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Association of an Evolutionary-concordance Lifestyle Pattern Score, Alone and Jointly with an Evolutionary-concordance Diet Pattern Score, with Biomarkers of Systemic Oxidative Stress and Inflammation

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An abstract of
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2022

Abstract

Association of an Evolutionary-concordance Lifestyle Pattern Score, Alone and Jointly with an Evolutionary-concordance Diet Pattern Score, with Biomarkers of Systemic Oxidative Stress and Inflammation

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Consistent with the evolutionary-discordance hypothesis, lifestyle and dietary patterns considered more evolutionary-concordant were associated with lower risk for colorectal adenoma and cancer, and all-cause, all-cancer, and all-cardiovascular disease mortality. Important mechanisms underlying these associations may involve the collective effects of multiple lifestyle and dietary exposures on inflammation and oxidative stress. Indeed, a more evolutionary-concordant, 14-component, diet pattern score (EC diet score) was associated with lower circulating concentrations of biomarkers of systemic inflammation (high sensitivity C-reactive protein [hsCRP]) and oxidative stress (F_2-isoprostanes [FiP]). To investigate whether a more evolutionary-concordant lifestyle pattern score (EC lifestyle score; comprising smoking status, body mass index, and physical activity), alone and combined (total EC score) with the previously-reported more evolutionary-concordant 14-component EC diet score, is associated with circulating hsCRP and FiP concentrations, we analyzed data from two pooled cross-sectional studies among 30–74-year-old men and women (n=677). We categorized the EC scores as low, moderate and high, and from general linear regression models, mean circulating hsCRP and FiP concentrations among those in the highest (most evolutionary-concordant) relative to the lowest EC lifestyle score, were 45.5% (P<0.01) and 19.2% (P<0.01) lower, respectively; for the total EC score they were 51.1% (P<0.01) and 19.8% (P<0.01) lower, respectively. The findings were similar by sex and regular use of non-steroidal anti-inflammatory drugs and multivitamin/mineral supplements. Our results suggest that more evolutionary-concordant lifestyle patterns, alone and combined with more evolutionary-concordant diet patterns, may be associated with lower systemic inflammation and oxidative stress.

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Chapter 1: Background

Globally, noncommunicable diseases (NCD) causes 41 million deaths each year, which is equivalent to 71% of all deaths [1]. The leading causes of NCD deaths are cardiovascular disease (17.9 million), cancers 99.3 million), respiratory diseases (4.1 million), and diabetes (1.5 million). Chronic inflammation and oxidative stress have been implicated in the etiology of cardiovascular diseases, some cancers, and other chronic diseases [2, 3] that collectively constitute the leading causes of deaths globally [4].

Oxidative stress is caused by an imbalance between production and accumulation of reactive oxygen nitrogen species (RONS) in cells and tissues [5]. The RONS are free radicle molecules with unpaired electrons and contribute to oxidative stress[5, 6]. These free radicals interact with nucleic acids and cause mutations by nucleic acid oxidation. Oxidation of lipids by these free radicle molecules cause damage to phospholipid cellular membrane structures. The free radicals interact with proteins by oxidation of amino acids, which can adversely affect enzymatic functions [6]. Another mechanism for causing oxidative stress is an imbalance in redox potential[7]. Oxidative stress can cause inflammation and vice versa.

Various lifestyle and dietary factors were reported to contribute to chronic systemic inflammation [8, 9] and oxidative stress [10], which in turn have been associated with the incidence of chronic diseases in epidemiologic studies [11].

The individual components of the evolutionary-concordance (EC) lifestyle score—physical activity, BMI, and current smoking status—all plausibility can affect inflammation and oxidative stress. Physical activity decreases contraction of skeletal muscles and in turn decreases overall inflammatory biomarkers [12]. The anti-inflammatory cytokines suppress other proinflammatory cytokines which contributes to lower levels of systemic inflammation in association increase in levels of physical activity. Also, physical activity may reduce inflammation by decreasing body weight and fatness [13]. Current cigarette smoking can lead to higher levels of proinflammatory marker–CRP [14]–and a positive association was reported for current smoking and CRP levels in a dose-response manner [15]. Also, smoking delivers

carcinogens and is a potent producer of free radicals that cause oxidative stress [16]. Free fatty acids associated with high BMI cause higher levels of oxidative stress markers, impaired serum redox balance, and increased lipid peroxidation [17, 18]. Higher intakes of fats and processed meats are associated with high BMIs and higher levels of CRP, while diets with higher intakes of whole grains, vegetables, and fruits were associated with lower levels of CRP [19]. Diets containing more fruits and vegetables rich in antioxidants, carotenoids, flavonoids, and folate have been associated with lower levels of inflammation and oxidative stress [20]. In relation to oxidative stress, cigarette smoke contains free radicles, including O^{-}_{2}, H_2O_2 and OH, that are inhaled directly into respiratory tract reduce antioxidant availability leading to oxidative stress levels [21, 22]. Oxidative stress is also associated with obesity, old age, and other chronic diseases, and regular exercise or physical activity can decrease lipid peroxidation, which an aspect of oxidative stress [23, 24].

The EC diet pattern score comprises components that plausibly affect inflammation and oxidative stress. This diet includes higher intake of fruits, nuts, and vegetables, which contain several dietary antioxidants—carotene, flavonoids, lutein, and lycopene—which activate the transcription factor, nuclear factor erythroid, which plays a key role in cellular protection against oxidative stress and inflammation [25-28]. This diet rich in plant-based foods that also contain vitamin C and vitamin E, which prevent lipid peroxidation and protects against oxidative stress [29, 30]. This diet pattern also is characterized by low consumption of high-fat red and processed meats, which contain dietary prooxidants, such as heme iron, which catalyzes oxidative reactions in the colon that increases the production of free-radicals that damage lipids, proteins, and DNA [31, 32].

The evolutionary discordance hypothesis postulates that changes in lifestyle and diet factors compared to those of our Paleolithic ancestors may be the reason for higher incidences of chronic diseases during modern times. In a cross-sectional study of 30–74 old men and women in an elective colonoscopy population, participants in the highest tertiles of an EC diet pattern (AKA “Paleolithic diet pattern”) score and a Mediterranean diet score had 37% and 29% lower odds, respectively, of having a higher hsCRP

concentration with respect to reference diet score quintile , and 49% and 61% lower odds, respectively, of having a higher plasma FiP concentration [33] with respect to reference diet score quintile. There is no published study that reported associations of a lifestyle EC score, alone or combined with a diet EC score, with biomarkers of inflammation and oxidative stress.

In this study, we report an investigation of cross-sectional associations of a lifestyle EC score, alone and combined with a diet EC score, with biomarkers of inflammation and oxidative stress.

