Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Ziling Mao Date

Dietary and Lifestyle Oxidative Balance Scores and Incident Colorectal Cancer Risk Among Older Women; the Iowa Women's Health Study

By

Ziling Mao

Master of Public Health

Epidemiology

_____

Roberd M. Bostick, MD, MPH

(Thesis Advisor)

Dietary and Lifestyle Oxidative Balance Scores and Incident Colorectal Cancer Risk Among Older Women; the Iowa Women's Health Study

By

Ziling Mao

B.S.

Capital Medical University

2018

Thesis Committee Chair: Roberd M. Bostick, MD, MPH

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Epidemiology

2020

Abstract

Dietary and Lifestyle Oxidative Balance Scores and Incident Colorectal Cancer Risk Among Older Women; the Iowa Women's Health Study

By Ziling Mao

Background: Basic science literature strongly supports a role of oxidative stress in colorectal cancer (CRC) etiology, but in epidemiologic studies, associations of most individual exposures with CRC have been weak or inconsistent. However, recent epidemiologic evidence suggests that the collective effects of these exposures on oxidative balance and CRC risk may be substantial.

Methods: Using food frequency and lifestyle questionnaire data from the prospective Iowa Women's Health Study (1986-2012), we calculated 11-component dietary and 4-component lifestyle oxidative balance scores (OBS), and investigated their associations with incident CRC using multivariable Cox proportional hazards regression.

Results: Of the 34,135 cancer-free women aged 55-69 years at baseline, 1,635 developed CRC during follow-up. Among participants in the highest relative to the lowest dietary and lifestyle OBS quintiles (higher anti-oxidant relative to pro-oxidant exposures), the adjusted hazard ratios (HRs) and their 95% confidence intervals (CI) were, respectively, 0.77 (0.64-0.94) and 0.63 (0.54-0.73) (both $P_{trend} < 0.01$). Risk was lowest risk among those in the highest joint dietary/lifestyle OBS quintile (HR = 0.47; 95% CI, 0.29-0.76).

Conclusions: Our findings suggest that a predominance of antioxidant over pro-oxidant dietary and lifestyle exposures—separately and especially jointly—may be inversely associated with CRC risk among older women.

Dietary and Lifestyle Oxidative Balance Scores and Incident Colorectal Cancer Risk Among Older Women; the Iowa Women's Health Study

By

Ziling Mao

B.S.

Capital Medical University

2018

Thesis Committee Chair: Roberd M. Bostick, MD, MPH

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Epidemiology

2020

Dietary and Lifestyle Oxidative Balance Scores and Incident Colorectal Cancer Risk Among Older Women; the Iowa Women's Health Study

Authors and Affiliations:

Ziling Mao[a], Anna E. Prizment[b,c], DeAnn Lazovich[c,d], David C. Gibbs[a], Roberd M. Bostick[a,e]

[a]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia. [b]Division of Hematology, Oncology and Transplantation, Medical School, University of Minnesota, Minneapolis, Minnesota. [c]Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota. [d]Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota. [e]Winship Cancer Institute, Emory University, Atlanta, Georgia.

Corresponding Author: Roberd M. Bostick

Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road NE # 1518-002-3BB, Atlanta, Georgia 30322, USA

Phone: 404-727-2671 Fax: 404-727-8737 Email: rmbosti@emory.edu

Running Title: Oxidative Balance and Colorectal Cancer Incidence

Word Count:

Abstract: 199

Main Text (Introduction to Discussion): 4,452

Number of Tables: 4

Number of Figures: 0

Number of Supplemental Tables: 11

Introduction

Colorectal cancer (CRC) remains the second leading cause of cancer death in the United States (1). Large international incidence rate differences coupled with rapid changes in incidence among populations who migrate from low- to high-risk countries point to the importance of diet and lifestyle in the disease's etiology (2). A substantial basic science literature suggests that oxidative stress may play an important role in CRC etiology (3-6); however, epidemiologic literature regarding associations of specific dietary and lifestyle anti- and pro-oxidant factors that may contribute to oxidative balance with CRC is inconsistent. It was proposed that contributions of such exposures individually to oxidative balance and CRC risk may be small, but collectively may be substantial (7-10). To address this, oxidative balance scores (OBS) have been reported (7-11), but reports of their associations with CRC have been limited to two epidemiologic studies, which found substantial, statistically significant inverse associations of a combined dietary and lifestyle OBS (a higher score indicates a higher balance of anti- relative to pro-oxidant exposures) with incident CRC (7, 11). Only one (7) of the two studies was prospective, and neither study reported potential interaction between dietary and lifestyle scores.

Accordingly, to clarify whether oxidative balance may be associated with incident CRC, herein we report an investigation of dietary and lifestyle OBS, separately and jointly, with CRC in the prospective Iowa Women's Health Study (IWHS).

Methods

Study Population

Details of the IWHS were previously published (12). Briefly, the IWHS is a prospective cohort study conducted in Iowa beginning in 1986, with follow-up for the present analysis through 2012. A total of 41,836 women aged 55 to 69 years completed mailed questionnaires. After the baseline survey, follow-up surveys were mailed in 1987, 1989, 1992, 1997, and 2004. The study was approved by the Minnesota Institutional Review Board (IRB), and the current analysis was approved by the Emory University IRB.

Collection of Exposure Information

Detailed information on demographics, lifestyle, diet, self-measured anthropometrics, and family history were collected at baseline. A Willett 127-item food frequency questionnaire (FFQ) was used to measure dietary and supplement intakes over the previous 12 months; the validity and reliability in the study population were previously reported (13). Total energy and nutrient intakes were calculated by adding energy and nutrients from all food and supplement sources using the dietary database developed by Willett, et al. (13). Physical activity was assessed via two questions regarding participants' usual frequency of moderate and vigorous activity, and categorized as high (vigorous activity twice a week or moderate activity > 4 times/week), medium (vigorous activity once a week plus moderate activity once a week, or moderate activity 2 – 4 times/week), and low (14). Self-measured anthropometrics were validated in the study population (13). Height and weight were used to calculate body mass index (BMI) as

weight divided by height squared (kg/m^2). Waist and hip circumferences were used to calculate a waist:hip ratio (WHR). Diet and physical activity were not comprehensively reassessed until 2004, at which time only 68.3% of participants remained alive. So, we used only baseline exposure information for our primary analyses, but included 2004 data in one of two sensitivity analyses described further below that supported the validity of this choice. Information on aspirin and other nonsteroidal anti-inflammatory drug (NSAID) use was not collected until 1992, and was used in sensitivity analyses described further below.

Collection of Outcome Information

Cancer diagnoses were collected through 2012 via linkage with the State Health Registry of Iowa, which is a participant in the National Cancer Institute's Surveillance, Epidemiology, and End Results Program. Ascertainment of cancer diagnoses was nearly 100% (15). Colorectal cancer was defined as adenocarcinoma of the colon or rectum (International Classification of Diseases-Oncology-3 (ICD-O-3) codes C18.0 – 18.9, C19.9, and C20.9). Deaths were identified through the State Health Registry of Iowa and the National Death Index. Follow-up time was calculated as the time between the date of baseline questionnaire completion and the date of a first CRC diagnosis, the date of moving from Iowa, the date of death, or the end of the last follow up (December 31, 2012), whichever was first.

OBS Components and Calculations

Before calculating the scores and conducting the statistical analyses, we excluded participants who had a history of cancer (other than non-melanoma skin cancer) at baseline (*n* = 3,830), left > 10% of the FFQ questionnaire items blank (*n* = 2,499), had self-reported implausible energy intakes (< 600 or > 5,000 kcal/day; *n* = 286), had an invalid contributed person-time (*n* = 4), or were missing data on any lifestyle OBS component (*n* = 1,082), leaving an analytic cohort of 34,135.

We calculated questionnaire-based, multi-component OBS as previously reported (7, 8) and described below. The OBS were previously validated via their associations with circulating F_2-isoprostane concentrations (8). In addition, associations of the OBS with colorectal adenoma (8) and with CRC (7) were both comparable regardless of which of four different component weighting schemes used (equal-weight, literature review-derived, study data-based, and a Bayesian method that combines prior knowledge with study data) (7, 8). Accordingly, for the current analysis we used the more straightforward equal-weight method, as described below.

The 11 dietary OBS components and four lifestyle OBS components were determined *a priori* based on their literature-supported physiological effects on oxidative processes as previously reported in detail (7, 8) (also see Table 1). The dietary OBS components included carotene (α and β), lutein/zeaxanthin, lycopene, flavonoids, vitamin C, vitamin E, selenium, and omega-3 fatty acids as antioxidants; and iron, saturated fats, and omega-6 fatty acids as pro-oxidants. The lifestyle OBS components included physical activity as

having antioxidant effects, and smoking, alcohol intake, and adiposity as having pro-oxidant effects.

