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.
Keywords
Epidemiology; Oxidative stress; Inflammation; Lipoproteins

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.\(^5\)

\textit{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 \textit{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 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. In its 2003 scientific statement, the American Heart Association (AHA) classified WBC count as an inflammatory marker. Hypoalbuminemia serves as a marker of inflammation because chronic inflammation has been shown to reduce rate of albumin synthesis. CRP, an acute-phase reactant, is a reliable biomarker of inflammation that has been shown to increase in the presence of oxidative stress. 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.

Materials and Methods

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 3,140 US counties, with an oversampling of blacks and residents of the Stroke Belt (noncoastal 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.

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). Venous total WBC counts were measured using an automated analyzer (Beckman Coulter, Inc., Fullerton, California). 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.

Definitions

Elevated CRP was defined as >3 mg/L. Hypoalbuminemia was defined as <3.5 g/dL. Cutoffs for lipid biomarkers were defined using the National Cholesterol Education

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Program’s (NCEP) Adult Treatment Panel III Guidelines (elevated total cholesterol: ≥200 mg/dL, elevated LDL: >100 mg/dL, elevated triglycerides: ≥150 mg/dL, and low HDL for males and females: <40 mg/dL). Elevated total WBC count was defined as being above the 75th percentile (>6.86 × 10^9 cells/L).

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 (≥4 times/week, 1–3 times/week, or none).

**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. Nutrient contents of foods were determined using the Block nutrient database with composition values from the U.S. Department of Agriculture and other sources. 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.

**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 the association of OBS with abnormal biomarker levels. The results were expressed as adjusted odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) adjusted for age, sex, total energy intake, BMI, race, educational level, region, and physical activity. The potential confounders were selected based on evidence in the literature and other *a priori* considerations. All models were examined for collinearity among independent variables and for interaction between the...
OBS and each covariate. When a statistically significant interaction was found, a stratified analysis was conducted to determine whether the OBS-biomarker association was appreciably modified by the covariate. Sensitivity analyses were conducted to examine the impact of individual OBS components by removing each OBS component from the score and controlling for it as a covariate and evaluate the impacts of different OBS categorization approaches (5 equal intervals, 4 equal intervals, or quartiles) and different outcome definitions on the results. We examined the results for low HDL using cutoffs from the American Heart Association’s guidelines for cardiovascular disease prevention (<50 mg/dL in females and <40 mg/dL in males). All analyses were conducted using SAS statistical software version 9.2 (SAS institute, Cary North Carolina).

Results

Seventy-two percent of participants (n=21,636) returned the completed FFQ. Individuals were excluded if no biomarker measurements were recorded (n =770), OBS components were missing (n = 433), or if they had a body mass index (BMI) of < 18.5 kg/m² (n = 336) or incomplete covariate information (n = 272). This resulted in an analytical cohort of 19,825 participants with at least one biomarker measurement. The number of participants with measurements for individual biomarkers varied as follows: 19,531 for serum CRP; 19,790 for serum total cholesterol, 19,685 for HDL-cholesterol, 19,416 for LDL-cholesterol, 19,782 for serum triglycerides,14,475 for serum albumin, and 13,716 for total venous WBC count. Albumin and WBC count were measured only in a subset of participants (n = 21,658) who were enrolled in an ancillary study.

The characteristics of participants in the analysis cohort are presented by equal interval OBS categories in Table 1. Compared to those in the lowest OBS interval (range 3–7), participants in the highest OBS interval (range 22–26) were older and had a higher energy intake. The proportion of participants who were female, Caucasian, college educated, exercised ≥4 times/week, and resided outside the stroke belt increased with increasing OBS. There was no significant difference in BMI across OBS intervals.

Associations of the OBS with each of the biomarkers expressed a continuous variables, are shown in Supplementary Table 2. The results for HDL are presented separately for males and females because there was a statistically significant (p<0.01) OBS-sex interaction. The associations were in the hypothesized direction for CRP, total cholesterol, HDL (among females), LDL, triglycerides, albumin, and WBC count. Only the associations for CRP, total cholesterol, LDL, triglycerides, serum albumin, WBC, and HDL among females were statistically significant. The standardized regression coefficients indicate that among all the biomarkers, OBS was most strongly associated with CRP and WBC.