Chapter 2: Manuscript for Submission for Publication in a Peer-Reviewed Journal

Abstract

Consistent with the evolutionary-discordance hypothesis, lifestyle and dietary patterns considered more evolutionary-concordant were associated with lower risk for colorectal adenoma and cancer, and all-cause, all-cancer, and all-cardiovascular disease mortality. Important mechanisms underlying these associations may involve the collective effects of multiple lifestyle and dietary exposures on inflammation and oxidative stress. Indeed, a more evolutionary-concordant, 14-component, diet pattern score (EC diet score) was associated with lower circulating concentrations of biomarkers of systemic inflammation (high sensitivity C-reactive protein [hsCRP]) and oxidative stress (F_2-isoprostanes [FiP]). To investigate whether a more evolutionary-concordant lifestyle pattern score (EC lifestyle score; comprising smoking status, body mass index, and physical activity), alone and combined (total EC score) with the previously-reported more evolutionary-concordant 14-component EC diet score, is associated with circulating hsCRP and FiP concentrations, we analyzed data from two pooled cross-sectional studies among 30–74-year-old men and women (n=677). We categorized the EC scores as low, moderate and high, and from general linear regression models, mean circulating hsCRP and FiP concentrations among those in the highest (most evolutionary-concordant) relative to the lowest EC lifestyle score, were 45.5% (P<0.01) and 19.2% (P<0.01) lower, respectively; for the total EC score they were 51.1% (P<0.01) and 19.8% (P<0.01) lower, respectively. The findings were similar by sex and regular use of non-steroidal anti-inflammatory drugs and multivitamin/mineral supplements. Our results suggest that more evolutionary-concordant lifestyle patterns, alone and combined with more evolutionary-concordant diet patterns, may be associated with lower systemic inflammation and oxidative stress.

Introduction

Chronic inflammation and oxidative stress have been implicated in the etiology of cardiovascular diseases, some cancers, and other chronic diseases [2, 3] that collectively constitute the leading causes of deaths globally [4]. Various lifestyle and dietary factors were reported to contribute to chronic systemic inflammation [9] and oxidative stress [10], which in turn have been associated with the incidence of chronic diseases in epidemiologic studies [11].

The evolutionary-discordance hypothesis is that diets and lifestyles that are more evolutionarily concordant may be associated with lower risk for cardiovascular diseases (CVD), some cancers, and other chronic diseases that have been particularly prevalent during the 20th and 21st centuries [34, 35]. Lifestyles considered more evolutionary-concordant include those that are more physically active, exclude smoking, and result in energy balances that maintain a lean body mass index (BMI) [34, 35]. Diets considered more evolutionary-concordant include those containing higher amounts and diversity of fruits and vegetables; higher amounts of lean meats, fish, nuts, and calcium; and low amounts of alcohol, refined sugars, salt, grains, dairy foods, and red and processed meats that contain high amounts of fats, especially saturated fats [34, 35]. Previous epidemiologic studies found that a more evolutionary-concordant lifestyle pattern score, which included physical activity, BMI, and smoking status, alone and in combination with a more evolutionary-concordant diet score was inversely associated with incident colorectal cancer [36] and adenoma [37], and all-cause, all-CVD, and all-cancer mortality [38].

Collectively, more evolutionary-concordant diets and lifestyles may contribute to lower risk for chronic diseases and premature mortality via lower systemic inflammation and oxidative stress. Indeed, a more evolutionary-concordant dietary pattern score was inversely associated with biomarkers of inflammation and oxidative stress [33]; however, there are no reports of more evolutionary-concordant lifestyle patterns, nor of total (dietary + lifestyle) patterns, with biomarkers of inflammation and oxidative stress in humans. Accordingly, herein we report an investigation of associations of an evolutionary-concordance

lifestyle score (comprising smoking status, BMI, and physical activity), with and without a 14-component evolutionary-concordance diet score, with circulating concentrations of biomarkers of systemic oxidative stress (F_2-isoprostanes [FiP]) and inflammation (high sensitivity C-reactive protein [hsCRP]) in humans.

Materials and Methods

Study population and data source

We pooled de-identified questionnaire and laboratory data from two previously-conducted cross-sectional studies of patients scheduled for elective outpatient colonoscopies: Markers of Adenomatous Polyps studies (MAP) I and II conducted in North Carolina (1994–1997) and South Carolina (2002), respectively. The methods of and questionnaires used in the MAP I and MAP II studies were virtually identical, as previously published [39, 40]. Briefly, the study participants were ages 30–74 years, English-speaking, in general good health, and had no history of colorectal adenomatous polyps, inflammatory bowel disease, or previous cancer diagnosis (other than non-melanoma skin cancer). The MAP I and MAP II consent rates were 67% and 76%, respectively, and the respective sample sizes were 474 and 203, yielding an initial pooled sample of 677.

Each study was approved by the Institutional Review Board of the institution from which it was conducted: Wake Forest University School of Medicine for MAP I and University of South Carlina for MAP II. All data analyses were conducted using de-identified data. The MAP I and MAP II studies are hereinafter referred to as the pooled MAP studies.

Data collection, blood collection, and biomarker assays

The participants completed self-reported questionnaires at home prior to their colonoscopy visit to provide detailed information about their demographics, medical history, anthropometrics, and lifestyle. Diet was assessed using Willett food frequency questionnaires (FFQs) [41]. Dietary total energy and nutrients from food, beverage, and supplement sources were calculated using the nutrient database

developed and maintained by Willett [42]. Physical activity was assessed using modified Paffenbarger physical activity questionnaires [43, 44].

Fasting peripheral venous blood samples were collected from participants at their colonoscopy visit, prior to the procedure, into red-coated, pre-chilled Vacutainer tubes, then placed in ice and shielded from light to prevent sample degradation, and immediately taken to the laboratory. In the laboratory, blood fractions were immediately separated in refrigerated centrifuges and aliquoted into amber-colored cryo-preservation vials, the air was displaced with inert gas (nitrogen in MAP I and argon in MAP II), and then the aliquots were stored in -80° C freezers until analysis [45]. The biomarker assay protocols were previously described [33]. Briefly, hsCRP was measured using latex-enhanced immunonephelometry on a Bering nephelometer II analyzer (Behring Diagnostics; the inter-assay coefficient of variation (CV) was 4%. FiP was measured using a highly specific and quantitative gas chromatography-mass spectrometry (GC-MS)-based method [33, 46]. An internal standard, [2H4] 8-iso-PGF2α (>98% pure; Cayman Chemical), was added to plasma prior to analysis. The quality control procedures involved analysis of two control pools with varying ranges of FiP concentrations (CVs 9.5% and 11%) [33].

Calculation of the evolutionary-concordance lifestyle score

As previously reported [37], the evolutionary-concordance (EC) lifestyle score comprised three equally-weighted components: smoking status, BMI, and physical activity (**Supplemental Table 1**). We categorized BMI and physical activity into three categories. We categorized physical activity according to tertiles of MET-hours/week of moderate plus vigorous physical activity in the study analytic population, and assigned the tertiles values of 1–3, from lowest to highest. We categorized BMI, calculated by dividing body weight (kilograms) divided by height squared (meters), according to World Health Organization criteria for underweight/normal weight (<25.0 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥30.0 kg/m^2), and assigned the three categories' values of 3–1, from lowest to highest. We categorized current smoking as yes/no, and assigned them values of 1 and 3, respectively. For each

person, we then summed the point values for their lifestyle components to constitute their EC lifestyle score, which could range from 3 to 9, with a higher score indicating higher evolutionary concordance.