All dietary OBS components were continuous variables derived from the FFQ. For all nutrients except selenium, we used total (i.e., from foods plus supplements) nutrient values; for selenium we used only supplement values since selenium intake from foods depends on the soils in which selenium's plant sources are grown. Prior to inclusion in the score, macronutrients were energy-adjusted as percentage of total energy contributed by the macronutrient, and micronutrients were energy-adjusted using the density method (e.g., IU of vitamin E/1,000 kcal of total energy intake). All lifestyle OBS components, derived from the lifestyle questionnaire, initially were 3-category categorical variables as follows: physical activity (low, medium, or high), smoking status (non-smoker, former smoker, or current smoker), alcohol intake (< 1 drinks/week, 1 – 7 drinks/week, or ≥ 7 drinks/week), adiposity (BMI < 30 and WHR < 0.8; *either* BMI ≥ 30 *or* WHR ≥ 0.8; or BMI ≥ 30 and WHR ≥ 0.8). The lifestyle OBS categories were then assigned initial values of 0, 1, or 2 for each category from the lowest to the highest level.

Next, all components' values were standardized to a mean of zero and standard deviation of one by subtracting a participant's value from the study population mean, and dividing it by the population standard deviation. These values were then multiplied by +1 or -1 for antioxidants or pro-oxidants, respectively. The resulting values for each of the two OBS, dietary and lifestyle, were summed to constitute a person's dietary and lifestyle OBS, such that a higher score would be considered more anti-inflammatory. A total OBS

was also calculated as the sum of the dietary and lifestyle OBS. For subsequent analyses, the OBS were categorized according to quintiles of their distributions in the analytic population at baseline.

Statistical Analyses

Participants' baseline characteristics were summarized across quintiles of the dietary and lifestyle OBS in the entire analytic population at baseline using descriptive statistics. To investigate OBS-incident CRC associations, we used multivariable Cox proportional hazards models to calculate hazard ratios (HR) and their 95% confidence intervals (CI). Trend tests across quintiles of dietary, lifestyle, and total OBS were conducted using the median value of each OBS as a continuous variable in its respective model. Covariates were chosen *a priori* based on biological plausibility and previous literature. Minimally-adjusted models included as covariates only age (years), family history of CRC in a first degree relative (yes/no), and total energy intake (kcal/day). Covariates in all fully-adjusted models for all OBS were age (years), family history of CRC in a first degree relative (yes/no), high school education or higher (yes/no), current use of hormone replacement therapy (yes/no), and intakes of total vegetables and fruit (servings/week), red meat (servings/week), processed meats (servings/week), and total energy intake (kcal/day). Because vegetables and fruit and red and processed meats contain dietary OBS components and have potential anti- and pro-colorectal carcinogenesis effects apart from their anti- and pro-oxidant effects, we assessed removal of these foods as model covariates; however, since their removal/inclusion had minimal effects on our estimated OBS-CRC associations, we retained them in the final models. Fiber intake was not

included as a covariate because of its high correlation with total vegetable and fruit intake in the IWHS. The dietary OBS models additionally included physical activity, smoking status, alcohol intake, and our above-described adiposity variable, and the lifestyle OBS models additionally included the dietary OBS. Proportional hazard assumptions were tested using Schoenfeld residuals for each exposure and covariate. Correlation between the lifestyle and dietary OBS was assessed via a Pearson correlation coefficient.

To assess potential interaction between the dietary and lifestyle OBS in relation to CRC risk, we conducted a joint/combined (cross-classification) analysis in which the lowest joint category of the two scores was considered the reference category. We calculated $P_{interaction}$ by including a dietary x lifestyle OBS interaction term in the multivariable Cox proportional hazards model and assessing its significance using the Wald test.

To assess potential differences in OBS-CRC associations across categories of selected participants' baseline characteristics, we conducted analyses stratified on age (</≥ 65 years), family history of CRC in a first degree relative (yes/no), education (</≥ high school education), current use of hormone replacement therapy (yes/no), and low/high intakes (based on the study population medians) of total vegetables and fruit (</≥ 40.5 servings/week), total calcium (</≥ 1,031.3 mg/day), and total energy (</≥ 1,718.6 kcal/day). Due to sample size constraints, we categorized all OBS according to tertiles for these analyses. We calculated $P_{interaction}$ by including stratification factor x OBS interaction terms in the multivariable Cox proportional hazards regression models.

We also assessed potential differences in OBS-CRC associations according to CRC site. CRCs in the cecum through the transverse colon (ICD-O-3 codes C18.0 – 18.4) were categorized as proximal colon cancer (*n* = 895); CRCs in the splenic flexure through the sigmoid colon (ICD-O-3 codes C18.5 – 18.7) were categorized as distal colon cancer (n = 419); and cancers in the recto-sigmoid junction and rectum (ICD-O-3 codes C19.9 and 20.9) were categorized as rectal cancer (*n* = 321). Due to sample size constraints, we categorized the OBS according to tertiles for these analyses.

We also conducted several sensitivity analyses. Since our primary analyses were based on baseline data for calculating the OBS, and some participants could have changed their exposures during follow up, we conducted two sensitivity analyses. First, we assessed potential differences in OBS-CRC associations considering study end dates of 5, 10, 15, 20, and 25 years after baseline. Second, we assessed incorporating exposure data from the 2004 follow-up questionnaire in two ways. Among those who were not censored prior to 2004, for their OBS, we used (i) the average of their baseline (1986) and 2004 follow-up OBS, and (ii) only their 2004 OBS.

Other sensitivity analyses included, first, because NSAID use is consistently inversely associated with CRC, and data on aspirin and other NSAID use were not collected until 1992 (i.e., 6 years after baseline), we also assessed OBS-CRC associations considering 1992 as baseline (i.e., excluded those who were diagnosed with CRC or censored prior to 1992 or did not complete the 1992 questionnaires), with and without inclusion of aspirin/other NSAID use as a model covariate, as well as stratified by aspirin/other

NSAID use. Second, to reduce ambiguity in the temporal relation between the OBS and incident CRC, we excluded participants who died or were diagnosed with CRC within 2 or 6 years after baseline. Third, we censored participants when they reached 75 years of age. Fourth, because some recent evidence suggested a U-shaped alcohol-oxidative stress association (16), we assessed the following alternative alcohol intake scoring: < 1 drink/week and ≥ 7 drinks/week were each assigned a value of 2, and 1 – 7 drinks/week was assigned a value of 0. Fifth, to assess whether the OBS-CRC associations were driven by particularly influential components, we removed individual components from the dietary and lifestyle OBS with replacement one at a time, and then examined the associations of the remaining 13-component dietary OBS and 3-component lifestyle OBS with incident CRC separately, adjusted for the removed component as a covariate.

All statistical tests were two-sided. A *P*-value < 0.05 or a 95% CI that excluded 1.0 was considered statistically significant. All analyses were conducted using SAS, version 9.2 (SAS Institute Inc.).

Results

Of the 34,135 cancer-free women at baseline, 1,635 developed CRC over a mean/median 20.5/24.0 person-years of follow up. The correlation between the dietary and lifestyle OBS was $r = 0.10$.

Selected baseline characteristics of the participants according to quintiles of the lifestyle and dietary OBS are summarized in Table 2. On average, study participants were 61 years of age, and > 99% were white. Study participants in the upper quintile of the lifestyle and dietary OBS were slightly more likely to take hormone replacement therapy and, on average, to have higher mean total calcium and total vegetables and fruit intakes, and lower red meat intakes. Exclusive of components of a given score, women in the upper relative to the lower lifestyle OBS quintiles also had, on average, higher intakes of total carotenes, lutein/zeaxanthin, selenium, and total vitamin C; and those in the upper relative to the lower dietary OBS quintiles were more likely to report higher physical activity, consuming ≥ 7 drinks of alcohol/week, and not currently smoking, and, on average, to consume less total energy and red and processed meats.

Associations of lifestyle, dietary, and total OBS with incident CRC risk are summarized in Table 3. The risk estimates from the minimally- and fully-adjusted models differed minimally. In the fully-adjusted analyses, CRC risk tended to decrease with a higher lifestyle OBS ($P_{trend} < 0.0001$), and among women in the highest relative to the lowest lifestyle OBS quintile, risk was statistically significantly 37% less. Similarly, there were statistically significant trends for decreasing risk with increasing dietary and total OBS, and among women in the upper relative to the lowest dietary and total OBS, risk was statistically significantly 23% and 35% lower, respectively.

In the joint/combined (cross-classification) analysis of the lifestyle and dietary OBS (Table 4), there was a pattern of decreasing CRC risk with an increasing lifestyle OBS

among women in the lowest dietary OBS quintile, culminating in statistically significant 40% lower risk, and a more modest pattern of decreasing risk with an increasing dietary OBS among women in the lowest lifestyle OBS quintile, culminating in non-statistically significantly 9% lower risk. Among those in the highest relative to the lowest joint lifestyle/dietary OBS quintile, risk was statistically significantly 53% lower ($P_{interaction}$ = 0.27).

There were no clear or consistent differences in the OBS-CRC associations according to selected participant characteristics (Supplemental Table 1), and the 95% CIs for corresponding HRs across strata overlapped considerably. However, there were some suggestions that the inverse lifestyle OBS-CRC association tended to be stronger among participants with no family history of CRC in a first degree relative, those who were less formally educated, and those with lower total energy intakes, and that the inverse dietary OBS-CRC association tended to be stronger among those who were younger and those with a family history of CRC in a first degree relative.

