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Oxidative Balance and Colorectal Neoplasms

By

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Doctor of Philosophy

Epidemiology

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Oxidative Balance and Colorectal Neoplasms

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Abstract

Oxidative Balance and Colorectal Neoplasms

by Chiranjeev Dash

Colorectal cancer (CRC), a multifactorial disease, is the second leading cause of cancer deaths in the US. Preventive approaches aimed at specific, known pathways of CRC causation, such as oxidative stress, might be effective in reducing CRC morbidity and mortality. Although oxidative stress is implicated in colorectal carcinogenesis, human studies that evaluated associations of individual pro- and antioxidants with CRC have been inconclusive. The goals for this dissertation were to develop, compare and evaluate comprehensive "oxidative balance scores (OBS)", comprised of individual dietary, and environmental exposures that are known to affect physiologic oxidative processes, and to investigate the association of oxidative balance with risk of colorectal neoplasms and biomarkers of oxidative stress.

Four OBS were created to reflect combined summary measures of dietary and non-dietary anti- and pro-oxidant exposures. A higher score represents a predominance of anti- over pro-oxidant exposures. In a pooled analysis of three colonoscopy-based case-control studies, a substantial, statistically significant lower risk of incident, sporadic colorectal adenomas was found with higher levels of OBS. The results also suggested a dose dependent decrease in F2-isoprostanes, a sensitive and specific marker of oxidative stress *in vivo*, with increasing levels of OBS, providing support for OBS as a valid measure of oxidative balance. In a large prospective cohort study, higher OBS were associated with lower risk of CRC. In a biomarker-based pooled analysis of two case-control studies of incident, sporadic colorectal adenomas, results suggested that lipid peroxidation, as indicated by circulating F2-isoprostanes, may be 1) positively associated with risk for incident, sporadic colorectal adenoma in women, but not men, and 2) inversely associated with antioxidant micronutrient exposures, with some differences according to sex.

In conclusion, I introduced three novel methods for constructing OBS in this dissertation. Results of this dissertation support the use of pathway exposure scores to measure complex multicomponent exposures, provide evidence to suggest that oxidative balance is strongly associated with colorectal adenoma and cancer incidence, and support further investigations of oxidative balance with other chronic diseases. My dissertation also provides a framework for the development of oxidative balance-based interventions to reduce colorectal cancer risk.

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CHAPTER 1. BACKGROUND

Background

Epidemiology of Colorectal Cancer

Colorectal cancer (CRC) is the third leading cause of cancer death among men and women in the US [1]. In 2014, it is estimated that 136,830 new cases of CRC will be diagnosed and 50,310 deaths from the disease will be recorded in the US [1]. Since 2000, incidence and mortality rates from CRC have steadily decreased in the US, primarily due to prevention and early detection of CRC through screening methods [2]. The lifetime risk of CRC is estimated to be 5% for Americans [1].

It is well established that CRC risk and risk of death from CRC increase with age, with more than 90% of new cases and deaths occurring after the age of 50 [3]. Overall, median age at diagnosis is lower for rectal than colon cancers in both men and women [2]. CRC incidence and mortality rates also show gender-related differences. Men are 30% to 40% more likely to be diagnosed with or die from CRC [1]. Although the exact cause(s) for higher risk of CRC in men is unknown, it is believed that gender-related differences in exposures to hormones and other CRC risk factors might be implicated [4]. Black men and women have higher rates of colorectal cancer than other races/ethnicities [5]. During 2006-2010, CRC incidence rates in Blacks were 25% higher than Whites [1]. Blacks also bear the burden for the largest CRC mortality disparity with Blacks having a 50% higher mortality rate than Whites [1].