Calculation of the evolutionary-concordance diet score

Calculation of the EC diet score was previously reported [37]. Briefly, the evolutionary-concordance diet score comprises 14 equally-weighted components (**Supplemental Table 1**). As previously, in the present study, all components were derived from FFQ responses. As previously, we created two unique score components: 1) a fruit and vegetable diversity score that was the sum of the total number of different fruits and vegetables a person consumed >1–3 times/month, and 2) because of evidence that Paleolithic diets were high in calcium but low in dairy foods, calcium residuals from the regression of total calcium intake on total dairy intake to account for calcium intake fully adjusted for dairy intake. All dietary components initially were continuous variables, most of which we categorized into quintiles based on the study- and sex-specific distributions. For alcohol, sugar-sweetened beverages, and fish consumption we used alternative cut points to create five categories because their distributions were not conducive to quintile categorization. We assigned to each component's categories values of 1–5, from lowest to highest for exposures we considered more evolutionary concordant (calcium, nuts, fruit, vegetables, fruit and vegetable diversity, fish, lean meats), and values of 5–1, from lowest to highest, for exposures we considered less evolutionary concordant (alcohol, baked goods and sweets, dairy foods, grains and starches, red and processed meats, sodium, and sugar-sweetened beverages). For each person, we then summed the point values for their dietary components to constitute their EC diet pattern score, which could range from 14 to 70, with a higher score indicating higher evolutionary concordance.

Calculation of the total evolutionary-concordance score

For the four-component total EC score (**Supplemental Table 1**), we first categorized the above-described EC diet pattern score according to tertiles of its distribution in the study population, and assigned the tertiles point values of 1–3, from the lowest to the highest tertiles. Next, for each study participant, we

added the point values for their EC diet score to those from their EC lifestyle components to constitute their total EC score, which could range from 4 to 12, with a higher score indicating higher overall evolutionary concordance.

Statistical analyses

Prior to score calculations and subsequent statistical analyses, we excluded participants missing >15% of their FFQ responses (*n*=77) or data on lifestyle factors (*n*=30); and those who reported implausible total energy intakes (<600 or >6,000 kcal/d) (*n*=14) or extreme (>10,000 IU/day) supplemental carotene intakes (*n*=38). For analyses in which plasma hsCRP concentrations were the primary endpoint, we also excluded those with missing or extreme (>3 standard deviations [SD] of the study population mean) hsCRP values (*n*=7). For analyses for which plasma FiP concentrations were the primary endpoint, we also excluded those with missing values (*n*=117, almost all due to insufficient remaining plasma for the assays). Thus, the final sample sizes for the hsCRP and FiP assays were 468 (MAP I = 310, and MAP II = 158) and 358 (MAP I = 253, and MAP II = 105), respectively.

We summarized and compared selected characteristics of the participants across the three categories of the EC lifestyle score using descriptive statistics and analysis of variance for continuous variables (transformed by the natural logarithm to meet normality assumptions when indicated) and chi-square tests for categorical variables. We assessed correlation between FiP and hsCRP concentrations with a Pearson correlation coefficient and between the lifestyle and diet scores with a Spearman correlation coefficient.

To estimate associations of the EC scores with the biomarkers, we used general linear models to calculate and compare crude and multivariable-adjusted mean circulating hsCRP and FiP concentrations across categories of the scores. We normalized the distributions of the circulating hsCRP and FiP concentrations via transformation by the natural logarithm, and so present geometric means and their 95% confidence intervals (CI). To provide perspective on the mean differences, we calculated proportional differences as the biomarker mean in the second (or third) category of the respective score minus the mean in the first

(reference) category divided by the mean in the first category times 100%. For all multivariable models, we considered the following potential covariates, based on biological plausibility, previous literature, and differences in participant characteristics across tertiles of the evolutionary-concordance lifestyle score: age, sex, hormone replacement therapy among women, education, regular nonsteroidal anti-inflammatory drug (NSAID) use, regular multivitamin use, study (MAP I or MAP II), and prevalent adenoma status. For the lifestyle score models, we also considered the diet score as a covariate, and for the diet score models, we also considered the lifestyle score as a covariate. Inclusion/exclusion of the potential covariates in the models made virtually no changes in the estimated proportional differences in the mean biomarker values across categories of the scores, so we present only the results from the crude (unadjusted) models.

To assess potential effect modification of the associations of the scores with the biomarkers, we stratified the above-described models by categories of selected participant characteristics (sex, regular NSAID use [<4 days/wk. vs. ≥4 days/wk.] regular multivitamin use [<3 days/wk. vs. ≥3 days/wk.], and diagnosis of a first-ever colorectal adenoma at colonoscopy [yes/no]). We tested for multiplicative interaction using the likelihood ratio test.

We used SAS version 9.4 software (SAS Institute, Inc, Cary, North Carolina) to perform all statistical analyses. All statistical tests were two-sided, and we considered *P*-values ≤0.05 statistically significant.

Results

The characteristics of the study participants, by EC lifestyle score categories, are presented in **Table 1**. Participants in the higher relative to the lowest EC lifestyle score categories, were more likely to be female, less likely to have a colorectal adenoma, and as would be expected from how the score was constructed, less likely to smoke or to be overweight or obese. On average, they had higher total calcium and total fruit and vegetable intakes and an EC diet score; as would be expected from how the score was constructed, they were also more physically active. Although not statistically significant, they also tended

to have lower mean circulating hsCRP and FiP concentrations. The Spearman correlation coefficient between the lifestyle and diet scores was 0.11 (P=0.01), and the Pearson correlation coefficient between the circulating FiP and hsCRP concentrations was 0.08 (P=0.12).

Crude mean plasma hsCRP and FiP concentrations by EC lifestyle score categories are presented in **Table 2**. Multivariable adjustment made no appreciable differences in the results, so only the unadjusted results are shown. There were decreasing mean hsCRP and FiP concentrations with higher EC lifestyle score categories. Among those in the highest relative to the lowest EC lifestyle score category, mean hsCRP and FiP concentrations were statistically significantly, proportionately 45.5% and 19.2% lower, respectively.

We previously reported statistically significant, lower mean hsCRP and FiP concentrations across EC diet score quintiles [37]. For our present paper, we categorized the diet score according to tertiles in order to make the categorization more comparable to the 3-category lifestyle score and of the subsequent 3-category total EC score. We present the crude mean hsCRP and FiP concentrations by EC diet score categories in **Supplemental Table 2**. Multivariable adjustment made no appreciable differences in the results, so only the unadjusted results are shown. There were decreasing mean FiP concentrations with higher EC diet score categories, and among those in the highest relative to the lowest EC diet score category, the mean FiP concentration was statistically significantly, proportionately 11.1% lower. Among those in the highest relative to the lowest EC diet score category, the mean hsCRP concentration was estimated to be 14.6% lower, although the finding was not statistically significant.

