HHS Public Access

Author manuscript

*Biomarkers*. Author manuscript; available in PMC 2015 March 20.

Published in final edited form as:

*Biomarkers*. 2014 September ; 19(6): 471–480. doi:10.3109/1354750X.2014.937361.

# Oxidative balance score and oxidative stress biomarkers in a study of Whites, African Americans, and African immigrants

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## Abstract

**Context—**Oxidative balance score (OBS) is a composite measure of multiple pro- and antioxidant exposures.

**Objective—**To investigate associations of OBS with F2-isoprostanes (FIP), mitochondrial DNA copy number (mtDNA), and fluorescent oxidative products (FOP), and assess inter-relationships among the biomarkers.

**Methods—**In a cross-sectional study, associations of a thirteen-component OBS with biomarker levels were assessed using multivariable regression models.

**Results—**Association of OBS with FIP, but not with FOP, was in the hypothesized direction. The results for mtDNA were unstable and analysis-dependent. The three biomarkers were not inter-correlated.

**Conclusions—**Different biomarkers of oxidative stress may reflect different biological processes.

### Keywords

Oxidative balance; oxidative stress; race

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**Declaration of interest**: The authors report no declarations of interest.

## Introduction

Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants, resulting in macromolecular damage and disruption of redox signaling and control (Sies & Jones, 2007). Pro-oxidants are factors that help to generate reactive oxygen species (ROS), which in turn interact with macromolecules and cause protein oxidation, lipid peroxidation, and DNA damage (Wu & Cederbaum, 2003). By contrast, antioxidant factors act to counter or reduce the effects of ROS thereby reducing oxidative stress (Valko et al., 2007).

Oxidative stress is affected by intrinsic factors, such as oxidative phosphorylation (Wallace, 1994), cellular antioxidant enzyme activity (Valko et al., 2007), and macromolecular damage (Pascucci et al., 2011). In addition, various extrinsic and presumably modifiable factors such as diet and medications also act as pro- and antioxidants. Although experimental biology evidence has demonstrated that antioxidants can slow disease pathogenesis (Padayatty et al., 2003; Pietta, 2000; Stahl & Sies, 2003), clinical trials of antioxidant supplementation have not shown benefits (Goodman et al., 2011; Steinhubl, 2008).

Studies of diet and health have demonstrated that nutrients do not act in isolation, and a combination of factors can be more strongly associated with disease risk than any single nutrient considered individually (Duthie et al., 1996; Slattery et al., 1998; Trichopoulou et al., 1995). By analogy, it appears possible that a combination of oxidative stress-related factors may be more strongly associated with health outcomes than can any individual pro-oxidant or antioxidant exposure.

To investigate this hypothesis, an oxidative balance score (OBS) that combines oxidative stress-related exposures based on the summed intake of various pro- and antioxidants, with a higher score indicating lower oxidative stress has been proposed in this work as well in other studies. Previous studies found that a higher OBS was associated with lower risk of colorectal adenoma (Goodman et al., 2008; Trichopoulou et al., 1995), colorectal cancer (Dash, 2010), and mortality (Van Hoydonck et al., 2002). By contrast, an OBS was not associated with prostate cancer risk (Agalliu et al., 2011), indicating that the role of oxidative stress in human chronic disease pathophysiology may be organ or disease specific.

Many known pro- and antioxidants act through a variety of mechanisms that may be independent of oxidative stress. For example, lycopene has anti-proliferative effects *in vitro* (Heber & Lu, 2002). Other carotenoids were found to regulate gene expression (Bertram, 1999) and immune response (Chew & Park, 2004). Similarly, tobacco smoke, in addition to its known pro-oxidant activity, has direct carcinogenic effects in many tissues and organ systems (Pryor, 1997). These examples illustrate that associations between an OBS and health outcomes may or may not be attributable to changes in oxidative stress. To resolve this uncertainty it is important to assess the relation of an OBS to blood levels of various biomarkers of oxidative stress, several of which have been used in population studies.

$F_2$-isoprostanes (FIP) are products of arachidonic acid peroxidation and a biomarker of oxidative stress (Montuschi et al., 2004). Although FIP can be measured in plasma and urine, plasma measurements are preferred because oxidative stress biomarkers in urine are

influenced by renal metabolism (Catella et al., 1986; Morrow, 2000). High levels of FIP have been associated with cardiovascular disease (Vassalle et al., 2003) and Alzheimer's disease (Reich et al., 2001).

Another relatively new marker that may increase in response to oxidative stress is mitochondrial DNA (mtDNA) copy number (Hosgood et al., 2010; Lee et al., 2000; Wang et al., 2011). Mitochondria are organelles that contain their own circular genome lacking introns. Their primary function is to generate adenine triphosphate through cellular respiration, a process that also produces ROS (Wallace, 1994). Unlike nuclear DNA, which is protected by elaborate repair mechanisms (Sancar et al., 2004), mtDNA responds to damage by increasing the number of its copies. High levels of mtDNA copy number have been linked to certain cancer outcomes (Hosgood et al., 2010; Lynch et al., 2011).

The use of florescent oxidation products (FOPs) as a measure of oxidative stress began in the food industry, but is now being proposed for population-based human studies (Wu et al., 2007a). FOPs are comprised primarily of fluorescent conjugated Schiff bases that are formed when malonaldehyde, a byproduct of lipid peroxidation, reacts with amino groups (Dillard & Tappel, 1984). In population-based studies FOP was directly associated with hypertension (Wu et al., 2007b) and may serve as an independent predictor of coronary heart disease (Wu et al., 2007a).

The use of each biomarker has distinct advantages and disadvantages. At present, FIP are considered the “gold-standard” biomarker of oxidative stress, but an accurate and reliable analysis of FIP requires careful handling of samples to prevent *in vitro* oxidation (Wu et al., 2004). Wu et al. found FOPs to be a stable measure, with levels from blood specimens remaining constant over 36 hours, whereas FIP in the same samples increased at each time measured (Wu et al., 2004). The main disadvantage of FIP and FOP as biomarkers is that they both represent short-term oxidative stress levels (Cracowski, 2006). By contrast, mtDNA copy number is a stable biomarker that is presumed to indicate long-term, cumulative, oxidative stress-induced damage.

