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# Oxidative stress, inflammation, and markers of cardiovascular health

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ABSTRACT

*Objective:* To investigate associations of a oxidative balance score (OBS) with blood levels of total cholesterol, low-density lipoprotein- (LDL)-cholesterol, high-density lipoprotein- (HDL) cholesterol and triglycerides, and biomarkers of inflammation (serum C-reactive protein [CRP], albumin and venous total white blood cell [WBC] counts) among 19,825 participants in a nationwide study.
*Methods:* Using cross-sectional data 14 dietary and lifestyle components were incorporated into the OBS and the resulting score (range 3–26) was then divided into five equal intervals. Multivariable-adjusted odds ratios (ORs) for abnormal biomarker levels and 95% confidence intervals (CIs) were calculated using logistic regression models.
*Results:* The ORs (95% CIs) comparing those in the highest relative to those in the lowest OBS equal interval categories were 0.50 (0.38–0.66) for CRP, 0.50 (0.36–0.71) for the total WBC count, and 0.75 (0.58–0.98) for LDL-cholesterol; all three p-values for trend were <0.001. The OBS-HDL-cholesterol association was statistically significantly inverse among females, but not among males. The OBS was not associated with serum albumin or triglycerides.
*Conclusion:* Our findings suggest that an OBS may be associated with some, but not all, circulating lipids/lipoproteins and biomarkers of inflammation.



## 1. Introduction

Oxidative stress is an imbalance between pro-oxidants and antioxidants, which results in macromolecular damage and disruption of redox signaling and control [1]. It is a complex physiological process, closely interrelated with inflammation [2].

Several exogenous factors may act as pro-oxidants by increasing levels of reactive oxygen species (ROS). ROS are present in the tar and smoke of cigarettes, and smoking also produces a secondary release of ROS from inflammatory cells [3]. Another important pro-oxidant is iron, which is consumed along with heme in large quantities as part of a red meat-rich diet. Iron may increase oxidative stress by catalyzing the production of highly reactive hydroxyl radicals via the Haber–Weiss reaction [4]. Alcohol induces oxidative stress through its metabolism, by inhibiting antioxidant enzymes, and by causing inflammation [5].

*In-vitro* evidence indicates that the effects of ROS and oxidative stress-induced inflammation can be reversed by certain antioxidant nutrients [6]. Carotenoids, lutein, lycopene, vitamin C, vitamin E, and flavonoids, can protect against lipid peroxidation and terminate free radical chain reactions [7]. Selenium and manganese are critical components of antioxidant enzymes [7]. Other nutrients can also indirectly contribute to a reduction in ROS. Omega-3 fatty acids contribute to oxidative stress through peroxidation [8], but also induce electrophile-responsive element (EpRE), which regulates genes responsible for transcribing antioxidant enzymes [9]. Moreover, omega-3 fatty acids have anti-inflammatory properties and therefore indirectly decrease oxidative stress [10].

Although oxidative stress and inflammation are implicated in the pathogenesis of numerous diseases [2,11], and antioxidants slow down these processes *in-vitro* [6], clinical trials of antioxidants as disease prevention agents have produced null or adverse results [12,13]. Other studies of chronic diseases found that a combination

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of factors may be more strongly associated with disease risk than any nutrient considered individually [14,15]. This led to a hypothesis that a combination of pro-oxidant and antioxidant exposures incorporated into a composite measure of oxidative balance may be more strongly associated with health outcomes more than would any one factor considered individually [16,17].

To address this issue we, and others, proposed using an oxidative balance score (OBS), an overall measure of oxidative stress-related exposures based on the summed intakes of various pro- and anti-oxidants, with a higher score indicating lower oxidative stress [16,17]. Previous studies found that a higher OBS was associated with lower risk of colorectal adenoma [16,18] and mortality [17], but not prostate cancer [19], indicating that the role of oxidative stress in human pathophysiology may be organ- or disease-specific. To better understand the specific roles of oxidative stress-modifying exposures in various health outcomes, the potential mechanisms represented by an OBS should be examined using biomarkers, which can act as upstream indicators of future health events [20].

We examined associations between an OBS and circulating biomarkers of inflammation including C-reactive protein (CRP), albumin, and total white blood cell (WBC) count. Previous epidemiological studies have used WBC count as a marker of inflammation [21]. In its 2003 scientific statement, the American Heart Association (AHA) classified WBC count as an inflammatory marker [22]. Hypoalbuminemia serves as a marker of inflammation because chronic inflammation has been shown to reduce rate of albumin synthesis [23]. CRP, an acute-phase reactant, is a reliable biomarker of inflammation [24] that has been shown to increase in the presence of oxidative stress [25]. We also assessed the association between OBS and blood levels of lipids/lipoproteins including total, LDL-, and HDL-cholesterol, and triglycerides. We examined these associations in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study cohort, with the hypothesis that a beneficial balance of pro-/anti-oxidants will be inversely related to abnormal biomarker levels.