Crude mean plasma hsCRP and FiP concentrations by total EC score categories are presented in **Table 3**. Multivariable adjustment made no appreciable differences in the results, so only the unadjusted results are shown. There were decreasing mean hsCRP and FiP concentrations with higher total EC score categories. Among those in the highest relative to the lowest total EC score category, mean hsCRP and FiP concentrations were statistically significantly, proportionately 51.1% and 19.8% lower, respectively.

The results of the analyses of mean plasma hsCRP and FiP concentrations across categories of the lifestyle and total EC scores, stratified by sex, regular NSAID use, regular multivitamin supplement use, and colorectal adenoma status are shown in **Supplemental Tables 3 – 6**. Briefly, with one exception, the decreasing concentrations of both biomarkers across both scores did not differ substantially or statistically significantly by categories of the stratification variables. The one exception was that among those in the highest relative to the lowest total EC score category, the mean circulating FiP concentrations were estimated to be, proportionately, 33.9% and 7.1% lower among women and men, respectively ($P_{\text{interaction}}$ <0.01) (**Supplemental Table 6**).

Discussion

Our findings suggest that more evolutionary-concordant lifestyle patterns, alone and combined with more evolutionary-concordant dietary patterns, may be associated with lower systemic inflammation and oxidative stress. Our findings were similar across categories of sex, regular NSAID and multivitamin/mineral supplement use, and prevalent colorectal adenoma status. This is the first study to report associations of an EC lifestyle score, alone and combined with an EC diet score, with circulating biomarkers of inflammation and oxidative stress.

Inflammation and oxidative stress, which may be viewed as an imbalance between pro-oxidants and antioxidants favoring the former [47, 48] and redox potential changes causing impaired redox balance [7], may increase each other [49] and are hypothesized to be important contributors to risk for several chronic diseases, such as CVD and colorectal neoplasms, and with premature mortality [50]. FiP is the most widely used, reliably measured biomarker of oxidative stress in epidemiologic studies. However, FiP is a biomarker of lipid peroxidation [46], and does not directly reflect oxidative damage to proteins or DNA. Likely due to cost, few studies have investigated associations of FiP with chronic diseases and mortality; however, circulating FiP concentrations were directly associated with incident sporadic colorectal adenoma [51], and oxidative balance scores, validated via their associations with FiP [51, 52], were

associated with risk for colorectal adenoma [52] and cancer [10], and all-cause, all-CVD, and all-cancer mortality [53]. Circulating concentrations of hsCRP, an acute phase reactant protein produced in the liver and released into the circulation [54], is also reliably measured and widely used as a biomarker of inflammation in epidemiologic studies [55]. However, for some applications, hsCRP has some limitations (e.g., increases in response to infection and other inflammatory medical conditions [56]), but it is highly correlated with other biomarkers of inflammation in generally healthy populations [55] and nevertheless has been associated with risk for various chronic diseases [57, 58] and mortality [59, 60].

The individual components of the EC lifestyle score—physical activity, BMI and current smoking status—all plausibly affect inflammation and oxidative stress (**Supplemental Table 7**). Regular physical activity results in increased adaptive responses to oxidative stress through activating cellular antioxidant systems and increasing the expression of antioxidant enzymes [61]. Moderate physical activity increases concentrations of anti-inflammatory cytokines and lowers vascular wall inflammation [62]. Physical activity may also reduce inflammation by decreasing body weight and fatness [13]. A high BMI reflects long-term positive energy balance [63], and free fatty acids associated with a high BMI impair redox balance and increase lipid peroxidation [17, 18]. An increase in adipose tissue is accompanied by increased synthesis and release of pro-inflammatory adipokines, such as plasminogen activator inhibitor-1 (PA-1) and TNF-α [17]. Smoking delivers carcinogens and other pro-inflammatory exposures, and is a potent producer of free radicals that cause oxidative stress [16]. Toxins from smoking injures tissues, upregulating cytokines and acute phase reactants causing inflammation and oxidative stress [64].

As reviewed elsewhere, diets may influence inflammation and oxidative stress multiple ways [9, 10, 33], and the EC diet pattern score contains components that plausibly affect inflammation and oxidative stress. As examples, a more evolutionary-concordant diet includes higher intakes of fruits, nuts, and vegetables, which contain multiple dietary antioxidant/anti-inflammatory constituents (e.g., carotenoids, flavonoids, lutein, and lycopene, vitamin C, vitamin E , polyphenols, polysaccharides, and triterpenoids [25-30, 65]) and have been associated with lower circulating CRP [9] and FiP [10] concentrations in epidemiologic

studies. A more evolutionary-concordant diet pattern also contains less high-fat red or processed meats, which have pro-oxidant/pro-inflammatory effects. As examples, heme iron catalyzes oxidative reactions in the colon, increasing the production of free-radicals that damage lipids, proteins, and DNA [31, 32]. Processed meats and red meats high in saturated fats can cause oxidative damage through increased production of bile acids in the colon [66]. Furthermore, higher intakes of fats and processed meats are associated with higher BMIs [67] and higher circulating CRP [9] and FiP [10] concentrations in epidemiologic studies.

Previous epidemiologic studies reported that smoking, BMI, and physical activity, separately and combined various ways and with dietary factors, were associated with biomarkers of inflammation [9] and oxidative stress [10]. Smoking as an individual exposure has been strongly associated with biomarkers of inflammation [68-70] and oxidative stress [10, 71] in several studies. Similarly, BMI as an individual exposure has been directly associated with circulating hsCRP concentrations [72, 73] and with plasma and urinary FiP concentrations [74, 75]. Physical activity as an individual exposure was strongly, inversely associated with hsCRP [76, 77] and oxidative stress biomarkers [10, 78, 79] in several studies. Several studies combined multiple individual exposures that may affect inflammation and/or oxidative stress. In a cross-sectional analysis of baseline data from a subset (*n*=639) of Black and White men and women from the United States' 48 contiguous in the Reasons for Geographic and Racial Differences in Stroke study (REGARDS), circulating concentrations of hsCRP, IL-6, IL-8 and IL-10 were measured and used to calculate inflammation biomarker panel-weighted 19-component, predominantly whole foods-based dietary (DIS) and 4-component (smoking, physical activity, BMI, alcohol intake) lifestyle (LIS) inflammation scores [9]. In the remainder of the REGARDS population (*n*=14,210) on whom hsCRP concentrations were measured, those in the highest (most pro-inflammatory) relative to the lowest DIS and LIS quintiles had statistically significant 1.66- and 4.29-fold higher odds of a high (>3 mg/dL) hsCRP concentration. The findings for the DIS and LIS were similar in two other validation populations. Also, the findings for another diet-related inflammation score, the predominantly nutrient-based Dietary

Inflammation Index (DII), in those same study populations were similar to, but somewhat weaker than, those for the DIS. Using similar methodology, in a pooled cross-sectional study (n=386 men and women), multiple dietary and lifestyle characteristics, individually, and especially collectively, were associated with circulating FiP concentrations [10]. In that study, circulating FiP concentrations-weighted lifestyle (four components: smoking, physical activity, BMI, alcohol intake), dietary (13 components), and total (lifestyle plus diet) oxidative balance scores (OBS) were created. Among men and women combined in the highest relative to the lowest tertiles of the lifestyle, diet, and total OBS, mean circulating FiP concentrations were, proportionately, 19.6% (P<0.001), 19.7% (P<0.001), and 29.7% (P<0.001) lower, respectively. Collectively, these results support 1) that lifestyle and diet contribute to systemic inflammation and oxidative stress, and 2) the findings of the present study.