Rates of CRC incidence have been steadily decreasing since the mid-1980s with steeper declines in the last decade [2]. From 2008 to 2010 rates of new cases of CRC decreased by 4% per year for both sexes [2]. However, a disparity exists in the rates of decrease of incidence trends for CRC with Blacks having much slower rates of decrease than Whites [1]. The decreasing trends for CRC incidence and the disparity in trends across races are largely attributable to detection and removal of adenomatous polyps and disparities in access to and utilization of CRC screening services, respectively [6]. Although CRC incidence has been decreasing for older adults (above 50 years of age), rates have been increasing in younger adults, especially for diseases of the distal colon and rectum [1]. Reasons for this trend are unknown but unfavorable changes in dietary and physical activity behaviors and the increasing obesity prevalence in younger adults are thought to be partly responsible for this trend [7]. CRC mortality rates have also been steadily but modestly decreasing in both men and women primarily attributable to CRC screening and better treatments [6]. As with incidence rates, mortality rates have declined steeply in recent years in both genders [1]. However, rates of decline in recent years have accentuated an increasing disparity between survival among White patients and other racial groups, especially Blacks [8]. The disparity in mortality trends between Whites and other racial and ethnic groups are believed to be related to higher screening rates and stage-specific survival rates among Whites [8].

Geographically, CRC rates vary widely in the world and even within the United States [9]. CRC is common in the US, Australia, New Zealand, Western Europe, and, more recently, in some parts of Asia including Japan, Singapore, and South Korea [10]. Conversely, other parts in Asia and most African countries have very low rates of CRC

[9]. Ecologic studies and studies among migrants to developed countries, where CRC is more common, suggest the influence of a Western lifestyle, primarily a Western dietary pattern, as the primary cause of this geographic disparity, although, given the design of such studies a causal association cannot be confirmed [11, 12]. However, evidence on trends of increasing CRC incidence being correlated with increased Westernization of diet and lifestyle behaviors in countries that have traditionally had lower rates of CRC (e.g. Singapore) supports the role of diet and other lifestyles in CRC risk [10, 13]. CRC rates also vary widely in the US. Rates of CRC are highest in the Midwest and Southern states and lowest in the Northeast [14, 15]. As with explanations for the change in incidence trends and disparity in CRC trends, differences in risk patterns, and availability and utilization of CRC screening and treatment are thought to be responsible [14].

Even with improvements in screening and treatment, 5-year survival rates of CRC have averaged about 65% since the mid-1990s [2]. Racial/ethnic and geographic disparities in survival have increased primarily associated with socioeconomic disparities that lead to disparities in early diagnosis and treatment rates for localized cancer [16-18]. Further decreases in CRC incidence and mortality and reduction of health disparities in CRC might be achieved by changing risk factor patterns in the population, especially by modification of dietary and non-dietary lifestyle behaviors.

Most colorectal cancer cases are sporadic non-familial cancers whose risk is primarily related to diet and other lifestyle associated factors [19, 20]. Less than 10% of CRCs are hereditary or familial and the following well-described syndromes commonly

account for most of these CRCs: familial adenomatous polyposis (FAP), hereditary nonpolyposis CRC (HNPCC), Peutz-Jeghers syndrome and juvenile polyposis [21].

Most sporadic CRCs begin as noncancerous growths in the colorectal epithelium, called adenomatous polyps or adenomas, and develop slowly over a period of 10 to 20 years [22, 23]. Lifetime probability of developing an adenoma in the US is estimated to be 30-50% [24]. Although all adenomas are considered precancerous and may develop into CRCs, only a small fraction (< 10%) progress to invasive cancer [25, 26]. Various factors, such as size, appearance, and dysplastic features, affect the likelihood of an adenoma developing into a cancerous growth [26]. Adenomas greater than 10 mm in size, flat or sessile adenomas as opposed to pedunculated ones, and adenomas with villous components are more likely to progress to invasive cancer [27]. Recent evidence suggests that some non-neoplastic polyps that have previously not been considered precancerous, such as hyperplastic and serrated polyps might lead to CRC [28].

Early detection and prevention of CRC through screening methods relies on the identification of a precancerous condition (adenoma), a slow course of growth from an adenoma to CRC, and removal of the adenoma thus preventing progression to CRC in that particular growth [22]. Availability of a screening method, such as colonoscopy that can not only detect precancerous adenomas but can also remove them has had a significant impact on reducing CRC incidence [29]. However, colonoscopy is an expensive procedure that requires considerable expertise and can have serious side-effects [29]. Diagnosis of an adenoma requires more frequent colonoscopies (every 3-5 years) leading to higher costs and side effects [29]. Additionally, colonoscopy screening

rates have plateaued in recent years and screening has only had a modest effect on CRC mortality [30]. Approaches that identify CRC pathways related to the most common CRC risk factors, i.e., diet and other lifestyle factors, and can target biomarkers of risk on such pathways will allow development of primary prevention interventions and will aid in reducing the risk of CRC. Measurement and modification of risk pathways, as has been demonstrated for cardiovascular diseases, should prove to be effective in reducing CRC incidence and mortality [31].

