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Oxidative Balance Score and Chronic Kidney Disease

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Oxidative Balance Score and Chronic Kidney Disease

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Abstract

Oxidative Balance Score and Chronic Kidney Disease

By

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Background and Objectives: The Oxidative balance score (OBS) is a composite estimate of the overall pro- and antioxidant exposure status in an individual. The aim of this study was to determine the association between OBS and renal disease: albuminuria, chronic kidney disease (CKD) and End Stage Renal Disease (ESRD).

Design, Setting, Participants and Measurements: The study was conducted with data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort study. Data were collected from 2003 to 2007 by phone questionnaire and in-home examination. OBS was calculated by combining 13 *a priori*-defined pro- and antioxidant factors using baseline dietary and lifestyle assessment. The OBS was divided into quartiles (Q1-Q4) with the lowest, Q1 (predominance of pro-oxidants) as the reference. Multivariable logistic regression and cox proportional hazards models were used to estimate adjusted odds ratios (ORs) for albuminuria defined as urine albumin/creatinine ratio $\geq$ 30mg/g and CKD defined as estimated glomerular filtration rate < 60ml/min as calculated by the CKD-EPI equation and hazard ratios (HRs) for ESRD, respectively.

Results: Of the 19,461 participants analyzed, 2,519 (12.9%) had albuminuria and 1,957 (10.1%) had CKD defined as estimated glomerular filtration rate (eGFR) < 60ml/min/1.73m^2 at baseline; over a median follow-up of 3.5 years (range 2.14-4.32 years), 90 (0.46%) developed ESRD. Higher OBS quartiles were associated with lower prevalence of CKD (OR vs. Q1: Q2=0.93, (95% CI, 0.80-1.08); Q3=0.90, (95% CI, 0.77-1.04; and Q4= 0.79, (95% C.I 0.67-0.92), p for trend <0.01). The associations between OBS and albuminuria (p for trend 0.31) and incident ESRD (p for trend 0.56) were not significant in the fully adjusted models.

Conclusions: These findings suggest that higher OBS is associated with lower prevalence of CKD. Lack of association with ESRD incidence in the multivariable analyses indicates that temporal relation between OBS and renal damage remains unclear.

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INTRODUCTION

Oxidative stress has been implicated both in the pathogenesis of chronic kidney disease (CKD) and in cardiovascular complications of CKD such as atherosclerosis (1-4). Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants in favor of pro-oxidants (5). CKD is associated with increased levels of reactive oxygen species and nitrogen species (RONS) (5, 6). This process is observed in early CKD and may contribute to both progressive decline in the glomerular filtration rate and vascular complications (6-8). Individuals with CKD and compromised nutritional status have more evidence of oxidative stress than well-nourished ones (9, 10).

The balance between pro-oxidants and antioxidants is largely determined by an endogenous enzymatic mechanism (11, 12). However, exogenous factors such as diet, medications and lifestyle are major environmental sources of both antioxidants and pro-oxidants (13). Ascertaining the individual effects of oxidative balance related exposures is difficult because they are small, may be correlated, and there may be existing biological interactions involving pro-oxidant and antioxidant factors (14). In 2002 Van Hoydonck et al proposed an Oxidative Balance Score (OBS) as a composite measure of oxidative stress-related exposures (15). OBS uses information about the dietary micronutrient antioxidants and pro-oxidants, antioxidant medication use and pro-oxidative smoking status to summarize pro-oxidant and antioxidant exposure status (15, 16). Data from population-based studies on pro-oxidant and antioxidant exposure status in individuals with CKD are sparse and focus on individual micro and macronutrients (17, 18) or dietary patterns (19) and their association with outcomes such as end-stage renal disease (ESRD) and mortality .

The OBS has been shown to correlate inversely with all-cause mortality (15), and the risk of sporadic colorectal adenoma (12, 20, 21), and colorectal cancer (22) . However, the associations between OBS and measures of CKD, including estimated glomerular filtration rate

(eGFR), albuminuria and incident End Stage Renal Disease (ESRD), have not been previously studied. We used data from a large national prospective cohort study to estimate the associations between OBS and renal outcomes.