Associations of the OBS with risk of proximal colon, distal colon, and rectal cancer are summarized in Supplemental Table 2. The estimated associations did not differ substantially across colorectal sites, although there was some suggestion that the dietary OBS-distal colon cancer association was inverse (23% non-statistically significant lower risk), but with proximal colon cancer very close to null; however, the 95% CIs for corresponding HRs across sites overlapped considerably.

In the sensitivity analyses to assess the validity of using only baseline data for our primary analyses, first, for each OBS, the OBS-CRC associations estimated after considering study end dates at 5-year intervals were similar across the end dates (Supplemental Table 3). Second, the OBS-CRC associations estimated after incorporating 2004 data in two ways were similar to those from our primary analyses (Supplemental Table 4).

In other sensitivity analyses, when we used 1992 as the baseline for follow-up, additional adjustment for aspirin and other NSAID use had minimal effect on our results (Supplement Table 5); however, the inverse associations of each OBS with CRC tended to be stronger among those who regularly took a non-aspirin NSAID, although the 95% CIs for corresponding HRs across strata overlapped and the $P_{interactions}$ were not statistically significant (Supplemental Table 6). Also, excluding participants who died or were diagnosed with CRC within the first 2 (Supplemental Table 7) or 6 years of follow-up (Supplemental Table 5), or censoring participants when they reached age 75 (Supplemental Table 8) had minimal effects on the estimated OBS-CRC associations. Use of alternative alcohol intake scoring had minimal effects on the estimated OBS-CRC associations (Supplemental Table 9). Removal of any one component from either the lifestyle (Supplemental Table 10) or the dietary (Supplemental Table 11) OBS tended to result in a slightly weaker inverse association with CRC. Removal of physical activity or adiposity from the lifestyle score tended to attenuate the lifestyle score-CRC association the most, but removal of any dietary component from the dietary OBS tended to have minimal effect on the dietary OBS-CRC association.

Discussion

Our findings suggest that a predominance of antioxidant over pro-oxidant dietary and lifestyle exposures—separately and especially jointly—may be inversely associated with CRC risk among older women.

Oxidative stress may be an important etiologic factor for carcinogenesis, especially colorectal carcinogenesis (4, 17-19). Oxidative stress is defined as an imbalance between the production of free radicals and reactive metabolites (i.e., oxidants or reactive oxygen and nitrogen species [RONS]) (20), and their elimination by protective mechanisms (i.e., antioxidants) (3). High levels of RONS can damage DNA, alter signaling pathways, and affect the development and progression of colon cancer in general (3-6, 21). In addition, a disruption of thiol-redox circuits can lead to aberrant cell signaling and dysfunctional redox control without involving macromolecular damage caused by RONS (22).

The biological plausibility for the components of our lifestyle and dietary OBS contributing to oxidative balance is summarized in Table 1. Lifestyle factors are important exogenous exposures that contribute to RONS production (17). Physical activity acts as an antioxidant by resulting in an increase in an adaptive response to oxidative stress (23). Tobacco smoking (24, 25), alcohol intake (26, 27), and adiposity (28, 29), which are associated with increased oxidative stress markers and RONS production, are considered powerful sources of pro-oxidants. In addition to their effects

on oxidative stress, lifestyle factors likely affect CRC through other mechanisms (2), which may, in part, account for the lifestyle OBS-CRC association being more strongly inverse than the dietary OBS-CRC association. Dietary nutrients can also induce multiple genes involved in oxidative stress responses. Antioxidants in foods or dietary supplements are thought to be chemopreventive via reducing genotoxicity and slowing cancer progression (5). Evidence suggested that carotene (29), flavonoids (30, 31), lutein (29), lycopene (32), and selenium (33) may reduce cancer risk by limiting the genotoxic insults caused by RONS; vitamins C and E can protect against lipid peroxidation (34, 35), and omega-3 fatty acids induce electrophile-responsive element regulated genes responsible for regulation of antioxidant enzymes (36, 37). Dietary prooxidants, including dietary iron (38, 39), omega-6 fatty acids (37, 40, 41), and saturated fat (42, 43), are associated with increased colorectal oxidative stress and DNA damage through increased free-radical production in the colon.

Findings from previous studies support an overall (dietary plus lifestyle) OBS as a valid measure of oxidative balance. Results from a cross-sectional analysis of a large, pooled outpatient, elective colonoscopy population suggested a dose-dependent decrease in plasma F_2-isoprostane concentrations—the best available biomarker of oxidative stress *in vivo* (44, 45)—with an increasing OBS (8). A nested case-control study (46) reported a positive association of pre-diagnostic serum oxidized low density lipoprotein concentrations with CRC risk. The results agreed with those from another nested case-control study in the European Prospective Investigation into Cancer and Nutrition cohort

(20), in which reactive oxygen metabolites and the ferric reducing ability of plasma were used as indicators of oxidative stress.

Consistent with our results, inverse associations of an OBS with colon or rectal cancer were observed in other epidemiologic studies. In the Cancer Prevention Study II (CPS-II) cohort (7), a total OBS (i.e., composed of dietary and lifestyle components) was calculated using four weighting methods; among participants in the highest relative to the lowest OBS quintile, CRC risk was statistically significantly 41% – 53% less. A population-based case-control study (11) found a decreasing trend of both colon and rectal cancer risk with an increasing OBS, and among participants in the upper relative to the lowest OBS quartile, risks of colon and rectal cancer were statistically significantly 48% and 36% lower, respectively. Our results are also consistent with those from studies that observed inverse associations of oxidative stress/balance scores with colorectal adenoma (9, 10, 47-49). However, the results of a cohort study (50) that measured total antioxidant capacity of food (TAC) intakes—estimated using the ferric reducing ability of plasma assay, which measures the reduction of Fe_3^+ (ferric ion) to Fe_2^+ (ferrous ion) in the presence of antioxidants (51, 52)—suggested a null association of the TAC intakes with colorectal and colon cancer risk. In that study, the TAC intakes did not involve pro-oxidant exposures, and no lifestyle factors were considered.

Although in previous studies, both dietary and lifestyle exposures were included as OBS components, we are the first to report a joint/combined (cross-classification) analysis to assess potential interaction between dietary and lifestyle OBS in relation to CRC. The

pattern of findings from the joint/combined analysis was consistent with a multiplicative interaction, although the *P* for interaction was not statistically significant. Of interest is that the total OBS-CRC association was intermediate to the dietary and lifestyle OBS-CRC associations. Given the very modest correlation (Pearson $r = 0.10$) between the dietary and lifestyle OBS, that the dietary OBS comprises 11 components that each contribute modestly to risk and the lifestyle OBS comprises only 4 components that each contribute substantially to risk, it would be expected that the findings for the total OBS would represent an averaging of those from the separate lifestyle and dietary OBS, rather than reflecting any additive or synergistic effects of lifestyle and diet. On the other hand, the joint/combined analysis allows a more valid look at whether having both a strong antioxidant lifestyle and a strong antioxidant diet may put someone at the lowest risk. Thus, we recommend not folding lifestyle and dietary OBS components into a single score.

Our results also suggested that our estimated inverse associations of dietary, lifestyle, and total OBS with CRC tended to be stronger among women who regularly took a non-aspirin NSAID; however, in that analysis, the corresponding 95% CIs across strata overlapped and the $P_{interaction}$ was not statistically significant. Plausibility for this latter finding includes that inflammation, which NSAIDs reduce, can increase oxidative stress, and oxidative stress can increase inflammation (53), suggesting that exposures that can decrease inflammation or yield a more anti-oxidant relative to pro-oxidant exposure balance could synergistically reduce both inflammation and oxidative stress and thus risk for certain diseases, such as CRC. Only one previous study (7) stratified an OBS-CRC

association by NSAID use, finding a stronger inverse OBS-CRC association among those who regularly took an NSAID (consistent with our findings). Further investigation of potential interaction between OBS and non-aspirin NSAID use in relation to CRC is warranted.

Our study had several limitations. For our primary analyses, all OBS components were derived from information collected at baseline. Some participant's diets and lifestyle could have changed somewhat during follow-up. However, it has been reported that the quintile rankings of cohort participants' dietary intakes estimated from FFQs repeated over time mostly remain in the same or adjacent quintiles, supporting that estimates from a single FFQ adequately categorize study participants' long-term dietary exposures (54). In addition, in a prospective cohort study, it is expected that changes occur before a participant has knowledge of their outcome, thus resulting in non-differential error that would be expected to attenuate associations. Consistent with these findings and expectations, in our sensitivity analyses, we found that the estimated OBS-CRC associations were similar 1) at follow-up intervals of 5, 10, 15, 20, and 25 years, and 2) when we incorporated 2004 follow-up data in two ways. These findings support that any changes in diet or lifestyle during follow up likely had minimal effects on our risk estimates. We did not collect data on aspirin/other NSAID use at baseline; however, in our sensitivity analyses, when we considered 1992 as baseline, inclusion/exclusion of aspirin/other NSAID use as a model covariate did not materially affect our risk estimates. Other limitations include the general limitations of food frequency questionnaires (e.g., recall error and limited food choices); however, these types of error are also considered

non-differential in a prospective study. The lack of data on CRC screening is a limitation. CRC screening can detect colorectal adenomas, and if individuals have their adenomas removed as a consequence of CRC screening, they are less likely to get CRC. This could attenuate results; however, the CRC screening rate was low in Iowa in the 1980s, thus missing this information may not have affected our results substantially. Finally, unlike in previous studies (7, 8), we lacked data on glucosinolates, and thus did not include them as an OBS component. However, based on the results of our past (7) and present sensitivity analyses in which we removed/replaced each score component one at a time, removal of any one dietary OBS component did not materially affect our estimated dietary OBS-CRC associations.