The odds of an elevated CRP or WBC count were 50% lower for participants in the highest versus the lowest OBS with evidence of a statistically significant inverse dose-response relationship (both p_trend <0.01). None of the OBS interval-specific ORs for low albumin was statistically significant and there was no evidence of a dose-response association (Table 2).
As shown in Table 3, for total cholesterol, the ORs (95% CIs) comparing those in the second through fifth OBS intervals to those in the lowest (first) interval were all approximately 15% lower, with the test for trend and the estimates for the third and fourth intervals being statistically significant, whereas the essentially same magnitude estimate in the upper interval (in which there were fewer persons) was not. The corresponding analyses for LDL similarly revealed a statistically significant inverse trend and statistically significant 25% lower odds of an elevated LDL among those in the highest versus those in the lowest OBS interval. None of the OBS interval-specific ORs for elevated triglycerides were statistically significant and there was no evidence of a dose-response relationship.

In the analyses for low vs. normal HDL (Table 4) there was again a statistically significant interaction between OBS and sex (p<0.01); these analyses are presented separately for males and females. Using the NCEP definition, the odds of a low HDL were 63% higher among males (OR=1.63; 95% CI: 1.09–2.45; p-trend = 0.13) but 52% lower among females (OR=0.48; 95% CI: 0.28–0.83; p-trend = 0.05) in the highest versus the lowest OBS category. No significant differences in the observed associations for the other biomarkers when stratified by race or sex (data not shown).

Sensitivity analyses found that removing an OBS component resulted in OR estimates within 5% of the original model result (data not shown). Multiple categorizations of OBS, results using quartiles or 4 equal interval categories of OBS were essentially the same for all biomarkers. The only exception was HDL, where the interaction with sex was no longer statistically significant. More robust results for albumin were observed using four equal interval categories due to the low number of participants with hypoalbuminemia, but 5 equal intervals were used for consistency. Under the AHA guidelines, which had a higher low HDL cutoff for females, the OBS-HDL association among females was also inverse and most pronounced when comparing those in the highest to those in the lowest OBS interval (OR=0.65; 95% CI: 0.46 – 0.92; p-trend < 0.01).

Discussion

The idea that a score may be more strongly associated with health-related outcomes than are individual factors is well accepted in nutrition research. In the ATTICA study, a Mediterranean diet score was calculated by assigning higher points to frequent consumption of food items adhering to the pattern and lower points to items not adhering to it. Participants in the highest tertile of total adherence had 20% lower CRP levels and 14% lower total white blood cell counts, relative to those in the lowest tertile of adherence. A separate analysis from the ATTICA study also found a statistically significant inverse association between adherence to the Mediterranean diet and serum total cholesterol.

Other studies have examined associations between an OBS and biomarkers of oxidative stress and inflammation, and observed a similar inverse associations between OBS and inflammatory markers such as CRP and F2-isoprostanes. This indicates that low OBS is associated with low-grade inflammation, which has a role in the pathobiology of obesity and metabolic syndrome. We found that sex modified the association between the OBS and low serum HDL. On average, females have higher HDL levels than males. Environmental

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factors may explain some, but not all, of this sex difference. Several studies found that an association between smoking and HDL level is greater among females than among males. Possible mechanisms are not well understood, but may include sex differences in lipid metabolism and the antiestrogenic effect of cigarette smoking. In an analysis of a population from six different countries smoking was associated with lower HDL levels among females (−0.15 mmol/L) than among males (−0.05 mmol/L). Similarly, in the Framingham Offspring Study, compared to non-smokers of the same sex, female smokers had a significantly lower HDL level than did male smokers. We also found that the magnitude of the association between smoking and low HDL was greater among females than males. However, analyses assessing the association between each individual component and low HDL indicated that no single factor could fully explain the interaction between OBS and sex (data not shown). At this time, there is only epidemiological evidence supporting our findings on effect modification by sex. Basic science/clinical studies can be conducted to elucidate mechanistic explanations.