BACKGROUND

Oxidative stress has been implicated as a causative factor in a number of conditions such as atherosclerosis, cardiomyopathy, heart failure and various cardiovascular diseases (1-4). Oxidative stress is a crucial pathognomonic process in Chronic Kidney Disease (CKD) where an excessive production of reactive oxygen and nitrogen species occurs(6). Chronic kidney disease is of increasing public health concern due to its increasing prevalence, the increased risk of cardiovascular disease (CVD) and the risk of progression to End Stage Renal Disease (ESRD)(23).

In the early stages of CKD, there is a significant gradual increase in the level of oxidative stress. As the glomerular filtration rate of the kidney falls and renal function gradually declines, there is a progressive gradual buildup of uremic toxins. The accumulation of toxins results in an imbalance of the redox status and pro-oxidant accumulation leading to chronic inflammation and oxidative stress (7). This process is perpetuated by the symptoms and signs that characterize the later stages of CKD, resulting in volume overload, buildup of uremic toxins, metabolic disturbances and hormonal imbalance resulting in a cascade of cellular reactions that activate inflammation and perpetuate oxidative stress (7). Cardiovascular risk is increased in patients with ESRD on dialysis compared to the general population. This risk is as much as 3.5-50 times greater in patients on dialysis compared to the general population (24). The micro-inflammatory state associated with oxidative stress has been postulated to be one of the non-traditional risk factors that may explain increased CVD risk seen in ESRD patients and could also represent new targets for therapeutic intervention (25) .

With the emerging evidence on the importance of oxidative stress in the kidney, it becomes imperative to be able to measure levels in the kidney. However, oxidants are highly reactive compounds with half-lives of only seconds which makes it difficult to measure them in-vivo (25). In addition, there is the need to identify which markers are most important in CKD. Currently

there is no consensus on which specific markers to monitor in measuring oxidative stress and what methods to employ in measurement in order to increase reliability and specificity (6). Individual measures and markers have been used in CKD but there is no consensus as to which of these can determine oxidative-stress related exposure. No composite measure of pro-and anti-oxidant exposure has been used to assess the association between oxidative-stress related exposure and kidney disease

It is difficult to accurately determine the independent effects of antioxidant nutrients because intakes of many of these nutrients are highly correlated with one another (14). The introduction of an integrated scoring system that includes pro-oxidant and anti-oxidant exposure may be more reflective of an individual's overall oxidative stress related exposure (15, 26).

Van Hoydonck investigated the association of OBS with all-cause, cardiovascular disease (CVD) and total cancer mortality in 2814 male Belgian smokers(15). In this study, OBS was defined as a combined intake of antioxidants (vitamin C and β-carotene) pro-oxidant (iron). In multivariable-adjusted Cox models, compared with men in the lowest score group, men in the highest OBS group had a significantly higher relative risk (RR) of all-cause mortality (RR = 1.44, 95% CI: 1.13, 1.82) and total cancer mortality (RR = 1.62, 95% CI: 1.07, 2.45) but not CVD mortality risk (RR = 1.31, 95% CI: 0.86, 2.00) (15).

Goodman et al used the OBS to characterize the pro-oxidant and anti-oxidant exposures of 2,305 participants in a case–control study of colorectal adenoma (oxidative stress related neoplasm). In this study, OBS included 12 lifestyle and dietary factors There was a significant inverse association between OBS (continuous variable) and colorectal adenoma in cases and controls (p-trend=0.01) (13).

Slattery et al. also studied OBS in colon and rectal cancer and found significant interaction between a composite oxidative balance score and a polygenic model for both colon (p-interaction< 0.001) and rectal cancer (p-interaction=0.002)(22).

OBS has been validated in previous studies. Kong et al. validated the OBS in all-cause, cancer and non-cancer mortality in the REGARDS cohort (27). Dash et al. also validated the OBS in a pooled Case-Control Study of incident, sporadic colorectal adenoma (21). OBS was also found to be associated with circulating biomarkers of oxidative stress F2-isoprostanes and C-reactive protein in colorectal adenoma (28).

METHODS

Research Goal

Our hypothesis is that higher OBS are associated with a reduced prevalence of albuminuria as defined as albumin/creatinine ratio (≥30 mg/g), a reduced prevalence of CKD (eGFR< 60ml/min/1.73m^2) and with reduced incident ESRD.

