-risk populations, such as smokers and asbestos workers[11,15,20,21]. One possible explanation for these results is that the high doses used during the trial may have resulted in pro-oxidant activity in the radical-rich environment of a smoker's lung[23].

In the United States (US), African Americans are at disproportionately higher risk for many oxidative stress-related medical conditions, such as diabetes, cardiovascular disease, hypertension, and they have the highest cancer burden of any US racial or ethnic group[2,104,105]. Moreover, survey data suggest that African Americans consume fewer daily

fruits and vegetables (i.e., antioxidant-rich foods) than do Whites[80,106] and tend to have lower blood levels of antioxidant nutrients. For example, according to data from the 2002 Behavioral Risk Factor Surveillance Survey (BRFSS), less than 19% of African Americans in North Carolina consumed the recommended 5 fruit and vegetable servings per day, which is lower than the median for the US (22.6%) and NC White populations (24.7%) and only 38% of African Americans reported current use of multivitamins, compared to 51% of Whites[79]. Similarly, African Americans had the lowest concentrations of serum α-tocopherol among all racial/ethnic groups in the Third National Health and Nutrition Examination Survey (NHANES III)[59] and in a North Carolina-based case-control study, serum levels of α-carotene, lycopene, and vitamin E were significantly lower for African Americans than Whites[107]. Based on this data, it appears that African Americans, including those in North Carolina, have dietary patterns that may put them at higher risk for oxidative stress and oxidative stress-related medical conditions, including cancer.

Using data from a convenience sample of Whites and African Americans in North Carolina, the aims of this report are to 1) determine whether dietary intakes and blood levels of antioxidant nutrients (carotenoids, vitamin C, and vitamin E) and oxidative DNA damage levels differ between African Americans and Whites, and 2) examine associations between antioxidants and oxidative DNA damage, and whether the associations differ by race. This study is among the first to examine these relationships in a sample with adequate representation of African Americans and Whites and may provide mechanistic support for the higher cancer burden in African Americans compared to Whites.

B. Methods

1. Study population Data are from the DIet, Supplements, and Health (DISH) Study, which enrolled 168 generally healthy African American and White adults (approximately equal by race and gender) from the Research Triangle Area of North Carolina between March and December 2005. Participants were recruited via flyers displayed in public venues, such as local churches, gyms, campus-wide emails, and on campus buildings throughout the Research Triangle Area. Eligible participants were 20 to 45 years of age, generally healthy, free of diseases related to oxidative stress (i.e., cancer, diabetes, heart disease, or Alzheimer's disease), and fluent in written and spoken English. Persons likely to have high levels of oxidative stress, such as current smokers and those with a self-reported body mass index (BMI) of 30 or greater were ineligible. Other exclusion criteria included anorexia or bulimia nervosa, large weight change (more than 15 pounds) in the past year, inability to fast for 6 hours, and pregnancy. Of the 191 respondents deemed eligible during the screening interview, 168 (88.0%) were enrolled and 164 (85.9%) completed all aspects of the study. Data for nine participants were excluded because of serum cotinine levels that were consistent with active smoking (≥15 ng/mL), leaving a total of 155 participants (76 African American, 79 White).

2. Data collection Participants completed four unannounced telephone-administered 24 hour dietary recall interviews and a self-administered demographic, health, antioxidant questionnaire. During a one-time visit to UNC's General Clinical Research Center (GCRC), participants had height, weight, and waist circumference measured, provided urine and semi-fasting (≥6 hours) blood samples, participated in a dietary

supplement inventory, and answered questions about the use of NSAIDS and lipid-lowering drugs, current occupation, outdoor exposure, and last menstrual cycle (women only). Blood samples were analyzed for plasma levels of antioxidant nutrients, cholesterol, oxidative DNA damage, hemoglobin A1C (to confirm self-reported absence of diabetes), and serum cotinine (to validate self-reported smoking status). Each participant received $100 compensation for his/her time upon completion of all study activities. This study was approved by the University of North Carolina at Chapel Hill (UNC)'s Institutional Review Board and written (signed) informed consent was obtained from all participants.

3. Dietary Recalls Four unannounced telephone-administered 24-hour dietary recalls were conducted by trained nutritionists from UNC's Clinical Nutrition Research Core using a computerized multiple pass approach with the Nutrition Data System (NDS) software (version 5.0.35, University of Minnesota, Minneapolis) over a *one month period*. Two recalls each were conducted on weekdays and weekend days (i.e., Saturday or Sunday) to account for variability in eating patterns. The consumed foods, beverages, preparation methods, amounts, and recipes reported by the participant were entered by a trained nutritionist into the NDS-R software package to obtain an estimate of intakes of various nutrients. The NDS-R database contains over 18,000 foods, 8,000 brand name products, and many ethnic foods.

4. Demographic, Health, and Antioxidant Questionnaire All participants completed a self-administered 12-page questionnaire, which included 37 questions pertaining to general health and diet and a newly developed antioxidant food frequency questionnaire

(FFQ). The questionnaire contained sections on general health, physical activity, attitudes and beliefs regarding diet, medical history, smoking and alcohol use, demographic characteristics, dietary supplement use, and the new antioxidant FFQ. We conducted a small pilot study in a convenience sample with representative demographic characteristics (i.e., equally divided by race and gender) to test the questionnaire for feedback about the design, content, and ease of completion and made the necessary modifications.

Antioxidant FFQ. We developed a semi-quantitative FFQ designed to capture *usual* dietary and supplemental intakes of carotenoids, vitamin C, and vitamin E. The 92-item questionnaire includes more than 80 foods that either are natural sources of carotenoids, vitamin C, and vitamin E (e.g., fruits and vegetables) or fortified sources (e.g., cold cereals). Participants were asked to report how often they ate each listed food *in the past month* and selected from the following choices: never or less than once per month, once per month, 2-3 per month, 1-2 per week, 3-4 per week, 1 per day, or 2+ per day. Participants also recorded whether they usually consumed a small, medium, or large amount (medium serving size was shown as a reference). The Fred Hutchinson Cancer Research Center Nutrition Assessment Shared Resource (FHCRC- NASR) analyzed all nutrient intake records using Nutrition Data System (NDS), which combines USDA's Nutrient Database for Standard Reference, information from scientific literature, and food manufacturers, to maintain the most accurate and comprehensive nutrition calculation software available in the US.

Dietary supplement use. A closed-ended format was used to quantify self-reported use (frequency and dose) of various antioxidant nutrients *in the past month*. Specifically, for

multivitamin use, participants selected from a list of common multivitamins or wrote in their brand if it was not listed, and indicated the usual frequency of use (number of days per week). Next, they reported whether they took a single nutrient supplement of β-carotene, vitamin A, vitamin C, or vitamin E, and if yes, the frequency and usual dose (amount per day). Daily intake of each nutrient was calculated as "frequency (days per week) x dose per day / 7 (days)" [72]. For these analyses, participants are categorized as "non-users" or "users" of dietary supplements from this instrument. Capturing supplemental intakes of antioxidants is crucial as supplements can contribute a large percentage of the total intake. This is especially true for vitamin E, as typical dietary intake (8-10 mg) is much smaller than typical doses in dietary supplements (e.g., 180 mg from single supplements)[71].

5. Dietary Supplement Inventory Participants were instructed to bring the bottles for all vitamin, mineral, and herbal supplement(s) taken (even once) in the past month to the GCRC visit, during which an in-person interview was conducted. For each supplement, a trained nutritionist recorded the brand name, type of supplement (multivitamin, single-, multi-nutrient), usual frequency of use, total number of pills taken each time, amount of *each* "nutrient" per pill, when usually taken (morning, afternoon, evening), and when the supplement was last taken. This open-ended approach has been shown to be more valid than self-administered questionnaires[72,74,78]. Average daily nutrient intake from the inventory was calculated as "frequency (days per week) x number of pills taken each time x dose per pill / 7"[72]. We then summed intakes of each individual nutrient from all multivitamins and single supplements reported to determine a total average daily intake for each nutrient. β-carotene,

retinol, and vitamin E were converted into activity units as follows: 1 IU of vitamin A = 0.3 μg of retinol and 0.6 μg of β-carotene; and 1 IU of vitamin E = 0.45 mg of α-tocopherol.[92]

6. Plasma nutrients Semi-fasting (≥ 6 hours) blood samples that were protected from heat and light were analyzed for plasma concentrations of carotenoids, retinols, tocopherols, vitamin C, and cholesterol. The aliquot of plasma designated for ascorbic acid assessment was preserved with a 6% weight/volume metaphosphoric acid (MPA) solution added in a 1:4 ratio plasma to MPA to stabilize vitamin C. Plasma concentrations of retinols, tocopherols (α-tocopherol, γ-tocopherol, and δ-tocopherol), and carotenoids (lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene) were measured using high performance liquid chromatography (HPLC) with multiwavelength photodiode-array absorbance detection[93]. Plasma cholesterol was measured by enzymatic/colorimetric analyses ("Trinder" procedure) using adaptations of commercially available kits[93]. Quality control samples and 10% duplicates were included in each batch. These assays were performed by Craft Technologies Inc. (Wilson, NC).

7. Oxidative DNA Damage Oxidative DNA damage was assessed using the single cell gel electrophoresis or comet assay. The comet assay is a widely used method for measuring DNA strand breaks at the level of a single cell in which lymphocytes are digested with lesion-specific repair endonucleases[45,54]: the comet assay used here was a slightly modified version in which formamidopyrimidine DNA glycosylase (FPG) (provided by Dr. A.R. Collins, Aberdeen, Scotland, UK) was added to convert oxidized purines into strand breaks[30,94]. Peripheral whole blood lymphocytes were washed in PBS, counted using a

hemacytometer, and cryopreserved in 1 ml RPMI-1640 + 15% BSA+ 10% DMSO. All samples were processed within 2 hours of collection and stored at –80ºC until assays were performed. Lymphocytes were sandwiched between 0.5% agarose and 0.5% low-melting-point (37°C) agarose (Fisher, Fair Lawn, NJ). The resulting slides were placed into cold, freshly made lysis solution [10 mmol Tris/L (pH 10), 2.5 mol NaCl/L, 100 mmol EDTA/L, 1% sodium sarcosinate, 10% DMSO, and 1% Triton X-100] at 4 °C for 1 hour and then treated for 20 min in electrophoresis buffer [300 mmol NaOH/L, 1 mmol EDTA/L (pH 13)][53]. After electrophoresis was performed at 25Vand 300mAfor 20 min, slides were incubated 3 times for 5 min in neutralization buffer [0.4 mol Tris/L (pH 7.5)] with FPG, washed with methanol, and stained with SYBR Green. Comet tail length (the distance of DNA migration from the body of the nuclear core) was visualized by using a fluorescence microscope (typically, 100 cells/sample) and SCION IMAGE software[53]. The comet tail moment (defined as the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail) was calculated by using the NIHIMAGEANALYSISMACRO language software.

8. Statistical analyses Data analyses were performed using Stata (version SE 8.2, STATACorp, College Station, TX). Descriptive statistics (means and percentages for continuous and categorical variables, respectively) were calculated for all variables. Missing data were excluded from analyses; on average less than one percent of data were missing. For each demographic characteristic, chi-square tests were used to test for equality by race. Antioxidant nutrient intakes were assessed in four main ways: as 1) biomarker (plasma) levels 2) average daily dietary intakes from the FFQ in the past month; 3) mean intakes

across the 4 dietary recalls; and 4) the average daily intake from supplements as reported in the supplement inventory. Oxidative DNA damage was quantified as the comet tail moment. Log transformations were applied to the dietary and oxidative DNA damage distributions to meet the normality distribution assumptions, as they were right-skewed. Crude mean levels of antioxidant nutrient and oxidative DNA damage were reported separately by sex and race and potential differences between African Americans and Whites were evaluated using analysis of variance. Plasma cholesterol was included in all analyses evaluating fat-soluble plasma antioxidant levels, as it affects bioavailability[67]. Multiple linear regression analyses[102] and partial Pearson's correlations were computed separately for each race to assess associations between the dietary estimates and blood levels of the antioxidant nutrients and oxidative DNA damage, controlling for relevant covariates. Age, sex, body mass index (calculated using measured weight and height as kilograms divided by meters squared), income, physical activity, education, serum cotinine, and alcohol consumption were evaluated as potential confounders, as these factors have been found to be associated with both antioxidant intakes/blood levels and oxidative DNA damage[58,67,82,98-101]. Tertiles of the dietary estimates were also computed and compared to oxidative DNA damage using multiple regression analyses and *p* for linear trend was calculated. To approximate total antioxidant concentration, z-scores were calculated for each antioxidant biomarker value and averaged. Hypothesis tests and 95% confidence intervals were used to make inferences about the regression coefficients. Statistical tests were two-sided and *p* values ≤ 0.05 were considered statistically significant.

C. Results

The distributions of demographic and lifestyle characteristics, stratified by race and sex (n=155) are given in Table 1. The mean age of African Americans was 30.9 years (7.9 SD) and 53% were female; in comparison, the mean age for Whites was 32.5 years (7.9 SD) and 52% were female. African Americans had statistically significantly lower formal educational levels, physical activity, and alcohol consumption than Whites and were also more likely to be obese (BMI $\geq$30kg/m^2). African American males were somewhat younger (20-28 years) than White males (58% vs. 34%), and females of both races tended to have higher BMI and lower alcohol consumption than men.

Table 2 gives the mean antioxidant levels for vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (α-tocopherol), and carotenoids measured from plasma biomarkers, mean of four dietary recalls, average daily intakes from the FFQ, and the supplement inventory, by race and sex. Compared to Whites, African Americans had statistically significantly lower plasma concentrations and dietary intakes of most of the antioxidant nutrients. Specifically, they had lower plasma levels of α-carotene, β-carotene, lutein + zeaxanthin, α-tocopherol, and retinols and lower intakes of α-carotene, β-carotene, lutein + zeaxanthin (FFQ only), α-tocopherol, and retinols (recalls only). In addition, African Americans had significantly lower dietary recall-based lycopene than did Whites. There were no statistically significant differences by race in supplemental intakes of any of the antioxidants examined. Intake of all antioxidants, except α-carotene for African American men, was higher for men than women of both races. Mean antioxidant estimates were also evaluated controlling for sex, age, BMI,

cotinine, physical activity, education, income, and alcohol intake and adjusted estimates were comparable to the unadjusted estimates shown.

Oxidative DNA damage levels, measured as the mean tail moment of 100 cells using the comet assay, are given in Table 3. Overall, African Americans had significantly lower crude mean oxidative DNA damage than Whites (1.404 vs. 1.559), p=0.005. Both African American men and African American women had lower oxidative DNA damage than their White counterparts, although the difference was not statistically significant for men. Estimates of oxidative DNA damage changed only slightly when adjusted by age, BMI, cotinine levels, alcohol intake, physical activity level, income, education and days since last menses for women.

Table 4 gives mean oxidative DNA damage levels by antioxidant plasma concentrations, dietary, and supplemental intakes. Antioxidant intakes were categorized into tertiles and mean oxidative DNA damage values were calculated for African Americans and Whites, adjusting for sex, age, BMI, cotinine levels, physical activity level, education, income, and alcohol intake. Although few associations were statistically significant, oxidative DNA damage was generally lower for the highest tertiles of plasma antioxidants compared to the lowest, with the exception of α-carotene, lutein+zeaxanthin, and ascorbic acid in Whites. This inverse relationship was evident for most of the self-reported estimates of antioxidant intakes (i.e., recalls, FFQ, and dietary supplement use). For almost all nutrients, mean levels of oxidative DNA damage were higher for non-users than users of

dietary supplements (based on the self-reported instrument); however, the only statistically significant association was with supplemental lycopene intake in Whites (p=0.01).

Pearson partial correlations between antioxidant plasma concentrations and oxidative DNA damage, stratified by race and sex, are given in Table 5. For the total study population, only lycopene and α-tocopherol were statistically significantly associated with oxidative DNA damage; however, lycopene was inversely associated with oxidative DNA damage (Pearson r=-0.20, p=0.03), whereas the association with α-tocopherol was positive (r=0.21, p=0.02). Although not significant when examined separately by race, associations with lycopene and α-tocopherol were in the same direction and of similar magnitude in both African Americans and Whites. Other racial and gender differences were noted, although not all were statistically significant. For example, Vitamin C was inversely associated with oxidative DNA damage in African Americans; in contrast, associations tended to be positive in Whites. α-tocopherol was positively associated with oxidative DNA damage in men (r=0.63, p=0.01 for African American men), but was inversely associated among women of both races. Oxidative DNA damage was not statistically significantly associated with all antioxidants combined (based on Z scores).

D. Discussion

In this cross-sectional study of generally healthy adults in North Carolina (NC), African Americans had significantly lower plasma levels of α-carotene, β-carotene, lutein + zeaxanthin, α-tocopherol, and retinols than Whites. In addition, African Americans also had

lower levels of oxidative DNA damage, as assessed by the mean comet tail moment. The only statistically significant inverse association between plasma antioxidants and oxidative DNA damage was found for lycopene in the combined study population. Rather unexpectedly, there were also positive associations of α-tocopherol with oxidative DNA damage in the total population and in African American men.

The lower self-reported intakes and plasma concentrations of antioxidants seen here among African Americans compared to Whites are in agreement with national and NC-specific data[59,62,79,80,106,107]. For example, Ford et al. reported statistically significant lower serum concentrations of α-tocopherol in African Americans compared to Whites using data from the National Health and Nutrition Examination Survey (NHANES III) for those 20 years and older[59]. In a study of seventh-day Adventists, African Americans had lower blood levels and dietary intakes of vitamins C (137 mg/day) and E (9 mg/day) than Whites[62]. The values for vitamins C and E are similar to the self-reported intakes reported here; however, the intakes of carotenes are substantially higher. Also, mean intakes of β-carotene, lutein, α-tocopherol, and ascorbic acid reported here were similar to those of healthy controls in a recent population-based case-control study of African Americans and Whites in NC[107]. We found statistically significant differences by race in plasma antioxidant concentrations and at least one method of self-reported dietary intake (i.e., FFQ or recalls) for retinols, α-carotene, β-carotene, lutein + zeaxanthin, and α-tocopherol. This suggests these levels can be attributed to differences in dietary intake of antioxidant-rich foods rather than dietary supplement use. Although we saw no difference in dietary supplemental intake by race, other studies have observed racial differences in supplement use[62,107]. Thus, our findings are

in agreement with other published data suggesting that African Americans have dietary patterns that may lead to increased oxidative stress.

We found statistically significantly lower oxidative DNA damage levels in African Americans compared to Whites, and among African American women compared with White women; however, there was no difference by race among men. Studies that have investigated the relationship between oxidative DNA damage and sex have found higher oxidative DNA damage levels in men than women, which was attributed to lower fruit intake in men[108,109]. Men in our study had higher total self-reported intakes but similar plasma levels of antioxidants than women, which may explain (at least in part) why we did not observe significant differences in oxidative DNA damage levels by sex. We were unable to find any prior studies in which the potential interaction between race and sex had been specifically investigated but, our findings are consistent with other similar studies. For example, the overall mean levels of oxidative DNA damage we observed are similar to baseline data in a recent study of choline depletion in African American and White healthy adults; however, oxidative DNA damage levels were not reported separately by race[53]. In a randomized controlled study of vitamins C and E supplements by Huang et al.[65] oxidative DNA damage (assessed by urinary 7-hydroxy-8-oxo-2'-deoxyguanosine) was lower in African American than White participants at baseline; however, final levels were not reported by race. The authors noted that these differences were not explained by diet or lifestyle factors and that all participants were non-smokers[65]. Similarly, Toraason et al. found statistically significant lower oxidative DNA damage levels in African Americans than Whites in a study of female dry cleaners[66]. Also, our results are comparable to those

published by Hininger et al.[110] for non-smokers 24 to 51 years (1.23 ± 0.2). It should be noted that the present study had a relatively small sample size and the study population was exceedingly healthy (non-smoking, non-obese, disease free). Considering that oxidative DNA damage is a potential mechanism associated with cancer risk, the relationship between race and oxidative DNA damage needs to be explored further in other studies that also include those at elevated risk of oxidative DNA damage.