One of the strengths of the present study was the ability to incorporate both dietary and lifestyle components into the score, allowing a more comprehensive view of various determinants of oxidative stress. Besides demographic diversity, there was substantial variability in the intake of dietary components, which allowed us to compare extremes of the OBS.

A limitation of this study was the lack of information about genotypes that may influence the metabolism of OBS components. For example, polymorphisms modify the association between alcohol consumption and HDL level. We did not have information on infection status of participants, and it is possible that the inverse association between OBS and inflammatory markers could be attributed to unmeasured confounding. However, this is unlikely, as a number of studies in different populations have observed similar associations.

We measured OBS components based on FFQs, a dietary assessment method that has known limitations. OBS, measured by FFQs, has been used in a number of peer-reviewed publications. Similar associations were observed when OBS was measured by replacing questionnaire responses with biomarkers of pro- and antioxidant exposures.

Oxidative stress is a complex and multifactorial process that is influenced by a number of modifiable pro- and anti-oxidant factors. The purpose of OBS is to examine the balance of these modifiable factors; this approach has been used in a number of previous publications. In addition to extrinsic modifiable factors, oxidative stress is also influenced by intrinsic factors such as antioxidant enzymes and cellular energy balance. These factors are not included in the OBS. Further, oxidative stress itself can be measured using a variety of biomarkers. These biomarkers serve as in vivo measures of redox signaling and oxidation of macromolecules. Individual biomarkers of oxidative stress may reflect different aspects of this process, and for this reason, several studies explored combining various measures in a single score.
In summary, we found that OBS was statistically significantly associated with several, but not all of the circulating markers of inflammation and lipids/lipoproteins that we measured. The association between the OBS and serum HDL-cholesterol substantially differed by sex, an observation that requires confirmation, and if confirmed, exploration of the underlying mechanism(s). Our study’s cross-sectional design cannot establish mechanisms, but can generate hypotheses and may help stimulate future research. Our findings provide further support for studying oxidative stress-related dietary and lifestyle factors in combination, rather than as individual exposures. This study implies that clinical interventions to improve biomarker levels should focus on multiple extrinsic sources of oxidative stress, rather than any individual source.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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34. United states department of agriculture: Nutrient database for standard reference [releases 10 and 11].


Highlights

- Associations of oxidative balance score (OBS) with lipids/lipoproteins, and inflammatory biomarkers were investigated in a nationwide study
- High OBS indicates a presumably beneficial balance of pro- and anti-oxidants
- In the analyses of inflammatory biomarkers, OBS was inversely associated with high CRP and total WBC counts, but not low albumin
- For lipids/lipoproteins, an inverse association was found with high LDL, total cholesterol, but not high triglycerides
- Sex modified the association between OBS and low HDL
Table 1
REGARDS (2003–2007) cohort characteristics and biomarker measurements by Oxidative Balance Score categories