This analysis is based on the cross-sectional Study of Race, Stress, and Hypertension (SRSH), which provided data and samples from a racially and ethnically diverse group of men and women residing in Georgia, USA. The primary goals of the present study were to examine associations between an OBS and three biomarkers – FIP, mtDNA, and FOP – each thought to reflect different aspects of oxidative stress, to compare the magnitude and the direction of the OBS-biomarker associations in different racial/ethnic groups, and to assess how the three biomarkers may relate to each other. The relation between OBS and two oxidative stress markers (FIP and FOP) was examined previously only once – in a study of colorectal adenoma that was limited to non-Hispanic whites (Kong et al., 2014). A notable unexpected finding in that study was the opposite of the associations of OBS with FIP and FOP. This observation requires confirmation in a different population, which is the secondary aim of the present study.

## Methods

### Study participants

SRSH was designed to assess racial disparities in dietary, lifestyle, and psychosocial exposures in relation to blood pressure. The study includes participants from three groups – US non-Hispanic Whites (NHW), African-Americans (AA), and native West Africans (WA), all residing in Georgia at the time of data collection. The NHW and AA were selected from 800 participants in a previously completed feasibility phase of the Georgia Cohort Study (GCS). The WA subjects were recruited *de novo* using previously established ties with Atlanta churches that include large proportions of West African immigrants. After the recruitment of the WA was complete, the sample of GCS participants was selected with frequency matching to the WA participants on age and sex. Study eligibility included 25–74 years of age, self-identification as NHW or AA (for those recruited from GCS) or as WA (for those recruited *de novo*), and being a permanent Georgia resident. Subjects were excluded if they did not give informed consent. All methods were approved by the Institutional Review Board of the Emory University.

For the current analysis we excluded participants for whom no biomarker measurements of interest were recorded ($n$ = 14). Of the remaining 321 subjects, the numbers of participants with measurements for each biomarker were as follows: FIP ($n$ = 227), mtDNA copy number ($n$ = 182), and FOP ($n$ = 272).

### Data and blood sample collection procedures

Recruitment and data collection occurred after church services for WA participants and at community events for NHW and AA participants. Following informed consent, blood was drawn by a phlebotomist into five 10mL vacutainer tubes (2 sodium heparin tubes, 1 EDTA tube, and 2 red top tubes) and immediately placed on ice. Plasma, serum, and buffy coats were separated within 4–8 hours of sample collection by refrigerated (4°C) centrifuge, aliquoted, and frozen at 80°C. The aliquots were then shipped overnight on dry ice for analysis to the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota.

Study-specific questionnaires were used to elicit data on demographic, medical history and lifestyle characteristics. Physical activity was assessed using a Paffenbarger questionnaire (Paffenbarger et al., 1993). Other data elements were obtained using instruments from previous studies (Potter et al., 1999).

### Laboratory analysis

Plasma lycopene, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, α-tocopherol, and γ-tocopherol were measured via high performance liquid chromatography (HPLC) as originally described by Bieri et al. (1985) with several modifications for the analysis of tocopherols, and using calibration methods described by Craft et al. (1988). The method and its modifications were described previously by Gross et al. (1995). Serum ferritin was measured by an antibody-based Roche immunoturbidimetric assay (Pfeiffer et al., 2007).

Plasma free FIP were measured by gas chromatography-mass spectrometry (GCMS) as described by Gross (Gross et al., 2005). This method, considered the gold standard for measuring FIP, measures a distinct set of FIP isomers. FIP were extracted from the plasma samples using deuterium (4)-labeled 8-iso-prostaglandin $F_2$ alpha as an internal standard. Unlabeled, purified FIP was used as a calibration standard.

The details of the procedure to measure mtDNA copy number are described elsewhere (Shen et al., 2010). Briefly DNA was extracted from venous white blood cells. Two pairs of primers were used in the two steps of relative quantification for mtDNA content: one for amplifying the mtDNA MT-ND1 gene, and another for amplifying the single-copy nuclear gene human globulin (HGB). In the first step, the ratio of mtDNA copy number to HGB copy number, which is also referred to as the mtDNA index, was determined for each sample from standard curves. This ratio was proportional to the mtDNA copy number in each cell and, for each sample, normalized to a calibrator DNA. All samples were assayed using 96-well plates with an Applied Biosystems StepOne Plus System. The PCRs for ND-1 and HGB were performed on separate 96-well plates with the same samples in the same well positions to avoid possible position effects. A standard curve of a diluted reference DNA, one negative control, and one calibrator DNA were included in each run. For each standard curve, one reference DNA sample was serially diluted 1:2 to produce a seven-point standard curve between 0.3125 and 20 ng of DNA (Shen et al., 2010).

The method of measuring FOP was modified from Shimasaki (1994). The procedures were described in detail previously (Wu et al., 2004). Briefly, plasma was extracted with ethanol-ether (3/1, v/v) and mixed on a vortex mixer. The mixed solution was centrifuged for 10 minutes at 3000 rpm, 1.0 mL of supernatant was added to cuvettes for spectro-fluorometric readings, and the readings were expressed as a relative fluorescence intensity units per milliliter of plasma at 360/430 nm wavelength (excitation/emission) (Wu et al., 2004). The wavelength we used is within the spectrum, but not the same as that used by Wu et al. (2004). All samples were calculated against 1.0 ppm fluorescent reference standard quinine in 0.1 $NH_2SO_4$.

Using two different controls, the coefficients of variation (CVs) ranges were 10.3–12.4% for zeaxanthin, 3.3–5.8% for β-cryptoxanthin, 26.4–31.9% for lycopene, 1.1–3.0% for α-carotene, 4.8–9.4% for β-carotene, 0.6–0.7% for α-tocopherol, 0.1–0.2% for γ-tocopherol, 11.9–12.3% for FIP, and 5.4–5.6% for FOP. Using one control, the CVs were 7.2% for ferritin and 5.9% for mtDNA copy number.