We found significant associations with oxidative DNA damage for two antioxidant nutrients, although one relationship was not in the hypothesized direction. In the combined sample, there was a significant positive association for α-tocopherol and an inverse association for lycopene with oxidative DNA damage. Although not significant when analyzed separately by race, the directions of these associations were consistent for both African Americans and Whites. There appear to be differences by sex in the association between α-tocopherol and oxidative DNA damage among African Americans, as there is a strong positive association in men and a non-statistically significant inverse association in women. Other studies comparing α-tocopherol and oxidative DNA damage have not reported a positive association in men[109,112] and α-tocopherol supplementation has been associated with lower oxidative stress levels in healthy young adults[112]. There is some evidence that in the presence of copper[113] or in smokers consuming a high fat diet[112], α-tocopherol can act as a strong pro-oxidant, but it is somewhat surprising to see a positive association of α-tocopherol in this sample of healthy, nonsmoking young adults. Conversely, the inverse association with lycopene is not surprising as intervention trials with lycopene or tomatoes (the richest food source of lycopene) have consistently demonstrated lower levels

of oxidative DNA damage[31,33,34,36]. One trial of 5 participants showed decreased levels of oxidative DNA damage after consuming only a single serving of tomatoes[36]. Oxidative DNA damage was also statistically significantly decreased after 3 weeks of consuming tomato sauce-based pasta dished in a study of 32 men with prostate cancer[33].

Although we found few significant associations of antioxidant nutrients with oxidative DNA damage, other investigations have reported associations of vitamin C and several carotenoids with oxidative DNA damage. For example, two intervention studies that showed a reduction in endogenous DNA damage with supplemental doses of antioxidants, including vitamin C, vitamin E, and carotenoids, were conducted in study populations over age 50 years[40,41]; in contrast, two studies that found no association examined younger, healthy adults ages 25-45 years and 35-64 years[109,114]. In our study, only vitamin C was inversely associated with oxidative DNA damage in African Americans. There are several possible reasons why we did not observe more significant associations. First, our study population consisted of healthy, non-smoking, non-obese young adults that were likely to have low levels of oxidative stress relative to other populations. Second, while we did not find a significant association between oxidative DNA damage and total antioxidant plasma concentration as measured by Z-scores, it is possible that antioxidants that were not assayed may be more strongly related to oxidative DNA damage and/or that a synergistic effect exists among all antioxidants not seen for each individual antioxidant. For example, a recent study modeled the "total antioxidant capacity" (TAOC) and found that uric acid was the greatest independent predictor of TAOC[115]. Third, it is plausible that associations between some of the antioxidants we examined and oxidative DNA damage may be better captured using other

measures of oxidative DNA damage. Finally, it is possible that the distributions of antioxidant concentrations and/or oxidative DNA damage in this study sample were not variable enough to detect associations or that associations do not exist.

Comparing results of oxidative damage across studies can be problematic. Although the comet assay is widely used, oxidative DNA damage may be assessed qualitatively by visual scoring, a subjective method whereby comets are classified into categories of damage by eye, or quantified using computer-based image analysis, which can be expressed as the tail length, relative tail intensity (% of DNA in tail), or the comet tail moment[54]. There is potential for inter-study variation with visual scoring as readers differ across studies; however, visual scoring was shown to correspond well to the percentage of DNA in tail within a study by Collins et al.[54]. Objective measures are generally preferred when feasible by time and cost. Both the percentage of DNA in tail and comet tail moment (used in the present study) have been described as optimal measures[54,55]. The percentage of DNA in tail is linearly related to break frequency and is scale-independent[48], whereas the comet tail moment was shown to be the most sensitive approach for low levels of damage, such as those seen here in healthy participants[55].

It is important to note that we only assessed oxidative DNA damage using the comet assay with FPG, a measure of direct oxidation of purines; however, there are other sources of oxidative DNA damage including oxidation of the sugar backbone and lipid peroxidation that form additional types of DNA damage, such as malondialdehyde-derived adducts and etheno adducts. These DNA lesions are repaired by different pathways, which could affect the

results. Oxidative stress alters many other biomolecules, including glutathione and isoprostanes, which have not been evaluated. Future studies could benefit from incorporating several measures of oxidative stress that reflect these divergent pathways.

Our study has several strengths. To our knowledge, it is among the first to examine associations of antioxidant nutrient levels and oxidative DNA damage in a sample of generally healthy African American and White adults. We collected dietary intake data using two self-report methods (diet recalls and food frequency questionnaire) and biological markers, which has been suggested as the optimal approach for capturing dietary intake[13]. In addition to self-administered queries in the food frequency questionnaire, information about dietary supplement intake was collected during an open-ended interview and recorded directly from the supplement bottles, a method shown to be superior to self-administered queries[72]. Finally, oxidative DNA damage was measured using a modified comet assay with FPG, which is considered to be an optimal measure for oxidative stress[56].

This study also has some limitations. First, self-reported dietary data are subject to both random and systematic bias[67] and since blood was collected at only one time point, seasonal variability in antioxidant intakes could not be assessed. Nonetheless, the results using self-reported and biological measures of diet were comparable. Second, the capacity for DNA repair activity was not measured; thus these estimates represent the oxidative DNA damage level only at the time of collection. It is also worth pointing out that oxidative DNA damage may not be an optimal intermediate marker of cancer risk, as it is possible that oxidative DNA damage is induced by carcinogenesis. As noted by Loft and Moller,

ascertaining whether oxidative DNA is a risk factor for, or a result of, carcinogenesis (or both) would be best examined in a prospective cohort investigation[46]. Third, although we controlled for a number of covariates, residual confounding is still a concern. Fourth, the fact that our study population consisted of generally healthy volunteers may limit generalizability, particularly since adults willing to participate in a research study may be more health conscious than the general public. Finally, due to the cross-sectional nature of this study, we were unable to examine changes in oxidative DNA damage over time and no inferences about causality can be drawn.

In summary, this is among the first studies to examine the relationship between antioxidants (from self-report and biomarkers) and oxidative DNA damage in African Americans and Whites. It has been suggested that oxidative DNA damage is associated with elevated cancer risk and that antioxidants may mitigate the effects of oxidative DNA damage. Also, diets high in fruits and vegetables, and which are also rich in antioxidants, have consistently been linked to lower risk of many cancers, including those of the breast, colon/rectum, and prostate, all of which disproportionately affect African Americans (22). Our findings are in agreement with other studies suggesting that African Americans may have dietary patterns that put them at higher risk for cancer and oxidative DNA damage. However, we found that oxidative DNA damage levels were actually lower among African Americans than Whites in this study population, which has also been reported in several other studies. Participants were healthy and young (20 to 45 years), and it is possible that the DNA repair activity can compensate for diets low in antioxidants in healthy, non-smoking young adults. Continued research, optimally involving prospective cohort investigations, is

needed to assess the relationship among antioxidant nutrients, oxidative damage, and cancer risk, especially in minority populations who suffer a disproportionately high cancer burden.

Table 1. Demographic, lifestyle, and other characteristics of study participants, by race and sex (n=155)

Characteristic	African Americans			Whites			
	Males (n=36)	Females (n=40)	Total (n=76)[1]	Males (n=38)	Females (n=41)	Total (n=79)	*p* value[2]
Age							
20-28	21 (58%)	13 (33%)	34 (45%)	13 (34%)	13 (32%)	26 (33%)	
29-37	8 (22%)	14 (35%)	22 (29%)	13 (34%)	14 (34%)	27 (34%)	
38-45	7 (19%)	13 (33%)	20 (26%)	12 (32%)	14 (34%)	26 (33%)	0.32
BMI[3]							
Normal (18.5–24.9 kg/m^2)	23 (58%)	9 (25%)	32 (42%)	34 (83%)	24 (63%)	58 (73%)	
Overweight (25–29.9 kg/m^2)	16 (40%)	22 (61%)	38 (50%)	7 (17%)	12 (32%)	19 (24%)	
Obese (≥30 kg/m^2)	1 (3%)	5 (14%)	6 (8%)	0 (0%)	2 (5%)	2 (3%)	<0.0001
Education							
Some College or less	12 (33%)	18 (45%)	30 (39%)	10 (24%)	10 (26%)	20 (25%)	
College graduate	19 (53%)	14 (35%)	33 (43%)	13 (32%)	19 (50%)	32 (41%)	
Advanced Degree	5 (14%)	8 (20%)	13 (17%)	18 (44%)	9 (24%)	27 (34%)	0.03
Marital Status							
Single/Separated or Divorced	20 (56%)	25 (63%)	45 (59%)	19 (46%)	20 (53%)	39 (49%)	
Married/Living with partner	16 (44%)	15 (38%)	31 (41%)	22 (54%)	18 (47%)	40 (51%)	0.22
Income							
Less than $20,000	6 (19%)	8 (22%)	14 (21%)	7 (19%)	7 (18%)	14 (19%)	
$20,000-39,000	7 (23%)	9 (25%)	16 (24%)	9 (24%)	9 (24%)	18 (24%)	
$40,000-79,000	10 (32%)	10 (28%)	20 (29%)	14 (38%)	13 (34%)	27 (36%)	
$80,000 or more	8 (26%)	9 (25%)	17 (25%)	7 (19%)	9 (24%)	16 (22%)	0.86
Dietary Supplement Use							
None	22 (61%)	28 (70%)	50 (66%)	21 (55%)	18 (44%)	39 (49%)	
Multivitamin Only	9 (25%)	6 (15%)	15 (20%)	10 (26%)	6 (15%)	16 (20%)	
Single Nutrient Only	1 (3%)	2 (5%)	3 (4%)	2 (5%)	2 (5%)	4 (5%)	
2 or More Supplements	4 (11%)	4 (10%)	8 (11%)	5 (13%)	15 (13%)	20 (25%)	0.09

[1] Numbers may not add up to 76 for African Americans and 79 for Whites and percentages may not add up to 100% due to rounding and missing data.
[2] Overall p values determined by chi-square tests for differences between "total African Americans" and "total Whites."
[3] BMI calculated as kg/m^2, based on measured weight (kg) and height (m^2).

Table 1. (cont'd) Demographic, lifestyle, and other characteristics of study participants, by race and sex (n=155)

Characteristic	African Americans			Whites			
	Males (n=36)	Females (n=40)	Total (n=76)[1]	Males (n=48)	Females (n=41)	Total (n=79)	*p* value[2]
Passive Smoke Exposure							
Lives with a smoker	3 (8%)	3 (8%)	6 (8%)	0 (0%)	4 (10%)	4 (5%)	
No one at home smokes	33 (92%)	37 (93%)	70 (92%)	38 (100%)	36 (88%)	74 (95%)	0.49
Physical Activity							
Less than once/week	4 (11%)	11 (28%)	15 (20%)	1 (3%)	0 (0%)	1 (1%)	
1-2 times per week	12 (33%)	14 (35%)	26 (34%)	8 (21%)	12 (34%)	20 (25%)	
3-4 times per week	17 (47%)	11 (28%)	28 (37%)	14 (37%)	15 (37%)	29 (38%)	
5+ times per week	3 (8%)	4 (10%)	7 (9%)	15 (39%)	14 (34%)	29 (35%)	0.002
Alcohol Consumption							
Never	12 (33%)	22 (55%)	34 (45%)	5 (13%)	10 (24%)	15 (19%)	
Less than 1 per week	8 (22%)	14 (35%)	22 (29%)	9 (24%)	15 (37%)	24 (30%)	
1-6 times per week	13 (36%)	4 (10%)	17 (22%)	18 (47%)	15 (37%)	33 (42%)	
1 or more per day	3 (8%)	0 (0%)	3 (4%)	6 (16%)	1 (2%)	7 (9%)	0.002
Self-Rated Health Status							
Excellent	11 (31%)	7 (18%)	18 (24%)	14 (37%)	12 (29%)	26 (33%)	
Very Good	16 (44%)	21 (53%)	37 (49%)	16 (42%)	22 (54%)	38 (48%)	
Good /Fair	9 (25%)	12 (30%)	21 (27%)	8 (21%)	7 (17%)	15 (19%)	0.49
County of Residence							
Urban	30 (83%)	35 (88%)	65 (86%)	33 (87%)	35 (85%)	68 (86%)	
Rural	6 (17%)	3 (8%)	9 (12%)	3 (8%)	3 (7%)	6 (8%)	
Not Specified	0 (0%)	2 (5%)	2 (3%)	2 (5%)	3 (7%)	5 (6%)	0.39

[1] Numbers may not add up to 76 for African Americans and 79 for Whites and percentages may not add up to 100% due to rounding and missing data.
[2] Overall p values determined by chi-square tests for differences between "total African Americans" and "total Whites."
[3] BMI calculated as kg/m^2, based on measured weight (kg) and height (m^2).

Table 2. Unadjusted[1] antioxidant intakes and plasma levels among study participants, stratified by race and sex (n=155)

	African Americans			Whites			
	Males (n=36)	Females (n=40)	Total (n=76)	Males (n=48)	Females (n=41)	Total (n=79)	*p* value[2]
Vitamin A (retinols)							
Biomarkers (µg/ml)	0.42	0.39	0.40	0.47	0.41	0.44	0.002
Dietary Recalls (mg/day)	513.7	344.1	424.4	608.3	467.4	535.2	0.02
Food Frequency Questionnaire (mg/day)	1435.2	481.4	933.2	1893.9	880.3	1367.8	0.48
Supplements Only (mg retinol equivalents /day)[3]	117.4	217.5	170.0	140.5	119.2	129.4	0.66
Vitamin C (ascorbic acid)							
Biomarkers (µg/ml)	8.38	8.82	8.61	9.02	8.24	9.06	0.90
Dietary Recalls (mg/day)	124.0	84.2	103.1	138.7	104.7	121.1	0.11
Food Frequency Questionnaire (mg/day)	191.0	124.4	156.0	210.7	139.5	173.7	0.13
Supplements Only (mg/day)	105.7	61.9	82.7	86.1	132.0	109.9	0.57
Vitamin E (α -tocopherol)							
Biomarkers (µg/ml)	7.64	7.35	7.49	9.81	10.43	10.13	<0.001
Dietary Recalls (mg/day)	9.0	7.0	8.0	12.4	9.9	11.1	<0.001
Food Frequency Questionnaire (mg/day)	12.1	8.0	9.9	16.8	12.8	14.7	0.004
Supplements Only (mg α–tocopherol equivalents /day)	62.9	34.3	47.9	28.9	84.0	57.5	0.69
α -Carotene							
Biomarkers (µg/ml)	0.05	0.05	0.05	0.05	0.07	0.06	0.006
Dietary Recalls (µg/day)	336.4	361.0	349.3	622.9	516.9	567.9	0.01
Food Frequency Questionnaire (µg/day)	600.5	517.5	556.8	1037.5	712.8	869.0	0.04
β-Carotene							
Biomarkers (µg/ml)	0.18	0.18	0.18	0.19	0.27	0.23	0.007
Dietary Recalls (µg/day)	3044.4	2249.1	2625.8	4134.1	3096.1	3595.4	0.02
Food Frequency Questionnaire (µg/day)	3900.5	3392.5	3633.1	5337.7	4430.3	4866.8	0.03
Supplements Only (µg β-Carotene equivalents /day)	704.2	1305.0	1020.4	843.0	715.2	776.7	0.66
β-Cryptoxanthin							
Biomarkers (µg/ml)	0.11	0.10	0.10	0.10	0.09	0.10	0.68
Dietary Recalls (µg/day)	257.4	169.8	211.3	270.4	236.6	252.9	0.38
Food Frequency Questionnaire (µg/day)	339.0	171.6	250.9	372.8	177.4	271.4	0.76
Lutein + Zeaxanthin							
Biomarkers (µg/ml)	0.12	0.12	0.12	0.12	0.14	0.13	0.05
Dietary Recalls (µg/day)	3064.1	2075.6	2543.8	3637.3	2774.5	3189.5	0.21
Food Frequency Questionnaire (µg/day)	3028.1	2563.1	2783.4	3791.3	3577.6	3680.4	0.04
Supplements Only (µg/day)	34.7	34.1	34.4	24.1	24.4	24.3	0.52
Lycopene							
Biomarkers (µg/ml)	0.48	0.40	0.44	0.44	0.38	0.41	0.73
Dietary Recalls (µg/day)	4819.7	4152.3	4468.4	10690.8	5861.9	8184.7	0.005
Food Frequency Questionnaire (µg/day)	7655.7	4152.9	5812.1	7890.8	4994.8	6387.8	0.60
Supplements Only (µg/day)	148.2	20.0	80.7	39.6	44.2	42.0	0.29

[1] No adjusted were made, except for total cholesterol levels for biomarkers values for fat soluble nutrients only.
[2] Tests for differences between total African Americans and Whites were calculated by ANOVA using log-transformed variables.
[3] Data for "supplements only" based on in-person dietary supplement inventory. Conversions into activity units were made as follows: 1 IU of vitamin A = 0.3 µg of retinol and 0.6 µg of β-carotene; 1 IU of vitamin E = 0.45 mg of α-tocopherol. No values were presented for α -carotene and β-cryptoxanthin because dietary supplements contributed only negligible amounts to intake.

Table 3. Mean oxidative DNA damage levels (comet tail moment), by race and sex (n=155[1])

	Total Population			Men			Women		
Comet Assay Mean Tail Moment (SD)	**African American (n=74)**	**White (n=77)**	***p* value[2]**	**African American (n=35)**	**White (n=37)**	***p* value**	**African American (n=39)**	**White (n=40)**	***p* value**
Crude Model	1.404 (0.298)	1.559 (0.359)	0.005	1.410 (0.312)	1.534 (0.351)	0.12	1.399 (0.289)	1.582 (0.370)	0.02
Adjusted[3] Model	1.398 (0.147)	1.563 (0.196)	0.01	1.399 (0.206)	1.535 (0.249)	0.24	1.396 (0.176)	1.587 (0.321)	0.03

[1] Comet assay results were not available for four participants due to missing samples.
[2] Overall p value calculated for by analysis of variance (ANOVA) using log-transformed oxidative DNA damage estimates.
[3] Mean values adjusted for age, BMI, cotinine levels, alcohol intake, physical activity level, income, education and for women, days since last menses.