Importantly, evolutionary-concordance scores have been associated with incident sporadic colorectal adenoma [37], incident colorectal cancer [36], and all-cause, all-cancer, and all-CVD mortality risks [38, 80]. In a pooled case-control study (n=771 cases, 1,990 controls) of incident, sporadic colorectal adenoma, higher (more evolutionary-concordant) EC diet and lifestyle scores were independently and jointly associated with lower risk for colorectal adenomas [37]. The multivariable-adjusted odds ratios (95% CI) comparing those in the highest relative to the lowest diet and lifestyle score quintiles were 0.84 (0.62, 1.12; P_{trend} 0.03) and 0.41 (95% CI 0.29, 0.59; P_{trend} <0.0001), respectively. In the prospective Iowa Women's Health Study (IWHS; n=35,221), the multivariable-adjusted hazards ratio (HR) for incident colorectal cancer comparing women in the highest relative to the lowest EC lifestyle score quintile was 0.66 (95% CI, 0.56–0.78; P_{trend}< 0.01) [36]. In the same IWHS population, the adjusted HRs and their 95% CIs for all-cause, all-cardiovascular disease, and all-cancer mortality among participants in the highest relative to the lowest EC lifestyle score quintile were, respectively, 0.52 (0.50, 0.55), 0.53 (0.49, 0.57), and 0.51 (0.46, 0.57) [36]. In the REGARDS cohort study (n=17,465), the multivariable-adjusted HRs (95% CI) for those in the highest relative to the lowest total evolutionary-concordance score quintiles for all-cause, all-CVD, and all-cancer mortality were, respectively, 0.45 (0.40, 0.50), 0.47 (0.39,

0.58), and 0.42 (0.34, 0.52) (all P_{trend} <0.01) [38]. Results similar to these have been found for associations of inflammation [70, 81, 82] and oxidative balance scores [53, 83, 84] with all-cause, all-cancer, and all-CVD mortality risks.

This study has several strengths. It is the first to report associations of an EC lifestyle score alone and combined with an EC diet score, with biomarkers of inflammation and oxidative stress in humans. Other strengths include the extensive data collection (including on potential confounding and effect modifying exposures), the pooled study design, and the use of validated biomarkers of inflammation and oxidative stress, using strict protocols and high measurement reliability.

This study also has several limitations. The sample size was relatively small, especially for stratified analyses; however, the findings for our primary analyses were statistically significant. The study population was limited to mostly White participants who were scheduled for outpatient, elective colonoscopies, thus potentially limiting the generalizability of our findings. The study design was cross-sectional, so the temporality of the associations could not be evaluated; however, all participants were in general good health, the questionnaires referred to past usual exposures, and our results were robust to potential confounding and effect modifying exposures. FFQs have known limitations, such as limited food choices and recall error by the respondents; however, since participants were not aware of their inflammation and oxidative stress measurements, these limitations likely were non-differential and resulted in attenuation of true associations. Finally, we had only single biomarkers of inflammation and oxidative stress, each with certain limitations; more comprehensive panels of biomarkers of inflammation and oxidative stress may have yielded more accurate representations of systemic inflammation and oxidative stress. However, on balance, the results from our study—the first to report associations of an EC lifestyle score alone and combined with an EC diet score, with biomarkers of inflammation and oxidative stress in humans—strongly support further investigation in larger, more diverse, more generally representative populations using more comprehensive panels of biomarkers of inflammation and oxidative stress.

In summary, our results along with previous literature, support that more evolutionary-concordant lifestyle patterns, alone and combined with more evolutionary-concordant diet patterns, may be associated with lower systemic inflammation and oxidative stress in humans. These results also may help explain previously reported associations of evolutionary-concordance pattern scores with colorectal neoplasms and all-cause and cause specific mortality. Finally, our results, taken with those of previous studies, support further investigation of associations of lifestyle, dietary, and total evolutionary-concordance pattern scores with systemic inflammation and oxidative stress and risk for relevant chronic diseases and premature mortality.

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Table 1. Selected characteristics of participants, by evolutionary-concordance lifestyle score[1] tertiles, in the pooled MAP cross-sectional studies

	Evolutionary-concordance lifestyle score tertiles[1]			
Characteristics[2]	**Low** (< 6) (*n* = 113)	**Medium** (6 – 7) (*n* = 242)	**High** (> 7) (*n* = 120)	***P***[3]
Demographics				
Age (yrs.)	55.2 ± 9.2	56.7 ± 8.6	57.7 ± 9.4	0.11
Male (%)	56.6	55.8	35.8	<0.01
White (%)	92.9	90.1	93.1	0.49
College degree or higher (%)	19.5	29.3	26.7	0.35
Prevalent colorectal adenoma (%)	52.4	39.2	36.9	0.04
Lifestyle				
Regular NSAID use[4] (%)	24.8	28.9	30.8	0.57
Regular multivitamin use[5] (%)	8.0	14.1	15.8	0.17
Current smoker (%)	61.1	20.3	0	<0.01
Physical activity[6] (MET-hrs./wk.)	141 ± 134	250 ± 159	333 ± 120	<0.01
Body mass index, kg/m^2 (%)				
< 25	11.5	24.5	68.3	<0.01
25 – 29.9	27.4	44.0	31.7	
> 30	61.1	31.5	0	
Current ethanol intake (g/1,000 kcal/day)	3.9 ± 7.9	2.9 ± 5.4	2.3 ± 4.8	0.13
Dietary intakes				
Total energy (kcal/day)	1,984 ± 775	1,946 ± 799	1,925 ± 839	0.85
Total fat (%kcal/day)	78.6 ± 30.0	75.2 ± 31.9	83.1 ± 29.3	0.07
Total[7] vitamin E (mg/1,000 kcal/day)	12.0 ± 8.7	14.8 ± 11.8	17.3 ± 12.7	0.18
Total[7] calcium (mg/1,000 kcal/day)	402 ± 217	459 ± 281	498 ± 300	0.03
Red & processed meats (servings/day)	1.3 ± 1.0	1.1 ± 0.8	1.1 ± 1.1	0.53
Fruits & vegetables (servings/day)	5.0 ± 3.1	5.2 ± 3.4	6.4 ± 4.2	<0.01
Evolutionary-concordance diet score[8]	40.2 ± 6.5	40.9 ± 6.6	43.1 ± 7.2	<0.01
Plasma biomarker concentrations[9]				
hsCRP (μg/mL)	6.5 ± 6.3	6.5 ± 16.7	3.6 ± 4.5	0.10
F_2-isoprostanes (ng/mL)	99.7 ± 47.9	89.3 ± 44.8	82.0 ± 62.0	0.06