Study Design

The REasons for Geographic and Racial Differences in Stroke (REGARDS) Study is a national, population-based prospective study to examine reasons for variation in stroke incidence and mortality in the United States. Details on recruitment and data collection were reported previously (29). Briefly, between January 2003 and October 2007, more than 30,000 healthy volunteers over the age of 45 were randomly selected and recruited with planned equal recruitment of men and women and oversampling of African Americans and residents of the eight southeastern US states because they have higher stroke mortality than the rest of the United States. These states are divided into the "stroke buckle" (coastal plain regions of Georgia, North and South Carolina) and the "stroke belt" (the remaining regions of Georgia, North and South Carolina, Mississippi, Alabama, Tennessee, Louisiana and Arkansas) (19). Trained personnel collected data by using a computer-assisted telephone survey to obtain socio-demographic factors and other information. Subsequently, a health professional visited the participants' homes to collect anthropomorphic variables as well as blood and urine samples. At this appointment, a 1998 Block Food Frequency Questionnaire (98-block FFQ; Nutrition Quest, Berkeley, CA) was left with the participant to be self-administered and returned to the data-coordinating center. The REGARDS study was approved by the institutional review boards at all the participating centers and all participants provided informed consent.

Participants were then contacted by telephone every 6 months to capture outcomes of interest such as stroke. ESRD status was assessed by linkage with the United States Renal Data

System (USRDS). The USRDS is a registry of ESRD, and it captures over 95% of incident cases in the United States (30).

Characteristics of the Study Population

From a total number of 30,183 participants, we excluded 8,630 individuals who did not have data from the block FFQ (28.6%). Of the remaining 21,553 individuals, we excluded individuals who were found on record linkage to have already been treated for ESRD (n=23) at the time of baseline interview, those with missing albumin-to-creatinine ratio (ACR) (n=943), then those with missing eGFR (n= 576) and those with missing data on any OBS component (n=550). A total of 19,461 participants were included in the final analysis. Excluded individuals differed by race and were predominantly black (57.6%), there were no other differences noted in the excluded population and those included in the study.

Measurements

Oxidative Balance Score

The main exposure (OBS) was calculated by summing up 13 *a priori*-defined pro- and antioxidant exposure factors (table 1) as described previously (13, 15). These factors include intake of polyunsaturated fatty acid (PUFA), iron, selenium, vitamin C, vitamin E, α carotene, β -carotene, lutein, lycopene, and cryptoxanthine ; and use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDS), and alcohol (15). Smoking was excluded from the original OBS score because smoking is a well-known strong risk factor for CKD (31-36). A higher OBS is reflective of higher antioxidant levels and low pro-oxidant levels and a lower score is reflective of a lower antioxidant levels and higher pro-oxidant levels. The dietary data were collected at baseline using the FFQ, which consists of 107 items that assess dietary intake over the previous year. The FFQ has been described in detail in previous studies (19, 37). Completed questionnaires were scanned and sent to Nutrition Quest ™ (Berkeley, CA) for analysis of nutrition content.

The continuous dietary variables reflecting pro-oxidant (unsaturated fat and iron) and antioxidant (vitamin C, lycopene, α-carotene, β-carotene, lutein, β-cryptoxanthine, vitamin E and selenium) exposures were divided into low, medium, and high categories based on each exposure's sex-specific tertiles. For antioxidants, the first through third tertiles were assigned 0 through 2 points, respectively, whereas the corresponding point assignment for pro-oxidants was the reverse (0 points for the highest tertile and 2 points for the lowest tertile).

A similar scoring approach was used for pro-oxidant and antioxidant categorical variables. For antioxidant aspirin and NSAID use, 0 points were assigned to participants with no regular use, 1 point to those with unknown or missing data, and 2 points to those with regular use. For pro-oxidant alcohol consumption, non-drinkers, moderate drinkers (1-7 drinks/week for women and 1-14 drinks/week for men), and heavy drinker (>7 drinks/week for women and >14 drinks/week for men) received 2, 1 and 0 points, respectively (table 1).