Table 4. Adjusted[1] mean oxidative DNA damage level (comet tail moment) by antioxidant intakes and plasma levels, by race

	Vitamin A (retinols)		Vitamin C (ascorbic acid)		Vitamin E (α-tocopherol)		α-Carotene		β-Carotene		β-Cryptoxanthin		Lutein + Zeaxanthin		Lycopene	
	African American	White	African American	White	African American	White	African American	White	African American	White	African American	White	African American	White	African American	White
Biomarkers																
Highest Tertile	1.348	1.472	1.309	1.602	1.337	1.526	1.465	1.584	1.444	1.456	1.287	1.521	1.276	1.555	1.296	1.523
Middle Tertile	1.373	1.571	1.327	1.545	1.408	1.543	1.247	1.420	1.217	1.641	1.399	1.542	1.427	1.619	1.434	1.457
Lowest Tertile	1.394	1.584	1.496	1.439	1.367	1.560	1.439	1.566	1.491	1.542	1.515	1.550	1.373	1.422	1.430	1.673
p for linear trend	0.98	0.84	0.08	0.03	0.52	0.84	0.75	0.77	0.70	0.54	0.03	0.35	0.96	0.34	0.73	0.65
Dietary Recalls																
Highest Tertile	1.406	1.409	1.307	1.515	1.485	1.515	1.381	1.590	1.433	1.587	1.273	1.587	1.455	1.502	1.363	1.468
Middle Tertile	1.335	1.667	1.424	1.577	1.571	1.577	1.354	1.533	1.345	1.517	1.538	1.563	1.365	1.559	1.315	1.556
Lowest Tertile	1.389	1.597	1.374	1.511	1.593	1.511	1.385	1.433	1.356	1.468	1.345	1.430	1.335	1.554	1.429	1.629
p for linear trend	0.33	0.39	0.81	0.84	0.59	0.32	0.81	0.81	0.61	0.65	0.54	0.10	0.29	0.18	0.92	0.61
Food Frequency Questionnaire																
Highest Tertile	1.369	1.461	1.346	1.574	1.378	1.499	1.421	1.561	1.403	1.550	1.385	1.536	1.405	1.555	1.372	1.528
Middle Tertile	1.431	1.544	1.426	1.518	1.373	1.590	1.300	1.533	1.392	1.567	1.360	1.575	1.432	1.538	1.410	1.510
Lowest Tertile	1.322	1.664	1.341	1.501	1.377	1.529	1.387	1.469	1.333	1.466	1.374	1.470	1.305	1.497	1.349	1.578
p for linear trend	0.17	0.54	0.38	0.33	0.27	0.63	0.34	0.34	0.31	0.68	0.88	0.64	0.40	0.76	0.24	0.99
Supplements Only [2]																
Users	1.386	1.545	1.392	1.534	1.386	1.532	NA[3]	NA	1.386	1.545	NA	NA	1.469	1.693	1.385	1.730
Non-user	1.380	1.539	1.378	1.553	1.380	1.553	NA	NA	1.380	1.539	NA	NA	1.365	1.520	1.377	1.511
Overall *p* value	0.72	0.85	0.76	0.69	0.91	0.33			0.72	0.85			0.63	0.69	0.64	0.01

[1] Associations adjusted for sex, age, BMI, cotinine, physical activity, education, income, alcohol intake, and plasma cholesterol for the fat soluble nutrients.
[2] Dietary supplement estimates from open-ended in-person dietary supplement inventory.
[3] NA=Not Available. Estimate is not available due to limited number of observations (cell size <5).

Table 5. Pearson's Partial Correlations[1] of antioxidant nutrient plasma levels and oxidative DNA damage[2], by race

	Total Population			Men		Women	
	Total (n=136)	African American (n=66)	White (n=70)	African American (n=31)	White (n=35)	African American (n=35)	White (n=35)
Vitamin A (retinols)	-0.01	-0.13	0.13	0.45	-0.14	-0.41	0.05
Vitamin C (ascorbic acid)	-0.02	-0.19	0.15	-0.27	0.01	-0.36	0.27
Vitamin E (α-tocopherol)	0.21*	0.13	0.17	0.63*	0.20	-0.05	-0.17
α -Carotene	0.47	0.01	0.06	0.35	0.08	-0.20	-0.06
β-Carotene	0.01	0.05	-0.08	0.15	0.02	-0.24	-0.33
β-Cryptoxanthin	-0.11	-0.13	-0.05	-0.13	-0.23	-0.12	-0.10
Lutein + Zeaxanthin	0.12	0.02	0.08	0.01	-0.23	-0.10	0.08
Lycopene	-0.20*	-0.16	-0.12	-0.34	0.10	-0.26	-0.05
All antioxidants combined[3]	-0.05	-0.15	0.02	0.18	-0.13	-0.36	-0.05

[1] Associations adjusted for age, BMI, cotinine levels, physical activity level, education, income, alcohol intake, plasma cholesterol for the fat soluble nutrients, and sex, where applicable.
[2] Oxidative DNA Damage measured as mean comet tail moment of 100 cells via the comet assay.
[3] Z-scores for the distribution of each antioxidant were calculated and averaged to provide a relative estimate of total antioxidant concentrations.
*p value <0.05

V. Demographic, Behavioral, and Psychosocial Correlates of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults

A. Introduction

There is considerable interest in the roles of antioxidant nutrients and oxidative DNA damage in carcinogenesis. Antioxidants are substances within many foods that decrease the adverse effects of reactive oxygen species (ROS) on normal physiological functions[6]. High ROS levels can lead to oxidative stress, in which the imbalance of radical-generating agent concentrations exceeds the body's defense mechanisms[24,103]. Excess oxidative stress can lead to oxidative damage of DNA, causing significant base damage, strand breaks, and ultimately mutagenesis[25,26]. Continuous oxidative damage to DNA is a significant contributor to the age-related development of cancer[28-30]. Most observational studies provide support for a protective association between high dietary intakes and/or supplemental doses of antioxidant vitamins with cancer risk[3,11,19]; however, two notable randomized trials reported elevated risk of lung cancer with high-dose supplementation in high-risk populations, such as smokers and asbestos workers[15,20,21].

Given the associations of antioxidants and oxidative DNA damage with cancer, identifying factors that may influence these levels is important for several reasons. First, it will provide information on key factors that need to be included in the design of research studies examining antioxidant nutrients and oxidative DNA damage. Second, identification of these potential confounders is important for the statistical analyses and appropriate interpretation of study results. Third, this information may identify mediating variables that could be targeted in cancer prevention initiatives, particularly intervention and education

programs. Studies that have investigated factors related to antioxidant and oxidative DNA damage levels have most often focused on demographic factors, such as age and gender, or behavioral factors, such as smoking, diet, and alcohol use[100,116,117]. While knowledge of these factors may be adequate for studies related to oxidative DNA damage, psychosocial factors have been found to explain a modest amount of variation in fruit and vegetable consumption. For example, in a survey of the National Cancer Institute's (NCI) 5 A Day for Better Health Program, psychosocial factors were shown to explain more of the variability in fruit and vegetable intake than demographic factors alone[84]. Considering that fruit and vegetable intake is a strong determinant of blood antioxidant levels[67], it is essential that psychosocial factors be examined in addition to demographic and behavioral factors in studies of antioxidant nutrient concentrations. To date, investigations of the various participant characteristics that affect antioxidant and oxidative DNA damage have been conducted in largely White populations.

Considerable evidence exists that plasma antioxidant concentrations and oxidative DNA damage levels differ in African Americans and Whites. In the Third National Health and Nutrition Examination Survey (NHANES III), African Americans had the lowest concentrations of serum α-tocopherol among all racial/ethnic groups[59] and serum levels of α-carotene, lycopene, and vitamin E were significantly lower for African Americans than Whites in a North Carolina-based case-control study[107]. Oxidative DNA damage also differs by race. Two studies of healthy adults have reported significantly lower oxidative DNA damage levels in African Americans compared to Whites[65,66]. Furthermore, African Americans are at disproportionately higher risk for many oxidative stress-related medical

conditions and have the highest cancer burden of any racial or ethnic group in the United States[2].

The relationships for demographic, behavioral, and diet-related psychosocial with antioxidants and oxidative DNA damage can be complex. Smoking has been consistently inversely associated with antioxidant concentrations[67,70,100], age and body mass index (BMI) are typically positively associated with antioxidants[67,100], and women often have higher antioxidant concentrations than do men[67,100,118]. Generally, heavy drinking is associated with lower antioxidant concentrations[67], but Svilaas et al. reported a positive association of β-carotene with red wine[119]. For oxidative DNA damage, there is less of a consensus regarding these factors. Older adults generally have higher DNA damage levels[42,116]; however, several large cross-sectional studies of healthy adults showed no difference by age[120,121]. Overall, men tend to have higher oxidative DNA damage levels than women[42,108,109], but some small studies have reported higher levels in women[50]. Physical activity, although generally beneficial, is associated with elevated oxidative DNA levels[50,65]. Although smoking is widely reputed to be associated with elevated oxidative DNA damage[21,121,122], some studies have failed to show an association[66,108]. Clearly further investigation is warranted, especially considering that many of these factors have not been studied within racially diverse populations.

The purpose of this study was to examine potential racial differences in demographic, behavioral, and diet-related psychosocial correlates of plasma antioxidant (carotenoids, vitamin C, and vitamin E) concentrations and oxidative DNA damage in a sample of healthy

African American and White adults in North Carolina. The demographic variables included age, sex, anthropometrics, education, income, marital status, and urban/rural residence; the behavioral variables captured were physical activity, self-reported health status, dietary supplement and non-steroidal anti-inflammatory drug (NSAID) use, passive smoke exposure, alcohol intake, and outdoor exposure; and the diet-related psychosocial factors measured personal beliefs about the benefits of antioxidants and diets high in fruits and vegetables, knowledge of dietary guidelines, ability to afford healthy foods, personal taste preferences, and self-efficacy. As noted above, identification of various factors and characteristics related to antioxidants and oxidative DNA damage, and whether they differ by race, has important implications for the design and implementation of research studies investigating antioxidant nutrients and/or oxidative stress, particularly those conducted in racially diverse populations.

B. Methods

1. Study population Data are from the DIet, Supplements, and Health (DISH) Study, which enrolled 168 generally healthy African American and White adults (approximately equal by race and gender) from the Research Triangle Area of North Carolina between March and December 2005. Participants were recruited via flyers displayed in public venues, such as local churches, gyms, campus-wide emails, and on campus buildings throughout the Research Triangle Area. Eligible participants were 20 to 45 years of age, generally healthy, free of diseases related to oxidative stress (i.e., cancer, diabetes, heart disease, or Alzheimer's disease), and fluent in written and spoken English. Persons likely to

have high levels of oxidative stress, such as current smokers and those with a self-reported body mass index (BMI) of 30 or greater were ineligible. Other exclusion criteria included anorexia or bulimia nervosa, large weight change (more than 15 pounds) in the past year, inability to fast for 6 hours, and pregnancy. Of the 191 respondents deemed eligible during the screening interview, 164 (85.9%) participants enrolled and completed all aspects of the study. Data for nine participants were excluded due to levels of cotinine, a metabolite of nicotine, which were consistent with active smokers (≥15 ng/mL); 155 participants remained (76 African American, 79 White).

2. Data collection Participants completed four unannounced telephone-administered 24 hour dietary recall interviews and a self-administered demographic, health, and antioxidant questionnaire. During a one-time visit to UNC's General Clinical Research Center (GCRC), participants had height, weight, and waist circumference measured, provided urine and semi-fasting (≥6 hours) blood samples, participated in a dietary supplement inventory, and answered questions about the use of NSAIDS drugs, current occupation, outdoor exposure, and last menstrual cycle (women only). Blood samples were analyzed for plasma levels of antioxidant nutrients, cholesterol, oxidative DNA damage, hemoglobin A1C (to confirm self-reported absence of diabetes), and serum cotinine (to validate self-reported smoking status). Each participant received $100 compensation for his/her time upon completion of all study activities. This study was approved by the University of North Carolina at Chapel Hill (UNC)'s Institutional Review Board and written (signed) informed consent was obtained from all participants.

3. Demographic, Health, and Antioxidant Questionnaire All participants completed a self-administered 12 page questionnaire, which included 37 questions pertaining to general health and diet and a newly developed antioxidant food frequency questionnaire (FFQ). The semi-quantitative FFQ was designed to capture usual dietary and supplemental intakes of carotenoids, vitamin C, and vitamin E. The questionnaire contained sections on general health, physical activity, attitudes and beliefs regarding diet, medical history, smoking and alcohol use, demographic characteristics, dietary supplement use, and the new antioxidant FFQ. Whenever possible, questions were adapted from items used in previous studies[81,96,97,123]. We conducted a small pilot study in a convenience sample with representative demographic characteristics (i.e., equally divided by race and gender) to test the questionnaire for feedback about the design, content, and ease of completion and made the necessary modifications. Although both self-reported dietary intakes and plasma concentrations of antioxidants were available, we selected to use plasma concentrations because biomarker measures obviate many of the limitations of self-reported instrument[13].

Demographic Characteristics. Various demographic characteristics were assessed using information from the demographic, health, and antioxidant questionnaire, including sex, age, education (some college or less, college graduate, or advanced degree), marital status (married/living with partner, never married, or divorced/separated/ widowed), income (ranging from <$20,000 to more than $80,000), and county of residence (urban or rural). During the in-person visit at the GCRC, height, weight, and waist circumference were measured. Anthropometrics were assessed two ways: body mass index (BMI) and waist circumference. Using height and weight measurements, BMI was calculated in kg/m^2 and further categorized as normal (18.5 to 24.9), overweight (25.0 to 29.9), or obese ($\geq$30.0)[124].

The average value of three repeated waist circumference measurements was calculated; tertiles of waist circumference were then computed for each sex separately and combined to create sex-specific tertiles.

Behavioral Characteristics. All behavioral factors, except NSAID use and outdoor exposure, were assessed using data from the demographic, health, and antioxidant questionnaire. Usual physical activity was captured using a 2-item question asking if s/he engages in physical activity and if so, how many times per week (none, 1-2, 3-4, 5+ times/week). Single item questions about general health and usual frequency of alcohol intake were used for self-rated health status (response options: excellent, very good, good, fair, or poor) and alcohol consumption (none, <1/week, 1-6/week, 1-2/day, 2-4/day, 4+/day). Passive smoke exposure was assessed by asking whether *anyone in the household smokes now* as a proxy of environmental smoke exposure (yes/no response). Dietary supplement use was queried in a closed-ended format that quantified use (frequency and dose) of multivitamins and herbal supplements. For these analyses, participants were categorized as "non-users" and "users" of multivitamin and herbal supplements separately, if they had used the supplement (even once) *in the past month.* During an in-person interview during the GCRC visit, participants reported how many hours they spent outdoors in the past month (outdoor exposure), as a proxy for environmental exposures, and how often, if ever, they used NSAIDs in the past month.

Diet-Related Psychosocial Factors. Questions adapted from previous studies that examined psychosocial variables as mediating factors in interventions aimed at increasing

fruit and vegetable intake[81,96,97,123] were used to assess several psychosocial factors regarding knowledge, attitudes, taste preferences, ability to afford healthy foods, and self-efficacy. Participants were asked whether they believe a diet and cancer relationship exists, and if so, whether the relationship is strong, moderate, or weak; whether they believed antioxidants were good for health (yes, no, not sure/don't know); how many servings of fruits and vegetables one *should* eat each day for good health (5+, 3-4, 1-2, not sure/don't know); how important it is for them personally to eat a diet high in fruits and vegetables (very, somewhat, or not important); and self-efficacy. Healthful eating self-efficacy was assessed by a Likert-scale item about respondents' confidence (very confident, somewhat confident, not confident, not sure/don't know) in their ability to eat more fruits and vegetables. Participants were also asked whether they felt they could afford healthy foods, such as fruits and vegetables (yes, no, sometimes, not sure/don't know). Taste preference was assessed by asking whether s/he likes the taste of vegetables (yes, no, sometimes).

4. Plasma nutrients Semi-fasting (≥ 6 hours) blood samples were protected from heat and light and analyzed for plasma concentrations of carotenoids, retinols, tocopherols, vitamin C, and cholesterol. The aliquot of plasma designated for ascorbic acid assessment was preserved with a 6% weight/volume metaphosphoric acid (MPA) solution added in a 1:4 ratio plasma to MPA to stabilize vitamin C. Plasma concentrations of retinols, tocopherols (α-tocopherol, γ-tocopherol, and δ-tocopherol), and carotenoids (lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene) were measured using high performance liquid chromatography (HPLC) with multiwavelength photodiode-array absorbance detection[93]. Plasma cholesterol was measured by enzymatic/colorimetric

analyses ("Trinder" procedure) using adaptations of commercially available kits[93]. Quality control samples and 10% duplicates were included in each batch. These assays were performed by Craft Technologies Inc. (Wilson, NC).

5. Oxidative DNA Damage Oxidative DNA damage was assessed using the single cell gel electrophoresis or comet assay. The comet assay is a widely used method for measuring DNA strand breaks at the level of a single cell in which lymphocytes are digested with lesion-specific repair endonucleases[45,54]: the comet assay used here was a slightly modified version in which formamidopyrimidine DNA glycosylase (FPG) (provided by Dr. A.R. Collins, Aberdeen, Scotland, UK) was added to convert oxidized purines into strand breaks[30,94]. Peripheral whole blood lymphocytes were washed in PBS, counted using a hemacytometer, and cryopreserved in 1 ml RPMI-1640 + 15% BSA+ 10% DMSO. All samples were processed within 2 hours of collection and stored at –80ºC until assays were performed. Lymphocytes were sandwiched between 0.5% agarose and 0.5% low-melting-point (37°C) agarose (Fisher, Fair Lawn, NJ). The resulting slides were placed into cold, freshly made lysis solution [10 mmol Tris/L (pH 10), 2.5 mol NaCl/L, 100 mmol EDTA/L, 1% sodium sarcosinate, 10% DMSO, and 1% Triton X-100] at 4 °C for 1 hour and then treated for 20 min in electrophoresis buffer [300 mmol NaOH/L, 1 mmol EDTA/L (pH 13)][53]. After electrophoresis was performed at 25Vand 300mAfor 20 min, slides were incubated 3 times for 5 min in neutralization buffer [0.4 mol Tris/L (pH 7.5)] with FPG, washed with methanol, and stained with SYBR Green. Comet tail length (the distance of DNA migration from the body of the nuclear core) was visualized by using a fluorescence microscope (typically, 100 cells/sample) and SCION IMAGE software[53]. The comet tail

moment (defined as the integrated density in the comet tail multiplied by the distance from the center of the nucleus to the center of mass of the tail) was calculated by using the NIHIMAGEANALYSISMACRO language software.

6. Statistical analyses Data analyses were performed using Stata (version SE 8.2, STATACorp, College Station, TX). Descriptive statistics were calculated for all variables. Missing data were excluded from analyses; on average less than one percent of data were missing. For each study population characteristic, chi-square tests were used to test for equality by race. Antioxidant nutrient levels were assessed as biomarker (plasma) concentrations. Oxidative DNA damage was quantified by the comet tail moment. Log transformations were applied to the dietary and oxidative DNA damage distributions to meet the normality distribution assumptions, as they were right-skewed. Mean levels of antioxidant nutrients and oxidative DNA damage were reported separately by race for each demographic, diet-related psychosocial, and behavioral factor. Potential differences between African Americans and Whites were evaluated using analysis of variance for dichotomous variables and *p* for linear trend and spearman's correlations were calculated for categorical variables. Plasma cholesterol was included in all analyses evaluating fat-soluble plasma antioxidant levels, as it affects bioavailability[67]. Forward stepwise regression analyses, with an addition criteria of 0.05 and plasma cholesterol forced into all models of fat soluble nutrients, were computed separately for each race to determine associations between the demographic, behavioral, and diet-related psychosocial correlates and plasma antioxidant concentrations and between the demographic and behavioral correlates and oxidative DNA

damage. Statistical tests were two-sided and p values ≤0.05 were considered statistically significant.