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Equal-interval OBS categories</th>
<th>3–7 (n=861)</th>
<th>8–12 (n=6,050)</th>
<th>13–17 (n=8,862)</th>
<th>18–21 (n=3,682)</th>
<th>22–26 (n=370)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.), Mean (SD)</td>
<td></td>
<td>61 (8.9)</td>
<td>64 (9.2)</td>
<td>65 (9.2)</td>
<td>66 (9.1)</td>
<td>67 (8.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male, (%)</td>
<td></td>
<td>416 (48.3)</td>
<td>2,789 (46.1)</td>
<td>3,901 (44.0)</td>
<td>1,581 (42.9)</td>
<td>142 (38.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Caucasian, (%)</td>
<td></td>
<td>555 (64.5)</td>
<td>3,937 (65.1)</td>
<td>5,957 (67.2)</td>
<td>2,543 (69.8)</td>
<td>271 (73.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Education, (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>College education or higher</td>
<td></td>
<td>212 (24.6)</td>
<td>1,942 (32.1)</td>
<td>3,489 (39.4)</td>
<td>1,647 (44.7)</td>
<td>178 (48.1)</td>
<td></td>
</tr>
<tr>
<td>Some college</td>
<td></td>
<td>269 (31.2)</td>
<td>1,682 (27.8)</td>
<td>2,402 (27.1)</td>
<td>989 (26.9)</td>
<td>92 (24.9)</td>
<td></td>
</tr>
<tr>
<td>High school graduate</td>
<td></td>
<td>275 (31.9)</td>
<td>1,731 (28.6)</td>
<td>2,187 (24.7)</td>
<td>787 (21.4)</td>
<td>82 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td></td>
<td>105 (12.3)</td>
<td>695 (11.5)</td>
<td>784 (8.8)</td>
<td>299 (7.0)</td>
<td>18 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Geographical region, (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stroke Belt</td>
<td></td>
<td>327 (38.0)</td>
<td>2,187 (36.2)</td>
<td>3,014 (34.0)</td>
<td>1,199 (32.6)</td>
<td>125 (33.8)</td>
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</tr>
<tr>
<td>Stroke Buckle</td>
<td></td>
<td>181 (21.0)</td>
<td>1,413 (23.4)</td>
<td>1,921 (21.7)</td>
<td>763 (20.7)</td>
<td>68 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>3,353 (41.0)</td>
<td>2,450 (40.4)</td>
<td>3,927 (44.3)</td>
<td>1,720 (46.7)</td>
<td>177 (47.8)</td>
<td></td>
</tr>
<tr>
<td>Exercise, (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>≥4 times/week</td>
<td></td>
<td>206 (23.9)</td>
<td>1,655 (27.4)</td>
<td>2,780 (31.4)</td>
<td>1,299 (35.3)</td>
<td>131 (35.4)</td>
<td></td>
</tr>
<tr>
<td>1–3 times/week</td>
<td></td>
<td>301 (35.0)</td>
<td>2,150 (35.5)</td>
<td>3,390 (38.3)</td>
<td>1,365 (37.1)</td>
<td>133 (36.0)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>354 (41.1)</td>
<td>2,245 (37.1)</td>
<td>2,692 (30.3)</td>
<td>1,018 (27.6)</td>
<td>106 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kcal/day), Mean (SD)</td>
<td></td>
<td>1,444 (360)</td>
<td>1,508 (624)</td>
<td>1,751 (705)</td>
<td>1,977 (768)</td>
<td>1,915 (644)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²), Mean (SD)</td>
<td></td>
<td>28.1 (5.5)</td>
<td>29.2 (6.0)</td>
<td>29.2 (5.9)</td>
<td>29.3 (6.0)</td>
<td>29.4 (6.4)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Population: any participant with at least one biomarker (n=19,825).

Analysis for biomarker limited to participants on whom the biomarker was measured: CRP, n=19,531; total cholesterol, n=19,790; HDL, n=19,685; LDL, n=19,416; triglycerides, n=19,782; albumin, n=14,475; WBC count, n=13,716.

Values presented as mean (SD) or number (%).
Table 2

Associations between the Oxidative Balance Score and biomarkers of inflammation in the REGARDS cohort