Abbreviations: hsCRP, high sensitivity C-reactive protein; MAP, Markers of Adenomatous Polyps; MET, metabolic equivalents of task; NSAID, nonsteroidal anti-inflammatory drug

[1] Evolutionary-concordance lifestyle score comprises smoking, physical activity, and body mass index, as described in the text; a higher score indicates higher evolutionary concordance; unequal sample sizes across tertiles due to ranking ties
[2] Data presented as mean ± standard deviation unless otherwise specified
[3] *P*-values for categorical variables based on chi-square test, and for continuous variables (transformed by the natural logarithm to meet normality assumptions, when indicated) they were based on ANOVA
[4] Regularly take ≥ 4 times/wk.
[5] Regularly take ≥ 3 times/wk.
[6] Moderate + vigorous physical activity
[7] Total = dietary + supplemental
[8] Evolutionary-concordance diet score comprises 14 unweighted components as described in the text; a higher score indicates higher evolutionary concordance
[9] Sample sizes for F_2-isoprostanes smaller than those for hsCRP due to insufficient remaining plasma; $n = 468$ for hsCRP and $n = 358$ for F_2-isoprostanes

Table 2. Mean[a] plasma hsCRP and F_2-isoprostanes concentrations by evolutionary-concordance lifestyle score[b] categories; pooled MAP cross-sectional studies

EC lifestyle score categories	Biomarkers									
	hsCRP					F_2-isoprostanes				
	n[c]	Means (μg/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values	*n*[c,e]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values
Low (< 6)	110	3.9	(3.2, 4.8)	Ref.	-	83	90.8	(83.1, 99.3)	Ref.	-
Moderate (6 – 7)	241	3.1	(2.7, 3.6)	-21.4	0.06	182	81.5	(76.7, 86.7)	-10.2	0.05
High (> 7)	117	2.1	(1.8, 2.6)	-45.5	< 0.01	93	73.4	(67.4, 79.9)	-19.2	< 0.01

Abbreviations: CI, confidence interval; EC, evolutionary concordance; hsCRP, high sensitivity C-reactive protein; MAP, Markers of Adenomatous Polyps; Prop. diff., proportional difference

[a] Crude geometric means, 95% confidence intervals, and *P*-values from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises three equally-weighted lifestyle components (smoking, physical activity, and body mass index) as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] Sample sizes for F_2-isoprostanes smaller than those for hsCRP due to insufficient remaining plasma

Table 3. Mean[a] plasma hsCRP and F_2-isoprostanes concentrations by total evolutionary-concordance score[b] categories; pooled MAP cross-sectional studies

Total EC score categories	Biomarkers									
	hsCRP					F_2-isoprostanes				
	n[c]	Means (μg/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values	*n*[c,e]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values
Low (< 8)	126	4.0	(3.3, 4.8)	Ref.	-	99	89.9	(82.9, 97.5)	Ref.	-
Moderate (8 – 9)	205	3.3	(2.9, 3.9)	-16.3	0.15	148	83.4	(78.0, 89.1)	-7.3	0.16
High (> 9)	137	2.0	(1.6, 2.3)	-51.1	< 0.01	111	72.2	(66.8, 77.9)	-19.8	< 0.01

Abbreviations: CI, confidence interval; EC, evolutionary concordance; hsCRP, high sensitivity C-reactive protein; MAP, Markers of Adenomatous Polyps; Prop. diff., proportional difference

[a] Crude geometric means, 95% confidence intervals, and *P*-values from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises five equally-weighted components, including smoking, physical activity, body mass index, and an evolutionary-concordance diet score, as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] Sample sizes for F_2-isoprostanes smaller than those for hsCRP due to insufficient remaining plasma

Supplemental Table 1. Lifestyle, diet, and total evolutionary-concordance scores'[a] components and point assignments

Components of scores	Scores' components' point assignments	Possible score ranges
Lifestyle only		3 – 9
Physical activity,[b] MET-hrs/wk	Tertiles 1 – 3 scored 1 – 3 from lowest to highest	
Body mass index, kg/m^2	< 25.0: 3 points	
	25.0 – 29.99: 2 points	
	≥ 30.0: 1 point	
Currently smoke	No: 3 points	
	Yes: 1 point	
Diet only[c]		14 – 70
Calcium[d]	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Fish[e]	Five categories scored 1 – 5 from none to highest	
Fruit & vegetable diversity[f]	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Fruits	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Lean meats[g]	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Nuts	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Vegetables	Quintiles 1 – 5 scored 1 – 5 from lowest to highest	
Alcohol[h]	Five categories scored 5 – 1 from none to highest	
Baked goods & sweets[i]	Quintiles 1 – 5 scored 5 – 1 from lowest to highest	
Dairy foods	Quintiles 1 – 5 scored 5 – 1 from lowest to highest	
Grains & starches	Quintiles 1 – 5 scored 5 – 1 from lowest to highest	
Red & processed meats[j]	Quintiles 1 – 5 scored 5 – 1 from lowest to highest	
Sodium[k]	Quintiles 1 – 5 scored 5 – 1 from lowest to highest	
Sugar-sweetened beverages[e,l]	Five categories scored 5 – 1 from none to highest	
Total (lifestyle + diet)		4 – 12
Physical activity,[b] MET-hrs/wk	Tertiles 1 – 3 scored 1 – 3 from lowest to highest	
Body mass index, kg/m^2	< 25.0: 3 points	
	25.0 – 29.99: 2 points	
	≥ 30.0: 1 point	
Smoking status	No: 3 points	
	Yes: 1 point	
Diet score	Tertiles 1 – 3 scored 1 – 3 from lowest to highest	

Abbreviations: MET, metabolic equivalents of task

[a] Scores are sums of values given to the components listed in this table and as described in the text; higher components' values, and thus the scores, reflect higher evolutionary concordance
[b] Moderate + vigorous activity from modified Paffenbarger Physical Activity Questionnaires
[c] From Willett Food Frequency Questionnaires; all categories study- and sex-specific
[d] Calcium intake considered independently of non-calcium components of dairy foods, calculated as the residuals from the linear regression of total calcium (mg/day) on dairy food intake
[e] Alternative cut points used because distribution was not conducive to quintile categorization
[f] Fruit & vegetable diversity calculated by summing the total number of types of fruit and vegetables that were consumed > 1 – 3 times/month
[g] Lean meats include skinless chicken or turkey, lean and extra lean hamburger beef
[h] Alcohol points assigned based on five sex-specific categories; men: 0, 0 – < 14, 14, > 14 – 21, and > 21 drinks/week; women: 0, 0 – < 7, 7, > 7 – 14, and > 14 drinks/week
[i] Baked goods & sweets includes candy, cookies, brownies, donuts, cake, and pie
[j] Red & processed meats includes bacon, hotdogs, bologna, liver, regular hamburger beef, pork, and cold cuts
[k] Measured as mg/day
[l] Sugar-sweetened beverages measured in servings/day and points assigned based on five categories: 0, 0.5, 1, 2, > 2