C. Results

The distributions of demographic and lifestyle characteristics, stratified by race and sex (n=155) are given in Table 6. The mean ages of African American participants was 30.9 years (7.9 SD) and 53% were female; in comparison, the mean age for Whites was 32.5 years (7.9 SD) and 52% were female. African Americans had statistically significantly lower formal educational levels, physical activity, and alcohol consumption than Whites and were also more likely to be obese (BMI >30kg/m^2). African American males were somewhat younger (20-28 years) than White males (58% vs. 34%), and females of both races tended to have higher BMI and lower alcohol consumption than men.

Table 7 gives the mean plasma antioxidant concentrations of vitamin C, vitamin E, carotenoids (β-carotene, lutein + zeaxanthin, and lycopene), and the levels of oxidative DNA damage for each of the demographic correlates examined, presented separately for African Americans and Whites. Potential differences by race were compared using analysis of variance for dichotomous variables and Spearman's correlations and p for linear trend tests for categorical variables. Age was positively associated with plasma concentrations in Whites (p for trend <0.05 for vitamin E, β-carotene, and lutein + zeaxanthin); however, there were no associations in African Americans. Although not statistically significant, antioxidant concentrations tended to be highest for those of normal weight (BMI=18.5-24.9 kg/m^2); with statistically significant inverse associations of BMI with Vitamin C for both

races. Similar associations were seen for waist circumference, except that there were also significant positive associations with vitamin E (r=0.27, p=0.02 in African Americans and *p* for trend=0.01 in Whites). Higher concentrations of almost all antioxidants and oxidative DNA damage were seen for those participants who were married/living with a partner compared to those who were single/ separated or divorced, with statistically significant differences for vitamin E, β-carotene, and lycopene concentrations in Whites. Both income and education were positively associated with antioxidant concentrations for most nutrients; however statistically significant results were seen only in Whites for vitamin E, β-carotene, and lycopene. For oxidative DNA damage, there were no associations in Whites; however BMI and waist circumference were positively associated with oxidative DNA damage in African Americans.

Mean plasma antioxidant concentrations and oxidative DNA damage levels for the behavioral and psychosocial correlates are presented in Table 8. Physical activity was generally positively associated with plasma antioxidant concentrations, with statistically significant associations seen for vitamin C (Whites only), β-carotene (Whites only), and lutein + zeaxanthin (both races). All antioxidant plasma concentrations, except lycopene, were higher for those who took multivitamins in both races; similar trends were seen for herbal supplement use. Passive smoke exposure was associated with greater oxidative DNA damage (*p*=0.009 in African Americans) and alcohol consumption was significantly positively associated with oxidative DNA damage in Whites only.

Those who *believe there is a "strong" relationship between diet and cancer risk* had statistically significant higher lutein + zeaxanthin (African Americans only) and β-carotene (both races) concentrations. Those who believed *antioxidants were good for health* had higher vitamin C concentrations (p=0.01 for African Americans) and lower oxidative DNA damage (p=0.02 for African Americans). African Americans who *knew that 5 or more FV servings are recommended for health* had statistically significant higher lutein + zeaxanthin, whereas White participants who knew *5 or more FV servings are recommended for health* had statistically significantly higher vitamin E concentrations. The *importance of a diet high in FV* was positively associated with vitamin E concentrations in African Americans (spearman's r=0.24, p=0.04) and with vitamin E, β-carotene, and lutein + zeaxanthin in Whites. In Whites only, those *able to afford healthy foods* had statistically significant higher vitamin E concentrations. Finally, those White participants who *liked the taste of vegetables* had statistically significantly higher vitamin E, β-carotene, and lutein + zeaxanthin concentrations.

Table 9 gives the results from the stepwise regression analyses (criteria of 0.05 for addition to model) examining demographic, behavioral, and psychosocial correlates with antioxidant plasma concentrations, stratified by race. For vitamin C, herbal supplement use, *belief that antioxidants are healthy* and *self-efficacy to eat a high FV diet* accounted for 29% of the variation of plasma concentrations in African Americans and herbal supplement use alone accounted for 10% in Whites. The beta-coefficients presented should be interpreted accordingly (note, all plasma concentrations and oxidative DNA damage variables were log-transformed): those participants who used herbal supplements had vitamin C concentrations

30.8% higher (African Americans) and 28.8% higher (Whites) than those who did not use herbal supplements. For vitamin E, cholesterol alone, which was forced into all models for fat-soluble nutrients, explained 21% of the variance for African Americans. Cholesterol, combined with age and multivitamin use, accounted for 46% of the variance in vitamin E concentrations in Whites. For β-carotene, African Americans who believed *antioxidants are good for health* had plasma concentrations 49.6% higher than those who did not (R^2=0.11). In Whites, *belief in the diet and cancer link*, income, and physical activity explained 43% of β-carotene concentrations. For lutein+zeaxanthin in African Americans, the final model included cholesterol, *belief in the diet and cancer link,* and *knowledge of the recommended FV servings* (R^2=0.27); those who believed *the diet and cancer link is "moderate" or "strong"* had plasma concentrations 28% higher than those who felt the link was "weak/did not exist." In Whites, the final model for lutein+zeaxanthin included *knowledge of the recommended FV servings,* herbal supplement use, waist circumference, physical activity, and "living with a smoker" (R^2=0.50). For lycopene, age was inversely related to plasma concentrations in Whites; whereas, cholesterol, waist circumference, and *belief in the diet and cancer link* were significantly correlated with lycopene in African Americans.

The regression analyses results examining demographic and behavioral correlates with oxidative DNA damage (measured as mean comet tail moment) are given in Table 10. For African Americans, only passive smoke exposure was included in the model, which explained 9% of the variation in oxidative DNA damage levels. Based on these results, those participants who lived with a smoker had oxidative DNA damage levels 24.8% higher than those who did not live with a smoker. For Whites, only age (categorized into approximate

tertiles) remained in the model (R^2= 0.14); those aged 38 to 45 had oxidative DNA damage levels 8.2% higher than the youngest age group (20 to 28 years) and those 29 to 37 years had oxidative DNA damage levels 7.9% lower than the youngest age group.

D. Discussion

In this cross-sectional study of healthy African American and White adults in North Carolina (NC), we examined: 1) demographic, behavioral, and diet-related psychosocial correlates of plasma antioxidant concentrations, and 2) demographic and behavioral correlates of oxidative DNA damage. Based on these results, the salient demographic, behavioral, and psychosocial correlates differed by races. The sole demographic characteristic associated with antioxidant concentrations in African Americans was age, whereas in Whites, age, waist circumference, and income were each statistically significantly associated with at least one antioxidant. The only significant behavioral correlate for African Americans was herbal supplement use; however, herbal supplement use and several other behavioral variables, including physical activity, multivitamin use, and passive smoke exposure were associated in Whites. The psychosocial correlates with antioxidant concentrations for both races were *belief in the diet and cancer link* and *knowledge of recommended FV servings.* B*elief that antioxidants are good for health* and *self-efficacy to eat a high FV diet* were also statistically significantly associated with plasma concentrations in African Americans. For oxidative DNA damage, only passive smoke exposure in African Americans and age in Whites had demonstrated significant associations.

1. Demographic correlates and antioxidant concentrations

Of the demographic correlates examined, only three (age, income, and waist circumference) were significant for either race. Age was *inversely* associated with plasma lycopene in African Americans and *positively* associated with vitamin E concentrations in Whites. Generally, age is positively associated with antioxidant concentrations[67,100]. The inverse association with lycopene in African Americans may be an anomaly in this sample; however, it supports the need to examine potential confounders separately by race. Income was significantly positively correlated with β-carotene (Whites only), lutein+zeaxanthin (Whites only), and vitamin E (both races) concentrations (spearman's r=0.25-0.34). This association is likely due to a difference in fruit and vegetable intake, as national survey data indicate that fruit and vegetable intake is lower for those with low incomes[125].

Waist circumference was significantly associated with several antioxidants; however in different directions (positively for lycopene and vitamin E, while inversely for lutein+zeaxanthin and vitamin C). Similar results were seen in a cross-sectional study in Sweden, where β-carotene concentrations were inversely associated and vitamin E concentrations were positively associated with waist circumference[70]. Two explanations were offered for this inverse association: 1) since β-carotene is stored in fat tissue, those with excess tissue would store more β-carotene and thus, have lower circulating plasma levels, or 2) obese persons likely consume fewer FV, which are antioxidant rich foods[70]. Whereas, waist circumference is a measure of abdominal adiposity, BMI estimates total body fat[126]. Although not significant in regression models, there appeared to be an inverse relationship with BMI with mean antioxidant concentrations, especially for vitamin C, β-carotene, and

lutein+zeaxanthin. The relationships seen here for waist circumference and BMI are especially notable considering those with a self-reported BMI above 30 kg/m^2 were ineligible to enroll in this study. These associations of antioxidant concentrations with BMI and waist circumference would likely be even more striking in samples with a wider range of anthropometric values.

2. Behavioral correlates and antioxidant concentrations

Four of the behavioral correlates examined, i.e., physical activity, passive smoke exposure, herbal supplement use, and multivitamin use, were significantly associated with at least one antioxidant concentration in the regression analyses. Although not all statistically significant, there were positive associations of physical activity frequency with each mean plasma antioxidant concentrations examined, except lycopene, and statistically significant associations in Whites for vitamin C, β-carotene, and lutein+zeaxanthin (both races). These results are consistent with previous work that reported associations of physical activity with elevated antioxidant concentrations[67]. Smoking has been consistently shown to be inversely associated with various antioxidant concentrations[67,70,100] including lutein+zeaxanthin[118]. We found that lutein+zeaxanthin concentrations were 44% lower for those living with a smoker in Whites. Considering this sample was restricted to nonsmokers and few participants (6%) lived with smokers, one would expect minimal effect from smoking. The association seen here for lutein+zeaxanthin provides support to studies showing smoking as an important factor in antioxidant concentrations.

Vitamin E concentrations were 18% higher among White participants who took a multivitamin, compared to those who did not. Dietary supplements can contribute large amounts to total antioxidant intake, especially for vitamin E. On average 8-10 mg of vitamin E comes from food, yet dietary supplement doses are often much larger (e.g., 180 mg from single supplements)[71,74,75]. It is somewhat surprising that the same association was not seen in African Americans. Perhaps, this reflects differences in supplement use patterns, as 66% of African Americans in this study reported taking no supplements compared to only 49% of Whites. There were consistently higher mean plasma concentrations of all antioxidants except lycopene for multivitamin users compared to non-users. Trends seen for herbal supplement use were similar to those for multivitamin use. In both races, vitamin C concentrations were approximately 30% higher in those who used herbal supplements. This association is not unexpected as many herbal supplements also include vitamins and minerals. Furthermore, the herbal supplements most frequently reported in this sample were ginseng, which naturally contains vitamin C[127], and glucosamine/chondritin, which can be packaged with vitamin C.

3. Diet-related psychosocial correlates and antioxidant concentrations

The most salient psychosocial factors based on the regression analyses appeared to be: *belief in the link between diet and cancer*, *belief that antioxidants are good for health*, and *the knowledge of recommended FV servings*. Those with a *"strong" belief in the link between diet and cancer* had plasma concentrations approximately 20% higher than those with a "*weak" belief* for β-carotene and lycopene in Whites and lutein+zeaxanthin in African Americans. This provides supports for studies that have shown that those who believe in the

association between diet and disease have statistically significantly higher fruit and/or vegetable intakes[88,90]. In African Americans, *believing that antioxidants are good for health* was associated with statistically significant higher vitamin C and β-carotene concentrations, but was not significant for Whites. Fewer White participants were either "not sure" or felt antioxidants were "not" good for health than African Americans. For both races, *knowledge of the recommended FV servings* was associated with lutein+zeaxanthin concentrations approximately 20% higher compared to those who believed <5 FV servings were recommended, which mirrors results from a study that found that knowledge of recommended FV servings resulted in 22% increase in fruit and vegetable (antioxidant rich foods) intakes[84]. Surprisingly, *self-efficacy to eat a diet high in FV* was inversely related to vitamin C in African Americans, based on the regression analyses. However, very few numbers of participants, i.e., less than five per race, responded that they were "not" confident. Thus, these results are likely an anomaly of this study sample as self-efficacy, defined as the extent to which one believes s/he can successfully perform a given behavior, has consistently been shown to positively influence healthy dietary behavior[82,84,87,88]. Psychosocial factors allow for evaluation of mediating factors, which are variables that explain how two variables are related and help explain dietary patterns[128]. For example, *knowledge of FV servings,* as discussed above, is related to fruit and vegetable intake and also lutein+zeaxanthin concentrations. By measuring these diet-related psychosocial factors, one may be able to gain insight into the motivations of a dietary pattern.

4. Demographic and behavioral correlates with oxidative DNA damage

Of the demographic and behavioral correlates considered for inclusion in the regression analyses with oxidative DNA damage, only passive smoke exposure was significant in African Americans and age in Whites. Each model explained less than 15% of the variance, suggesting there are additional important variables either not considered here or that were not captured in these analyses. Our results in African Americans support results in other studies showing that smoking is associated with elevated oxidative DNA damage levels[21,121,122]. Since our sample was restricted to non-smokers, we used whether *they lived with a smoker* as a proxy for passive smoke exposure.

The relationship with age and oxidative DNA damage in Whites was not linear, as the middle age category (29 to 37) had the lowest oxidative DNA damage. It is possible that this sample was too young to see the effects of aging, considering that in studies that found associations of age with oxidative DNA damage, differences were usually seen for those approximately 60 years and older[42,116]. Interestingly, when mean oxidative DNA damage was compared by passive smoke exposure without adjusting for any covariates, damage was statistically significantly *lower* for African Americans living with a smoker. However, these results were confounded by age as all but one person who reported living with a smoker was in the youngest age category, which was significantly correlated with oxidative DNA damage (r=0.24). Considering the correlates we identified, age and smoke exposure are among the most strongly associated factors in the literature, it suggests that oxidative DNA damage and the correlates were accurately captured here.

Although the diet-related psychosocial factors were not considered in the regression analyses for oxidative DNA damage, it is worth noting that in African Americans, those who *believed that antioxidants were good for health* had statistically significantly lower oxidative DNA damage. However, the results of the regression analyses were the same whether or not psychosocial factors were considered: age and passive smoke exposure were the only significant variables.

Obesity has also been associated with elevated oxidative DNA damage levels[50,100]. Although not included in the final regression model, both BMI and waist circumference were statistically significantly correlated with oxidative DNA damage (r=0.27 and 0.25) in African Americans; however, there appeared to be no association in Whites. These associations are remarkable given the exclusion of participants with a self-reported BMI <30; one would one expect to see even stronger associations if examined in a sample with a greater range in obesity. Furthermore, the oxidative DNA damage levels here are relatively homogenous and low, as this is a young and healthy sample. Associations may be more apparent in a sample with a greater distribution of values.

We also explored the regression models for oxidative DNA damage with less conservative inclusion criteria to see which "marginal" correlates might be added to the model. Only education (at an inclusion criteria of 0.2) was added to passive smoke exposure in the model for African Americans. For Whites, alcohol intake, sex, and outdoor exposure were added to age in the regression analyses at an inclusion criteria of 0.2 (R^2=0.33). Although not significant here, these factors have been associated with oxidative DNA

damage[25,42,129] and may be important correlates in other populations. It is notable that none of these factors were associated with oxidative DNA damage in both African Americans and Whites, suggesting that correlates of oxidative DNA damage should be further examined separately by race.

Our study has several strengths. To our knowledge, it is the first study to examine the correlates of plasma antioxidant concentrations and oxidative DNA damage separately by race in a sample of healthy African American and White adults. Our survey instrument was adapted from questionnaires that have been used in other studies[81,82,90,130,131]. Plasma concentrations of antioxidant nutrients were assessed using biomarkers, which are objective measures unaffected by many of the biases associated with self-reported dietary intake and also may be more biologically relevant than self-report intake[13]. In addition, oxidative DNA damage was measured using a modified comet assay with FPG, which is considered to be an optimal measure for oxidative stress[56].

We also acknowledge some limitations. First, self-reported data are subject to both random and systematic bias[67]. Second, the limited sample size may obscure some of the associations examined, especially for those variables with multiple responses stratified by race. Third, the fact that our study population consisted of generally healthy volunteers may limit generalizability, particularly since adults willing to participate in a research study may be more health conscious than the general public. Fourth, some measures designed to capture complex behaviors, e.g., physical activity, were measured using one or two self-reported items. Fifth, the psychosocial factors we examined are not a complete sampling of

possible psychosocial variables that could be studied in this context. Last, due to the cross-sectional nature of this study, no inferences about causality can be drawn.

In summary, based on these results, the correlates of antioxidant concentrations and oxidative DNA damage differ for African Americans and Whites. Thus, it is important to include and measure these items as accurately as possible in future studies so that potential racial differences can be examined. Generally, the regression models here explained more of the variance in plasma concentration in Whites, 10% (vitamin C) to 50% (lutein+zeaxanthin), than in African Americans, 11% (β-carotene) to 29% (vitamin C). Less of the variance in oxidative DNA damage was also explained in regression analyses with demographic and behavioral correlates (R^2=0.09 in African Americans and R^2=0.14 in Whites). Considering that most studies have been conducted in largely White populations, this is not unexpected as many of these correlates were selected based on the literature. These results generally confirm other studies suggesting that demographic, behavioral, and psychosocial correlates potentially influence plasma antioxidant concentrations. Particular attention should be paid to age, physical activity, dietary supplement use (multivitamins and herbals), waist circumference, income, *knowledge of recommended servings of FV*, and *belief in the diet and cancer link* in Whites and age, dietary supplement use, and the diet-related psychosocial variables, *knowledge of recommended servings of FV*, *belief that antioxidants are good for health*, and *belief in the diet and cancer link* in African Americans. Based on the results presented, age and smoking (passive and active exposure) should be examined in all investigations of oxidative DNA damage. Additional studies using similar methods but with larger demographically-diverse samples containing sufficient ranges of important variables,

such as age, race, BMI, and smoking exposure, are needed so that data can be stratified and analyzed with adequate statistical power.