Outcome Measures

We defined albuminuria as urine albumin/creatinine ratio $\geq$30mg/g at baseline. The eGFR at baseline was calculated using the CKD-EPI (38) equation using isotope dilution mass spectrometry-calibrated creatinine. Incident ESRD was determined by linkage of the REGARDS study participants to the United States Renal Data System (USRDS) via personal identifiers. ESRD status was determined through August 31, 2009.

Covariates

Age, sex, race, smoking, income and educational attainment were self-reported. Region was classified as stroke belt, stroke buckle and other; caloric intake was obtained from the FFQ and analyzed as a continuous variable. Hypertension was defined as systolic blood pressure $\geq$ 140mm Hg or diastolic blood pressure $\geq$ 90mm Hg, a self-reported prior diagnosis of hypertension or current use of antihypertensive medications. Diabetes was defined as fasting blood glucose $\geq$ 126mg/dl, non-fasting blood glucose $\geq$ 200mg/dl or self-reported use of insulin or oral hypoglycemic agents. Statin use was self-reported. The waist circumference was measured

in cm during the in-home visit. Physical activity was assessed by asking how many times participants engaged in intense physical activity enough to work up a sweat (we assessed physical activity as 1-3 times a week and above vs other).

Analytic Plan

The baseline characteristics of the study population were reported using mean and standard deviation, median and interquartile range and number and percentages for the overall population and across OBS quartiles. Differences in baseline characteristics between the four OBS quartiles were tested using ANOVA for continuous variables and chi square statistics for categorical variables.

In all analyses the exposure was OBS (in quartiles and as a continuous variable) and the dependent variables of interest were albuminuria, CKD and ESRD. The first OBS quartile (predominance of pro-oxidants) was used as the reference group. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression for albuminuria and CKD. Adjusted hazard ratios (HR) and 95% CIs were estimated using Cox proportional hazards model for ESRD. We tested the PH assumption by examination of log-log survival curves, Schoenfield residuals, and the extended Cox models and found that the PH assumption was satisfied. The trend tests for OBS (from lower to higher quartiles) in the crude and adjusted models were analyzed by assessing the OBS quartiles as an ordinal variable.

We developed two models to adjust for various confounders based on prior literature review. Model 1 adjusted for age, sex, race, region and calories, and model 2 adjusted for variables in model 1 plus BMI (body mass index), smoking, waist circumference, physical activity, education, income, hypertension, diabetes and statin medications. The first model adjusted for factors that were general to most disease processes and the second model contained confounders which were more specific to kidney disease. In creating these models, we wanted to

appreciate the effect of adjusting for general factors with and without disease specific factors affecting kidney disease. All reported p values are two-sided with an alpha of 0.05.

RESULTS

There were 19,461 subjects included in the analyses. Their mean age (SD) was 64.8 (9.2) years, and 55.2% were female and 67.4% were white (Table 2). The mean (SD) OBS was 12.7 (3.7), with median of 13 and interquartile range (IQR) of 10-15. The proportion of white participants was lower in patients with lower vs. higher OBS (Q1=66.3% vs Q4=69.6%).

Higher OBS quartiles (i.e. those with increasing predominance of antioxidants and lower pro-oxidants) included lower proportions of participants in the stroke belt and buckle region. Higher OBS quartiles were associated with lower likelihood of smoking, greater income and education level, and higher likelihood of self-reported hypertension and statin and diabetic medication use.

Table 1 (supplemental) shows the distribution of the individual score components by OBS quartiles. Higher OBS quartiles reflect predominance of anti-oxidants and lower quartiles reflects predominance of pro-oxidants. NSAID use was higher in higher vs. lower OBS quartiles. Aspirin use also higher in higher vs lower OBS quartiles. The prevalence of heavy alcohol use was lowest in Q4. The intake of antioxidants (vitamin c, α-carotene, β-carotene, vitamin E, lutein, lycopene and cryptoxanthine levels) was highest in Q4. However, pro-oxidants polyunsaturated fatty acids and iron were also higher in Q4. Participants in the high OBS quartiles had a higher percentage of aspirin and non-steroidal anti-inflammatory drug use.