Major strengths of this study include the prospective study design, long follow up, large sample size and number of CRC cases, high quality outcome ascertainment, comprehensive collection and assessment of multiple potential confounding/effect modifying risk factors, and multiple sensitivity analyses. Also, we conducted a joint/combined (cross-classification) analysis to assess potential interaction between the dietary and lifestyle OBS in relation to CRC risk, and were able to conduct analyses by cancer site and various participant characteristics.

In summary, our findings taken together with previous literature, support that a predominance of antioxidant over pro-oxidant dietary and lifestyle exposures—separately and especially jointly—may be inversely associated with CRC risk.

Acknowledgements

None

Disclosure Statement

None of the authors has a conflict of interest to disclose. The findings and conclusions contained within are those of the authors and do not necessarily reflect positions or policies of the National Cancer Institute or the Wilson P. and Anne W. Franklin Foundation. The National Cancer Institute and the Wilson P. and Anne W. Franklin Foundation had no influence on the analysis and interpretation of the data, the decision to submit the manuscript for publication, or the writing of the manuscript.

Funding

This work was supported by the National Cancer Institute at the National Institutes of Health under Grant R01 CA039742, and the Wilson P. and Anne W. Franklin Foundation.

References

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2019. *CA Cancer J Clin* **69,** 7-34, 2019. doi: 10.3322/caac.21551
2. Potter J, Slattery M, Bostick RM, Gapstur S: Colon cancer: A review of the epidemiology. *Epidemiol Rev* **15,** 499–505, 1993
3. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB: Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* **49,** 1603-1616, 2010. doi: 10.1016/j.freeradbiomed.2010.09.006
4. Saha SK, Lee SB, Won J, Choi HY, Kim K, et al.: Correlation between Oxidative Stress, Nutrition, and Cancer Initiation. *Int J Mol Sci* **18**, 2017. doi: 10.3390/ijms18071544
5. Stone WL, Krishnan K, Campbell SE, Palau VE: The role of antioxidants and pro-oxidants in colon cancer. *World J Gastrointest Oncol* **6,** 56–67, 2014. doi: 10.4251/wjgo.v6.i3.55
6. Wang Z, Li S, Cao Y, Tian X, Zeng R, et al.: Oxidative stress and carbonyl lesions in ulcerative colitis and associated colorectal cancer. *Oxid Med Cell Longev* **2016,** 15, 2016. doi: 10.1155/2016/9875298
7. Dash C, Bostick RM, Goodman M, Flanders WD, Patel R, et al.: Oxidative balance scores and risk of incident colorectal cancer in a US prospective cohort study. *Am J Epidemiol* **181,** 584–594, 2015. doi: 10.1093/aje/kwu318
8. Dash C, Goodman M, Flanders WD, Mink PJ, McCullough ML, et al.: Using pathway-specific comprehensive exposure scores in epidemiology: application to

oxidative balance in a pooled case-control study of incident, sporadic colorectal adenomas. *Am J Epidemiol* **178,** 610-624, 2013. doi: 10.1093/aje/kwt007

9. Goodman M, Bostick RM, Dash C, Flanders WD, Mandel JS: Hypothesis: oxidative stress score as a combined measure of pro-oxidant and antioxidant exposures. *Ann Epidemiol* **17,** 394-399, 2007. doi: 10.1016/j.annepidem.2007.01.034
10. Goodman M, Bostick RM, Dash C, Terry P, Flanders WD, et al.: A summary measure of pro- and anti-oxidant exposures and risk of incident, sporadic, colorectal adenomas. *Cancer Causes Control* **19,** 1051-1064, 2008. doi: 10.1007/s10552-008-9169-y
11. Slattery ML, Lundgreen A, Welbourn B, Wolff RK, Corcoran C: Oxidative balance and colon and rectal cancer: interaction of lifestyle factors and genes. *Mutat Res* **734,** 30-40, 2012. doi: 10.1016/j.mrfmmm.2012.04.002
12. Folsom A, Kaye S, Potter J, Prineas R: Association of incident carcinoma of the endometrium with body weight and fat distribution in older women: early findings of the Iowa Women's Health Study. *Cancer Res* **49,** 6828–6831, 1989
13. Munger R, Folsom A, Kushi L, Kaye S, Sellers T: Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* **136,** 192–200, 1992
14. Kushi L, Fee R, Folsom A, Mink P, Anderson K, et al.: Physical activity and mortality in postmenopausal women. *J Am Med Assoc* **277,** 1287–1292, 1997

15. Zhang S, Folsom A, Sellers T, Kushi L, Potter J: Better breast cancer survival for postmenopausal women who are less overweight and eat less fat. The Iowa Women's Health Study. *Cancer Causes Control* **76,** 275–283, 1995
16. Shaper AG, Wannamethee G, Walker M: Alcohol and mortality in British men: explaining the U-shaped curve. *The Lancet* **332,** 1267–1273, 1988
17. Carini F, Mazzola M, Rappa F, Jurjus A, Geagea AG, et al.: Colorectal Carcinogenesis: Role of Oxidative Stress and Antioxidants. *Anticancer Res* **37,** 4759-4766, 2017. doi: 10.21873/anticanres.11882
18. Perse M: Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? *Biomed Res Int* **2013,** 1–9, 2013
19. Rossin D, Calfapietra S, Sottero B, Poli G, Biasi F: HNE and cholesterol oxidation products in colorectal inflammation and carcinogenesis. *Free Radic Biol Med* **111,** 186-195, 2017. doi: 10.1016/j.freeradbiomed.2017.01.017
20. Leufkens AM, van Duijnhoven FJ, Woudt SH, Siersema PD, Jenab M, et al.: Biomarkers of oxidative stress and risk of developing colorectal cancer: a cohort-nested case-control study in the European Prospective Investigation Into Cancer and Nutrition. *Am J Epidemiol* **175,** 653-663, 2012. doi: 10.1093/aje/kwr418
21. Gothai S, Muniandy K, Gnanaraj C, Ibrahim IAA, Shahzad N, et al.: Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms. *Biomed Pharmacother* **107,** 1514-1522, 2018. doi: 10.1016/j.biopha.2018.08.112
22. Jones DP: Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* **295,** C849-868, 2008. doi: 10.1152/ajpcell.00283.2008

23. Ji LL, Gomez-Cabrera MC, Vina J: Exercise and hormesis: activation of cellular antioxidant signaling pathway. *Ann N Y Acad Sci* **1067,** 425-435, 2006. doi: 10.1196/annals.1354.061

24. Thaiparambil JT, Vadhanam MV, Srinivasan C, Gairola CG, Gupta RC: Time-dependent formation of 8-oxo-deoxyguanosine in the lungs of mice exposed to cigarette smoke. *Chem Res Toxicol* **20,** 1737–1740, 2007

25. Vaart HV, Postma DS, Timens W, Ten Hacken NH, Vaart Hvd: Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax* **59,** 713–721, 2004

26. Das SK, Vasudevan DM: Alcohol-induced oxidative stress. *Life Sci* **81,** 177-187, 2007. doi: 10.1016/j.lfs.2007.05.005

27. Wu D, Zhai Q, Shi X: Alcohol-induced oxidative stress and cell responses. *J Gastroenterol Hepatol* **21 Suppl 3,** S26–29, 2006

28. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, et al.: Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* **114,** 1752–1761, 2004

29. Rao AV, Rao LG: Carotenoids and human health. *Pharmacol Res* **55,** 207-216, 2007. doi: 10.1016/j.phrs.2007.01.012

30. Silva MM, Santos MR, Caroço G, Rocha R, Justino G, et al.: Structure-antioxidant Activity Relationships of Flavonoids: A Re-examination. *Free Radical Research* **36,** 1219-1227, 2009. doi: 10.1080/198-1071576021000016472

31. Fraga CG: Plant polyphenols: how to translate their in vitro antioxidant actions to in vivo conditions. *IUBMB Life* **59,** 308-315, 2007. doi: 10.1080/15216540701230529

32. Rao AV, Ray MR, Rao LG: Lycopene. *Adv Food Nutr Res* **51,** 99–164, 2006. doi: 10.1016/s1043-4526(06)51002-2

33. Rayman MP: Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc Nutr Soc* **64,** 527–542, 2005

34. Burton G, Ingold K: Vitamin E as an in vitro and in vivo antioxidant. *Ann N Y Acad Sci* **570,** 7–22, 1989

35. Kojo S: Vitamin C: Basic metabolism and its function as an index of oxidative stress. *Curr Med Chem* **11,** 1041–1064, 2004

36. Takahashi M, suboyama-Kasaoka N, Nakatani T, Ishii M, TSUTSUMI S, et al.: Fish oil feeding alters liver gene expressions to defend against PPARα activation and ROS production. *Am J Physiol Gastrointest Liver Physiol* **282,** G338–348, 2002