Table 6. Characteristics of Study Participants Stratified by Race (n=155)

Characteristic	African Americans (n=76)[1]	White (n=79)	*p* value[2]
Sex			
Male	36 (47%)	41 (48%)	
Female	40 (53%)	38 (52%)	0.93
Age			
20-28	34 (45%)	26 (33%)	
29-37	22 (29%)	27 (34%)	
38-45	20 (26%)	26 (33%)	0.32
BMI[3]			
Normal (18.5–24.9 kg/m^2)	32 (42%)	58 (73%)	
Overweight (25–29.9 kg/m^2)	38 (50%)	19 (24%)	
Obese (³30 kg/m^2)	6 (8%)	2 (3%)	<0.0001
Education			
Some College or less	30 (39%)	20 (25%)	
College graduate	33 (43%)	32 (41%)	
Advanced Degree	13 (17%)	27 (34%)	0.03
Marital Status			
Single/Separated or Divorced	45 (59%)	39 (49%)	
Married/Living with partner	31 (41%)	40 (51%)	0.22
Income			
Less than $20,000	14 (21%)	14 (19%)	
$20,000-39,000	16 (24%)	18 (24%)	
$40,000-79,000	20 (29%)	27 (36%)	
$80,000 or more	17 (25%)	16 (22%)	0.86
Dietary Supplement Use			
None	50 (66%)	39 (49%)	
Multivitamin Only	15 (20%)	16 (20%)	
2 or More Supplements	11 (14%)	24 (30%)	0.05
Physical Activity			
Less than twice/week	41 (50%)	21 (26%)	
3-4 times per week	28 (37%)	29 (38%)	
5+ times per week	7 (9%)	29 (35%)	<0.0001
Alcohol Consumption			
Never	34 (45%)	15 (19%)	
Less than 1 per week	22 (29%)	24 (30%)	
1-6 times per week	17 (22%)	33 (42%)	
1 or more per day	3 (4%)	8 (10%)	0.0002
Self-Rated Health Status			
Excellent	18 (24%)	26 (33%)	
Very Good	37 (49%)	38 (48%)	
Good /Fair	21 (27%)	15 (19%)	0.49
County of Residence			
Urban	65 (86%)	68 (86%)	
Rural	9 (12%)	6 (8%)	
Not Specified	2 (3%)	5 (6%)	0.39

[1] Numbers may not total 76 for African Americans and 79 for Whites due to rounding and missing data.
[2] Overall p value for African Americans compared to Whites determined by chi-square.
[3] BMI calculated as kg/m^2, based on measured weight (kg) and height (m^2).

Table 7. Mean Plasma Antioxidant and Oxidative DNA Damage Levels for Demographic Correlates, by Race (n=155)

	Vitamin C (ascorbic acid) (µg/ml)		Vitamin E (α-tocopherol) (µg/ml)		β-carotene (µg/ml)		Lutein + Zeaxanthin (µg/ml)		Lycopene (µg/ml)		Oxidative DNA Damage[1]	
	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=74)	White (n=77)
Sex												
Male	8.38	9.02	7.64	9.81	0.18	0.19	0.12	0.12	0.48	0.44	1.41	1.53
Female	8.82	8.24	7.35	10.43	0.18	0.27	0.12	0.14	0.40	0.38	1.40	1.58
Overall *p* value[2]	0.45	0.12	0.72	0.54	0.67	0.15	0.61	0.20	0.10	0.02	0.90	0.55
Age												
20-28	8.38	8.30	7.17	9.01	0.17	0.17	0.11	0.12	0.44	0.37	1.35	1.53
29-37	9.49	8.63	7.94	10.52	0.19	0.26	0.13	0.14	0.47	0.43	1.42	1.43
38-45	8.03	8.92	7.64	10.89	0.19	0.28	0.12	0.15	0.40	0.44	1.49	1.71
p for linear trend	0.44	0.39	0.69	0.001	0.61	0.001	0.49	0.02	0.19	0.06	0.12	0.11
Spearman Correlation[3]	-0.01	0.04	0.14	0.28*	0.05	0.29**	0.05	0.20	-0.09	0.20	0.24*	0.19
BMI[4]												
Normal (18.5–24.9 kg/m^2)	9.45	8.81	7.08	10.20	0.20	0.24	0.13	0.14	0.40	0.42	1.35	1.57
Overweight (25–29.9 kg/m^2)	8.15	8.36	8.03	10.03	0.17	0.22	0.11	0.11	0.47	0.37	1.42	1.54
Obese (30 kg/m^2)	7.04	5.32	6.55	9.50	0.14	0.10	0.14	0.10	0.44	0.55	1.67	1.47
p for linear trend	0.002	0.04	0.38	0.71	0.29	0.11	0.63	0.02	0.61	0.77	0.02	0.72
Spearman Correlation	-0.35**	-0.09	0.06	-0.04	-0.10	-0.13	-0.05	-0.27**	0.18	-0.03	0.27**	-0.01
Waist Circumference												
Lowest Tertile (Sex-specific)	9.46	8.92	6.30	9.42	0.20	0.23	0.11	0.14	0.39	0.40	1.34	1.52
Middle Tertile (Sex-specific)	8.72	8.81	7.90	9.83	0.23	0.24	0.14	0.14	0.51	0.40	1.37	1.59
Highest Tertile (Sex-specific)	7.99	7.76	8.03	11.93	0.14	0.23	0.11	0.12	0.43	0.45	1.48	1.57
p for linear trend	0.001	0.11	0.33	0.01	0.09	0.77	0.67	0.31	0.38	0.43	0.05	0.63
Spearman Correlation	-0.30**	0.14	0.27**	0.21	-0.17	0.04	-0.07	-0.07	0.05	0.08	0.25**	0.07

[1] Oxidative DNA Damage measured as mean comet tail moment of 100 cells via the comet assay; results were unavailable for 4 participants due to missing samples.

[2] Differences between each demographic variable and the log-transformed distributions of plasma antioxidant concentration or oxidative DNA damage were calculated by t-test, separately for total African Americans and Whites. Plasma cholesterol was included in all models of fat soluble nutrients. *p <0.05, **p <0.01, ***p <0.001

[3] Spearman's correlations computed for all categorical variables with 3 or more responses.

[4] BMI calculated as kg/m^2, based on measured weight (kg) and height (m^2).

Table 7. (cont'd) Mean Plasma Antioxidant and Oxidative DNA Damage Levels for Demographic Correlates, by Race (n=155)

	Vitamin C (ascorbic acid) (µg/ml)		Vitamin E (α-tocopherol) (µg/ml)		β-carotene (µg/ml)		Lutein + Zeaxanthin (µg/ml)		Lycopene (µg/ml)		Oxidative DNA Damage	
	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=74)	White (n=77)
Education												
Some College or less	8.35	8.71	7.21	9.14	0.18	0.18	0.12	0.12	0.40	0.41	1.41	1.55
College graduate	9.02	8.30	7.59	9.70	0.18	0.21	0.13	0.12	0.45	0.38	1.45	1.59
Advanced Degree	8.16	8.93	8.01	11.41	0.19	0.31	0.12	0.16	0.51	0.46	1.27	1.53
p for linear trend	0.98	0.64	0.56	0.004	0.17	0.001	0.98	0.003	0.03	0.31	0.45	0.94
Spearman Correlation	-0.03	0.04	0.14	0.29**	0.17	0.36***	-0.06	0.34*	0.31**	0.05	-0.02	0.02
Marital Status												
Single/Separated or Divorced	8.34	8.15	7.20	9.50	0.19	0.20	0.12	0.13	0.44	0.38	1.38	1.50
Married/Living with partner	9.00	9.07	7.97	10.77	0.17	0.27	0.12	0.14	0.45	0.45	1.44	1.61
Overall *p* value	0.83	0.16	0.39	0.02	0.72	0.001	0.82	0.11	0.82	0.02	0.32	0.24
County of Residence												
Urban	8.66	8.62	7.55	10.21	0.19	0.23	0.12	0.14	0.43	0.40	1.41	1.56
Rural	7.89	8.43	7.47	9.43	0.17	0.18	0.16	0.10	0.52	0.50	1.34	1.56
Overall *p* value	0.69	0.85	0.74	0.88	0.97	0.58	0.01	0.19	0.15	0.02	0.44	0.74
Income												
Less than $20,000	9.27	7.83	8.33	8.92	0.20	0.18	0.11	0.11	0.43	0.37	1.32	1.51
$20,000-39,000	7.84	8.38	6.36	9.88	0.20	0.19	0.13	0.13	0.50	0.38	1.44	1.55
$40,000-79,000	9.32	9.02	7.32	10.23	0.20	0.24	0.12	0.13	0.37	0.46	1.44	1.53
$80,000 or more	8.58	9.41	8.53	11.52	0.16	0.33	0.13	0.18	0.49	0.43	1.39	1.69
p for linear trend	0.64	0.03	0.41	0.02	0.57	0.001	0.95	0.03	0.60	0.11	0.51	0.44
Spearman Correlation	-0.01	0.15	0.25**	0.28**	-0.07	0.34**	-0.01	0.25**	0.02	0.18	0.06	0.09

Table 8. Mean Plasma Antioxidant and Oxidative DNA Damage Levels for Behavioral and Psychosocial Correlates, by Race (n=155)

	Vitamin C (ascorbic acid) (µg/ml)		Vitamin E (α-tocopherol) (µg/ml)		β-carotene (µg/ml)		Lutein + Zeaxanthin (µg/ml)		Lycopene (µg/ml)		Oxidative DNA Damage[1]	
	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=74)	White (n=77)
Usual Physical Activity												
<2 times per week	8.77	7.63	7.45	9.64	0.19	0.18	0.11	0.12	0.44	0.44	1.47	1.57
3-4 times per week	7.86	8.61	7.20	10.23	0.16	0.23	0.12	0.14	0.48	0.40	1.36	1.62
5+ times per week	9.06	9.14	7.78	10.54	0.28	0.27	0.16	0.15	0.43	0.41	1.32	1.48
p for linear trend	0.75	0.08	0.84	0.11	0.20	0.02	0.11	0.02	0.36	0.75	0.16	0.28
Spearman Correlation	-0.05	0.19	0.03	0.11	0.11	0.24**	0.19	0.22**	0.09	-0.12	-0.12	-0.17
Self-Rated Health Status												
Good /Fair	7.77	8.30	7.71	11.06	0.15	0.25	0.11	0.12	0.46	0.41	1.44	1.59
Very Good	9.22	8.74	7.10	10.18	0.19	0.24	0.12	0.14	0.42	0.44	1.40	1.51
Excellent	8.34	8.62	8.13	9.57	0.21	0.22	0.14	0.13	0.45	0.37	1.38	1.61
p for linear trend	0.61	0.82	0.23	0.57	0.21	0.82	0.04	0.09	0.81	0.41	0.76	0.78
Spearman Correlation	0.10	<0.01	0.19	-0.15	0.07	-0.02	0.24*	0.08	-0.05	-0.18	-0.02	0.01
Multivitamin Use												
Yes	10.62	9.32	8.54	11.71	0.20	0.28	0.13	0.15	0.43	0.41	1.33	1.59
No	7.89	8.16	7.15	9.21	0.18	0.21	0.12	0.13	0.45	0.42	1.43	1.54
Overall *p* value	0.003	0.09	0.07	<0.0001	0.58	0.03	0.61	0.09	0.55	0.72	0.31	0.67
Herbal Supplement Use												
Yes	13.10	10.28	8.30	10.75	0.28	0.32	0.13	0.17	0.32	0.39	1.40	1.49
No	8.14	8.18	7.32	9.97	0.18	0.21	0.12	0.13	0.45	0.42	1.40	1.57
Overall *p* value	0.003	0.008	0.14	0.22	0.31	0.02	0.61	0.03	0.13	0.52	0.96	0.43
NSAIDs[2] Use												
Yes	8.38	8.58	7.71	10.13	0.15	0.23	0.12	0.13	0.45	0.41	1.41	1.57
No	9.03	8.82	7.15	10.23	0.24	0.23	0.12	0.17	0.43	0.44	1.40	1.50
Overall *p* value	0.10	0.56	0.83	0.50	0.004	0.63	0.50	0.10	0.78	0.89	0.98	0.60

[1] Oxidative DNA Damage measured as mean comet tail moment of 100 cells via the comet assay; results were unavailable for 4 participants due to missing samples.
[2] NSAIDs= Non steroidal anti-inflammatory drugs

Table 8. (cont'd) Mean Plasma Antioxidant and Oxidative DNA Damage Levels for Behavioral and Psychosocial Correlates, by Race

	Vitamin C (ascorbic acid) (µg/ml)		Vitamin E (α-tocopherol) (µg/ml)		β-carotene (µg/ml)		Lutein + Zeaxanthin (µg/ml)		Lycopene (µg/ml)		Oxidative DNA Damage	
	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=74)	White (n=77)
Passive Smoke Exposure												
Lives with a smoker	8.61	9.00	6.99	9.81	0.15	0.36	0.12	0.23	0.39	0.45	1.13	1.41
No one at home smokes	8.61	8.65	7.56	10.19	0.19	0.23	0.12	0.13	0.45	0.41	1.43	1.56
Overall *p* value	0.52	0.78	0.97	0.64	0.46	0.22	0.96	0.04	0.56	0.62	0.009	0.48
Alcohol Consumption												
Never	8.56	8.81	7.15	9.71	0.21	0.22	0.12	0.14	0.40	0.39	1.43	1.44
Less than 1 per week	9.28	8.66	6.95	10.90	0.17	0.33	0.11	0.14	0.46	0.47	1.35	1.53
1-6 times per week	7.73	8.39	8.69	9.41	0.15	0.19	0.12	0.12	0.45	0.40	1.41	1.57
1 or more per day	9.24	9.11	9.11	11.95	0.25	0.15	0.13	0.16	0.69	0.33	1.35	1.87
p for linear trend	0.73	0.92	0.16	0.76	0.35	0.05	0.38	0.93	0.07	0.19	0.75	0.02
Spearman Correlation	0.07	0.05	-0.16	0.01	0.14	0.25*	0.09	0.07	-0.22*	0.15	0.06	-0.20
Outdoor Exposure[1]												
< 30 hours / month	8.41	6.76	7.34	9.27	0.16	0.18	0.12	0.12	0.44	0.41	1.45	1.28
30-59 hours / month	8.21	9.23	7.01	10.43	0.16	0.23	0.12	0.14	0.45	0.40	1.34	1.59
60-89 hours / month	7.57	9.12	7.62	10.38	0.19	0.27	0.11	0.14	0.44	0.41	1.50	1.60
90+ hours / month	9.76	7.82	7.97	9.78	0.21	0.21	0.13	0.12	0.43	0.43	1.37	1.56
p for linear trend	0.45	0.83	0.51	0.77	0.30	0.84	0.93	0.82	0.61	0.51	0.75	0.31
Spearman Correlation	0.10	-0.08	0.05	-0.11	0.14	0.02	0.07	-0.10	-0.03	0.01	-0.04	0.13
The link between diet & cancer is:												
Weak/None	7.97	8.37	7.44	9.72	0.15	0.19	0.10	0.12	0.41	0.42	1.44	1.60
Moderate	9.32	8.28	7.15	10.52	0.21	0.28	0.14	0.15	0.51	0.39	1.30	1.49
Strong	9.23	10.66	8.24	10.18	0.23	0.21	0.14	0.12	0.41	0.48	1.46	1.69
p for linear trend	0.09	0.07	0.24	0.27	0.04	0.03	0.002	0.40	0.67	0.56	0.96	0.93
Spearman Correlation	0.21	0.14	0.16	0.12	0.20	0.29*	0.37***	0.15	0.06	-0.02	<0.01	<0.01

[1] Outdoor Exposure assessed during in-person interview by asking participants how many hours they spent outdoors in the past month.

Table 8. (cont'd) Mean Plasma Antioxidant and Oxidative DNA Damage Levels for Behavioral and Psychosocial Correlates, by Race

	Vitamin C (ascorbic acid) (µg/ml)		Vitamin E (α-tocopherol) (µg/ml)		β-carotene (µg/ml)		Lutein + Zeaxanthin (µg/ml)		Lycopene (µg/ml)		Oxidative DNA Damage	
	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=76)	White (n=79)	African American (n=74)	White (n=77)
Believe antioxidants are good for health?												
Yes	9.04	8.72	7.68	10.02	0.18	0.24	0.12	0.14	0.45	0.40	1.35	1.56
Not Sure/Don't Know	7.19	7.94	7.02	11.39	0.19	0.19	0.12	0.13	0.43	0.49	1.56	1.57
Overall *p* value	0.01	0.62	0.87	0.43	0.64	0.44	0.90	0.87	0.92	0.09	0.02	0.87
Knowledge of FV servings												
5 or More	9.22	8.61	7.16	10.85	0.23	0.25	0.14	0.15	0.43	0.41	1.40	1.58
4 or less	8.07	8.93	8.07	9.60	0.18	0.24	0.11	0.12	0.46	0.40	1.33	1.54
Overall *p* value	0.13	0.98	0.51	0.02	0.27	0.12	0.03	0.62	0.82	0.50	0.81	0.67
Importance of High FV diet												
Not Important	7.57	9.58	7.05	7.12	0.11	0.12	0.12	0.10	0.38	0.59	1.65	1.46
Somewhat Important	8.47	8.69	7.05	9.67	0.16	0.21	0.11	0.12	0.44	0.42	1.37	1.50
Very Important	8.88	8.52	8.27	10.57	0.21	0.26	0.13	0.15	0.43	0.41	1.41	1.60
p for linear trend	0.45	0.86	0.13	0.02	0.08	0.03	0.23	0.005	0.48	0.38	0.79	0.19
Spearman Correlation	0.11	-0.02	0.24*	0.28*	0.18	0.26*	0.16	0.37***	-0.03	-0.03	0.07	0.14
Self Efficacy to Eat FV												
Not Confidant	15.66	7.64	7.91	8.07	0.12	0.18	0.10	0.10	0.32	0.35	1.43	1.52
Somewhat Confidant	8.36	8.38	7.20	10.84	0.16	0.24	0.13	0.14	0.42	0.49	1.47	1.65
Very Confidant	8.29	8.81	7.70	10.13	0.20	0.24	0.12	0.14	0.46	0.39	1.36	1.53
p for linear trend	0.08	0.22	0.75	0.32	0.18	0.22	0.67	0.10	0.23	0.40	0.21	0.62
Spearman Correlation	-0.09	0.12	0.09	0.18	0.14	0.11	-0.08	0.17	0.16	-0.06	-0.09	-0.06
Able to Afford Healthy Foods?												
Yes	8.55	8.44	7.84	10.57	0.18	0.25	0.12	0.14	0.43	0.42	1.41	1.54
No or Sometimes	8.79	9.21	6.52	8.99	0.19	0.20	0.12	0.11	0.48	0.40	1.41	1.59
Overall *p* value	0.70	0.59	0.21	0.04	0.83	0.07	0.78	0.007	0.30	0.46	0.60	0.27
Like the Taste of Vegetables?												
Yes	8.66	8.73	7.70	10.30	0.19	0.24	0.12	0.14	0.45	0.42	1.38	1.57
No	8.78	7.81	6.23	9.06	0.14	0.18	0.09	0.13	0.33	0.38	1.59	1.45
Overall *p* value	0.40	0.22	0.11	0.04	0.31	0.01	0.14	0.05	0.06	0.16	0.08	0.38

Table 9. Results of Regression Models[1] Relating Demographic, Behavioral, and Psychosocial Correlates with Plasma Antioxidant Concentrations (n=155)

	African Americans				Whites			
	Variable	β coef	*p* value	R^2	Variable	β coef	*p* value	R^2
Vitamin C (ascorbic acid)	Herbal Supplement Use: (User vs. Non-user)	0.308	0.05	0.29	Herbal Supplement Use: (User vs. Non-user)	0.288	0.009	0.10
	Antioxidants are good for health: (yes vs. not sure/no)	0.298	0.01					
	FV self-efficacy (somewhat vs. not confidant)	-0.550	0.01					
	FV self-efficacy (very vs. not confidant)	-0.598	0.006					
Vitamin E (α -Tocopherol)	Cholesterol (mg/dl)	0.004	<0.0001	0.21	Cholesterol (mg/dl)	0.004	<0.0001	0.46
					Multivitamin Use: (User vs. Non-user)	0.184	0.001	
					Age: (29-37 vs. 20-28)	0.130	0.04	
					Age: (38-45 vs. 20-28)	0.211	0.002	
β-Carotene	Cholesterol (mg/dl)	-0.002	0.48	0.11	Cholesterol (mg/dl)	0.00004	0.98	0.43
	Belief that antioxidants are good for health	0.496	0.01		Belief in the diet and cancer link: (moderate vs. weak/no)	0.512	<0.001	
					Belief in the diet and cancer link: (strong vs. weak/no)	0.187	0.30	
					Income (20-39,000 vs. <20,000)	0.052	0.77	
					Income (40-79,000 vs. <20,000)	0.445	0.01	
					Income (80,000+ vs. <20,000)	0.500	0.01	
					Usual physical activity (3-4x/wk vs. <2/wk)	0.296	0.05	
					Usual physical activity (5x+/wk vs. <2/wk)	0.435	0.01	

[1] Forward stepwise regression models, using an addition criteria of 0.05, were computed for each nutrient and oxidative DNA Damage separately by race. Cholesterol was automatically retained in all models for the fat soluble nutrients (all nutrients here, except vitamin C).