## OBS components and their assessment

The OBS is comprised of 13 components that were selected based on *a priori* knowledge about their relation to oxidative stress (Table 1). The score combined plasma micronutrient measurements, serum ferritin measurements, and questionnaire derived information on lifestyle/medical factors. Continuous variables were divided into categories based on fertile values. Participants who had low exposure to a particular antioxidant (1st fertile) were assigned zero points, and those in the medium (2nd fertile) or high (3rd tertile) exposure category, received one or two points, respectively. Antioxidant OBS components expressed as continuous variables included plasma lycopene, α-carotene, β-carotene, β-cryptoxanthin,

zeaxanthin, lutein, α-tocopherol, and γ-tocopherol plus physical activity. For serum ferritin, the only continuous variable reflecting pro-oxidant exposure, two points were given for low exposure (1st tertile), one point for medium exposure (2nd tertile), and zero points for high exposure (3rd tertile).

Categorical variables (all lifestyle/medical) were assigned scores from 0 to 2 to maintain consistency with the continuous OBS components. For smoking and alcohol use non-smokers and non-drinkers received 2 points, while current smokers and current drinkers received zero points. For NSAID and aspirin use, zero points were assigned to participants who reported never using any of these medications, and two points to those who reported regular (at least once a week) use. The points assigned to each component were summed to calculate the overall OBS.

## Statistical analysis

Correlation coefficients were calculated for oxidative stress biomarkers and for plasma levels of OBS components. Using median values as the cutoffs, high FIP, mtDNA copy number, and FOP were defined as ≥48.37 pg/mL, ≥3.05 (relative copy number), and ≥0.04 (average standard reference adjusted units), respectively. Participants with high and low levels of FIP, mtDNA copy number, and FOP were compared with respect to various demographic and lifestyle characteristics using chi-square tests for categorical variables and *t*-tests for continuous variables.

Multivariable linear regression models were constructed to examine associations between the OBS and each biomarker. The results of the linear regression models were expressed as regression coefficients and their corresponding 95% confidence intervals (CIs) adjusted for age, sex, BMI, and race/origin. The biomarker measurements were not normally distributed, and so were log transformed when used in the linear regression analyses. The OBS-biomarker associations were examined both overall and separately for NHW, AA, and WA participants.

Linear regression models for investigating continuous outcome variables may have greater statistical power; however: (1) it cannot be used to identify a threshold or an asymptotic dose-response relationship, and (2) the clinical or biological significance of results from analyses in which exposures and outcomes are categorized may be more apparent because they allow a quantitative comparison of risks (or prevalence estimates) in persons at different ends of the exposure distribution. For these reasons, in a separate analysis, the OBS was also divided into tertiles, and blood levels of oxidative stress biomarkers were dichotomized as high versus low using median values as the cutoffs. Multivariable logistic regression models were used to examine the association between OBS and high biomarker levels, controlling for age, sex, BMI, and race/origin. The results of logistic regression models were expressed as adjusted odds ratios (ORs) and 95% CIs. Potential confounders were selected based on literature evidence and other *a priori* considerations. All models were examined for collinearity among the independent variables and for interaction between the OBS and each covariate. In all analyses the default approach was to calculate the measures of association from the dataset restricted to participants with non-missing values for all OBS components (Method 1). Sensitivity analyses were conducted to evaluate the

impact of imputing missing values. Imputations for missing score components were performed using two methods: first by assigning random values (Method 2); and then by assigning the median estimates for the same age, sex, and race/ethnicity category (Method 3). Additional sensitivity analyses were conducted to examine the impact of individual OBS components by removing each component from the score and controlling for it as a covariate. Adjusted ORs and 95% CIs were also calculated for the individual OBS components. All analyses were conducted using SAS statistical software version 9.2 (SAS Institute, Cary, NC).

## Results

### Distributions of biomarkers in the study population

The demographic and lifestyle characteristics of the study participants according to high and low biomarker levels are summarized in Table 2. Participants with higher FIP levels had, on average, a higher BMI, and were more likely to be NHW, AA, or non-drinkers. In the high mtDNA copy number category, there was a lower proportion of males, and a higher proportion of WA. In the high FOP category, there was a higher proportion of WA.

The dietary characteristics of the SRSH participants by high and low biomarker levels are summarized in Table 3. Plasma levels of zeaxanthin, cryptoxanthin, lycopene, α-carotene, and β-carotene were greater in the high FOP group than in the low FOP group. By contrast, plasma levels of these nutrients were lower in the high FIP group than in the low FIP group. Serum ferritin levels were greater in all three high biomarker groups than in the low biomarker groups.

The two-way correlations involving the individual dietary OBS components and the biomarkers of oxidative stress are presented in Table 4. The strongest positive Pearson correlation was observed between α-carotene and β-carotene ($r = 0.88$), both of which were negatively correlated with γ-tocopherol ($r = -0.35$ and $-0.30$, respectively). Spearman correlation coefficients were somewhat larger. There was no evidence of a positive correlation for biomarkers of oxidative stress, with Pearson coefficients ranging from −0.17 to 0.00 and Spearman coefficients ranging from −0.01 to −0.32.

### Associations of OBS with biomarkers

Associations between the OBS and the oxidative stress biomarkers expressed as continuous variables are shown in Table 5. Among participants with complete information on each of the OBS components (Method 1), the associations were in the hypothesized direction for FIP (inverse), but not for FOP (direct), and, among all participants combined, these associations were statistically significant. The association for mtDNA copy number was not statistically significant. The estimated associations did not substantially differ by race/ethnicity, and the tests for interaction between race and each of the three biomarkers were not statistically significant. Accordingly, all remaining results shown are for the combined population, with race/ethnicity included in the models as a covariate. In the sensitivity analyses (Methods 2 and 3), the measures of associations between the OBS and both FIP and FOP obtained by imputing values for missing score components were not substantially

different from the original results (Method 1). However, the positive association with mtDNA copy number observed using the original approach was no longer evident after imputation of the missing values, and the regression coefficients were in the opposite direction (Table 5).

Associations between OBS tertiles and the oxidative stress biomarkers expressed as binary (high versus low) variables are presented in Table 6. There was a statistically significant inverse trend ($p<0.01$), indicating that the odds of having an elevated FIP level were progressively lower with a progressive higher OBS. Using the lowest OBS fertile as reference, the OR (95% CIs) for high FIP in the middle and upper OBS tertiles were 0.34 (0.11–1.08) and 0.04 (0.01–0.17), respectively. None of the tertile-specific ORs for high mtDNA copy numbers was statistically significantly different from the null and there was no evidence of a dose-response. There was a statistically significant trend for higher odds of having a higher FOP level with a higher OBS ($p<0.01$), with a statistically significant OR of 5.64 (2.35–13.54) among those in the third (relative to the first) OBS fertile.