37. van Beelen VA, Aarts JM, Reus A, Mooibroek H, Sijtsma L, et al.: Differential induction of electrophile-responsive element-regulated genes by n-3 and n-6 polyunsaturated fatty acids. *FEBS Lett* **580,** 4587-4590, 2006. doi: 10.1016/j.febslet.2006.07.028

38. Glei M, Latunde-Dada G, Klinder A, Becker TW, Hermann U, et al.: on-overload induces oxidative DNA damage in the human colon carcinoma cell line HT29 clone 19A. *Mutat Res* **519,** 151–161, 2002

39. Tappel A: Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases. *Med Hypotheses* **68,** 562-564, 2007. doi: 10.1016/j.mehy.2006.08.025

40. Toborek M, Barger S, Mattson M: Linoleic acid and TNF-alpha cross-amplify oxidative injury and dysfunction of endothelial cells. *J Lipid Res* **37,** 123–135, 1996

41. Ghosh S, Kewalramani G, Yuen G, Pulinilkunnil T, An D, et al.: Induction of mitochondrial nitrative damage and cardiac dysfunction by chronic provision of dietary omega-6 polyunsaturated fatty acids. *Free Radic Biol Med* **41,** 1413-1424, 2006. doi: 10.1016/j.freeradbiomed.2006.07.021
42. Venturi M, J.Hambly R, Glinghammar B, J.Rafter J, R.Rowland1 I: Genotoxic activity in human faecal water and the role of bile acids: a study using the alkaline comet assay. *Carcinogenesis* **18,** 2353–2359, 1997
43. Rosignoli P, Fabiani R, De Bartolomeo A, Fuccelli R, Pelli MA, et al.: Genotoxic effect of bile acids on human normal and tumour colon cells and protection by dietary antioxidants and butyrate. *Eur J Nutr* **47,** 301-309, 2008. doi: 10.1007/s00394-008-0725-8
44. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, et al.: Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radic Biol Med* **38,** 689–101, 2005. doi: 10.1016/j.freeradbiomed.2004.09.017
45. Milne GL, Musiek ES, Morrow JD: F2-Isoprostanes as markers of oxidative stressin vivo: An overview. *Biomarkers* **10,** 10-23, 2008. doi: 10.1080/13547500500216546
46. Suzuki K, Ito Y, Wakai K, Kawado M, Hashimoto S, et al.: Serum oxidized low-density lipoprotein levels and risk of colorectal cancer: a case-control study nested in the Japan collaborative cohort study. *Cancer Epidemiol Biomarkers Prev* **13,** 1781–1787, 2004
47. Kong SY, Bostick RM, Flanders WD, McClellan WM, Thyagarajan B, et al.: Oxidative balance score, colorectal adenoma, and markers of oxidative stress and

inflammation. *Cancer Epidemiol Biomarkers Prev* **23,** 545-554, 2014. doi: 10.1158/1055-9965.EPI-13-0619

48. Labadie J, Goodman M, Thyagarajan B, Gross M, Sun Y, et al.: Associations of oxidative balance-related exposures with incident, sporadic colorectal adenoma according to antioxidant enzyme genotypes. *Ann Epidemiol* **23,** 223-226, 2013. doi: 10.1016/j.annepidem.2012.12.001
49. Wang T, Goodman M, Sun YV, Thyagarajan B, Gross M, et al.: DNA base excision repair genetic risk scores, oxidative balance, and incident, sporadic colorectal adenoma. *Mol Carcinog* **56,** 1642-1652, 2017. doi: 10.1002/mc.22620
50. Mekary RA, Wu K, Giovannucci E, Sampson L, Fuchs C, et al.: Total antioxidant capacity intake and colorectal cancer risk in the Health Professionals Follow-up Study. *Cancer Causes Control* **21,** 1315-1321, 2010. doi: 10.1007/s10552-010-9559-9
51. Benzie IF, Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* **239,** 70–76, 1996
52. Halvorsen BL, Holte K, Myhrstad MCW, Barikmo I, Hvattum E, et al.: A systematic screening of total antioxidants in dietary plants. *J Nutr* **132,** 461–471, 2002
53. Bruce W, Giacca A, Medline A: Possible mechanisms relating diet and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* **9,** 1271-1279, 2000
54. Nagel G, Zoller D, Ruf T, Rohrmann S, Linseisen J: Long-term reproducibility of a food-frequency questionnaire and dietary changes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg cohort. *Br J Nutr* **98,** 194-200, 2007. doi: 10.1017/S0007114507691636

Table 1. Components of the oxidative balance score in the Iowa Women's Health Study and their rationales

Components	Rationale for inclusion
Lifestyle antioxidants	
Physical activity	Although acute bouts of exercise increase RONS production, regular exercise results in increased adaptive responses to oxidative stress, via activating cellular antioxidant signaling systems and enhancing expression of antioxidant enzymes through a process termed "hormesis" (23)
Lifestyle prooxidants	
Adiposity	Independently associated with increased oxidative stress markers, impaired serum redox balance, and increased lipid peroxidation; source of free fatty acids, which can lead to oxidative stress through increased RONS production (28)
Alcohol intake	Chronic intake results in oxidative stress through oxidation of ethanol to acetaldehyde, which can lead to RONS production, nucleic acid oxidation, and decreased activity of antioxidant enzymes (26, 27)
Smoking	Potent producer of free radicals, associated with increase in blood/tissue markers of oxidative stress (24, 25)
Dietary antioxidants	
Carotene (α and β)	Precursors of vitamin A, potent antioxidants (29)
Flavonoids	Plant polyphenols with multiple antioxidant functions: phenolic groups donate hydrogen to free radicals, prevent metal-catalyzed free-radical formation, and integrate with cell membranes to protect against lipid peroxidation (30, 31)
Lutein + zeathanxin	Antioxidant (29)
Lycopene	Antioxidant (32)
Omega-3 fatty acids	Induced electrophile-responsive element regulated genes responsible for transcription regulation of antioxidant enzymes (36, 37)
Vitamin C	Prevents lipid peroxidation, helps regenerate α-tocopherol (35)
Vitamin E	Membrane-bound antioxidant, protects against lipid peroxidation (34)
Selenium	Trace element that is part of important antioxidant selenoproteins (33)
Dietary prooxidants	
Iron	Available from red meat; preferentially catalyzes oxidative reactions in the colon through production of free radicals, resulting in lipid, protein, and DNA and other nucleic acid damage (38, 39)

Omega-6 fatty acids	Higher intakes associated with increased oxidative stress through increased free-radical production; unlike omega-3 fatty acids, they do not induce electrophile-responsive elements (37, 40, 41)
Saturated fats	Oxidative DNA damage through increased production of known prooxidant bile acids in the colon (42, 43)

Abbreviations: RONS, reactive oxygen and nitrogen species.

Table 2. Selected baseline participant characteristics[a] according to lifestyle and dietary oxidative balance score quintiles; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Characteristics	Dietary oxidative balance score quintiles[b]			Lifestyle oxidative balance score quintiles[b]		
	1 (*n* = 6,923)	**3 (*n* = 6,878)**	**5 (*n* = 7,682)**	**1 (*n* = 6,827)**	**3 (*n* = 6,827)**	**5 (*n* = 6,827)**
	Mean ± SD or %	Mean ± SD or %	Mean ± SD or %	Mean ± SD or %	Mean ± SD or %	Mean ± SD or %
Age (years)	61.2 ± 4.1	61.5 ± 4.2	61.6 ± 4.2	61.1 ± 4.1	61.7 ± 4.2	61.7 ± 4.2
High school graduate or higher (%)	79.0	83.6	82.6	80.1	81.4	83.8
1st degree relative with CRC (%)	3.2	3.0	3.3	3.0	2.9	3.0
Currently use hormone therapy (%)	10.0	11.7	13.2	10.1	11.1	12.3
Total energy intake (kcal/day)	1,920 ± 661	1,828 ± 580	1,743 ± 553	1,825 ± 625	1,794 ± 603	1,800 ± 583
Total vegetables and fruit intake (servings/week)	31.3 ± 14.0	43.9 ± 17.6	58.1 ± 28.7	40.7 ± 20.8	42.8 ± 20.8	47.2 ± 21.9
Red meats intake (servings/week)	7.2 ± 4.9	6.0 ± 3.6	4.4 ± 3.2	6.0 ± 4.0	6.1 ± 4.1	5.7 ± 3.9
Processed meats intake (servings/week)	2.7 ± 3.3	2.0 ± 2.1	1.2 ± 1.7	2.3 ± 2.7	2.1 ± 2.7	1.8 ± 2.2
Total[c] calcium intake (mg/day)	1,031 ± 547	1,103 ± 544	1,184 ± 590	1,024 ± 546	1,065 ± 540	1,167 ± 558
Dietary antioxidant intakes						
Total[c] carotene (α and β), (IU/1,000 kcal/day)	3,075 ± 1,518	4,881 ± 2,164	8,801 ± 6,747	4,658 ± 3,428	5,124 ± 4,150	5,812 ± 4,468
Flavones (mg/1,000 kcal/day)	4 ± 3	7 ± 5	14 ± 11	7 ± 8	8 ± 7	9 ± 7
Total[c] lutein + zeathanxin (mcg/1,000 kcal/day)	957 ± 652	1,497 ± 782	3,134 ± 3,153	1,663 ± 1,516	1,582 ± 1,401	1,843 ± 1,493