Table 9. (cont'd) Results of Regression Models Relating Demographic, Behavioral, and Psychosocial Correlates with Plasma Antioxidant Concentrations (n=155)

	African Americans				Whites			
	Variable	β coef	*p* value	R^2	Variable	β coef	*p* value	R^2
Lutein + Zeaxanthin	Cholesterol (mg/dl)	0.001	0.66	0.27	Cholesterol (mg/dl)	0.002	0.07	0.50
	Belief in the diet and cancer link: (moderate vs. weak/no)	0.280	0.003		Herbal Supplement Use: (User vs. Non-user)	0.191	0.04	
	Belief in the diet and cancer link: (strong vs. weak/no)	0.279	0.008		Knowledge of rec. FV servings (5+ vs. <5)	0.251	0.001	
	Knowledge of rec. FV servings (5+ vs. <5)	0.174	0.05		Waist Circumference (in)	-0.031	0.003	
					Usual physical activity (3-4x/wk vs. <2/wk)	0.279	0.003	
					Usual physical activity (5x+/wk vs. <2/wk)	0.259	0.004	
					Lives with a smoker	-0.441	0.008	
Lycopene	Cholesterol (mg/dl)	0.005	0.01	0.26	Cholesterol (mg/dl)	0.003	0.001	0.27
	Age: (29-37 vs. 20-28)	-0.130	0.23		Waist Circumference (in)	0.024	0.03	
	Age: (38-45 vs. 20-28)	-0.295	0.007		Belief in the diet and cancer link: (moderate vs. weak/no)	-0.096	0.24	
					Belief in the diet and cancer link: (strong vs. weak/no)	0.216	0.06	

Table 10. Results of Regression Models[1] Relating Demographic and Behavioral Correlates with Oxidative DNA Damage Levels (n=155)

	African Americans				Whites			
	Variable	β coef	*p* value	R^2	Variable	β coef	*p* value	R^2
Oxidative DNA Damage	Lives with a smoker	0.248	0.02	0.09	Age: (29-37 vs. 20-28)	-0.079	0.22[2]	0.14
					Age: (38-45 vs. 20-28)	0.082	0.22	

[1] Forward stepwise regression models, using an addition criteria of 0.05, were computed for each nutrient and oxidative DNA Damage separately by race. Cholesterol was automatically retained in all models for the fat soluble nutrients

[2] Overall *p* value for age category variable= 0.04. The *p* value presented here tests each individual age category (e.g. 29 to 37 years), given all other age categories (e.g., 20 -28 and 38-45 years) are included in the model.

VI. Associations of Psychosocial Factors with Fruit and Vegetable Intake in African Americans

A. Introduction

Diets high in fruits and vegetables are associated with lower risks of obesity and several chronic illnesses[3,7,9,132,133]. In the United States (US), African Americans are at disproportionately higher risk for many diet-related medical conditions, such as diabetes[105] and cardiovascular disease[104] and have the highest cancer burden of any US racial or ethnic group[2]. Approximately 70% of African Americans are overweight or obese, considerably higher than the national average (57% for the total population)[134]. Underscoring these disparate health risks are survey data showing that African Americans do not meet the recommended 5 to 9 servings of fruits and vegetables daily[80]. According to the 2002 Behavioral Risk Factor Surveillance Survey (BRFSS), less than 19% of African Americans in North Carolina (NC) consumed at least 5 fruit and vegetable servings per day, which is lower than the median for the US (22.6%) and NC White populations (24.7%)[79]. Baseline data from the National Cancer Institute's (NCI) 5 A Day program indicate that African Americans consume more fruit (mostly via fruit juice) but fewer vegetables than Whites[106]. On average, African American men and women consume 3.3 and 3.5 servings of fruits and vegetables per day, respectively, far less than the recommended 5 to 9 servings[106]. A variety of demographic and environmental factors, including age, gender, education, socioeconomic status, childhood eating patterns, and the local food environment, have been associated with lower fruit and vegetable intakes among African Americans[133-135] and although less studied, so have several key psychosocial variables, such as self-efficacy and social support[82,138,139].

Interventions to increase fruit and vegetable consumption in the general population have been conducted with varying levels of success, with most programs resulting in increases of 0.2 to 0.6 servings per day[85]. These interventions have typically examined sociodemographic characteristics, such as age, gender, education, and socioeconomic status, and a handful have considered psychosocial factors as potentially mediating variables[81-83]. However, psychosocial factors may be important predictors or correlates of dietary behavior, particularly fruit and vegetable consumption. For example, results from NCI's 5 A Day program showed that psychosocial factors were more important determinants of fruit and vegetable intake than demographic factors alone[84]. Three dietary interventions aimed at African American churches that incorporated both demographic and psychosocial factors in their design resulted in relatively large increases of 0.7 to 1.4 fruit and vegetable servings per day[85]. Even so, few studies have examined the possible influence of psychosocial factors on fruit and vegetable intake, and there is even less such data for African Americans. One recent study of psychosocial factors in a sample of African American men concluded that men were motivated by perceived benefits to consume fruits, whereas vegetable consumption was driven by extrinsic rewards[140]; we are not aware of a similar study in African American women. Clearly, additional knowledge regarding the possible impact of psychosocial factors on fruit and vegetable consumption is essential for designing optimal interventions to promote this behavior in African American men and women.

One particularly effective theory-based dietary intervention trial, the Black Churches United for Better Health Project, used the PRECEDE/PROCEED planning framework to organize concepts based on the Social Cognitive Theory, Stages-of-Change Transtheoretical Model, and Social Support Models[82]. This intervention resulted in an increase of 0.85 servings

of fruits and vegetables per day after 2 years. The PRECEDE (Predisposing, Reinforcing, and Enabling Constructs in Educational Diagnosis and Evaluation) planning framework, used to understand motivations for healthy dietary behaviors and mediating factors in dietary interventions, categorizes psychosocial factors into 3 main categories: predisposing, reinforcing, and enabling factors[86]. Predisposing factors are antecedents that influence the likelihood of how one will behave and include the individuals' knowledge, attitudes, beliefs, existing skills, personal preferences, and self-efficacy (i.e., the extent one believes he/she can successfully perform a given behavior)[86]. Reinforcing factors are incentives following a behavior that may affect the likelihood that this behavior will be repeated over time, such as social support, peer influence, significant others, and rewards[86]. Enabling factors help facilitate a behavior and may include programs, services, and resources necessary for a behavior to occur[86]. It has been noted that this model is particularly well-suited for studies of minority populations because it is amenable to adaptation to the population of interest[91].

In this report, we use the PRECEDE framework to 1) describe psychosocial (predisposing, reinforcing, and enabling) factors related to fruit and vegetable intake, and 2) examine associations of these factors with fruit and vegetable intake in a population-based sample of African American men and women in North Carolina. This work has important implications for the design of interventions to increase fruit and vegetable intake in African Americans.

B. Methods

1. Study population and data collection. Data presented here were collected as part of a study examining methods and strategies to recruit African Americans into cancer prevention studies. Detailed study design and data collection information are described elsewhere[141]. Briefly, 5,000 potential African American participants, 18-70 years, residing in 6 North Carolina counties (3 urban, 3 rural) were randomly selected from Department of Motor Vehicle rosters and assigned at random to one of five recruitment strategies, based on variations of approach letters and inclusion, non-inclusion, or promise of an incentive. Specifically, the five recruitment strategies were: generic letter only, culturally sensitive letter only, culturally sensitive letter plus promise of an incentive, generic letter plus included incentive, and culturally sensitive letter plus included incentive. All prospective participants were sent an 11-page questionnaire by mail with a pre-paid return envelope, as well as instructions for completing the survey via the Internet or by telephone. An advance postcard was sent to alert potential participants to the upcoming questionnaire mailing and a reminder letter was sent 2-3 weeks later with information for obtaining a replacement questionnaire and instructions for completing the survey by telephone or the internet. The questionnaire assessed various demographic, lifestyle, dietary, and behavioral cancer risk factors and was pretested in a small sample. The study had a 17.5% response rate (n=747): 87.7% by mail, 11.2% via the Internet, and 1.1% by telephone. Data were excluded from 89 respondents who did not meet eligibility criteria and whose questionnaires failed quality-control checks; data from the remaining 658 persons were used for the analyses presented here. The study was approved by the Institutional Review Board of the School of Public Health at the University of North Carolina - Chapel Hill.

2. Survey Instrument. Using the PRECEDE framework as a guide, an 11-page questionnaire was designed to measure demographic, psychosocial, lifestyle, and behavioral factors related to cancer prevention. Three sets of these questions were used in our analyses: diet-related psychosocial factors, demographic characteristics, and fruit and vegetable intake. All data are self-reported.

3. Diet-related psychosocial factors. Questions designed to capture psychosocial factors were adapted from previous studies that used the PRECEDE framework to examine psychosocial variables as mediating factors in interventions aimed at increasing fruit and vegetable intake[81,96,97]. PRECEDE organizes psychosocial factors into 3 main categories: predisposing, reinforcing, and enabling factors[86]. Predisposing factors included questions regarding knowledge, attitudes, taste preferences, and self-efficacy. Healthful eating self-efficacy was assessed by a Likert-scale (very confident, somewhat confident, or not very confident) item about respondents' confidence in their ability to eat more fruits and vegetables. Reinforcing factors addressed social support. Respondents were asked whether they felt they could count on those close to them: to encourage them to eat healthfully; to tell them about healthier foods and how to prepare them; to prepare healthier foods with them; and to eat healthier foods with them. Enabling factors included four items related to perceived barriers to healthy eating and queried respondents on whether: they can afford to purchase healthy foods and meals; it takes too much time and trouble to prepare healthy meals; it is easy for them to order healthy foods in restaurants; and they need more information on how to prepare healthy foods and meals. Scales were created for each set of factors by linearly summing responses to individual questions (least healthy responses scored the lowest and the healthiest responses

scored the highest). All questions had an equal number of possible responses and a summary score for each scale was computed as the mean of the non-missing responses. The distinctions "least healthy" and "most healthy" are used only to categorize the responses to each psychosocial factor. We do not intend to make any inference to actual behavior. Table 11 gives the questions, response options, and the distribution of participants' responses.

4. Demographic characteristics. Various demographic characteristics were assessed, including age (categorized approximately into tertiles), gender, education (less than or equivalent to high school, some college, college graduate, or advanced degree), marital status (never married, married/living with partner, or divorced/separated/widowed), self-rated health status (excellent, very good, good, fair, or poor), and county of residence (urban or rural). Using self-reported height and weight, body mass index (BMI) was calculated as kg/m^2 and further categorized as normal (18.5 to 24.9), overweight (25.0 to 29.9), or obese ($\geq$30.0)[124]. Information was collected about other lifestyle and behavioral characteristics, such as physical activity and smoking, but was not included in these analyses.

5. Fruit and vegetable intake. Fruit and vegetable consumption during the past 3 months was assessed using the seven-item fruit and vegetable screener developed at the National Cancer Institute[142,143]. Fruit intake was the sum of "fruit juice" and "fruit, not counting juice", and vegetable intake was calculated as the sum of green or lettuce salad, potatoes (boiled, baked, or mashed), other vegetables, beans and peas, and vegetables in mixed dishes. Fruit and vegetable intake was calculated as the sum of all seven items. The standard approach for evaluation in the 5 A Day program was used to calculate fruit and vegetable servings per day[144].

6. Statistical analyses. Data analyses were performed using Stata (version SE 8.2, STATACorp, College Station, TX). Descriptive statistics (means and percentages for continuous and categorical variables, respectively) were calculated for all demographic, psychosocial, and dietary variables. Missing data were excluded from analyses; on average less than two percent of data were missing. For each demographic characteristic, one-way ANOVA models were used to assess whether there were statistically significant differences between the mean values of each psychosocial (i.e., predisposing, reinforcing, and enabling) scale and mean fruit and vegetable consumption (servings per day). To examine associations between the psychosocial scales (categorized into approximate tertiles) and fruit and vegetable intake, we used multiple linear regression models to calculate unadjusted and adjusted (for age, gender, education, and BMI) means for fruit, vegetable, and total fruit and vegetable intake (servings per day) as well as overall p values. We also compared associations of each psychosocial factor (categorized by least healthy to most healthy response) with fruit and vegetable intake by using multiple linear regression models to generate mean values for fruit and vegetable intake, unadjusted and adjusted for age, gender, education, BMI, and the other predisposing, reinforcing, and enabling factors. The fruit and vegetable variables were not transformed because the data were not markedly skewed, based on recommendations in Curran, et al.[145]. Statistical tests were two-sided and p values ≤ 0.05 were considered statistically significant.

C. Results

Table 11 gives each predisposing, reinforcing, and enabling factor and the distributions of responses (n=658). Participants expressed healthy beliefs regarding many of, but not all, the psychosocial factors. Among predisposing factors, half of the participants believed it is

important to eat a diet high in fruits and vegetables and 60% were very confident they had the ability to increase their intake; however, only 26% knew that 5 or more daily servings of fruits and vegetables are recommended. The vast majority had heard of the Food Guide Pyramid (82%) and liked the taste of most fruits (91%) and vegetables (79%). Among reinforcing factors (social support), 88% of respondents could count on those around them "a lot" or "some" to encourage them if they tried to eat healthier foods. Approximately half could rely on their family and social referents "some" to: tell them about healthier foods (52%), prepare healthier foods with them (46%), and eat healthier foods with them (56%). Among enabling factors, most respondents (72%) could afford to purchase fruits and vegetables and 52% stated that it does not take a lot of time and trouble to prepare healthy foods. About a third believed it is easy to order healthy foods in restaurants (38%) and did not need more information on how to prepare healthy foods (30%).

Table 12 gives mean psychosocial scale scores and fruit and vegetable intakes by demographic characteristics. The mean age of participants was 43.9 years (11.6 SD); 57% were female, 40% had some college education, 76% were overweight or obese (BMI greater than 24.9 kg/m^2), 56% were married/living with partner, and 82% resided in an urban county. In comparison, based on 2000 NC census data for the six counties included here, 53% were female, 30% had some college education, 68% were overweight or obese (using BRFSS NC statewide data), 44% were married/living with partner, and 82% resided in an urban county[134,146]. Females had statistically significantly higher predisposing scale scores, lower reinforcing and enabling scores, and higher fruit and vegetable intakes than males. Higher education was positively associated with predisposing scale scores and fruit and vegetable intake; respondents with

advanced degrees reported eating almost one extra serving of fruits and vegetables each day compared to those with a high school degree or less. Excellent or very good self-rated health (43% of respondents) was inversely associated with the predisposing and enabling scales, whereas respondents with poor self-rated health had the highest fruit and vegetable intakes (all $p<0.001$).

Associations of individual psychosocial factors with fruit and vegetable intake are given in Tables 13-15. All analyses were adjusted for age, gender, education, BMI, and the other psychosocial (predisposing, reinforcing, and enabling) factors within its category. Table 13 presents the associations of fruit and vegetable intake with each individual predisposing factor. Three of the seven predisposing factors were statistically significantly associated with higher total fruit and vegetable intake, with differences between the healthiest and least healthy responses ranging from 0.5 to 0.9 serving per day. The two predisposing factors associated with the largest differences were *belief in the importance of a diet high in fruits and vegetables* (0.9 serving) and *high self-efficacy to eat more fruits and vegetables* (0.7 serving). The amount of variance in intakes explained by the demographic and predisposing factors ranged from 9% (adjusted R^2 for vegetable intake) to 11% (adjusted R^2 for total fruit and vegetable intake); only 2-3% of the variance is explained by demographic characteristics alone (data not shown).

As shown in Table 14, only one reinforcing factor was significantly associated with fruit and vegetable intake; specifically, total fruit and vegetable intake was approximately 0.8 serving per day higher for those who felt they *could count on those close to them to help prepare healthier foods* "a lot" (2.9 servings per day) compared to "not at all" (2.1 servings per day).

There were no significant associations for any of the enabling factors (Table 15). The variance in fruit and vegetable intakes explained by reinforcing, enabling, and/or demographic factors was small, ranging from 2-4%.

We also examined associations of fruit and vegetable intake with the predisposing, reinforcing, and enabling factor scale scores (data not shown). Individual scales were created by linearly summing the responses within each category and dividing by the number of factors within each category (i.e., predisposing, reinforcing, and enabling). Healthiest responses, as defined in Table 11, were scored the highest. Respondents in the healthiest tertile of the predisposing scale consumed almost 1.3 more daily servings of fruits and vegetables than those in the lowest tertile (3.2 vs.1.9 servings/day, $p<0.001$) after controlling for age, gender, education, and BMI. There were also slightly higher total fruit and vegetable intakes for those in the healthiest tertile of the enabling scale compared to the least healthy tertile (0.6 serving per day, $p=0.03$). There were no significant associations for the reinforcing scale.

Associations of each significant individual psychosocial factor (presented in Tables 13-15) with fruit and vegetable intake, adjusted for age, education, BMI, and all other statistically significant psychosocial factors are given in Table16. Associations are shown for the total study population and also stratified by gender. After adjustment, all four psychosocial (3 predisposing and 1 reinforcing) factors as above were still significantly associated with total fruit and vegetable intake: *belief in the importance of a diet high in fruits and vegetables, high self-efficacy to eat more fruits and vegetables, knowledge of recommended fruit and vegetable servings,* and *could count on those close to them to help prepare healthier foods*, with

differences between the healthiest and least healthy responses of 1.0, 0.7, 0.6, and 0.5 serving per day, respectively. For fruits only, 2 predisposing factors (*belief in the importance of a diet high in fruits and vegetables* and *high self-efficacy)* remained significant after adjustment, whereas for vegetables only, all 3 predisposing factors remained significant.

Since women reported higher intakes (Table 11), we explored whether there were gender differences in the associations of psychosocial factors with fruit and vegetable consumption. For total fruits and vegetables, both men and women with a strong *belief in the importance of a high fruit and vegetable diet* reported significantly higher intakes compared to those with a weak/no belief in this relationship (0.9 and 1.1 servings for men and women, respectively). Among men, no other factors were significantly associated with high fruit and vegetable intakes; however, for women, the following factors were statistically significant: *high self-efficacy* (0.9 serving), *having someone with whom to prepare healthy foods* (0.9 serving), and *knowledge of recommended servings* (0.7 serving). Similar trends were found for fruit intake. For vegetables, both men and women *who like the taste of vegetables* reported significantly higher intakes compared to those who did not (0.5, 0.2 and 0.6 serving for men and women, men only, and women only, respectively). One additional factor remained significant after adjustment in men (*knowledge of recommended servings)* and in women (*high self-efficacy)* (0.5 serving for each).