| Biomarker                  | LS mean (SD) | Normal biomarker levels (n) | Abnormal biomarker levels (n) | OR (95% CI) | p-trend  \\
|----------------------------|--------------|-----------------------------|-----------------------------|--------------|-----------
| Elevated serum CRP\(^c\)  |              |                             |                             |              |           \\
| OBS Interval               |              |                             |                             |              |           \\
| 3–7                       | 5.73 (7.75)  | 454                         | 383                         | 1.0          | <0.01     \\
| 8–12                      | 5.05 (8.16)  | 3,414                       | 2,504                       | 0.77 (0.66 – 0.90) |           \\
| 13–17                     | 4.44 (8.57)  | 5,370                       | 3,274                       | 0.62 (0.53 – 0.72) |           \\
| 18–21                     | 4.15 (8.31)  | 2,338                       | 1,255                       | 0.53 (0.45 – 0.62) |           \\
| 22–26                     | 3.88 (7.71)  | 236                         | 123                         | 0.50 (0.38 – 0.66) |           \\
| Elevated total WBC count\(^b\) |              |                             |                             |              |           \\
| OBS Interval               |              |                             |                             |              |           \\
| 3–7                       | 6.38 (3.01)  | 377                         | 214                         | 1.0          | <0.01     \\
| 8–12                      | 6.05 (3.19)  | 3,051                       | 1,148                       | 0.63 (0.53 – 0.76) |           \\
| 13–17                     | 5.83 (3.39)  | 4,688                       | 1,431                       | 0.51 (0.43 – 0.61) |           \\
| 18–21                     | 5.80 (3.26)  | 1,992                       | 558                         | 0.46 (0.38 – 0.56) |           \\
| 22–26                     | 5.69 (3.00)  | 196                         | 61                          | 0.50 (0.36 – 0.71) |           \\
| Low serum albumin\(^c\)   |              |                             |                             |              |           \\
| OBS Interval               |              |                             |                             |              |           \\
| 3–7                       | 4.15 (0.32)  | 612                         | 7                           | 1.0          | 0.65      \\
| 8–12                      | 4.16 (0.33)  | 4,379                       | 69                          | 1.23 (0.56 – 2.71) |           \\
| 13–17                     | 4.19 (0.35)  | 6,377                       | 70                          | 0.87 (0.39 – 1.92) |           \\
| 18–21                     | 4.19 (0.34)  | 2,653                       | 37                          | 1.08 (0.47 – 2.50) |           \\
| 22–26                     | 4.16 (0.31)  | 266                         | 5                           | 1.44 (0.44 – 4.67) |           \\

\(^a\)CRP cutoffs: normal CRP, ≤3.0 mg/L (n=11,812); high CRP, >3.0 mg/L (n=7,539)

\(^b\)WBC count cutoffs: normal WBC count, ≤6.86 × 10⁹ cells/L (n=10,304); high WBC count, >6.86 × 10⁹ cells/L (n=3,412)
c Albumin cutoffs: normal albumin, ≥3.5 g/dL (n=14,287); hypoalbuminemia, <3.5 g/dL (n=188)

d Adjusted for age, sex, race, BMI, total daily energy, education, region, and exercise.

e $X^2$ test
## Table 3

Associations between the Oxidative Balance Score and lipids/lipoproteins in the REGARDS cohort

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>LS mean (SD)</th>
<th>Normal biomarker levels (n)</th>
<th>Abnormal biomarker levels (n)</th>
<th>OR (95% CI)</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elevated serum total cholesterol</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS Interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–7</td>
<td>194 (39)</td>
<td>482</td>
<td>376</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>8–12</td>
<td>192 (41)</td>
<td>3,622</td>
<td>2,422</td>
<td>0.89 (0.77 – 1.03)</td>
<td></td>
</tr>
<tr>
<td>13–17</td>
<td>190 (43)</td>
<td>5,359</td>
<td>3,485</td>
<td>0.85 (0.74 – 0.99)</td>
<td></td>
</tr>
<tr>
<td>18–21</td>
<td>190 (41)</td>
<td>2,232</td>
<td>1,442</td>
<td>0.84 (0.72 – 0.99)</td>
<td></td>
</tr>
<tr>
<td>22–26</td>
<td>189 (38)</td>
<td>223</td>
<td>147</td>
<td>0.85 (0.66 – 1.10)</td>
<td></td>
</tr>
<tr>
<td><strong>Elevated serum LDL-cholesterol</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS Interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–7</td>
<td>116 (34)</td>
<td>263</td>
<td>585</td>
<td>1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>8–12</td>
<td>114 (36)</td>
<td>2,113</td>
<td>3,817</td>
<td>0.85 (0.73 – 0.99)</td>
<td></td>
</tr>
<tr>
<td>13–17</td>
<td>113 (38)</td>
<td>3,204</td>
<td>5,465</td>
<td>0.80 (0.69 – 0.93)</td>
<td></td>
</tr>
<tr>
<td>18–21</td>
<td>113 (36)</td>
<td>1,387</td>
<td>2,218</td>
<td>0.75 (0.64 – 0.89)</td>
<td></td>
</tr>
<tr>
<td>22–26</td>
<td>112 (34)</td>
<td>141</td>
<td>223</td>
<td>0.75 (0.58 – 0.98)</td>
<td></td>
</tr>
<tr>
<td><strong>Elevated serum triglycerides</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBS Interval</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–7</td>
<td>130 (88)</td>
<td>613</td>
<td>345</td>
<td>1.0</td>
<td>0.17</td>
</tr>
<tr>
<td>8–12</td>
<td>129 (87)</td>
<td>4,229</td>
<td>1,811</td>
<td>1.05 (0.89 – 1.23)</td>
<td></td>
</tr>
<tr>
<td>13–17</td>
<td>129 (92)</td>
<td>6,344</td>
<td>2,496</td>
<td>0.98 (0.83 – 1.15)</td>
<td></td>
</tr>
<tr>
<td>18–21</td>
<td>128 (90)</td>
<td>2,630</td>
<td>1,044</td>
<td>0.99 (0.83 – 1.18)</td>
<td></td>
</tr>
<tr>
<td>22–26</td>
<td>132 (83)</td>
<td>255</td>
<td>115</td>
<td>1.11 (0.84 – 1.47)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Cholesterol cutoffs: normal cholesterol, <200 mg/dL (n=11,918); High cholesterol, ≥200 mg/dL (n=7,872)