Risk Factors for Colorectal Cancer

Risk factors for CRCs have been previously reviewed in detail [10] and well-established risk factors for sporadic CRCs are summarized below:

Table 1.1. Risk factors for colorectal cancers

Factors that increase risk
Family history of CRC in first degree relative, more than 1 relative, or relative with diagnosis before age 45
Inflammatory bowel diseases : Crohn disease and Ulcerative colitis
Diabetes
Heavy alcohol consumption
Obesity
Red and processed meat consumption
Smoking
Factors that decrease risk
Physical activity
Dairy consumption
Fruit and vegetable consumption
High dietary fiber consumption
Use of NSAID medications
Use of postmenopausal hormones in women

Heredity and family history

Individuals with a first-degree relative diagnosed with CRC have a 2-4 fold higher risk of developing CRC compared to those with no family history [9]. The risk increases

if multiple family members have had CRC or if CRC was diagnosed at a younger age (< 45 years) in the relative [32, 33]. Prevalence of a family history of CRC in a close relative among CRC patients is about 20% [34]. As mentioned previously, about 5% of CRCs are considered to be hereditary with a well-defined genetic syndrome that causes the CRC [34]. The most common syndrome is HNPCC or the Lynch syndrome. It is estimated that 1 in 35 CRC patients has Lynch syndrome [34]. Patients with Lynch syndrome get CRC at a younger age than those with sporadic CRC and are also at higher risk for other cancers such as, endometrial, stomach, and ovarian cancers [34]. FAP is the second most common cause of hereditary CRCs and is characterized by development of hundreds of colorectal polyps at young ages [35]. Lifetime risk of CRC without intervention reaches about 100% by age 40 for most patients with FAP [36].

Personal medical history

Having a history of adenomas increases the risk for colorectal cancer and the risk is higher for larger and multiple (≥ 3) adenomas, flat adenomas, and adenomas with villous and dysplastic features [37]. Although a family history of adenomas is also thought to increase CRC risk the evidence is less convincing for this risk factor [38].

Patients with chronic inflammatory bowel diseases, such as Crohn disease and ulcerative colitis are at higher risk of CRC due to the long term and high levels of inflammation in the colon in these conditions [39]. Risk in inflammatory bowel disorders increases with the severity and duration of the disease [39].

Evidence from multiple studies suggests that diabetes, particularly type II diabetes, is associated with increased risk of CRC [40, 41]. Recent studies have suggested that this association is stronger in males than females with many analyses suggesting a null association between diabetes and CRC among females [41, 42]. Studies adjusting for common risk factors of CRC and diabetes (obesity, physical inactivity, and diet) have reported that diabetes is an independent risk factor of CRC [42, 43]. Diabetic patients on insulin therapy have higher risk of CRC than non-diabetics and patients not on insulin [41, 42, 44]. Conversely, use of metformin, an oral anti-diabetic medication, has shown to reduce the risk of CRC in some studies [44].

Behavioral risk factors

Physical activity

Physical activity is a risk factor that is one of the most consistently reported to be associated with lower risk of CRC. Compared to people who report the least amount of physical activity, regularly active people have a 25% lower risk of CRC [45]. Evidence from large epidemiologic studies also suggests that the association of CRC risk and exercise might be dose-dependent [45], that both occupational and recreational activity might be associated with lower CRC risk [46], and that sedentary people who increase their physical activity later in life might also reduce their CRC risk [46].

Overweight and obesity

Overweight and obesity are independent risk factors for colon cancer among both men and women but for rectal cancer only among men [47]. Associations of body mass

index (BMI) and waist circumference with CRC risk are stronger among men than women [47]. Abdominal obesity seems to be a more sensitive marker of CRC risk than BMI [48, 49].