The results of the sensitivity analyses in which we examined the associations between OBS tertiles and high levels of biomarkers are presented in Table 7, where missing OBS components were either assigned random values (Method 2) or the median estimates for the same age, sex, and race/ethnicity category (Method 3). For FIP and FOP, the results were not substantially different from the original analysis (Method 1, Table 5). For mtDNA copy number, the association changed the direction.

### Association of individual OBS components with biomarkers

Associations between the individual OBS components and each oxidative stress biomarker are shown in Table 8. For the most part, the estimated associations for FIP and mtDNA copy number were inverse for both the antioxidant and pro-oxidant exposures, except that, for FIP, the estimated associations were direct with α-tocopherol, γ-tocopherol, aspirin and other NSAID use, and for mtDNA copy number, the estimated associations with lycopene, α-tocopherol, and physical activity were direct; the only statistically significant departures from the null were the strong inverse associations of FIP with zeaxanthin, cryptoxanthin, lycopene, α-carotene, and β-carotene. For most of the parts, the estimated associations of FOP with the various exposures were opposite to those for FIP and mtDNA copy number; only the direct associations with zeaxanthin, lycopene, α-carotene, β-carotene, and a-tocopherol were strong and statistically significant.

In additional sensitivity analyses the associations between high levels of the biomarkers and the 13-component OBS (examined as a continuous variable) were compared to those from alternative models in which each component was removed from the score one at a time and included in the model as a covariate. For all alternative models, removing an OBS component resulted in few meaningful differences from the ORs found using the original model. The OR estimates in the alternative models were within 12% of the OR from the original model (data not shown).

## Discussion

In this cross-sectional study, we examined associations between an OBS and biomarkers of oxidative stress (FIP, mtDNA copy number, and FOP), hypothesizing that a high OBS would be inversely associated with all biomarker levels. We found a strong, statistically significant inverse association of the OBS with FIP, but the OBS-FOP association, which was also statistically significant, was in the opposite direction than was hypothesized. While these results for both FOP and FIP were essentially the same as were found in the sensitivity analyses, the corresponding results for MtDNA copy number were unstable and the association changed direction depending on the method of missing data handling. There was no indication that our findings differed substantially across non-Hispanic whites, African-Americans, and native West Africans. The three biomarkers were not inter-correlated.

Other studies reported associations between an OBS and these biomarkers in different populations. Dash et al. (2013) observed a significant inverse association between a questionnaire-derived OBS and FIP in a case-control study of colorectal adenoma. In the same population, Kong et al. (2014) performed a separate analysis using an OBS comprised of components measured by both food frequency questionnaires (FFQ) and blood markers. As in our study, Kong et al. (2014) found that those in the lowest relative to those in the highest interval category of the OBS had statistically significant lower levels of FIP but higher levels of FOP.

FIP is considered the gold-standard measure of oxidative stress in population-based studies (Yin et al., 2005). The results from several placebo-controlled, randomized clinical trials (RCTs) of the effects of limited numbers of supplemental antioxidant micronutrients on FIP have been inconsistent (Dietrich et al., 2002, Gokce et al., 1999, Patrignani et al., 2000). However, when nutrients were examined in combination as a dietary score (similarly as for the OBS) in observational studies, stronger associations with FIP were observed (Meyer et al., 2013). In the Coronary Artery Risk Development in Young Adults (CARDIA) study, a diet quality score was determined by assigning higher points to frequent consumption of foods beneficial to health, and lower points to frequent consumption of foods believed to be detrimental to health (all determined *a priori*) (Meyer et al., 2013). A significant inverse association was observed between the dietary score and plasma FIP (Meyer et al., 2013).

Our findings for an OBS-mtDNA copy number association were not consistent, and mtDNA copy number was not substantially or statistically significantly correlated with either FIP or FOP. Liu et al. (2003) found mtDNA copy number to be correlated with thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation. In an *in vitro* study, exposure of human lung fibroblasts to oxidative stress resulted in an increase in mtDNA copy number (Lee et al., 2000). However, as shown in mouse models, oxidative stress may not be solely responsible for increasing mtDNA copy number, since transcription factors also play a regulatory role (Ekstrand et al., 2004).

A higher OBS in our study was associated with higher FOP levels. This observation appears counterintuitive, but it is in agreement with the results previously reported by Kong (2013). Moreover, we found that FOP was not correlated with FIP. In addition, the associations

between many individual antioxidants and FOP were opposite in direction to those hypothesized and those found for FIP. Since FOP is purported to be a non-specific measure of global oxidative stress, it may also be comprised of non-oxidative products (Wu et al., 2007b). Considering that FOP was previously directly associated with coronary heart disease and hypertension, but inversely associated with colorectal adenoma (Kong et al., 2014; Wu et al., 2007a,b) future studies should be conducted to understand exactly what FOP is measuring in humans and what role(s) its specific components may play in human pathophysiology.

One of the strengths of this study was the diverse population that included similar numbers of non-Hispanic white, African-American, and native West African participants. This allowed us to better examine possible interactions between the OBS and race/ethnicity, although none was observed. Another distinguishing feature of the present study was the use of plasma measures of dietary OBS components. Circulating levels of nutrients more accurately represent their current intake and availability for metabolism than do FFQ-derived measures (Potischman, 2003).

A major limitation of this study was missing information in a substantial proportion of participants. In a sensitivity analysis conducted to examine the impact of missing information, the results for FIP and FOP were similar to the original ones, but the direction of the association for mtDNA copy number (for which there were more missing data) reversed. Thus, the results for FIP and FOP appear to be reasonably robust, but the interpretation of the findings for mtDNA copy number is problematic at this time.

In conclusion, we found that a higher oxidative balance score (OBS) – a composite measure that reflects predominantly antioxidant exposures – was strongly inversely associated with the currently most accepted biomarker of oxidative stress, F2-isoprostanes, thus, providing further support for the validity of the OBS. Also, as we found in a separate study (Kong et al., 2014), fluorescent oxidation products (FOP) were directly associated with the OBS and with circulating antioxidant micronutrient levels, thus raising serious questions about whether or not FOP is a true indicator of oxidative balance in humans. The observation that the three biomarkers measured in the current study were not inter-correlated suggests that they are unlikely to measure the same or similar biological processes.