Table 3 shows the crude association between the OBS quartiles and the outcomes of interest: albuminuria, CKD and ESRD. The crude ORs (95% CI) were estimated for CKD and albuminuria and the crude HRs (95% CI) were estimated for ESRD. A total of 2,519 (12.9%) had prevalent albuminuria, 1,957 participants had prevalent CKD (10.0%) and 90 (0.46%) participants developed ESRD over a median follow up period of 3.5 years (range 2.14-4.32 years). There was lower prevalence of albuminuria in the higher OBS quartiles. The incidence rate of

ESRD appeared lower in the higher OBS quartiles: Q3 and Q4, although it was somewhat higher in Q2 than in Q1 (table 3). In summary, the crude associations (OR and HR) reflecting the association between OBS quartile and each of the individual outcomes (albuminuria, CKD or ESRD) were not statistically significant. When we analyzed OBS as a continuous variable, we found that in the crude model, for every 5-unit increase in the OBS score, the odds of having albuminuria was 0.98 times the odds of not having albuminuria (OR 0.98 95% CI 0.93,1.04). The results for a 10-unit increase in OBS are also shown in table 3. In the crude model, for every 5-unit increase in OBS, the odds of CKD was 0.99 times the odds of not having CKD (OR 0.99 95% CI 0.93–1.06). For every 5-unit increase in OBS in the crude model, the likelihood of developing ESRD was 0.82 that of not developing ESRD (HR 0.82 95% CI 0.62-1.08). The crude associations between OBS score as a continuous variable and the outcomes (albuminuria, CKD and ESRD) were not statistically significant.

Table 4 shows the associations of OBS with albuminuria, CKD and ESRD, adjusted for various potential confounders. The highest vs. lowest OBS quartile was associated with decreased frequency of albuminuria (OR=0.89 95% CI = 0.77-1.01); however this association was not statistically significant (p_{trend}= 0.05) in model 1. There was also no significant association between OBS quartile and albuminuria in the fully adjusted model (table 4). OBS in the highest vs. lowest quartile was associated with significantly lower odds of CKD (OR = 0.83, 95% CI = 0.71-0.96) in model 1. The trend for OBS was also significant for CKD in model 1 (p_{trend}= 0.02). This statistically significant inverse association remained after adjusting for other covariates in model 2 (OR = 0.78, 95% CI = 0.66-0.91). Those in Q4 had 21% lower odds of having an eGFR <60ml/min. The trend test for OBS in model 2 was also statistically significant (p_{trend} <0.01).

When we analyzed OBS as a continuous variable, in the crude models, there was no association between the odds of having CKD and a 5-unit increase in OBS score (table 3). However in model 1, (table 4), the odds of having CKD vs no CKD for 5-unit increase in OBS

score was 0.93 and this was statistically significant (95% CI= 0.86-0.99). In model 1, for a 10-unit increase in OBS, the odds of having CKD vs no CKD was 0.86 with a 95% CI of 0.74-0.99. In the fully adjusted model, the odds of having CKD vs no CKD for a 5-unit increase in OBS was 0.90 (95% C.I 0.84-0.97) and for a 10-unit increase in OBS, the odds of having CKD vs no CKD was 0.81 (95% C.I 0.70-0.94).

The association between OBS quartiles and ESRD was not statistically significant in the crude model (table 3) and adjusted models. The trend tests were also not significant (model 1 p_{trend}= 0.55 and model 2, p_{trend}=0.50 respectively) (table 4). When we analyzed OBS as a continuous variable, there was no significant association between OBS and time to incident ESRD.

DISCUSSION

In this cohort, we examined the association between OBS, albuminuria, CKD and ESRD. We found that higher OBS quartiles (which reflect an increased antioxidant effect) were associated with lower prevalence of CKD, as measured by eGFR < 60ml/min/1.73 m^2 but not associated with albuminuria or incident ESRD.

It is well known that ESRD is associated with a high prevalence of oxidative stress and inflammation, both of which are thought to be related to increased cardiovascular morbidity and mortality (8, 39-41). Recent studies have suggested that the alterations in pro-and antioxidant effects start in the early stages of CKD and become more pronounced in patients on dialysis (23, 25, 42). CKD has been identified as an independent risk factor for cardiovascular outcomes (8, 43). Newer data have demonstrated that individuals with CKD show evidence of increased oxidative stress (44).