Total[c] lycopene (mcg/1,000 kcal/day)	1,636 ± 1,153	2,419 ± 1,463	4,204 ± 4,762	2,685 ± 2,473	2,532 ± 2,148	2,680 ± 2,228
Total[c] vitamin C (mg/1,000 kcal/day)	88 ± 93	148 ± 137	326 ± 311	164 ± 190	169 ± 189	186 ± 206
Total[c] vitamin E (mg/1,000 kcal/day)	22 ± 45	34 ± 65	109 ± 164	43 ± 90	46 ± 93	54 ± 106
Total[c] omega-3 fatty acids (g/1,000 kcal/day)	0.06 ± 0.05	0.10 ± 0.07	0.19 ± 0.18	0.10 ± 0.10	0.10 ± 0.11	0.11 ± 0.12
Selenium[d] (mcg/1,000 kcal/day)	5.0 ± 23.1	1.4 ± 7.2	2.1 ± 9.3	1.8 ± 10.6	2.2 ± 12.0	2.6 ± 13.7
Dietary prooxidant intakes						
Total[c] iron intake (mg/1,000 kcal/day)	14 ± 14	10 ± 8	11 ± 8	10 ± 9	11 ± 10	12 ± 9
Saturated fats (g/1,000 kcal/day)	15.5 ± 3.0	13.1 ± 2.2	11.2 ± 2.4	13.4 ± 3.0	13.4 ± 2.7	13.0 ± 2.8
Omega-6 fatty acids (g/1,000 kcal/day)	7.4 ± 2.1	6.4 ± 1.5	5.9 ± 1.6	6.5 ± 1.9	6.4 ± 1.6	6.4 ± 1.7
Lifestyle, antioxidant						
High physical activity[5] (%)	17.0	25.0	35.1	8.4	3.2	40.2
Lifestyle, prooxidants						
Current smoker (%)	19.9	13.8	12.8	51.3	5.8	2.5
≥7 alcoholic drinks/week (%)	7.2	10.5	10.4	37.6	0.0	2.8
High adiposity[6] (%)	20.2	19.7	18.7	34.2	13.6	11.9

Abbreviations: CRC, colorectal cancer; IU, international units; SD, standard deviation.

[a] Continuous variables presented as means (standard deviation); categorical variables presented as percentages.

[b] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in the table; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to prooxidant exposures.

[c] Total = diet plus supplements.
[d] Selenium intake from supplements only.
[e] Physical activity level derived from two questions regarding the frequency of moderate and vigorous physical activity (14), and categorized as high (vigorous activity twice a week or moderate activity > 4 times/week), medium (vigorous activity once a week plus moderate activity once a week, or moderate activity 2 – 4 times/week), and low.
[f] Body mass index (BMI; weight [kg]/height [m^2]) < 30 and waist:hip ratio (WHR) < 0.8 considered low adiposity; either BMI ≥ 30 or WHR ≥ 0.8 considered medium adiposity; BMI ≥ 30 and WHR ≥ 0.8 considered high adiposity.

Table 3. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer in the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Oxidative balance score[a]	**N (cases/total)**	**Minimally-adjusted associations**[b]		**Fully-adjusted associations**[c]	
		HR	**95% CI**	**HR**	**95% CI**
Dietary OBS, quintiles (quintile median)					
1 (-4.58)	(345/6,827)	1.00	Referent	1.00	Referent
2 (-2.58)	(353/6,827)	0.95	0.82, 1.10	0.94	0.81, 1.09
3 (-0.71)	(322/6,827)	0.92	0.79, 1.07	0.90	0.77, 1.06
4 (1.36)	(361/6,827)	0.96	0.83, 1.12	0.93	0.79, 1.10
5 (5.31)	(301/6,827)	0.80	0.68, 0.93	0.77	0.64, 0.94
P_{trend}		*0.007*		*0.01*	
Lifestyle OBS, quintiles (quintile median)					
1 (-2.69)	(358/6,923)	1.00	Referent	1.00	Referent
2 (-1.19)	(346/6,884)	0.87	0.75, 1.01	0.86	0.74, 1.00
3 (0.22)	(364/6,878)	0.86	0.75, 1.00	0.85	0.73, 0.98
4 (1.34)	(305/5,768)	0.86	0.74, 1.00	0.85	0.73, 1.00
5 (2.65)	(309/7,682)	0.63	0.54, 0.73	0.63	0.54, 0,73
P_{trend}		*< 0.0001*		*< 0.0001*	
Total OBS, quintiles (quintile median)					
1 (-5.70)	(338/6,827)	1.00	Referent	1.00	Referent
2 (-2.73)	(371/6,827)	0.98	0.84, 1.13	0.95	0.82, 1.10
3 (-0.54)	(349/6,827)	0.90	0.78, 1.05	0.86	0.74, 1.01
4 (1.89)	(331/6,827)	0.84	0.73, 0.98	0.79	0.67, 0.93
5 (6.15)	(329/6,827)	0.72	0.62, 0.84	0.65	0.54, 0.79
P_{trend}		*< 0.0001*		*< 0.0001*	

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, and total energy intake.

[c] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Table 4. Joint/combined (cross-classification) associations[a] of dietary and lifestyle oxidative balance scores[b] with incident colorectal cancer in the Iowa Women's Health Study (n = 34,135), 1986 – 2012

		Dietary OBS, quintiles														
		1			2			3			4			5		
		n	**HR**	**95% CI**	***n***	**HR**	**95% CI**	***n***	**HR**	**95% CI**	***n***	**HR**	**95% CI**	***n***	**HR**	**95% CI**
Lifestyle OBS, quintiles	**1**	1,596	1.00	Referent	1,433	0.94	0.67, 1.31	1,417	0.76	0.52, 1.11	1,286	0.98	0.65, 1.48	1,191	0.91	0.56, 1.48
	2	1,517	0.80	0.58, 1.11	1,425	0.69	0.49, 0.98	1,314	0.71	0.48, 1.06	1,322	0.79	0.53, 1.18	1,306	0.63	0.38, 1.06
	3	1,474	0.98	0.73, 1.33	1,499	0.75	0.53, 1.07	1,410	0.77	0.53, 1.11	1,337	0.63	0.40, 0.98	1,158	0.57	0.35, 0.94
	4	908	0.91	0.63, 1.30	1,067	0.75	0.51, 1.09	1,169	0.93	0.61, 1.41	1,271	0.48	0.30, 0.76	1,353	0.60	0.37, 0.97
	5	1,332	0.60	0.43, 0.85	1,403	0.57	0.40, 0.83	1,517	0.47	0.30, 0.72	1,611	0.48	0.31, 0.76	1,819	0.47[3]	0.29, 0.76

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

[b] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[c] P-interaction = 0.27.

Supplemental Table 1. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer, according to categories of selected participant characteristics; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Character-istics	OBS tertiles	Lifestyle OBS[a]		Dietary OBS[a]		Total OBS[a]	
		HR[b]	95% CI	HR[b]	95% CI	HR[b]	95% CI
Age, yrs.							
< 65	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.91	0.79, 1.05	0.93	0.81, 1.08	0.93	0.81, 1.08
	3	0.74	0.64, 0.86	0.86	0.72, 1.03	0.71	0.59, 0.84
≥ 65	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.79	0.64, 0.97	1.06	0.85, 1.33	0.96	0.77, 1.19
	3	0.66	0.54, 0.82	1.06	0.82, 1.37	0.80	0.62, 1.02
$P_{interaction}$		*0.44*		*0.64*		*0.85*	
CRC in a 1st degree relative							
No	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.85	0.75, 0.95	0.97	0.86, 1.10	0.93	0.82, 1.04
	3	0.70	0.62, 0.80	0.93	0.80, 1.08	0.74	0.64, 0.85
Yes	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	1.49	0.77, 2.88	0.79	0.40, 1.53	1.19	0.61, 2.29
	3	0.85	0.42, 1.71	0.42	0.18, 1.03	0.60	0.26, 1.40
$P_{interaction}$		*0.26*		*0.94*		*0.76*	
Education							
< High school graduate	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.67	0.51, 0.88	0.96	0.72, 1.28	0.78	0.59, 1.03
	3	0.55	0.41, 0.74	0.87	0.62, 1.22	0.60	0.42, 0.84
≥ High school graduate	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.92	0.81, 1.05	0.97	0.85, 1.11	0.97	0.85, 1.11
	3	0.75	0.66, 0.86	0.90	0.77, 1.06	0.75	0.64, 0.88
$P_{interaction}$		*0.08*		*0.83*		*0.76*	
Current use of hormone replacement therapy							
No	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.85	0.76, 0.96	0.97	0.85, 1.10	0.92	0.81, 1.04
	3	0.72	0.63, 0.81	0.92	0.79, 1.01	0.73	0.63, 0.85
Yes	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.97	0.65, 1.45	0.98	0.64, 1,51	1.25	0.81, 0.91
	3	0.71	0.47, 1.08	0.85	0.52, 1.40	0.80	0.49, 1.32
$P_{interaction}$		*0.82*		*0.62*		*0.96*	