## Acknowledgments

We would like to thank the study participants for allowing us to conduct this work. We appreciate the contribution of Loree Mincey for processing the biological specimens.

S. Lakkur was supported in part by a postdoctoral training grant from the National Heart, Lung, and Blood Institute (grant T32HL072757).

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## Table 1

Oxidative balance score (OBS) assignment scheme.

| OBS components | Score assignment scheme |
|---|---|
| Zeaxanthin[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| Cryptoxanthin[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| Lycopene[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| α-carotene[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| β-carotene[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| α-tocopherol[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| γ-tocopherol[a] | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| Ferritin[b] | 0=high (3rd tertile), 1=medium (2nd tertile); 2=low (1st tertile) |
| Physical activity | 0=low (1st tertile), 1=medium (2nd tertile); 2=high (3rd tertile) |
| Smoking history | 0=current smoker, 2=never smoker |
| Aspirin | 0=never, 2=regular user |
| Other NSAID | 0=never, 2=regular user |
| Alcohol | 0=current drinker, 2=never drinker |

NSAID = non-steroidal anti-inflammatory drug (not including aspirin); PUFA = polyunsaturated fatty acids.

[a] Plasma derived measurement.

[b] Serum derived measurement.

*Biomarkers*. Author manuscript; available in PMC 2015 March 20.

**Table 2**

Demographic and lifestyle characteristics of SRSH participants according to high versus low levels of FIP, mtDNA copy number, and FOP.

| | FIP[a] | | mtDNA[b] | | FOP[c] | |
|---|---|---|---|---|---|---|
| | **Low (*n*=113)** | **High (*n*=114)** | **Low (*n*=92)** | **High (*n*=90)** | **Low (*n*=132)** | **High (*n*=140)** |
| Age (years), Mean (SD) | 45.2 (12.7) | 48.2 (12.5) | 47.7 (12.2) | 45.2 (11.3) | 45.5 (11.7) | 47.3 (12.2) |
| Missing (%) | (0.0) | (4.4) | (5.4) | (5.6) | (2.3) | (1.4) |
| Male (%) | (38.1) | (36.8) | (58.7) | (38.9)¶ | (36.4) | (37.9) |
| Missing (%) | (4.4) | (4.4) | (6.5) | (7.8) | (3.8) | (3.6) |
| Race/ethnicity | | | | | | |
| NHW (%) | (11.5) | (62.3)¶ | (16.3) | (15.6)¶ | (49.3) | (35.7)¶ |
| AA (%) | (21.3) | (28.1) | (41.3) | (22.2) | (33.3) | (22.9) |
| WA (%) | (63.7) | (6.1) | (40.2) | (61.1) | (15.9) | (41.4) |
| Missing (%) | (3.5) | (3.5) | (2.2) | (1.1) | (1.5) | (0.0) |
| BMI (kg/m$^2$), Mean (SD) | 28.6 (5.4) | 31.3 (7.0)¶ | 31.1 (6.9) | 30.8 (6.5) | 30.4 (7.2) | 29.8 (5.5) |
| Missing (%) | (21.2) | (8.8) | (16.3) | (25.6) | (8.33) | (18.6) |
| Physical Activity, MET-mins./week | 3889.8 (3508.6) | 2979.0 (3013.4) | 3149.0 (3178.1) | 4019.8 (3582.1) | 3045.0 (2943.5) | 3665.2 (3798.2) |
| Missing (%) | (23.9) | (38.6) | (45.7) | (28.9) | (36.4) | (28.6) |
| Smoking history | | | | | | |
| Current (%) | (1.8) | (6.1) | (2.2) | (3.4) | (3.8) | (3.6) |
| Non-smoker (%) | (82.3) | (59.7) | (62.0) | (72.2) | (62.9) | (77.1) |
| Missing (%) | (15.9) | (34.2) | (35.8) | (24.4) | (33.3) | (19.9) |
| Aspirin | | | | | | |
| Current (%) | (12.4) | (17.6) | (16.3) | (15.6) | (12.9) | (21.4) |
| Never (%) | (72.6) | (49.1) | (48.9) | (63.3) | (54.5) | (60.0) |
| Missing (%) | (15.0) | (33.3) | (34.8) | (21.1) | (32.6) | (18.6) |
| Other NSAID | | | | | | |
| Current (%) | (15.0) | (21.9)¶ | (16.3) | (13.3) | (21.2) | (17.8) |
| Never (%) | (71.7) | (44.7) | (48.9) | (65.6) | (46.2) | (63.6) |
| Missing (%) | (13.3) | (33.3) | (34.8) | (21.1) | (32.6) | (18.6) |

| | FIP[a] | | mtDNA[b] | | FOP[c] | |
|---|---|---|---|---|---|---|
| | **Low (*n*=113)** | **High (*n*=114)** | **Low (*n*=92)** | **High (*n*=90)** | **Low (*n*=132)** | **High (*n*=140)** |
| Alcohol | | | | | | |
| Current (%) | (27.4) | (37.7)¶ | (16.3) | (27.8) | (31.0) | (35.0) |
| Non-drinker (%) | (55.8) | (27.2) | (48.9) | (45.6) | (34.1) | (44.3) |
| Missing (%) | (16.8) | (35.1) | (34.8) | (26.7) | (34.9) | (20.7) |

[a] FIP cutoffs: Low FIP,<48.37 pg/mL (n= 113); High FIP, ≥ 48.37 pg/mL (*n* = 114).

[b] mtDNA copy number: Low mtDNA copy number, <3.05 relative copy number (*n* = 92); High mtDNA copy number, ≥3.05 relative copy number (*n* = 90).

[c] FOP cutoffs: Low FOP, <0.04 average standard reference adjusted (*n* = 132); High FOP, ≥0.04 average standard reference adjusted (*n* = 140).

¶ $p<0.05$ based on *t*-test for continuous variables and chi-square test for categorical variables.

*Biomarkers*. Author manuscript; available in PMC 2015 March 20.