Oxidative stress therefore plays a vital role in the progression of CKD up until the development of ESRD (6, 45, 46). Our study suggests that the greater the shift from pro-oxidant to antioxidant exposures (as measured my higher OBS), the lower the odds of CKD as defined by an eGFR<60ml/min. To our knowledge, this is the first study that utilizes the OBS to determine the association between the balance of pro-oxidant and antioxidant exposure and kidney disease.

The lack of association between OBS and incident ESRD may have been due to the relatively low number of incident ESRD cases (n=89) in the cohort, which may indicate the need for a more extended follow up. Other possible explanations of this are that since the FFQ is measured at baseline, secular dietary changes, particularly those that may occur among participants with prevalent kidney disease who have many dietary restrictions, are not accounted for. It also shows that the temporal association between OBS and renal damage remains unclear.

There was a significant inverse association between OBS and CKD as defined by eGFR< 60ml/min that became more pronounced as we adjusted for potential confounders. Current evidence suggests that CKD is a pro-oxidant state as evidenced by the increase in oxidative markers in atherosclerotic lesions of patients with CKD as well as in circulating plasma of CKD patients (47-49). Our results suggest that the OBS score may be a measure of pro- and antioxidant exposure in CKD. Measuring the OBS may be the first step in detecting the pro-and antioxidant exposure in individuals at risk for development of an eGFR< 60ml/min. These results need to be further validated using incident CKD cases in a dedicated CKD cohort.

One strength of this study is the large population cohort. The OBS provides a measure whereby the effects of pro- and antioxidant factors in the diet and medications are summed up and this "effect" or "balance" can be used to determine outcomes. The *a priori* selection of cut-offs in the components of OBS reduces subjectivity in measurement (12). A validated FFQ was used to capture nutrient intake in a large population.

The limitations of the study include the small number of incident ESRD cases in the cohort; misclassification due to self-reported data, particularly dietary assessments from the food frequency questionnaire, to determine the OBS; the use of prevalent kidney function/albuminuria; selection bias from missing data; and residual confounding. The OBS was limited to dietary/lifestyle exposures and included no endogenous measures of antioxidant cell function (44, 50).

Ultimately, we would have to compare the OBS score with measured oxidative metabolites and investigate the utility of this score as a modifiable predictor of oxidative stress in CKD patients.

The goal of identifying the individuals at risk for higher oxidative stress is to institute aggressive risk modification. This study suggests that higher OBS may be independently associated with lower prevalence of CKD and may represent a modifiable risk factor for CKD.

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Table 1. Oxidative balance score (OBS) components[a]

1. Cryptoxanthine	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
2. Selenium supplements	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
3. Aspirin	0 = never, 1 = missing, 2 = current user
4. Other NSAID[b]	0 = never, 1 = missing, 2 = current user
5. Alcohol Female	0 = 8+ drinks/week, 1 = 1-7 drinks/week, 2 = <1 drink/week
Male	0 =15+ drinks/week,1 = 1-14 drinks/week, 2 =<1 drink/week
6. PUFA[b]	0 = high (3rd tertile), 1 = medium (2nd tertile), 2 = low (1st tertile)
7. Total (dietary and supplemental) iron	0 = high (3rd tertile), 1 = medium (2nd tertile), 2 = low (1st tertile)
8. Total (dietary and supplemental) vit. C	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
9. Total (dietary and supplemental) α-carotene	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
10. Total (dietary and supplemental) β -carotene	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
11. Total (dietary and supplemental) vit E.	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
12. Lutein	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)
13. Lycopene	0 = low (1st tertile), 1 = medium (2nd tertile); 2 = high (3rd tertile)

a. Low, medium and high categories correspond to sex-specific 1st, 2nd, and 3rd tertiles **b.** NSAID = non-steroidal anti-inflammatory drug (not including aspirin); PUFA = polyunsaturated fatty acid

TABLE 2: CHARACTERISTICS OF PARTICIPANTS IN THE REGARDS COHORT BY OBS QUARTILES AT BASELINE

(HIGHER QUARTILES REPRESENT HIGHER ANTIOXIDANT EXPOSURE)