Total energy intake[c] (kcal/day)							
< 1,718.6	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.82	0.70, 0.96	1.04	0.88, 1.25	0.87	0.74, 1.03
	3	0.66	0.56, 0.78	1.00	0.82, 1.21	0.73	0.60, 0.88
≥ 1,718.6	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.93	0.79, 1.09	0.92	0.78, 1.09	1.03	0.87, 1.21
	3	0.79	0.66, 0.94	0.89	0.72, 1.10	0.78	0.62, 0.97
$P_{interaction}$		*0.45*		*0.59*		*0.21*	
Total calcium intake[c] (mg/day)							
< 1,031.3	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.83	0.72, 0.97	1.01	0.86, 1.19	0.98	0.83, 1.14
	3	0.75	0.63, 0.87	0.93	0.76, 1.13	0.75	0.62, 0.91
≥ 1,031.3	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.93	0.77, 1.11	0.94	0.78, 1.14	0.92	0.76, 1.11
	3	0.71	0.60, 0.86	0.97	0.78, 1.20	0.77	0.62, 0.95
$P_{interaction}$		*0.50*		*0.85*		*0.63*	
Total vegetable and fruit intake[c] (servings/week)							
< 40.5	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.86	0.74, 1.01	0.96	0.82, 1.13	0.86	0.74, 1.01
	3	0.68	0.57, 0.81	0.97	0.79, 1.18	0.75	0.61, 0.92
≥ 40.5	1	1.00	Referent	1.00	Referent	1.00	Referent
	2	0.88	0.74, 1.04	1.02	0.84, 1.24	1.11	0.92, 1.34
	3	0.75	0.64, 0.89	0.93	0.76, 1.15	0.84	0.68, 1.02
$P_{interaction}$		*0.87*		*0.62*		*0.02*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

[c] Dichotomized on median intake among analytic population at baseline.

Supplemental Table 2. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer, by cancer site; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Oxidative balance scores[a]	Proximal colon[b]			Distal colon[c]			Rectum[d]		
	No. of Cases	HR[e]	95% CI	No. of Cases	HR[e]	95% CI	No. of Cases	HR[e]	95% CI
Lifestyle OBS, tertiles									
1	338	1.00	Referent	150	1.00	Referent	107	1.00	Referent
2	300	0.86	0.72, 1.03	141	0.99	0.75, 1.32	113	0.74	0.53, 1.05
3	257	0.80	0.66, 0.97	128	0.88	0.66, 1.17	101	0.91	0.63, 1.31
Dietary OBS, tertiles									
1	307	1.00	Referent	150	1.00	Referent	108	1.00	Referent
2	308	0.99	0.81, 1.20	143	0.85	0.62, 1.15	104	1.03	0.69, 1.53
3	280	1.03	0.82, 1.30	126	0.77	0.53, 1.11	109	0.88	0.56, 1.37
Total OBS, tertiles									
1	316	1.00	Referent	141	1.00	Referent	108	1.00	Referent
2	317	0.94	0.78, 1.13	157	0.71	0.53, 0.96	108	1.03	0.72, 1.46
3	262	0.87	0.64, 1.10	121	0.87	0.59, 1.30	105	0.82	0.53, 1.25

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] Proximal colon: cecum, appendix, ascending colon, hepatic flexure, and transverse colon.

[c] Distal colon: splenic flexure, descending colon, and sigmoid colon.

[d] Rectum: recto-sigmoid junction and rectum.

[e] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement

therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 3. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer, when stopping follow-up at 5, 10, 15, 20, and 25 years; the Iowa Women's Health Study (n = 34,135)

Oxidative balance score[a]	Follow-up intervals (No. of cases)				
	5 years (285)	10 years (602)	15 years (972)	20 years (1,362)	25 years (1,616)
Lifestyle OBS, 5th relative to 1st quintile					
HR[b]	0.96	0.65	0.64	0.63	0.62
(95% CI)	(0.67, 1.37)	(0.51, 0.84)	(0.53, 0.79)	(0.53, 0.75)	(0.53, 0.73)
P_{trend}	*0.68*	*0.002*	*< 0.0001*	*< 0.0001*	*< 0.0001*
Dietary OBS, 5th relative to 1st quintile					
HR[b]	0.70	0.77	0.79	0.76	0.82
(95% CI)	(0.44, 1.12)	(0.55, 1.08)	(0.61, 1.04)	(0.60, 0.95)	(0.67, 1.00)
P_{trend}	*0.29*	*0.14*	*0.006*	*0.01*	*0.06*
Total OBS, 5th relative to 1st quintile					
HR[b]	0.88	0.62	0.66	0.63	0.68
(95% CI)	(0.55, 1.40)	(0.45, 0.85)	(0.51, 0.85)	(0.50, 0.78)	(0.56, 0.83)
P_{trend}	*0.45*	*0.005*	*0.0006*	*< 0.0001*	*< 0.0001*

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 4. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer using alternative methods for OBS calculation; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Oxidative balance score[a]	**Fully-adjusted associations**[b,d]		**Fully-adjusted associations**[c,d]	
	HR	**95% CI**	**HR**	**95% CI**
Dietary OBS, quintiles				
1	1	Referent	1	Referent
2	0.98	0.84, 1.14	0.96	0.82, 1.11
3	0.90	0.77, 1.05	0.92	0.79, 1.07
4	0.91	0.77, 1.07	0.96	0.82, 1.12
5	0.81	0.67, 0.97	0.85	0.71, 1.00
P_{trend}	*0.02*		*0.09*	
Lifestyle OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.93	0.81, 1.08	0.96	0.83, 1.11
3	0.85	0.73, 0.99	0.85	0.73, 0.98
4	0.86	0.74, 1.00	0.85	0.73, 0.99
5	0.66	0.56, 0.77	0.67	0.57, 0.79
P_{trend}	*<0.0001*		*<0.0001*	
Total OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.98	0.84, 1.13	0.91	0.79, 1.06
3	0.85	0.73, 1.00	0.95	0.81, 1.10
4	0.77	0.65, 0.92	0.83	0.71, 0.97
5	0.65	0.52, 0.80	0.70	0.59, 0.85
P_{trend}	*<0.0001*		*0.0003*	

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] For participants censored prior to 2004, the baseline (1986) data were used for the OBS calculations; for participants not censored prior to 2004, the average of the baseline and follow-up (2004) data were used for the OBS calculations.

[c] For participants censored prior to 2004, the baseline data (1986) were used for the OBS calculations; for participants not censored prior to 2004, the follow-up data (2004) were used for the OBS calculations.

[d] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake,

education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 5. Associations of lifestyle, dietary, and total oxidative balance scores with incident CRC, after excluding participants who died or were diagnosed with CRC in the first six years of follow up, with and without adjustment for aspirin and other NSAID use; the Iowa Women's Health Study (n = 26,253), 1992 – 2012

Oxidative balance score[a]	Minimally-adjusted associations[b]		Fully-adjusted associations[c]		Fully-adjusted associations[d]	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Lifestyle OBS, quintiles						
1	1.00	Referent	1.00	Referent	1.00	Referent
2	0.89	0.74, 1.06	0.88	0.74, 1.05	0.87	0.72, 1.04
3	0.90	0.75, 1.09	0.89	0.74, 1.07	0.88	0.73, 1.05
4	0.79	0.66, 0.94	0.79	0.66, 0.94	0.78	0.65, 0.93
5	0.60	0.49, 0.74	0.60	0.49, 0.74	0.60	0.49, 0.74
P_{trend}	*< 0.0001*		*< 0.0001*		*< 0.0001*	
Dietary OBS, quintiles						
1	1.00	Referent	1.00	Referent	1.00	Referent
2	0.95	0.79, 1.14	0.95	0.78, 1.14	0.94	0.78, 1.13
3	0.96	0.80, 1.15	0.94	0.78, 1.14	0.95	0.78, 1.15
4	0.95	0.79, 1.14	0.93	0.76, 1.14	0.93	0.76, 1.15
5	0.80	0.66, 0.97	0.77	0.61, 0.98	0.76	0.60, 0.97
P_{trend}	*0.03*		*0.04*		*0.03*	
Total OBS, quintiles						
1	1.00	Referent	1.00	Referent	1.00	Referent
2	1.02	0.85, 1.22	0.99	0.82, 1.19	1.00	0.83, 1.20
3	0.97	0.81, 1.16	0.92	0.76, 1.11	0.93	0.77, 1.12
4	0.82	0.68, 0.99	0.77	0.63, 0.94	0.76	0.62, 0.94
5	0.71	0.58, 0.87	0.63	0.50, 0.80	0.64	0.50, 0.81
P_{trend}	*< 0.0001*		*< 0.0001*		*< 0.0001*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazards ratio; NSAID, non-steroidal anti-inflammatory drug; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, and total energy intake.

[c] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity,

smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

[d] From Cox proportional hazards models; covariates for all models same as in footnote [c] plus aspirin and other NSAID use.