D. Discussion

This study examined psychosocial correlates of fruit and vegetable intake, using the PRECEDE framework, in a population-based sample of 658 African American men and women in North Carolina. We found that items from the predisposing and reinforcing scales were

associated with fruit and vegetable consumption; however, the predisposing factors, specifically *belief in the importance of a high fruit and vegetable diet* and *high self-efficacy to eat more fruits and vegetables*, had the strongest associations with fruit and vegetable intake.

Several demographic factors were also associated with the psychosocial scales and fruit and vegetable intake. Women, those with higher education, and those with high self-rated health reported higher fruit and vegetable consumption, confirming previous work[82,90,106,137]. These groups of participants also had higher predisposing scale scores, supporting our finding that among the psychosocial factors, predisposing variables were most strongly associated with fruit and vegetable consumption. Also, more of the variance in fruit and vegetable intake was explained by the psychosocial (particularly predisposing) factors than by demographic characteristics. Men reported higher reinforcing and enabling scores than women, suggesting that men may focus more on external or environmental factors, rather than the individual, (intrapersonal) predisposing factors. Respondents 50-70 years, those with normal BMI, and those with higher self-rated health reported higher enabling scores; the latter group also had high fruit and vegetable intakes.

These relationships of psychosocial factors with fruit and vegetable intake have been reported in other studies that applied the PRECEDE framework[81,87,147]. In the Working Well Trial, a worksite intervention consisting of a largely White population, Kristal et al. reported that predisposing factors were stronger predictors of fruit and vegetable intake than were reinforcing or enabling factors and found greater differences (those with highest predisposing scale scores consumed 1.6 extra servings of fruit and vegetables compared to those with the lowest)[81] than in

the present study. Other investigations using different theoretical frameworks and conducted in largely White or Asian populations have also found that predisposing factors are associated with higher intakes of fruits and vegetables[84,147-150]. Regrettably, there are few such studies with sizeable numbers of African Americans to which we can compare our results.

The sole significant reinforcing factor, *could count on those close to them to help prepare healthier foods*, was significant for women but not for men, with a difference of approximately one fruit and vegetable serving for those who could and could not count on others. Similar results have been reported in other studies of African Americans, suggesting an important role of social support in dietary change[151] and preventive health practices[152] in African Americans. None of the enabling factors were significantly associated with fruit and vegetable consumption, perhaps suggesting that the specific variables we examined may not be salient in this study population. Nonetheless, other enabling factors may still be appreciable barriers to higher fruit and vegetable consumption in African Americans.

We also found that relationships of fruit and vegetable intake with psychosocial factors differed between men and women. Only two factors were salient for both men and women: s*trong belief in the importance of a high fruit and vegetable diet* (with total fruit/vegetable and fruit consumption) and *taste preference for vegetables* (with vegetable intake). *Knowledge of the recommended servings, self-efficacy,* and *having someone with whom to prepare healthy foods* were only associated with higher consumption in women, while *knowledge of fruit and vegetable recommendations* was only associated with higher vegetable intakes in men. These results in women are supported by a recent study of low-income African American mothers, in

which high self-efficacy and awareness of health benefits were associated with later stages of change[153]. High self-efficacy has consistently been shown to influence healthy dietary behavior in women[82,84,87,88,144]. The latter results are in agreement with those reported by Moser and colleagues who found that different factors influenced fruit versus vegetable consumption in African American men[140]. Specifically, intrinsic benefits and social norms influenced fruit consumption, whereas extrinsic benefits, such as tangible rewards, and preferences for other foods influenced vegetable consumption in men. However, in a racially diverse population, Van Duyn et al. found that perceived benefits (which Moser called intrinsic benefits) were associated with both fruit and vegetable intake in men, but were associated with neither in women[84]. Data from a cross-sectional survey in Washington State indicated that intrinsic motives were associated with fruit and vegetable intake in both men and women, but extrinsic motives were not associated with intake in either men or women[90].

Our results suggest specific psychosocial factors that may be prioritized in intervention design and planning, with an emphasis on factors that can be modified. Specifically, a sizeable portion of study participants reported "less healthy" responses for several important factors associated with fruit and vegetable intake. For example, only 26% of participants knew that 5 or more servings of fruits and vegetables are recommended for good health. Van Duyn et al.'s finding that knowledge of the 5 A Day program resulted in a 22% increase in fruit and vegetable intake in a nationwide sample[84] suggesting this factor is indeed modifiable and important. Similarly, only half of our respondents felt it was "very important" to eat a high fruit and vegetable diet, although it was consistently associated with higher fruit and vegetable intakes.

This study has a number of strengths. To our knowledge, this is the first study of psychosocial factors related to fruit and vegetable consumption in a population-based sample of African American men and women. Respondents represent a demographically diverse population and the sample size was large enough (n=658) to permit detection of associations that may be obscured in smaller studies. Also, our survey instrument was adapted from questionnaires that have been used in other studies[81,82,90,130,131].

We also acknowledge some limitations. The overall response rate was relatively low (17.5%), which may limit the generalizability of our findings and we are unable to compare responders and non-responders in this sample. Based on 2000 US Census data for the six counties included in this study and NC state data in the Behavioral Risk Factor Surveillance Survey (BRFSS), our sample is generally comparable to African Americans in NC (data not shown)[134,146]. In addition, all data are from self-report, which is subject to both random and systematic bias[67]. Fruit and vegetable intake was assessed using a brief seven-item screener, which may result in measurement error, underreporting, and/or misclassification[117,142,154]. Nonetheless, this instrument has been used extensively in other studies[90,142,143]. The psychosocial factors we examined are likely not a complete sampling of possible psychosocial variables that could be studied in this context. Finally, because this is a cross-sectional study, no inferences can be made regarding causality.

In conclusion, while many fruit and vegetable interventions focus on reinforcing (social support) and enabling (barriers) factors, the results of this study suggest that interventions in African Americans that target predisposing factors, such as knowledge, self-efficacy, and

attitudes, may be more effective. This does not mean, however, that reinforcing and enabling factors should be ignored; for example, social support in the provision and preparation of fruits and vegetables may be very helpful for increasing intake in women. Our finding of different associations of psychosocial factors with fruit and vegetable by gender, and specifically that there were fewer salient correlates for men compared to women, also has implications for intervention design. Programs aimed at increasing fruit and vegetable consumption in both men and women might focus on *increasing one's belief in the merits of a high fruit and vegetable diet* and *taste preferences,* and for women specifically, also incorporate *self-efficacy* and *social support*.

Table 11. Distribution of Participants by Response to Each Psychosocial Factor among African Americans in North Carolina (n=658)

	Healthiest Response	N (%)	Moderate Response	N (%)	Least Healthy Response	N (%)
Predisposing Factors						
Do you think what you eat and drink are related to your own chance of getting cancer? (Yes/No); Do you think this relationship between diet and cancer is:	Yes, Strong	324 (49%)	Yes, Moderate	198 (30%)	Yes, Weak Or No	136 (21%)
How many servings of fruits and vegetables should one eat each day for good health?	5 or more	173 (26%)	3 - 4	274 (42%)	1 - 2	211 (32%)
How important is it to you personally to eat a diet high in fruits and vegetables?	Very Important	326 (50%)	Somewhat Important	252 (39%)	Not Important	74 (11%)
If you wanted to eat more fruits and vegetables, how confident are you that you could do it?	Very Confident	389 (60%)	Somewhat Confident	208 (32%)	Not Confident	54 (8%)
Have you ever heard of the Food Guide Pyramid?	Yes	533 (82%)	Not Sure/Don't Know	94 (14%)	No	25 (4%)
Do you like the taste of most fruits?	Yes	591 (91%)	Sometimes	32 (5%)	No	30 (5%)
Do you like the taste of most vegetables?	Yes	514 (79%)	Sometimes	68 (10%)	No	70 (11%)
Reinforcing Factors						
If you tried to eat healthier foods, how much could you count on the people close to you to:						
Encourage you.	A lot	310 (48%)	Some	261 (40%)	Not at all	76 (12%)
Tell you about healthier foods and how to prepare them.	A lot	164 (26%)	Some	336 (52%)	Not at all	142 (22%)
Prepare healthier foods with or for you.	A lot	161 (25%)	Some	300 (46%)	Not at all	185 (29%)
Eat healthier foods with you.	A lot	198 (31%)	Some	361 (56%)	Not at all	89 (14%)
Enabling Factors						
Do you feel that you can afford to purchase healthy foods, such as fruits and vegetables?	Yes	463 (72%)	Sometimes	127 (20%)	No	55 (9%)
Do you feel that it takes a lot of time and trouble to prepare healthy foods and meals?	No	338 (52%)	Sometimes	146 (23%)	Yes	162 (25%)
Do you feel that it is easy for you to order healthy foods when you go out to eat at restaurants?	Yes	246 (38%)	Sometimes	205 (32%)	No	196 (30%)
Do you more need information on how to prepare healthy foods and meals?	No	196 (30%)	Sometimes	75 (11%)	Yes	379 (58%)

Table 12. Mean Fruit and Vegetable Intake By Participant Characteristics among African Americans in North Carolina (n=658)

		Mean Scale Score[1]			Fruit and Vegetable Intake		
Characteristic	N (%)[2]	Predisposing	Reinforcing	Enabling	Vegetables (servings/ day)	Fruits (servings/ day)	Total (servings/ day)
Gender							
Male	271 (41%)	2.35[a3]	2.24[a]	2.23[a]	1.46[a]	0.79[a]	2.25[a]
Female	378 (57%)	2.45[a]	2.05[a]	2.13[a]	1.76[a]	0.94[a]	2.70[a]
Overall *p* value		<0.001	<0.001	0.01	0.004	0.02	0.002
Age (years)							
20-34	154 (23%)	2.34[a,b]	2.04	2.13[a]	1.56	0.89	2.45
35-49	286 (43%)	2.44[a]	2.15	2.13[b]	1.67	0.89	2.56
50-70	218 (33%)	2.44[b]	2.17	2.26[a,b]	1.65	0.86	2.51
p for trend		0.005	0.08	<0.001	0.72	0.88	0.82
Education							
< High School	146 (23%)	2.26[a,b,c]	2.06	2.16	1.47[a]	0.67[a]	2.14[a]
Some College	256 (40%)	2.41[a,d]	2.13	2.13	1.56	0.88[b]	2.44
College graduate	168 (26%)	2.48[b]	2.17	2.22	1.74	0.94	2.69
Advanced Degree	74 (11%)	2.57[c,d]	2.15	2.23	2.01[a]	1.10[a,b]	3.11[a]
Overall *p* value		<0.001	0.44	0.26	0.02	0.001	0.001
BMI							
Underweight (<18.5 kg/m^2)	4 (1%)	2.32	2.19	2.25	2.05	1.48	3.52
Normal (18.5–24.9 kg/m^2)	147 (23%)	2.40	2.16	2.28[a]	1.65	0.90	2.55
Overweight (25–29.9 kg/m^2)	227 (35%)	2.44	2.11	2.18	1.71	0.97	2.68
Obese (≥30 kg/m^2)	266 (41%)	2.39	2.13	2.09[a]	1.58	0.79	2.37
p for trend		0.74	0.87	<0.001	0.68	0.05	0.21
Marital Status							
Single	177 (27%)	2.37	1.99[a]	2.11	1.43[a]	0.87	2.29[a]
Married/Living with partner	368 (56%)	2.43	2.22[a]	2.22	1.69	0.86	2.55
Separated or Divorced	88 (13%)	2.40	2.01	2.11	1.59	0.88	2.47
Widowed	19 (3%)	2.53	2.28	2.10	2.58[a]	1.19	3.77[a]
Overall *p* value		0.10	<0.001	0.03	0.002	0.38	0.01
Self-Rated Health Status							
Excellent	67 (10%)	2.50[a]	2.23	2.28	2.01	1.06	3.07
Very Good	214 (33%)	2.49[b]	2.13	2.24[a]	1.61[a]	0.96	2.57
Good	260 (40%)	2.39	2.12	2.14	1.60[b]	0.81	2.41[a]
Fair	93 (14%)	2.29[a,b]	2.08	2.04[a]	1.44[c]	0.70[a]	2.14[b]
Poor	13 (2%)	2.24	2.23	2.15	2.95[a,b,c]	1.48[a]	4.42[a,b]
Overall *p* value		<0.001	0.55	0.004	<0.001	<0.001	<0.001
County of Residence							
Urban	518 (82%)	2.43[a]	2.14	2.19	1.69[a]	0.90[a]	2.59[a]
Rural	97 (16%)	2.31[a]	2.10	2.09	1.34[a]	0.70[a]	2.04[a]
Overall *p* value		<0.001	0.49	0.06.	0.01	0.02	0.005

[1] Scales were created by combining responses to individual questions (least healthy responses scored the lowest and the healthiest responses scored the highest). Possible scores range from 1.00 to 3.00.
[2] Numbers may not add up to 658 and percentages may not add up to 100% due to rounding and missing data.
[3] Values with same superscript letters are significantly different (<0.05) from one another within characteristic category.

Table 13. Adjusted[1] Mean Fruit and Vegetable Intake By Individual Predisposing Factors among African Americans in North Carolina (n=658)

	Belief that diet is related to cancer risk	Knowledge of recommended FV servings	Belief in importance of a high FV diet	Self-efficacy to eat more FV	Awareness of FGP	Taste preferences for fruits	Taste preferences for vegetables	Unadj. R^2	Adj. R^2
Total Fruits & Vegetables (servings/day)								0.14	0.11
Healthiest Response	2.82	2.82	2.74	2.71	2.54	2.52	2.61		
Moderate Response	2.60	2.50	2.45	2.32	2.44	2.39	2.29		
Least Healthy Response	2.36	2.31	1.87	2.02	2.57	2.82	2.14		
p value	0.06	0.04	0.002	0.01	0.88	0.64	0.10		
Fruits (servings/day)								0.13	0.10
Healthiest Response	0.96	0.95	1.03	0.94	0.88	0.90	0.86		
Moderate Response	0.93	0.87	0.78	0.82	0.86	0.59	1.00		
Least Healthy Response	0.82	0.83	0.58	0.68	0.93	0.87	0.88		
p value	0.16	0.44	<0.001	0.05	0.94	0.13	0.45		
Vegetables (servings/day)								0.11	0.09
Healthiest Response	1.85	1.87	1.71	1.76	1.66	1.62	1.75		
Moderate Response	1.67	1.63	1.67	1.51	1.58	1.80	1.29		
Least Healthy Response	1.54	1.47	1.30	1.34	1.65	1.95	1.25		
p value	0.09	0.03	0.07	0.03	0.87	0.39	0.003		

[1] Mean values adjusted for all predisposing factors, BMI, education, age, and gender.

Table 14. Adjusted[1] Mean Fruit and Vegetable Intake By Individual Reinforcing Factors among African Americans in North Carolina (n=658)

	Can count on people close to you:					
	to encourage you to eat healthy foods	to tell you about healthier foods	to prepare healthier foods with you	to eat healthier foods with you	Unadj. R^2	Adj. R^2
Total Fruits & Vegetables (servings/day)					0.06	0.04
Healthiest Response	2.61	2.26	2.92	2.64		
Moderate Response	2.44	2.52	2.58	2.46		
Least Healthy Response	2.48	2.84	2.11	2.54		
p value	0.68	0.19	0.03	0.72		
Fruits (servings/day)					0.05	0.04
Healthiest Response	0.88	0.72	1.07	0.90		
Moderate Response	0.86	0.90	0.87	0.86		
Least Healthy Response	0.93	1.00	0.73	0.87		
p value	0.84	0.11	0.05	0.94		
Vegetables (servings/day)					0.05	0.03
Healthiest Response	1.73	1.54	1.85	1.73		
Moderate Response	1.59	1.62	1.71	1.60		
Least Healthy Response	1.55	1.83	1.38	1.67		
p value	0.54	0.40	0.08	0.66		

[1] Mean values adjusted for all reinforcing factors, BMI, education, age, and gender.

Table 15. Adjusted[1] Mean Fruit and Vegetable Intake By Individual Enabling Factors among African Americans in North Carolina (n=658)

	Can afford to purchase healthy foods, such as fruits and vegetables	It takes time and trouble to prepare healthy foods	Feel it is easy to order healthy foods at restaurants	Need information on how to prepare healthy foods	Unadj. R^2	Adj. R^2
Total Fruits & Vegetables (servings/day)					0.05	0.03
Healthiest Response	2.52	2.65	2.46	2.72		
Moderate Response	2.49	2.44	2.51	2.65		
Least Healthy Response	2.39	2.29	2.57	2.38		
p value	0.88	0.14	0.84	0.11		
Fruits (servings/day)					0.05	0.03
Healthiest Response	0.90	0.92	0.85	0.94		
Moderate Response	0.85	0.85	0.87	0.92		
Least Healthy Response	0.73	0.80	0.91	0.83		
p value	0.41	0.29	0.79	0.33		
Vegetables (servings/day)					0.04	0.02
Healthiest Response	1.63	1.72	1.61	1.78		
Moderate Response	1.63	1.58	1.64	1.73		
Least Healthy Response	1.66	1.50	1.66	1.54		
p value	0.99	0.21	0.84	0.14		

[1] Mean values adjusted for all enabling factors, BMI, education, age, and gender.

Table 16. Adjusted[1] Mean Fruit and Vegetable Intake by All Significant Psychosocial Factors by Gender for African Americans in North Carolina (n=658)

	Knowledge of recommended FV servings	Belief in importance of a high FV diet	Self-efficacy to eat more FV	Taste preference for vegetables	to prepare healthier foods with you	Unadj. R^2	Adj. R^2
Total Fruits & Vegetables (servings/day)							
Men and Women						0.13	0.11
Healthiest Response[2]	2.86	2.76	2.73	NS[3]	2.77		
Moderate Response	2.50	2.44	2.28	NS	2.55		
Least Healthy Response	2.26	1.80	2.01	NS	2.27		
p value	0.01	<0.001	0.002	NS	0.05		
Men						0.10	0.06
Healthiest Response	2.49	2.57	2.51	NS	2.37		
Moderate Response	2.47	2.24	2.10	NS	2.40		
Least Healthy Response	2.03	1.69	2.11	NS	2.22		
p value	0.09	0.02	0.14	NS	0.81		
Women						0.16	0.13
Healthiest Response	3.19	2.98	2.96	NS	3.35		
Moderate Response	2.62	2.73	2.55	NS	2.65		
Least Healthy Response	2.54	1.89	2.03	NS	2.41		
p value	0.02	0.01	0.02	NS	0.01		
Fruits (servings/day)							
Men and Women						0.11	0.10
Healthiest Response	NS	1.05	0.94	NS	0.95		
Moderate Response	NS	0.76	0.81	NS	0.86		
Least Healthy Response	NS	0.55	0.69	NS	0.83		
p value	NS	<0.001	0.04	NS	0.38		
Men						0.10	0.07
Healthiest Response	NS	0.96	0.86	NS	0.78		
Moderate Response	NS	0.72	0.72	NS	0.83		
Least Healthy Response	NS	0.47	0.77	NS	0.80		
p value	NS	<0.001	0.33	NS	0.86		

[1]Mean values adjusted for all other factors deemed significant in Tables 3-5, BMI, education, and age.
[2]Detailed description of healthiest, moderate, and least healthy responses can be found in Table 1.
[3]NS= Not significant. Factor was not significant after adjustment for BMI, education, age, gender, and other psychosocial factors in Table 3-5.