Characteristic	All	OBS Quartile			
		Q1	Q2	Q3	Q4
OBS Range	2-24	2-9	10-12	13-15	16-24
N	19462	4096	5090	5453	4823
Age (SD)	64.8 (9.2)	63.5 (9.3)	64.4 (9.3)	65.2 (9.1)	65.9 (8.9)*
Female	55.2	55.1	55.1	55.4	55.3
White	67.4	66.3	66.4	67.3	69.6*
Current Smoking	13.5	19.9	15.2	11.1	9.0*
Income less than $20k	15.2	16.7	15.3	15.3	13.8*
Education < high school	9.3	11.6	10.2	8.9	7.1*
HTN	56.3	52.9	55.9	56.9	58.7*
DM	17.8	17.3	17.3	18.4	18.0
Waist circumference (cm)	57.8	56.9	58.3	57.7	58.4
BMI (kg/m^2)	30.0 (6.0)	28.7 (5.9)	29.0 (6.1)	28.9 (5.9)	29.2 (6.0)
Calories (Kcal)	1709 (709)	1436 (573)	1610 (654)	1767 (702)	1981 (766)*
eGFR ml/min/1.73m^2	85.2 (18.8)	87.8 (19.5)	85.3 (19.2)	84.9 (18.6)	84.9 (17.9)
ACR (mg/g)	39.7 (234.8)	49.8(320)	42.1 (237)	37.4 (210)	31.3 (159)*
OBS mean (SD)	12.7 (3.7)	7.6 (1.4)	11.0 (0.81)	14.0 (0.82)	17.5 (1.5)*

BMI Body Mass Index; eGFR creatinine estimated glomerular filtration rate; ACR albumin-to-creatinine ratio; OBS oxidative balance score.

†Values for age, BMI, calories, hemoglobin, eGFR, ACR and OBS years are reported as mean (±SD). Race, sex, current smoking status, income, education, hypertension (HTN), diabetes (DM and waist circumference are reported as percent.

*$p<0.001$ based on the ANOVA for continuous variables and chi-square (X^2) test for categorical variables.

TABLE 3: CRUDE MODELS SHOWING THE ASSOCIATION BETWEEN OBS: ALBUMINURIA CKD AND ESRD

		Albuminuria		CKD			ESRD		
OBS	**Total (N)**	**Cases N (%)***	**OR (95%CI)**	**Cases N (%)***	**OR (95% CI)**	**Total PY**	**Cases**	**IR****	**HR(95% CI)**
Q1	4096	536 (13.1)	1	410(10)	1	12968.5	20	154.2	1
Q2	5090	667(13.1)	1.0(0.88-1.13)	519(10.2)	1.02(0.89-1.17)	16488.6	28	169.8	1.2(0.67-2.16)
Q3	5452	704(12.9)	0.99(0.87-1.11)	566(10.4)	1.04(0.91-1.19)	17851.5	25	140	0.93(0.51-1.71)
Q4	4823	611(12.7)	0.96(0.85-1.09)	462(9.6)	0.95(0.83-1.10)	16111.4	17	105.5	0.72(0.37-1.38)
p for trend			0.51		0.55				0.21
OBS (continuous)									
For a 5-unit increase in OBS			0.98(0.93-1.04)		0.99(0.93-1.06)				0.82(0.62-1.08)
For a 10-unit increase in OBS			0.96(0.86-1.08)		0.99(0.87-1.12)				0.67(0.38-1.17)
P value			0.51		0.85				0.15

OBS = oxidative balance score; OBS[#]=OBS continuous, OR = odds ratio, HR = hazards ratio; CI= 95% confidence interval. *% = Percentage of the entire quartile with the outcome PY= person years **IR = incident rate per 100,000 person-years, CKD= Chronic Kidney disease. ESRD= End Stage renal disease. Logistic regression was used to calculate odds ratio for Albuminuria and CKD while Cox proportional hazards models were used to calculate hazard ratios for ESRD.