Supplemental Table 6. Associations of lifestyle, dietary, and total oxidative balance scores with incident colorectal cancer, according to categories of aspirin and other NSAID use; the Iowa Women's Health Study (n = 26,253), 1992 – 2012

	Lifestyle OBS[a]		**Dietary OBS**[a]		**Total OBS**[a]	
	HR[b]	**95% CI**	**HR**[b]	**95% CI**	**HR**[b]	**95% CI**
Use of aspirin						
< 1/week	1.00	Referent	1.00	Referent	1.00	Referent
	0.81	0.67, 0.98	1.00	0.82, 1.23	0.86	0.71, 1.05
	0.65	0.53, 0.79	0.92	0.72, 1.17	0.66	0.52, 0.84
≥ 1/week	1.00	Referent	1.00	Referent	1.00	Referent
	0.99	0.80, 1.23	0.82	0.65, 1.03	0.98	0.78, 1.22
	0.71	0.56, 0.90	0.81	0.62, 1.07	0.67	0.51, 0.88
$P_{interaction}$	*0.41*		*0.40*		*0.52*	
Use of other non-aspirin NSAID						
< 1/week	1.00	Referent	1.00	Referent	1.00	Referent
	0.86	0.73, 1.01	0.94	0.79, 1.11	0.94	0.79, 1.10
	0.70	0.59, 0.83	0.92	0.75, 1.13	0.70	0.57, 0.86
≥ 1/week	1.00	Referent	1.00	Referent	1.00	Referent
	1.00	0.75, 1.34	0.87	0.63, 1.21	0.84	0.61, 1.15
	0.58	0.40, 0.83	0.70	0.47, 1.04	0.54	0.37, 0.80
$P_{interaction}$	*0.56*		*0.62*		*0.43*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; NSAID, nonsteroidal anti-inflammatory drugs; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models; model for Lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, use of aspirin and other NSAID, and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, adiposity (see text), use of aspirin and other NSAID; model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit

intake, red meat intake, processed meat intake, regular use of aspirin and other NSAIDs.

Supplemental Table 7. Associations of lifestyle, dietary, and total oxidative balance scores with incident CRC after excluding participants who died or were diagnosed with CRC within the first two years of follow-up; the Iowa Women's Health Study (n = 33,474), 1988 – 2012

Oxidative balance score[a]	**Minimally-adjusted associations**[b]		**Fully-adjusted associations**[c]	
	HR	**95% CI**	**HR**	**95% CI**
Lifestyle OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.84	0.72, 0.98	0.83	0.71, 0.97
3	0.84	0.73, 0.98	0.83	0.71, 0.96
4	0.85	0.73, 1.00	0.85	0.72, 0.99
5	0.59	0.50, 0.69	0.58	0.50, 0.68
P_{trend}	*< 0.0001*		*< 0.0001*	
Dietary OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.97	0.84, 1.13	0.97	0.83, 1.13
3	0.94	0.81, 1.10	0.92	0.78, 1.09
4	0.97	0.83, 1.13	0.94	0.79, 1.12
5	0.80	0.68, 0.94	0.77	0.63, 0.94
P_{trend}	*0.008*		*0.01*	
Total OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.96	0.82, 1.12	0.93	0.80, 1.09
3	0.91	0.78, 1.06	0.87	0.74, 1.01
4	0.82	0.70, 0.96	0.76	0.65, 0.91
5	0.71	0.60, 0.83	0.63	0.52, 0.77
P_{trend}	*< 0.0001*		*< 0.0001*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazards ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, and total energy intake.

[c] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total

vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 8. Associations of lifestyle, dietary, and total oxidative balance scores with incident CRC with additional censoring of participants when they reached the age of 75 years; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Oxidative balance score[a]	Minimally-adjusted associations[b]		Fully-adjusted associations[c]	
	HR	**95% CI**	**HR**	**95% CI**
Lifestyle OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.85	0.69, 1.06	0.86	0.69, 1.06
3	0.83	0.68, 1.03	0.84	0.68, 1.03
4	0.71	0.57, 0.90	0.72	0.57, 0.92
5	0.63	0.50, 0.78	0.64	0.51, 0.80
P_{trend}	*< 0.0001*		*< 0.0001*	
Dietary OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.95	0.76, 1.18	0.93	0.75, 1.16
3	0.87	0.70, 1.08	0.84	0.67, 1.07
4	0.96	0.77, 1.19	0.91	0.71, 1.16
5	0.70	0.55, 0.88	0.65	0.48, 0.87
P_{trend}	*0.005*		*0.006*	
Total OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.96	0.78, 1.18	0.94	0.76, 1.16
3	0.71	0.57, 0.89	0.68	0.54, 0.85
4	0.82	0.66, 1.02	0.77	0.61, 0.97
5	0.63	0.50, 0.80	0.57	0.43, 0.75
P_{trend}	*< 0.0001*		*< 0.0001*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; OBS, oxidative balance score.

[a] Oxidative balance scores (OBS) composed of the lifestyle or dietary or exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[b] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, and total energy intake.

[c] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity,

smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 9. Associations of lifestyle, dietary, and total oxidative balance scores with incident CRC using alternative scoring of alcohol intake[a]; the Iowa Women's Health Study (n = 33,474), 1988 – 2012

Oxidative balance score[b]	Minimally-adjusted associations[c]		Fully-adjusted associations[d]	
	HR	**95% CI**	**HR**	**95% CI**
Lifestyle OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.93	0.81, 1.08	0.93	0.81, 1.08
3	0.89	0.76, 1.03	0.89	0.77, 1.04
4	0.70	0.60, 0.81	0.71	0.61, 0.82
5	0.72	0.61, 0.84	0.74	0.63, 0.87
P_{trend}	*< 0.0001*		*< 0.0001*	
Dietary OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.95	0.82, 1.10	0.94	0.81, 1.09
3	0.92	0.79, 1.07	0.90	0.77, 1.06
4	0.96	0.83, 1.12	0.93	0.79, 1.10
5	0.80	0.68, 0.93	0.77	0.64, 0.94
P_{trend}	*0.008*		*0.01*	
Total OBS, quintiles				
1	1.00	Referent	1.00	Referent
2	0.94	0.81, 1.09	0.92	0.79, 1.07
3	0.93	0.81, 1.08	0.90	0.78, 1.05
4	0.82	0.70, 0.95	0.78	0.66, 0.92
5	0.73	0.63, 0.86	0.69	0.57, 0.83
P_{trend}	*< 0.0001*		*< 0.0001*	

Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazards ratio; OBS, oxidative balance score.

[a] Alcohol intake scored as follows: < 1 drinks/week assigned value of 3; alcohol intake 1 –7 drinks/week assigned value of 1, alcohol intake ≥ 7 drinks/week assigned value of 3.

[b] Oxidative balance scores (OBS) composed of the lifestyle or dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

[c] From Cox proportional hazards models, adjusted for age, family history of colorectal cancer in a first-degree relative, and total energy intake.

[d] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit

intake, red meat intake, processed meat intake and the dietary OBS; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, and adiposity (see text); model for total OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, and processed meat intake.

Supplemental Table 10. Multivariable-adjusted associations[a] of the lifestyle oxidative balance score[b] with risk for incident colorectal cancer, with removal/replacement of each score component one at a time; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Component removed	Associations[a] for upper relative to lowest lifestyle OBS[b] quintile	
	HR	95% CI
Physical activity	0.79	0.70, 0.90
Smoking status	0.70	0.60, 0.82
Alcohol intake	0.65	0.56, 0.76
Adiposity	0.80	0.69, 0.93

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] From Cox proportional hazards models; model for lifestyle OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake and the equal-weight dietary OBS.

[b] Lifestyle oxidative balance scores (OBS) composed of the lifestyle exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.

Supplemental Table 11. Multivariable-adjusted associations[a] of the dietary oxidative balance score[b] with risk for incident colorectal cancer, with removal/replacement of each score component one at a time; the Iowa Women's Health Study (n = 34,135), 1986 – 2012

Component removed	**Associations[a] for upper relative to lowest quintile of the dietary OBS[b]**	
	HR	**95% CI**
Carotene (α and β)	0.85	0.70, 1.02
Flavonoids	0.89	0.72, 1.06
Lutein and zeathanxin	0.83	0.68, 1.00
Lycopene	0.83	0.69, 1.01
Vitamin C	0.82	0.68, 1.10
Vitamin E	0.86	0.71, 1.05
Omega-3 fatty acids	0.86	0.71, 1.05
Selenium	0.79	0.65, 0.96
Iron	0.84	0.69, 1.01
Saturated fats	0.78	0.65, 0.94
Omega-6 fatty acids	0.83	0.68, 1.01

Abbreviations: CI, confidence interval; HR, hazard ratio; OBS, oxidative balance score.

[a] From Cox proportional hazards models; model for dietary OBS adjusted for age, family history of colorectal cancer in a first-degree relative, total energy intake, education, current use of hormone replacement therapy, total vegetable and fruit intake, red meat intake, processed meat intake, physical activity, smoking status, alcohol intake, adiposity (see text), and the removed component.

[b] Dietary oxidative balance scores (OBS) composed of the dietary exposures listed in Table 1; see the text for construction of the 'equal-weight' scores; a higher score represents a higher balance of antioxidant relative to pro-oxidant exposures.