<sup>b</sup> LDL cutoffs: normal LDL, <100mg/dL (n=7,108); high LDL, ≥100 mg/dL (n=12,308)
Triglycerides cutoffs: normal triglycerides, < 150 mg/dL (n=14,071); high triglycerides, ≥150 mg/dL (n=5,711)

Adjusted for age, sex, race, BMI, total daily energy, education, region, and exercise

X² test
Table 4

Associations between the Oxidative Balance Score and serum HDL-cholesterol level\textsuperscript{a} by sex in the REGARDS cohort

<table>
<thead>
<tr>
<th>Sex</th>
<th>OBS Interval</th>
<th>LS Mean (SD)</th>
<th>Normal HDL</th>
<th>Low HDL</th>
<th>OR (95% CI)\textsuperscript{d}</th>
<th>p-trend\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female\textsuperscript{b}</td>
<td>3–7</td>
<td>55.49 (0.75)</td>
<td>380</td>
<td>62</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>8–12</td>
<td>57.12 (0.29)</td>
<td>2,872</td>
<td>364</td>
<td>0.71 (0.53 – 0.95)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13–17</td>
<td>57.57 (0.24)</td>
<td>4,376</td>
<td>538</td>
<td>0.72 (0.54 – 0.96)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18–21</td>
<td>57.59 (0.36)</td>
<td>1,862</td>
<td>214</td>
<td>0.68 (0.50 – 0.93)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22–26</td>
<td>57.54 (1.04)</td>
<td>209</td>
<td>19</td>
<td>0.48 (0.28 – 0.83)</td>
<td></td>
</tr>
<tr>
<td>Male\textsuperscript{c}</td>
<td>3–7</td>
<td>47.06 (0.64)</td>
<td>277</td>
<td>136</td>
<td>1.0</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>8–12</td>
<td>45.99 (0.27)</td>
<td>1,725</td>
<td>1,050</td>
<td>1.22 (0.97 – 1.53)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13–17</td>
<td>45.65 (0.24)</td>
<td>2,420</td>
<td>1,464</td>
<td>1.25 (1.00 – 1.56)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18–21</td>
<td>45.56 (0.36)</td>
<td>997</td>
<td>579</td>
<td>1.22 (0.96 – 1.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22–26</td>
<td>45.10 (1.09)</td>
<td>83</td>
<td>58</td>
<td>1.63 (1.09 – 2.45)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Normal HDL (\geq 40 mg/dL); Low HDL (<40 mg/dL)

\textsuperscript{b}Normal HDL (n=9,699); Low HDL (n=1,197)

\textsuperscript{c}Normal HDL (n=5,502); Low HDL (n=3,287)

\textsuperscript{d}Adjusted for age, race, BMI, total daily energy, education, region, and exercise

\textsuperscript{e}X\textsuperscript{2} test