Supplemental Table 2. Mean[a] plasma hsCRP and F_2-isoprostanes concentrations by evolutionary-concordance diet score[b] tertiles; pooled MAP cross-sectional studies

	Biomarkers									
EC diet score categories	**hsCRP**					**F_2-isoprostanes**				
	n[c]	Means (μg/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values	*n*[c,e]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	*P*-values
Low (< 38)	147	3.1	(2.6, 3.7)	Ref.	-	107	86.1	(79.5, 93.3)	Ref.	-
Moderate (38 – 45)	161	3.4	(2.8, 4.0)	9.1	0.49	117	83.2	(77.9, 90.6)	-3.4	0.54
High (> 45)	160	2.6	(2.2, 3.1)	-14.6	0.22	134	76.6	(71.4, 82.2)	-11.1	0.03

Abbreviations: CI, confidence interval; EC, evolutionary concordance; hsCRP, high sensitivity C-reactive protein; MAP, Markers of Adenomatous Polyps; Prop. diff., proportional difference

[a] Crude geometric means, 95% confidence intervals, and *P*-values from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises 14 equally-weighted dietary components as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] Sample sizes for F_2-isoprostanes smaller than those for hsCRP due to insufficient remaining plasma

Supplemental Table 3. Mean[a] plasma hsCRP concentrations across evolutionary-concordance lifestyle score[b] categories, stratified by selected participant characteristics; pooled MAP cross-sectional study

Stratification variables and categories	Evolutionary-concordance lifestyle score categories													
	Low (< 6)				Medium (6 – 7)				High (> 7)					
	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	*P*[e]	$P_{interaction}$[f]
Sex														
Male	62	3.0	(2.3, 3.9)	Ref.	134	2.3	(1.9, 2.7)	-24.2	41	1.7	(1.3, 2.4)	-42.3	0.01	
Female	62	5.6	(4.1, 7.7)	Ref.	60	4.5	(3.6, 5.5)	-20.6	109	2.4	(19, 3.0)	-58.0	< 0.01	0.33
Regular NSAID use[g]														
No	82	3.5	(2.8, 4.5)	Ref.	172	2.7	(2.3, 3.2)	-23.1	81	2.1	(1.7, 2.8)	-39.3	< 0.01	
Yes	28	5.4	(3.8, 7.6)	Ref.	69	4.1	(3.3, 5.1)	-23.7	36	2.1	(1.5, 2.8)	-61.8	< 0.01	0.25
Regular multivitamin use[h]														
No	102	3.7	(3.0, 4.6)	Ref.	208	3.0	(2.6, 3.5)	-18.3	100	2.1	(1.7, 2.6)	-43.3	< 0.01	
Yes	8	8.0	(3.6, 17.7)	Ref.	33	3.2	(2.2, 4.7)	-60.2	17	2.2	(1.3, 3.7)	-73.0	< 0.01	0.34
Colorectal adenoma[i]														
Yes	52	4.3	(3.2, 5.7)	Ref.	85	2.9	(2.3, 3.6)	-21.3	38	2.8	(2.0, 3.9)	-24.4	0.01	
No	58	3.7	(2.8, 4.9)	Ref.	156	3.2	(2.7, 3.8)	-13.8	79	1.9	(1.5, 2.4)	-49.2	0.06	0.16

Abbreviations: MAP, Markers of Adenomatous Polyps; NSAID, nonteroidal anti-inflammatory drug; Prop. diff., proportional difference

[b] Comprises three equally-weighted lifestyle components (smoking, physical activity, and body mass index) as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as: (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] *P* for crude mean hsCRP differences across evolutionary-concordance score categories within stratum, from generalized linear models; multivariable adjustment made no appreciable difference in the results

[f] *P* for interaction across strata

[g] Defined as four or more times a week

[h] Defined as three or more times a week

[i] First ever diagnosed colorectal adenoma at time of colonoscopy visit

Supplemental Table 4. Mean[a] plasma F_2-isoprostanes concentrations across evolutionary-concordance lifestyle score[b] categories, stratified by selected participant characteristics; pooled MAP cross-sectional study

Stratification variables and categories	Evolutionary-concordance lifestyle score categories													
	Low (< 6)				Medium (6 – 7)				High (> 7)					
	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	P[e]	$P_{interaction}$[f]
Sex														
Male	43	74	(68, 81)	Ref.	101	72	(68, 77)	-2.5	30	65	(58, 72)	-12.7	0.05	
Female	40	113	(98, 130)	Ref.	81	95	(86, 105)	-16.1	63	78	(70, 87)	-31.1	<0.01	0.13
Regular NSAID use[g]														
No	63	90	(81, 100)	Ref.	123	81	(75, 87)	-10.2	66	76	(69, 84)	-15.9	0.02	
Yes	20	93	(78, 110)	Ref.	59	83	(75, 91)	-10.9	27	68	(58, 78)	-27.3	<0.01	0.44
Regular multivitamin use[h]														
No	75	92	(84, 102)	Ref.	157	83	(78, 89)	-9.8	119	76	(69, 83)	-17.6	<0.01	
Yes	8	78	(63, 97)	Ref.	25	72	(63, 81)	-8.2	15	61	(52, 71)	-22.0	0.07	0.88
Colorectal adenoma[i]														
Yes	43	93	(82, 105)	Ref.	70	83	(75, 92)	-10.3	31	68	(59, 79)	-26.0	0.06	
No	40	89	(78, 101)	Ref.	112	81	(74, 87)	-9.4	62	76	(69, 84)	-14.7	<0.01	0.43

Abbreviations: MAP, Markers of Adenomatous Polyps; NSAID, nonteroidal anti-inflammatory drug; Prop. diff., proportional difference

[a] Crude geometric means and 95% confidence intervals from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises three equally-weighted lifestyle components (smoking, physical activity, and body mass index) as described in the text; a higher score indicates higher e

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as: (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] P for crude mean F_2-isoprostanes differences across evolutionary-concordance score categories within stratum, from generalized linear models; multivariable adjustment made no appreciable difference in the results

[f] P for interaction across strata

[g] Defined as four or more times a week

[h] Defined as three or more times a week

[i] First ever diagnosed colorectal adenoma at time of colonoscopy visit

Supplemental Table 5. Mean[a] plasma hsCRP concentrations across total evolutionary-concordance score[b] categories, stratified by selected participant characteristics; pooled MAP cross-sectional study