**Table 3**

Selected dietary characteristics of SRSH participants.

| | FIP[a] | | mtDNA[b] | | FOP[c] | |
|---|---|---|---|---|---|---|
| | **Low (*n*=113)** | **High (*n*=114)** | **Low (*n*=92)** | **High (*n*=90)** | **Low (*n*=132)** | **High (*n*=140)** |
| Zeaxanthin (μg/dL), Mean (SD) | 22.87 (9.86) | 19.88 (11.42)¶ | 23.70 (9.94) | 22.06 (10.15) | 17.66 (10.31) | 22.90 (10.30)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| Cryptoxanthin (μg/dL), Mean (SD) | 9.96 (1.35) | 5.79 (4.01)¶ | 9.85 (16.06) | 7.05 (4.39) | 6.54 (5.13) | 7.36 (4.58) |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| Lycopene (μg/dL), Mean (SD) | 57.77 (27.79) | 36.18 (17.68)¶ | 50.95 (26.54) | 56.87 (27.11) | 32.58 (17.27) | 54.92 (26.09)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| α-carotene (μg/dL), Mean (SD) | 18.18 (17.91) | 4.05 (7.16)¶ | 13.75 (14.43) | 18.90 (19.60) | 6.02 (10.65) | 13.06 (16.50)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| β-carotene (μg/dL), Mean (SD) | 33.90 (28.24) | 12.74 (11.69)¶ | 25.97 (19.64) | 31.49 (30.17) | 13.74 (14.42) | 27.50 (26.61)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| α-tocopherol (mg/dL), Mean (SD) | 0.94 (0.29) | 1.00 (0.29) | 0.94 (0.26) | 0.94 (0.28) | 0.91 (0.30) | 1.00 (0.27)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| γ-tocopherol (mg/dL), Mean (SD) | 0.16 (0.06) | 0.32 (0.10)¶ | 0.19 (0.09) | 0.17 (0.08) | 0.21 (0.09) | 0.19 (0.08)¶ |
| Missing, *n* (%) | (8.9) | (6.1) | (20.7) | (8.9) | (10.6) | (7.9) |
| Ferritin (mg/dL), Mean (SD) | 127.50 (343.90) | 133.40 (125.80)¶ | 143.00 (111.20) | 158.40 (379.50) | 108.80 (105.70) | 138.20 (309.80) |
| Missing, *n* (%) | (10.6) | (5.3) | (13.0) | (7.8) | (7.6) | (4.3) |

FIP = F2-isoprostanes; mtDNA = mitochondrial DNA copy number; FOP = florescent oxidation products.

[a] FIP cutoffs: Low FIP,<48.37 pg/mL (*n* = 113); High FIP, ≥48.37 pg/mL (*n* = 114).

[b] mtDNA copy number: Low mtDNA copy number, <3.05 relative copy number (*n* = 92); High mtDNA copy number, ≥3.05 relative copy number (*n* = 90).

[c] FOP cutoffs: Low FOP, <0.04 average standard reference adjusted (*n*= 132); High FOP, ≥0.04 average standard reference adjusted (*n* = 140).

¶ $p<0.05$ based on *t*-test.

**Table 4**

Correlations among individual biomarkers.

| | Correlation coefficients (Pearson are above the diagonal and Spearman are below the diagonal) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Biomarkers** | **Zeaxanthin** | **Cryptoxanthin** | **Lycopene** | **α-carotene** | **β-carotene** | **α-tocopherol** | **γ-tocopherol** | **Ferritin** | **FIP** | **mtDNA** | **FOP** |
| Zeaxanthin[a] | - | 0.24‡ | 0.23‡ | 0.21‡ | 0.28‡ | 0.13‡ | 0.08 | 0.00 | −0.25‡ | −0.10 | 0.20‡ |
| Cryptoxanthin[a] | 0.55‡ | - | 0.05 | 0.11 | 0.22‡ | 0.09 | 0.17‡ | −0.04 | −0.19‡ | −0.09 | 0.17‡ |
| Lycopene[a] | 0.38‡ | 0.34‡ | - | 0.60‡ | 0.59‡ | 0.04 | −0.21‡ | −0.04 | −0.34‡ | 0.04 | 0.40‡ |
| α-carotene[a] | 0.48‡ | 0.51‡ | 0.63‡ | - | 0.88‡ | −0.11 | −0.35‡ | −0.04 | −0.39‡ | 0.12 | 0.24‡ |
| β-carotene[a] | 0.50‡ | 0.55‡ | 0.62‡ | 0.88‡ | - | 0.04 | −0.30‡ | −0.07 | −0.38‡ | 0.13 | 0.29‡ |
| α-tocopherol[a] | 0.18‡ | 0.18‡ | 0.08 | −0.06 | 0.07 | - | 0.21‡ | 0.12‡ | 0.09 | −0.07 | 0.17‡ |
| γ-tocopherol[a] | 0.02 | 0.00 | −0.21‡ | −0.44‡ | −0.40‡ | 0.24‡ | - | 0.03 | 0.35‡ | −0.16‡ | −0.15‡ |
| Ferritin[b] | 0.03 | −0.07 | −0.03 | −0.06 | −0.06 | 0.08 | 0.02 | - | −0.01 | −0.06 | 0.03 |
| FIP | −0.30 | −0.38 | −0.43‡ | −0.63‡ | −0.56‡ | 0.17‡ | 0.39‡ | 0.11 | - | −0.14 | −0.17‡ |
| mtDNA | −0.14 | −0.09 | 0.08 | 0.10 | 0.05 | −0.09 | −0.14 | −0.16‡ | −0.15 | - | 0.00 |
| FOP | 0.31‡ | 0.25‡ | 0.57‡ | 0.41‡ | 0.44‡ | 0.21‡ | −0.15‡ | 0.06 | −0.32‡ | −0.01 | - |

OBS = oxidative balance score; FIP = F2-isoprostanes; mtDNA = mitochondrial DNA copy number; FOP = florescent oxidation products.

[a]Plasma derived measurement

[b]Serum derived measurement.

‡$p<0.05$.