## 2. Materials and methods

### 2.1. Study population

The REGARDS prospective cohort study is designed to examine the causes of racial and geographic disparities in stroke, and offers an opportunity for ancillary research projects. The cohort is comprised of 30,239 black and white males and females, ages 45 or older, enrolled from January 2003 to October 2007. The institutional review boards of the multiple participating entities approved this study. Participants were recruited by telephone and mail from 1,842 (59%) of 3140 US counties, with an oversampling of blacks and residents of the Stroke Belt (non-coastal regions of Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee) and Stroke Buckle (coastal plain regions of North Carolina, South Carolina, and Georgia), and the remainder of the rest of the continental US. At baseline, an interview was conducted by telephone to obtain demographic and risk factor information, and blood samples and physical measurements were obtained during an in-home visit. A self-administered questionnaire food frequency questionnaire (FFQ) was left with the participant to be returned by self-addressed prepaid envelopes. Details of the study design can be found elsewhere [26].

### 2.2. Laboratory analyses

After an overnight fast, blood samples were drawn, centrifuged, and then shipped to the University of Vermont central laboratory for reprocessing and analysis. Plasma CRP was measured using particle-enhanced immunonephelometry (N High-Sensitivity CRP assay; Dade Behring, Inc., Deerfield, Illinois) [27]. Venous total WBC counts were measured using an automated analyzer (Beckman Coulter, Inc., Fullerton, California) [28]. Serum total and HDL-cholesterol, triglycerides, and albumin were measured by colorimetric reflectance spectrophotometry using the Ortho Vitros Clinical Chemistry System 950IRC instrument (Johnson & Johnson Clinical Diagnostics). Serum LDL-cholesterol concentrations were determined using the Friedewald equation [29].

### 2.3. Definitions

Elevated CRP was defined as >3 mg/L [22]. Hypoalbuminemia was defined as <3.5 g/dL [30]. Cutoffs for lipid biomarkers were defined using the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III Guidelines (elevated total cholesterol: $\geq$ 200 mg/dL, elevated LDL: >100 mg/dL, elevated triglycerides: $\geq$150 mg/dL, and low HDL for males and females: <40 mg/dL) [31]. Elevated total WBC count was defined as being above the 75th percentile (>6.86 $\times$ $10^9$ cells/L) [32].

Covariates included age, sex, total energy intake, BMI, self-reported race (black or white), educational level (college graduate or higher, some college, high school graduate or GED, or less than high school), region (Stroke Buckle, rest of the Stroke Belt, or other), and frequency of physical activity ($\geq$4 times/week, 1–3 times/week, or none).

### 2.4. OBS components and their assessment

The OBS was comprised of 14 components that were selected based on *a priori* knowledge about their relation to oxidative stress. Dietary components were derived from the self-administered 98-item Block FFQ [33]. Nutrient contents of foods were determined using the Block nutrient database with composition values from the U.S. Department of Agriculture and other sources [34]. The nutrient intakes were calculated by multiplying the reported frequency of consumption by the nutrient composition of the specified portion size for each food item. Nutrient values in this analysis represent the total dietary and supplemental intake for each nutrient.

The components of the OBS are summarized in Supplementary Table 1 and calculation of the OBS is available in Supplementary Methods. Briefly, the points assigned to each OBS component were summed to create the overall OBS, which was divided into equal interval categories. The cut points for the categories were determined using the distribution of the OBS within the analysis cohort, and are listed in Table 2. A high OBS indicates a presumably beneficial balance of pro- and anti-oxidants.

### 2.5. Statistical analysis

In descriptive analyses, the means, standard deviations, and frequencies were calculated for covariates and biomarker measurements within each OBS interval. To assess differences in various parameters across OBS intervals, the chi-square test was used for categorical variables and analysis of variance (ANOVA) was used for continuous variables. With the exception of serum albumin, the biomarker measurements were not normally distributed, and so were log transformed when used in linear regression analyses. Multivariable linear regression models were constructed to assess associations between the OBS and each biomarker expressed as a continuous measure. To calculate the standardized linear regression coefficients, the biomarker variables were standardized, so that their variances were equal to one.

Multivariable logistic regression models were used to examine