Diet

It is well accepted that diet plays an important role in colorectal carcinogenesis. However, research is still ongoing on the role of specific dietary components in CRC risk. Current evidence suggests that the following components have a role to play in CRC risk:

- High consumption of red meats, especially cooked at high temperatures, is fairly consistently associated with a higher risk of colorectal cancer [50]. Processed meat consumption has also been associated with CRC risk possibly due to the high level of nitrite additives [51].
- Dietary fiber, cereal fiber, and whole grain intakes are associated with lower risk of CRC [52].
- Evidence suggests that moderate, relative to low, fruit and vegetable consumption may provide some protection against colon but not rectal cancer [53, 54]. However, it is unclear which components in fruits and vegetables are primarily responsible for this association. It is also unclear whether very high consumption may provide any additional benefit in reducing CRC risk [54].
- Higher intakes of dairy products, milk, and calcium have been reported to be inversely associated with CRC risk [55].

- Higher blood levels of vitamin D are associated with moderately lower risk for CRC [56].
- Dietary folate intake has been reported to be inversely associated CRC risk but that the timing of high folate intake may determine the risk benefit achieved [57]. It has been hypothesized that high folate intake after cancer initiation might promote cancer growth, leading to concern about mandatory folate fortification in flour and cereals [58, 59]. Data from a folic acid supplementation trial reported increased adenoma recurrence and CRC incidence in the intervention group compared to the placebo control [59]. However, a recent study did not find any evidence of increased CRC risk with folic acid fortification and confirmed the inverse associations between total dietary folate and CRC risk seen in previous studies [60].

Although it is hypothesized that the oxidative stress pathway and inflammation might be important mediators of the diet-CRC association, epidemiologic studies have been unable to find consistent associations between components of these pathways (e.g., dietary antioxidants) and CRC risk, with the exception of calcium and vitamin D [61].

Smoking

There is considerable evidence to suggest that tobacco smoking is a risk factor for CRC [62-64]. Recent evidence suggest that the association might be stronger for rectal than colon cancer and also for certain molecular subtypes of CRC [64-66].

Alcohol

Compared to people who drink less than a drink a day, those who consume, on average, 2-4 drinks a day or more appear to have about 25% or higher risk of CRC [67, 68]. Moderate and heavy alcohol drinking are both considered risk factors for CRC [69].

Medications

Long-term, regular use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) lowers risk of CRC [70, 71]. However, use of these drugs for CRC prevention in the asymptomatic population is currently not recommended because of the risk of gastrointestinal bleeding from non-selective NSAIDs and the higher rates of cardiovascular side effects from selective COX-2 inhibitors seen in recent trials [1].

Decreased risk of CRC is seen in women who are long-term users of postmenopausal hormone therapy [72, 73]. However, postmenopausal hormones are not currently recommended to all women because of concerns of elevated breast cancer and cardiovascular disease risk [72, 73]. Studies have also shown that oral contraceptives [74] and oral bisphosphonates[75] might also reduce CRC risk but the risk-benefit information for CRC prevention in the general population has not been tested for these medications.

Colorectal adenoma has the same risk factors as colorectal cancer but the strengths of the associations between risk factors and colorectal neoplasia is weaker for adenomas than for CRC [76].

Colorectal carcinogenesis

CRC develops through a stepwise accumulation of genetic and epigenetic changes that lead to the transformation of the normal mucosa into adenoma and then to invasive cancer [77]. This normal mucosa to adenoma to carcinoma sequence has been traditionally recognized as the most accepted model of CRC development [78]. However, identification of different molecular pathways has since demonstrated the heterogeneous nature of CRC [77].