**Table 5**

Associations between OBS and biomarkers (continuous) in the SRSH population using linear regression.

| | Method 1[c] | | Method 2[d] | | Method 3[e] | |
|---|---|---|---|---|---|---|
| **Biomarker** | **Regression coefficient (95%CI)** | ***p* Value** | **Regression coefficient (95%CI)** | ***p* Value** | **Regression coefficient (95%CI)** | ***p* Value** |
| All participants[a] | | | | | | |
| Ln (FIP) | −0.02915 (−0.04998, −0.00832) | <0.01 | −0.02598 (−0.04241, −0.00955) | <0.01 | -0.02579 (-0.04383, -0.00775) | <0.01 |
| Ln (mtDNA) | 0.00349 (−0.01524, 0.02223) | 0.71 | −0.01675 (−0.02896, −0.00455) | 0.40 | -0.00644 (-0.02145, 0.00857) | 0.40 |
| Ln (FOP) | 0.03122 (0.00898, 0.04828) | <0.01 | 0.02641 (0.01412, 0.03869) | <0.01 | 0.03259 (0.01530, 0.04988) | <0.01 |
| NHW[b] | | | | | | |
| Ln (FIP) | −0.04358 (−0.07497, −0.01219) | <0.01 | −0.02932 (−0.05559, −0.00305) | 0.04 | −0.03042 (−0.05864, −0.00220) | 0.04 |
| Ln (mtDNA) | 0.01495 (−0.03571, 0.06562) | 0.52 | −0.01497 (−0.03920, 0.00926) | 0.16 | −0.01776 (−0.04288, 0.00735) | 0.16 |
| Ln (FOP) | 0.02954 (0.00994, 0.04913) | <0.01 | 0.02578 (0.01052, 0.04104) | <0.01 | 0.02907 (0.01005, 0.04808) | <0.01 |
| AA[b] | | | | | | |
| Ln (FIP) | −0.01638 (−0.06143, 0.02867) | 0.46 | −0.03722 (−0.07258, −0.00185) | 0.47 | −0.01390 (−0.05191, 0.02410) | 0.47 |
| Ln (mtDNA) | 0.00215 (−0.10550, 0.10979) | 0.96 | −0.02759 (−0.05451, −0.00067) | 0.04 | −0.00536 (−0.04482, 0.03410) | 0.79 |
| Ln (FOP) | 0.06208 (0.00143, 0.12273) | 0.05 | 0.03059 (0.00295, 0.05824) | 0.03 | 0.06502 (0.00668, 0.12336) | <0.01 |
| WA[b] | | | | | | |
| Ln (FIP) | 0.01071 (−0.02990, 0.05133) | 0.59 | 0.00220 (−0.02536, 0.02976) | 0.85 | 0.01657 (−0.03659, 0.03016) | 0.85 |
| Ln (mtDNA) | −0.00083 (−0.02326, 0.02160) | 0.94 | −0.00614 (−0.02446, 0.01218) | 0.95 | −0.00059 (−0.02055, 0.01937) | 0.95 |
| Ln (FOP) | 0.00109 (−0.05284, 0.05501) | 0.97 | 0.01383 (−0.02247, 0.05013) | 0.45 | 0.00729 (−0.04646, 0.06105) | 0.78 |

OBS = oxidative balance score; FOP = florescent oxidation products; FIP = F2-isoprostanes; mtDNA = mitochondrial DNA copy number; NHW = Non-Hispanic White; AA = African American; WA = West African, CI = confidence interval.

[a] Adjusted for age, sex, race/ethnicity, and BMI.

[b] Adjusted for age, sex, and BMI.

[c] Method 1 - analytical population only includes participants with complete information on OBS components.

[d] Method 2 - analytical population includes participants with missing values for OBS components. Imputed values for missing OBS components were randomly assigned.

[e] Method 3 - analytical population includes participants with missing values for OBS components. Imputed values for missing OBS components were the observed median values for the same age, gender, and race/ethnicity category.

## Table 6

Association between OBS and biomarkers of oxidative stress[f].

| Biomarker | Low biomarker levels | High biomarker levels | OR (95% CI)[d] | *p* Trend[e] |
|---|---|---|---|---|
| FIP[a] | | | | |
| OBS Tertile | | | | |
| 4–11 | 11 | 38 | 1.0 | <0.01 |
| 12–14 | 23 | 21 | 0.34 (0.11–1.08) | |
| 15–22 | 33 | 7 | 0.04 (0.01–0.17) | |
| MtDNA count[b] | | | | |
| OBS Tertile | | | | |
| 5–10 | 19 | 14 | 1.0 | 0.44 |
| 11–15 | 18 | 14 | 1.60 (0.39–6.65) | |
| 16–21 | 8 | 17 | 6.09 (1.09–34.02) | |
| FOP[c] | | | | |
| OBS Tertile | | | | |
| 4–12 | 44 | 18 | 1.0 | <0.01 |
| 13–15 | 16 | 21 | 2.06 (0.78–5.44) | |
| 16–23 | 14 | 46 | 5.64 (2.35–13.54) | |

OBS = oxidative balance score; OR = odds ratio; CI = confidence interval; FOP = florescent oxidation product; FIP = F2-isoprostanes; mtDNA = mitochondrial DNA relative copy number.

[a] FIP cutoffs: Low FIP, <46.44 pg/mL ($n$ = 67); High FIP, ≥ 48.34 pg/mL ($n$ = 66).

[b] MtDNA count: Low MtDNA count, >=3.19 ($n$ = 45); High MtDNA count, <3.19 ($n$ = 45).

[c] FOP cutoffs: Low FOP, <0.04 average standard reference adjusted ($n$ = 74); High FOP, ≥0.04 average standard reference adjusted ($n$ = 85).

[d] Adjusted for age, sex, origin, and BMI.

[e] $X^2$ test for linear trend.

[f] Included participants with complete information on OBS components (Method 1).