Stratification variables and categories	Total evolutionary-concordance score categories													
	Low (< 8)				Medium (8 – 9)				High (> 9)					
	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (µg/mL)	(95% CI)	Prop. diff.[d] (%)	*P*[e]	$P_{interaction}$[f]
Sex														
Male	68	2.8	(2.2, 3.6)	Ref.	116	2.6	(2.1, 3.1)	-7.7	53	1.4	(1.1, 1.9)	-48.2	< 0.01	
Female	58	6.0	(4.5, 7.9)	Ref.	89	4.5	(3.6, 5.7)	-23.8	84	2.3	(1.8, 2.9)	-61.3	< 0.01	0.52
Regular NSAID use[g]														
No	98	3.6	(2.9, 4.5)	Ref.	150	2.8	(2.3, 3.4)	-21.5	87	1.9	(1.5, 2.4)	-46.4	< 0.01	
Yes	28	5.6	(4.1, 7.7)	Ref.	55	5.1	(4.0, 6.4)	-9.5	50	1.9	(1.5, 2.5)	-65.6	< 0.01	0.06
Regular multivitamin use[h]														
No	118	3.9	(3.2, 4.7)	Ref.	182	3.2	(2.7, 3.7)	-17.3	110	1.9	(1.5, 2.3)	-51.4	< 0.01	
Yes	8	5.8	(2.6, 12.8)	Ref.	23	4.2	(2.7, 6.8)	-26.4	27	2.1	(1.4, 3.3)	-62.8	0.03	0.82
Colorectal adenoma[i]														
Yes	52	4.0	(3.0, 5.4)	Ref.	75	3.4	(2.7, 4.3)	-16.3	48	2.3	(1.7,3.1)	-43.5	< 0.01	
No	74	3.9	(3.0, 5.0)	Ref.	130	3.2	(2.7, 3.9)	-16.7	89	1.8	(1.4, 2.2)	-55.0	< 0.01	0.62

Abbreviations: hsCRP, high sensitivity C-reactive protein; MAP, Markers of Adenomatous Polyps; NSAID, nonteroidal anti-inflammatory drug; Prop. diff., proportional difference

[a] Crude geometric means and 95% confidence intervals from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises five equally-weighted components, including smoking, physical activity, body mass index, and an evolutionary-concordance diet score, as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as: (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] *P* for crude mean hsCRP differences across evolutionary-concordance score categories within stratum, from generalized linear models; multivariable adjustment made no appreciable difference in the results

[f] *P* for interaction across strata

[g] Defined as four or more times a week

[h] Defined as three or more times a week

[i] First ever diagnosed colorectal adenoma at time of colonoscopy visit

Supplemental Table 6. Mean[a] plasma F_2-isoprostanes concentrations across total evolutionary-concordance score[b] categories, stratified by selected participant characteristics; pooled MAP cross-sectional study

Stratification variables and categories	Total evolutionary-concordance score categories													
	Low (< 8)				Medium (8 – 9)				High (> 9)				*P*[e]	$P_{interaction}$[f]
	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)	n[c]	Means (ng/mL)	(95% CI)	Prop. diff.[d] (%)		
Sex														
Male	50	70	(65, 76)	Ref.	82	75	(71, 80)	7.4	42	65	(60, 71)	-7.1	0.23	
Female	49	116	(102, 131)	Ref.	66	94	(85, 105)	-18.5	69	77	(69, 85)	-33.9	< 0.01	< 0.01
Regular NSAID use[g]														
No	76	88	(80, 96)	Ref.	103	82	(76, 89)	-5.9	73	76	(69, 83)	-13.8	0.03	
Yes	23	98	(84, 114)	Ref.	45	86	(77, 95)	-12.8	38	66	(59, 74)	-32.8	< 0.01	0.11
Regular multivitamin use[h]														
No	90	91	(83, 99)	Ref.	131	85	(79, 91)	-6.4	89	75	(69, 82)	-17.1	< 0.01	
Yes	9	84	(69, 102)	Ref.	17	73	(64, 84)	-12.9	22	61	(54, 69)	-27.3	< 0.01	0.74
Colorectal adenoma[i]														
Yes	44	98	(87, 111)	Ref.	59	82	(73 ,91)	-16.6	41	69	(61, 78)	-29.3	< 0.01	
No	55	84	(75, 94)	Ref.	89	84	(77, 92)	0.0	70	67	(67, 81)	-12.1	0.08	0.12

Abbreviations: MAP, Markers of Adenomatous Polyps; NSAID, nonteroidal anti-inflammatory drug; Prop. diff., proportional difference

[a] Crude geometric means and 95% confidence intervals from general linear models; multivariable adjustment made no appreciable difference in the results

[b] Comprises five equally-weighted components, including smoking, physical activity, body mass index, and an evolutionary-concordance diet score, as described in the text; a higher score indicates higher evolutionary concordance

[c] Unequal sample sizes across categories due to ranking ties

[d] Proportional difference calculated as: (comparison group mean - reference group mean) / (reference group mean) x 100%

[e] *P* for crude mean F_2-isoprostanes differences across evolutionary-concordance score categories within stratum, from generalized linear models; multivariable adjustment made no appreciable difference in the results

[f] *P* for interaction across strata

[g] Defined as four or more times a week

[h] Defined as three or more times a week

[i] First ever diagnosed colorectal adenoma at time of colonoscopy visit

Supplemental Table 7. Components of the evolutionary-concordance lifestyle score and their biological plausibility

Components of lifestyle score	Biological plausibility
Prooxidants	
BMI	Associated with increased oxidative stress markers, impaired redox balance, and increased lipid peroxidation; source of free fatty acids, can increase free radicle-RONS production which leads to oxidative stress [17, 49, 85-87].
Currently smoke	Producer of free radicals, associated with increase in blood/tissue markers of oxidative stress, carcinogenic components also cause cytotoxicity and inflammation [64, 88, 89].
Antioxidants	
Physical activity	Intense exercise increases RONS production, but regular exercise can result in increased adaptive responses to oxidative stress through activated cellular antioxidant signaling systems and enhancing expression of antioxidant enzymes [90, 91]

Abbreviations: BMI, body mass index; RONS, reactive oxygen and nitrogen species

Chapter 3: Public Health Implications and Future Research Work

In this study about evolutionary concordance (EC) lifestyle and diet pattern scores in a cross-sectional study, we found that EC lifestyle and total EC scores were inversely associated with mean hsCRP and FiP concentrations, which suggests that closer adherence to more evolutionary-concordant diets and lifestyles may decrease systemic inflammation and oxidative stress. Previous studies found that these diet and lifestyle scores, separately and combined, were inversely associated with risk for colorectal neoplasms and all-cause, all-cardiovascular disease, and all cancer mortality. The findings of our present study support that these associations may have been mediated, at least in part, through the effects of more evolutionary-concordant diets and lifestyles on inflammation and oxidative stress. To advance these lines of research, future research work should include 1) larger observational studies (cross-sectional and prospective) of the EC diet and lifestyle pattern scores in more diverse, representative study populations and using panels of biomarkers of inflammation and oxidative stress, and 2) randomized controlled trials.