Table 16. Adjusted[1] Mean Fruit and Vegetable Intake by All Significant Psychosocial Factors by Gender for African Americans in North Carolina (n=658) *con't*

	Knowledge of recommended FV servings	Belief in importance of a high FV diet	Self-efficacy to eat more FV	Taste preference for vegetables	to prepare healthier foods with you	Unadj. R^2	Adj. R^2
Women						0.13	0.10
Healthiest Response[2]	NS[3]	1.14	1.03	NS	1.19		
Moderate Response	NS	0.83	0.93	NS	0.89		
Least Healthy Response	NS	0.65	0.66	NS	0.89		
p value	NS	0.001	0.09	NS	0.05		
Vegetables (servings/day)							
Men and Women						0.09	0.07
Healthiest Response	1.92	NS	1.77	1.74	NS		
Moderate Response	1.63	NS	1.49	1.26	NS		
Least Healthy Response	1.43	NS	1.32	1.26	NS		
p value	0.003	NS	0.01	0.001	NS		
Men						0.09	0.05
Healthiest Response	1.79	NS	1.64	1.64	NS		
Moderate Response	1.61	NS	1.44	1.02	NS		
Least Healthy Response	1.28	NS	1.41	1.49	NS		
p value	0.02	NS	0.36	0.02	NS		
Women						0.10	0.08
Healthiest Response	2.02	NS	1.90	1.86	NS		
Moderate Response	1.70	NS	1.57	1.48	NS		
Least Healthy Response	1.59	NS	1.38	1.24	NS		
p value	0.06	NS	0.04	0.02	NS		

[1]Mean values adjusted for all other factors deemed significant in Tables 3-5, BMI, education, and age.
[2]Detailed description of healthiest, moderate, and least healthy responses can be found in Table 1.
[3]NS= Not significant. Factor was not significant after adjustment for BMI, education, age, gender, and other psychosocial factors in Table 3-5.

VII. Synthesis

A. Overview of findings

This research investigated racial differences in antioxidant (vitamin C, vitamin E, and carotenoids) intakes/blood concentrations and oxidative DNA damage, as well as the association between plasma antioxidant concentrations and oxidative DNA damage in healthy African American and White adults. The data used were from the DIet, Supplements, and Health (DISH) Study, a cross-sectional study of 164 generally healthy non-smoking African Americans and Whites ages 20 to 45 living in North Carolina (NC). We also examined demographic, behavioral, and psychosocial correlates of individual antioxidant concentrations and oxidative DNA damage. In addition, data from a cross-sectional study of African Americans ages 18 to 70 (n=658) were used to study psychosocial correlates of fruit and vegetable (antioxidant rich foods) intake in African Americans. This research fills important gaps in the literature by contributing information about racial differences in 1) antioxidant intakes and plasma blood concentrations, 2) oxidative stress levels, 3) associations between antioxidant concentrations and oxidative stress, 4) demographic, behavioral, and psychosocial factors that influence blood concentrations of antioxidants and oxidative DNA damage levels and also those of antioxidant-rich foods. This work sought to improve our understanding of antioxidant intakes and oxidative stress levels in African Americans in North Carolina, as well as possible racial (African American-White) differences, which may contribute to higher cancer rates for African Americans. This section briefly summarizes this research and provides a synthesis of these findings.

1. Associations of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults

Using data from a cross-sectional study of generally healthy adults in North Carolina (NC), we determined antioxidant intakes and plasma concentrations, oxidative DNA damage, and the association between plasma antioxidant concentrations and oxidative DNA damage by race. Diet was assessed using two self-reported methods, a newly-developed antioxidant food frequency questionnaire and four 24-hour dietary recalls, and plasma biomarkers. Oxidative DNA damage was measure using the alkaline comet assay and reported as the mean comet tail moment. We found that African Americans had statistically significantly lower plasma levels of α-carotene, β-carotene, lutein + zeaxanthin, α-tocopherol, and retinols than Whites. In addition, African Americans also had lower levels of oxidative DNA damage. The only statistically significant inverse association between plasma antioxidants and oxidative DNA damage was found for lycopene in the combined study population (Pearson's r=-0.20, p=0.03). There were also positive associations for α-tocopherol and oxidative DNA damage in the total population (r=0.21, p=0.02) and in African American men (r=0.63, p=0.01) after controlling for sex, age, BMI, passive smoke exposure, physical activity, education, income, and alcohol intake.

This is among the first studies to examine the relationship between various antioxidants and oxidative DNA damage in African Americans and Whites. Oxidative DNA damage is thought to be associated with elevated cancer risk and antioxidants may mitigate the effects of oxidative DNA damage. Diets high in fruits and vegetables, which are rich in antioxidants, have consistently been linked to lower risk of many cancers, including those of the breast, colon/rectum, and prostate, all of which disproportionately affect African Americans. Our findings are in agreement with other studies suggesting that African

Americans may have dietary patterns that put them at higher risk for cancer and oxidative DNA damage. However, we found that oxidative DNA damage levels were actually lower among African Americans than Whites in this study population, which has also been seen in several other studies. It is possible that because participants were generally healthy and young (20 to 45 years), DNA repair activity can compensate for diets low in antioxidants. Continued research, optimally prospective cohort investigations, is needed to assess the relationship among antioxidant nutrients, oxidative damage, and cancer risk, especially in minority populations who suffer a disproportionately high cancer burden.

2. Demographic, Behavioral, and Psychosocial Correlates of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults

Using data from a cross-sectional study of healthy African American and Whites adults in North Carolina, we examined: 1) demographic, behavioral, and diet-related psychosocial correlates of plasma antioxidant concentrations, and 2) demographic and behavioral correlates of oxidative DNA damage. Using forward stepwise regression analyses, we identified salient correlates of plasma antioxidant concentrations and oxidative DNA damage and found they differed for African Americans and Whites. The correlates that were statistically significantly associated with at least one antioxidant in Whites were age, waist circumference, income, physical activity, multivitamin use, herbal supplement use, 'living with a smoker," *belief in the diet and cancer link,* and *knowledge of recommended FV servings*. Fewer correlates were significantly associated with plasma antioxidant concentrations in African Americans and included age, herbal supplement use, *belief in the diet and cancer link*, *belief that antioxidant are good for health*, *knowledge of recommended FV servings*, and *self-efficacy to eat a high FV diet.*

For oxidative DNA damage, only "living with a smoker" in African Americans and age in Whites were significantly correlated based on the regression analyses. The regression models presented here typically explained more of the variance in plasma concentrations and oxidative DNA damage in Whites (R^2=0.10 to 0.50) than African Americans (R^2=0.09 to 0.29). Considering that most studies have been conducted in largely White populations and we selected potential correlates based on the literature, this is not surprising. These results support the need to analyze factors related to antioxidant concentrations oxidative DNA separately by race. Additional studies using similar methods but with larger demographically-diverse samples containing sufficient ranges of critical variables, such as age, race, BMI, and smoking exposure, are needed so that data can be stratified and analyzed with adequate statistical power.

3. Associations of Psychosocial Factors with Fruit and Vegetable Intake in African Americans

We examined psychosocial correlates of fruit and vegetable intake, using the PRECEDE (Predisposing, Reinforcing, and Enabling Constructs in Educational Diagnosis and Evaluation) framework, in a population-based sample of 658 African American men and women in North Carolina. The PRECEDE planning framework categorizes psychosocial factors into 3 main categories: predisposing, reinforcing, and enabling factors. Predisposing factors include the individuals' knowledge, attitudes, beliefs, existing skills, personal preferences, and self-efficacy (i.e., the extent one believes he/she can successfully perform a given behavior). Reinforcing factors are incentives following a behavior, such as social support, peer influence, significant others, and rewards, and enabling factors help facilitate a behavior and may include programs, services, and resources necessary for a behavior to

occur[86]. We found that items from the predisposing and reinforcing scales were associated with fruit and vegetable consumption; however, the predisposing factors, specifically *belief in the importance of a high fruit and vegetable diet* and *high self-efficacy to eat more fruits and vegetables*, had the strongest associations with fruit and vegetable intake.

Our results suggest that specific psychosocial factors that may be prioritized in intervention design and planning, with an emphasis on factors that can be modified. While many fruit and vegetable interventions focus on reinforcing (social support) and enabling (barriers) factors, the results of this study suggest that interventions in African Americans that target predisposing factors, such as knowledge, self-efficacy, and attitudes, may be more effective. This does not mean, however, that reinforcing and enabling factors should be ignored; for example, social support in the provision and preparation of fruits and vegetables may be very helpful for increasing consumption in women. We found that associations of psychosocial factors with fruit and vegetable differed by gender. Specifically, there were fewer salient (and dissimilar) correlates for men compared to women, which has implications for intervention design. For example, programs aimed at increasing fruit and vegetable consumption in both men and women might focus on *increasing one's belief in the merits of a high fruit and vegetable diet* and *taste preferences,* and for women specifically, also incorporate *self-efficacy* and *social support*.

B. Strengths and Limitations

This section addresses the strengths and limitations of the data used for the work presented here. We utilized two datasets to examine racial differences in antioxidant

nutrients, antioxidant-rich foods, and oxidative DNA damage. For ease of presentation, each study is considered separately.

Perhaps, the most striking limitation in the cross-sectional survey of African Americans (n=658) is that the overall response rate was relatively low (17.5%), which may limit the generalizability of these findings. We were unable to compare responders and non-responders in this sample; however, based on 2000 US Census data for the six counties included in this study and NC state data in the Behavioral Risk Factor Surveillance Survey (BRFSS), our sample was generally comparable to African Americans in NC (data not shown)[134,146]. A low response rate, in itself, is not a limitation if those who responded reasonably reflect the general population. Based on these demographic variables, our sample had slightly more formal education, but otherwise was very similar to the general population of African American in NC and thus, should not greatly affect the generalizability. In addition, all data in this study were collected from a diet and health questionnaire mailed to each participant; thus, all analyses were conducted on self-reported data, which are subject to both random and systematic bias[67]. Fruit and vegetable intake was assessed using a brief seven-item screener, which may result in measurement error, underreporting, and/or misclassification[117,142,154].

This study also has a number of strengths. To our knowledge, this is the first study of psychosocial factors related to fruit and vegetable consumption in a sample of African American men and women. Respondents represent a demographically diverse population and the sample size was large enough (n=658) to permit detection of associations that may be

obscured in smaller studies. Finally, the survey instrument was adapted from questionnaires that have been used in previous studies examining psychosocial factors and healthy eating initatives[81,82,90,130,131].

We also acknowledge limitations in the DISH study. Again, self-reported data are subject to both random and systematic bias[67]. For our main outcome variable of diet, we also measured objective biomarker values. Regardless, almost all of the demographic, behavioral, and psychosocial factors considered were from self-report. Second, the relatively small sample size may obscure some of the associations examined, especially for some of the analyses examining variables with multiple responses stratified by race and sex. Third, the fact that our study population consisted of generally healthy volunteers may limit generalizability, particularly since adults willing to participate in a research study may be more health conscious than the general public, and oxidative DNA damage may be much lower in a younger, generally healthy population. Fourth, some measures designed to capture complex behaviors were measured using one or two self-reported items. For example, physical activity was assessed in these analyses as self-reported frequency per week, without measuring occupational activity and incidental activity. Similarly, the psychosocial factors we examined are not a complete sampling of possible psychosocial variables that could be studied in this context. However, these somewhat crude measures still captured these variables well enough that we were able to detect associations that were hypothesized based on published studies.

Due to the cross-sectional nature of this study, no inferences about causality can be drawn. All biological samples were collected and measured at one point in time. These measurements represent only the values on the day of the blood draw (or over several weeks for the fat-soluble antioxidant concentrations). It should be noted that measuring oxidative DNA damage does not account for DNA repair capacity. Also, seasonal differences in diet were not directly assessed. Although macronutrients have been shown to not vary significantly across seasons, there is a reduction in fruit intake, especially citrus fruits, during winter [155-157]. Cursory data analysis showed no difference in either race or sex by month of blood draw (data not shown) and thus, no adjustment for seasonal differences was made during analysis.

Despite of these potential limitations, this research has numerous strengths. This study is among the first to describe associations of antioxidant nutrient levels and oxidative DNA damage in a sample of healthy adults that included a sizeable sample of African Americans and the first, to our knowledge, to examine correlates of antioxidant nutrient concentrations and oxidative DNA damage. Additionally, we collected dietary intake data using two self-report methods (diet recalls and food frequency questionnaire) and biological markers, which has been suggested as the optimal approach for capturing dietary intake[13]. In addition to self-administered queries in the food frequency questionnaire, we collect information about dietary supplement intake directly from the supplement bottles, as this method has been shown to be superior to self-administered queries[72]. Our survey instrument was adapted from questionnaires that have been used in other studies[81,82,90,130,131]. Overall,

this work has made important substantive and methodological contributions to the field of nutrition-related cancer prevention, with special relevance for reducing health disparities.

C. Public Health Significance

Our research has important implications for advancing public health and generating new or different avenues for future research. Given the disparately high cancer burden experienced by African Americans in the US, identifying modifiable factors, such as diet, is critical for cancer prevention programs designed to reduce cancer among African Americans. There are several findings within this work with the potential for great public health impact.

1. Our findings support the need for programs designed to increase fruit and vegetable intake in African Americans

We found that African Americans have lower self-reported intake of antioxidants and also lower plasma antioxidant concentrations. There is considerable data from national and NC-specific studies that also found patterns of lower intake of antioxidant-rich foods among African Americans, compared to Whites. Given the association between fruit and vegetable intake and cancer, it appears that African Americans, including those in North Carolina, have dietary patterns that may put them at higher risk for oxidative stress-related medical conditions, including cancer. Thus, cancer prevention initiatives should consider focusing on programs designed to improve fruit and vegetable intake in African Americans. In addition, this work suggests that such programs would be most effective if the predisposing factors, *belief in the importance of a high fruit and vegetable diet* and *high self-efficacy to eat more fruits and vegetables*, are incorporated into the program.

2. Our findings suggest that antioxidant and oxidative DNA damage should be examined separately by race in future studies

Based on this work, we found that antioxidant concentrations and intakes as well as oxidative DNA damage levels were statistically significantly lower for African Americans than Whites. In addition, we examined the demographic, behavioral, and psychosocial correlates of plasma antioxidant concentrations and oxidative DNA damage (demographic and behavioral correlates only) and found that the correlates differed by race. To our knowledge, this is the first examination of the correlates of both antioxidant concentrations and oxidative DNA damage stratified by race in a sample of African Americans and Whites. When considering these results together, they suggest that not only do blood levels differ, but that demographic, behavioral, and psychosocial factors may also differ. Thus, these findings provide support for examining antioxidant concentrations and oxidative DNA damage separately for African Americans and Whites. Although there were some correlates that were associated with plasma concentrations in both races (i.e., age, herbal supplement use, belief in the diet and cancer link, and knowledge of recommended FV servings), many of the factors we examined were associated with one race but not the other. For example, BMI was statistically significantly correlated with oxidative DNA damage ($r=0.27$) in African Americans, whereas there was no association in Whites. This information could be used to generate hypotheses for future studies, such as examining whether racial differences in how BMI measures fatness affects measures of oxidative DNA damage. The implications of these analyses are that there are different (and possibly additional) factors that contribute to antioxidant concentrations and oxidative DNA damage levels in African Americans and Whites. If one does not examine these levels by race, potentially important difference may

remain hidden. Considering that the literature to date consists of investigations in largely White populations, additional studies in racially-diverse populations are needed.

D. Directions for Future Research

The research presented here could proceed in many natural directions that would contribute to the understanding of the associations of antioxidant nutrients and oxidative DNA damage, as well as the factors that influence these levels. However, I would like to focus on paths that contribute information about the etiology of cancer in African Americans or add to understanding and interpretation of oxidative DNA damage measures.

With the advantage of hindsight, there are a few modifications I would add to the collection of the data used in this work. First, we examined plasma concentrations of individual antioxidants because at the time of study design, this was the extent of the laboratory capabilities. However, there is now the possibility of measuring total antioxidant capacity in blood at a reasonable cost. Measuring total antioxidant capacity would allow for the possibility of a synergist relationship among nutrients. Although this has yet to be quantified, we know that certain nutrients are altered in the presence of others. For instance, iron is better absorbed in the presence of vitamin C[92] and conversely, vitamin E has pro-oxidant capabilities in the presence of copper[112]. It is reasonable then to expect that total antioxidant capacity may be different than the sum of its parts. Measuring total antioxidant capacity would allow for this investigation. Second, we assessed oxidative DNA damage using the comet assay and quantified results using the continuous measure, mean comet tail moment. As discussed in "Associations of Antioxidant Nutrients and Oxidative DNA Damage in Healthy African American and White Adults", comparing results of oxidative

DNA damage across studies is difficult. Simply for ease of comparison, it would have more been convenient to also have measured oxidative DNA damage using visual scoring, despite the limitations inherent in using a subjective measure.

With those two modifications, I would like to repeat this study in a much larger sample with fewer exclusion criteria. The elegance of the DISH study is that many of the potential confounders, i.e., older ages, obesity, smoking, and chronic-disease, are exclusion criteria and thus, there are fewer concerns about residual confounding. However, if the sample size is large enough that we are able to stratify on several measures and determine the contribution of these factors, this information would add greatly to the current literature, particularly for those factors affecting understudied groups, such as African Americans. There is also a gap in the literature about the period of exposure that the comet assay measures. Ideally, we would also measure DNA repair capacity. However, repeated oxidative DNA damage measurements would provide information about reliability and may also reveal how long "recovery" time is after exposure to a smoke-filled room or similar insult. Rehman et al. showed that a single serving of tomatoes statistically significantly altered endogenous DNA[36]. Thus, it would not necessarily need to be a very long time between measures. There are many smaller investigations that would be interesting, but few that would be as productive as capturing the same information as in the DISH study with a much larger sample size, which would preferably also come with greater variety in damage levels, demographic characteristics, behaviors, and psychosocial factors.

The motivation to study African Americans is based on the remarkable disparate cancer rates. As the US population grows and shifts, there may be additional racial/ethnic groups that also experience disproportional cancer burden. Proactive inclusion of racially diverse study populations sounds attractive, but would require very large sample sizes so that meaningful comparisons could be made. One population group of interest may be Hispanics. Although cancer rates are declining for Hispanics, the Hispanic population in the US is growing rapidly, and according to the American Cancer Society, they represent a group with unique and interesting psychosocial and behavioral factors that have not been explored.

Building upon this work could expand in almost limitless directions, as there is a dearth of information about potential racial differences, especially concerning oxidative stress and psychosocial factors. The identification modifiable factors (e.g., diet), mechanisms of carcinogenesis (e.g., oxidative DNA damage), or mediating factors that contribute to these factors (e.g., psychosocial factors) are critical for cancer prevention and control programs to reduce the disparate cancer burden among African Americans.

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