**Table 7**

Sensitivity analysis of associations between the OBS and biomarkers of oxidative stress using imputed values for missing OBS components.

| | Method 2[f] | | | | Method 3[g] | | | |
|---|---|---|---|---|---|---|---|---|
| **Biomarker** | **Low biomarker level** | **High biomarker level** | **OR (95% CI)[d]** | ***p* Trend[e]** | **Low biomarker level** | **High biomarker level** | **OR (95% CI)[d]** | ***p* Trend[e]** |
| FIP[a] | | | | | | | | |
| OBS tortile | | | | | | | | |
| 4–11 | 21 | 57 | 1.0 | <0.01 | 17 | 60 | 1.0 | <0.01 |
| 12–14 | 37 | 33 | 0.35 (0.15–0.82) | | 34 | 27 | 0.25 (0.11–0.59) | |
| 15–22 | 55 | 24 | 0.17 (0.07–0.38) | | 53 | 19 | 0.12 (0.05-0.28) | |
| mtDNA[b] | | | | | | | | |
| OBS tortile | | | | | | | | |
| 5–11 | 30 | 38 | 1.0 | 0.03 | 38 | 31 | 1.0 | 0.32 |
| 12–14 | 28 | 22 | 0.44 (0.16–1.17) | | 23 | 22 | 0.62 (0.23–1.63) | |
| 15–22 | 34 | 30 | 0.35 (0.13–0.91) | | 22 | 28 | 0.60 (0.21–1.71) | |
| FOP[c] | | | | | | | | |
| OBS tortile | | | | | | | | |
| 4–11 | 62 | 32 | 1.0 | <0.01 | 66 | 28 | 1.0 | <0.01 |
| 12–14 | 33 | 41 | 2.12 (1.05–4.28) | | 33 | 42 | 2.52 (1.28–4.97) | |
| 15–23 | 37 | 67 | 2.47 (1.30–4.69) | | 26 | 65 | 4.53 (2.27–9.06) | |

OBS = oxidative balance score; OR = odds ratio; CI = confidence interval; FOP = florescent oxidation product; FIP = F2-isoprostanes; mtDNA = mitochondrial DNA relative copy number.

[a] FIP cutoffs: Low FIP, <48.37 pg/mL ($n$ = 113); High FIP, ≥ 48.37 pg/mL ($n$ = 114).

[b] mtDNA copy number: Low mtDNA copy number <3.05 relative copy number ($n$ = 92); High mtDNA copy number, ≥3.05 relative copy number ($n$ = 90).

[c] FOP cutoffs: Low FOP, <0.04 average standard reference adjusted ($n$ = 132); High FOP, ≥0.04 average standard reference adjusted ($n$ = 140).

[d] Adjusted for age, sex, origin, and BMI.

[e] $X^2$ test for linear trend.

[f] Imputed values for missing OBS components were randomly assigned.

[g] Imputed values for missing OBS components were the observed median estimates for the same age, gender, and race/ethnicity category.

**Table 8**

Associations between individual OBS components and biomarkers.

| Variables | | FIP OR (95% CI)[a] | mtDNA OR (95% CI)[a] | FOP OR (95% CI)[a] |
|---|---|---|---|---|
| Zeaxanthin[b] | 1 point relative to 0 | 0.35 (0.15–0.84) | 0.53 (0.19–1.50) | 1.79 (0.88–3.65) |
| | 2 points relative to 0 | 0.22 (0.09–0.53) | 0.50 (0.18–1.37) | 3.19 (1.56–6.51) |
| Cryptoxanthin[b] | 1 point relative to 0 | 0.24 (0.10–0.56) | 0.81 (0.30–2.21) | 1.61 (0.80–3.24) |
| | 2 points relative to 0 | 0.14 (0.06–0.36) | 0.86 (0.32–2.33) | 1.70 (0.83–3.48) |
| Lycopene[b] | 1 point relative to 0 | 0.56 (0.26–1.36) | 0.82 (0.30–2.28) | 2.49 (1.20–5.14) |
| | 2 points relative to 0 | 0.20 (0.08–0.52) | 1.23 (0.38–3.92) | 11.72 (4.43–25.95) |
| α-carotene[b] | 1 point relative to 0 | 0.14 (0.14–0.77) | 0.75 (0.22–2.55) | 3.09 (1.53–6.24) |
| | 2 points relative to 0 | 0.04 (0.01–0.14) | 0.82 (0.20–3.37) | 8.87 (3.44–22.85) |
| β-carotene[b] | 1 point relative to 0 | 0.22 (0.09–0.57) | 0.72 (0.25–2.05) | 3.12 (1.54–6.33) |
| | 2 points relative to 0 | 0.03 (0.02–0.17) | 0.71 (0.38–3.84) | 5.34 (2.33–12.23) |
| α-tocopherol[b] | 1 point relative to 0 | 0.94 (0.40–2.12) | 0.25 (0.19–1.32) | 2.09 (1.00–4.34) |
| | 2 points relative to 0 | 1.12 (0.45–2.76) | 1.10 (0.37–3.27) | 3.44 (1.56–7.58) |
| γ-tocopherol[b] | 1 point relative to 0 | 0.82 (0.36–1.83) | 0.71 (0.26–1.93) | 1.75 (0.85–3.60) |
| | 2 points relative to 0 | 3.13 (1.25–7.83) | 0.81 (0.26–2.52) | 0.87 (0.40–1.87) |
| Ferritin[c] | 1 point relative to 0 | 1.01 (0.43–2.38) | 0.79 (0.32–1.97) | 0.91 (0.45–1.82) |
| | 2 points relative to 0 | 0.67 (0.26–1.76) | 0.80 (0.25–2.51) | 0.97 (0.45–2.09) |
| Smoking | 2 points relative to 0 | 0.15 (0.02–1.32) | 0.63 (0.09–1.22) | 1.12 (0.31–4.09) |
| Alcohol | 2 points relative to 0 | 0.31 (0.14–0.70) | 0.44 (0.17–1.16) | 0.89 (0.47–1.70) |
| Aspirin | 2 points relative to 0 | 1.71 (0.59–1.93) | 0.80 (0.27–2.36) | 0.48 (0.23–1.00) |
| Other NSAID | 2 points relative to 0 | 1.54 (0.66–3.58) | 0.53 (0.19–1.50) | 2.00 (0.86–4.66) |
| Physical activity | 1 point relative to 0 | 0.84 (0.33–2.12) | 2.49 (0.76–8.23) | 1.10 (0.57–2.14) |
| | 2 points relative to 0 | 0.65 (0.25–1.71) | 2.86 (0.74–10.98) | 1.68 (0.73–3.89) |

OBS = oxidative balance score; OR = odds ratio; CI = confidence interval; NSAID = non-steroidal anti-inflammatory drug; FOP = florescent oxidation product; FIP = F2-isoprostanes; mtDNA = mitochondrial DNA copy number.

[a] All results adjusted for age, sex, race/ethnicity, and BMI.

[b] Plasma derived measurement.

[c] Serum derived measurement.