TABLE 4: ADJUSTED ASSOCIATION BETWEEN OBS: ALBUMINURIA, CKD, AND ESRD

OBS	Total	Albuminuria		CKD		ESRD	
		Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
	N	OR (95%CI)	OR(95% CI)	OR(95% CI)	OR (95% CI)	HR(95% CI)	HR(95%CI)
Q1	4096	1	1	1	1	1	1
Q2	5090	0.96(0.85-1.09)	0.99(0.88-1.13)	0.95(0.82-1.09)	0.93(0.80-1.08)	1.21(0.67-2.18)	1.25(0.69-2.25)
Q3	5452	0.92(0.84-1.04)	0.96(0.84-1.09)	0.93(0.80-1.07)	0.90 (0.77-1.04)	0.99(0.53-1.83)	1.06(0.57-1.97)
Q4	4823	0.89(0.77-1.01)	0.94(0.82-1.07)	**0.83(0.71-0.96)**	**0.79(0.67-0.92)**	0.85(0.43-1.68)	0.85(0.43-1.71)
p for trend		0.05	0.31	0.02	<0.01	0.50	0.56
OBS (continuous)							
For a 5-unit increase in OBS		0.94(0.89-1.00)	0.96(0.90-1.03)	0.93(0.86-0.99)	0.90(0.84-0.97)	0.88(0.65-1.19)	0.89(0.66-1.22)
For a 10-unit increase in OBS		0.89(0.76-1.00)	0.93(0.82-1.05)	0.86(0.74-0.99)	0.81(0.70-0.94)	0.78(0.42-1.43)	0.80(0.43-1.48)
P value		0.05	0.23	0.03	<0.01	0.41	0.48

OBS=oxidative balance score; OBS# = OBS as a continuous variable, HR=hazards ratio; CI= 95% confidence interval. **IR = incident rate per 100,000 person-years. Logistic regression was used to calculate odds ratio (OR) for Albuminuria and CKD while cox proportional hazard was used to calculate hazard ratios for ESRD.

SUPPLEMENTAL TABLE

TABLE 1: DISTRIBUTION OF INDIVIDUAL OBS COMPONENTS BY OBS QUARTILE

	Mean (by OBS quartiles)			
Characteristics (Units)	**Q1**	**Q2**	**Q3**	**Q4**
Total	4096	5090	5453	4823
Regular Aspirin Use[#]	963 (23.5%)	2007(39.4%)	2522(46.2%)	3065(63.5%)
Regular NSAID Use[#]	264(6.5%)	609(12.0%)	856(15.7%)	1195(24.8%)
Alcohol				
Heavy	330(8.1%)	224(4.4%)	203(3.7%)	113(2.3%)
Moderate	1558(38.0%)	1883(37.0%)	2003(36.7%)	1585(32.9%)
None	2208(53.9%)	2983(58.6%)	3247(59.5%)	3125(64.8%)
Selenium (mcg)	69.23(30.6)	85.3(43.6)	102.1(53.0)	127.8(69.1)
Daily PUFA (g)	16.50(9.2)	17.80(10.0)	53.04(19.1)	69.12(20.5)
Iron (mg)	17.3(14.7)	22.0(16.9)	26.1(17.8)	30.5(20.2)
Vitamin C (mg)	118.43 (180.7)	247.00(317.6)	397.7(434.89)	614.7(536.7)
α-carotene (µg)	279.5 (213.35)	471.7(460.83)	764.9(753.54)	1220.24(1103.40)
β-carotene (µg)	2006.7(1349.1)	3468.4(3204.81)	5760.9(5300.25)	8960.5 (7327.4)
Vitamin E (α-TE)	31.4(78.7)	74.0(139.7)	120.4(176.2)	187.8(195.2)
Lutein (µg)	823.9(616)	1300.6(1038.7)	2003.6(1785.8)	3062.93(2639.4)
Lycopene (µg)	2379.8(2611.9)	3519.4 (3803.3)	4453.5 (4547.6)	6441.0(6255.5)
Cryptoxanthine (µg)	50.8(67.5)	100.0 (112.3)	139.3(134.5)	193.7 (158.5)

Values are presented as mean (SD) or number (%).SD =standard deviation Abbreviations PUFA = Polyunsaturated fatty acid, NSAID= Non-steroidal anti-inflammatory drug OBS= Oxidative balance score,