Fearon and Vogelstein proposed the first model for colorectal carcinogenesis that posited that: colorectal neoplasia arises as a result of the mutational activation of oncogenes coupled with the inactivation of tumor suppressor genes; "genomic instability" exemplified by mutations in at least 4 to 5 genes are required for CRC to develop; and finally, accumulation of the genetic alterations ("multiple hits") but not necessarily the order of those mutations determines tumor behavior [79]. Although the Fearon and Vogelstein model is still relevant to understanding CRC development, discovery of the Microsatellite Instability (MSI) caused by defective Mismatch Repair (MMR) genes in about 15% of sporadic CRC, and the discovery of the role of hypermethylation in silencing of gene function in the CpG Island Methylator Phenotype (CIMP) tumors have highlighted the complex molecular nature of CRC [77]. Jass *et al.* describe five molecular CRC subtypes based on the presence of MSA and CIMP, each with a different molecular profile and pathological features [80]:

1. CIMP high/MSI high (12% of CRC); originates in serrated adenomas and is characterized by BRAF mutation and MLH1 methylation.

2. CIMP high/MSI low or microsatellite stable (8%); originates in serrated adenomas and is characterized by BRAF mutation and methylation of multiple genes.
3. CIMP low/MSI low or microsatellite stable (20%); originates in tubular, tubulovillous, or serrated adenomas and is characterized by chromosomal instability (CIN), *K-ras* mutation, and *MGMT* methylation.
4. CIMP negative/microsatellite stable (57%); originates in traditional adenoma and is characterized by CIN.
5. Hereditary Non Polyposis Colorectal Cancer (HNPCC); CIMP negative/MSI high; negative for *BRAF* mutations.

Three distinct molecular pathways have been recognized for CRC: Chromosomal Instability (CIN) pathway, MMR pathway and the CIMP pathway [81, 82]. These pathways may co-exist in CRC tumors and are not mutually exclusive [81]. In addition to the pathways mentioned above, microRNA and inflammation pathways have also been reported to be involved in colorectal carcinogenesis [83]. The sections below summarize these pathways.

Chromosomal instability pathway

This is the most common cause of genomic instability in CRC and accounts for about 70% of sporadic CRC [83, 84]. CIN results from defects in chromosome segregation, telomere dysfunction, or defects in DNA damage response mechanisms [84]. This can lead to aneuploidy, chromosomal amplifications, and high frequency of loss of heterozygosity (LOH) [84]. CIN is characterized by broad amplifications (e.g.,

chromosomes 7 and 8q) and broad deletions (e.g. chromosomes 1, 4) on chromosomes [77]. In addition, more focal gains or losses are found in regions containing important cancer genes, e.g., *VEGF, MYC, MET,* and others [85]. In addition to these karyotypic abnormalities we also see accumulation of mutations in several oncogenes and tumor suppressor genes. The most common single gene mutations are in the *Adenomatous Polyposis Coli* (*APC)* and *K-ras* genes [86-88].

Somatic *APC* mutations are seen in 60-80% of CRCs and in a large percentage of adenomas, indicating that this is an early event in CRC carcinogenesis [88]. *APC* is described as the gatekeeper of cellular proliferation in the colon and belongs to the Wnt pathway, which plays an important role in intestinal epithelial renewal [89]. APC binds to β-catenin and induces its degradation [90]. Loss of APC function results in accumulation of cytoplasmic β-catenin, leading to nuclear translocation and binding of β-catenin to T-cell factor (TCF)/lymphoid enhancer factor (LEF) [90]. This induces dysregulation of multiple downstream events such as cell cycle progression (through effects on c-myc and cyclin D1), cell proliferation, angiogenesis, and apoptosis [90]. Therefore, the Wnt pathway is important for both initiation and progression of CRC.

The *K-ras* proto-oncogene is mutated in about 30-60% of CRC and large adenomas [86, 87]. It is believed that activated K-ras may play an important role in transforming adenoma to carcinoma through activation of multiple downstream targets such as *BCL-2* and *MMP1* [91].

Other major alterations in the CIN pathway are loss of the 8p allele (50% of CRC) associated with advanced stage disease and increased metastatic potential [92]; loss of

17p allele (75% of CRC but not in adenoma) that is associated with *p53* mutations and is thought to mediate the transition of adenoma to carcinoma [93]; and 18q LOH (50-70% of CRC) which is marker of poor prognosis in stage II and III CRC [94, 95].

Microsatellite instability pathway

Microsatellites are short repeat nucleotide sequences that are prone to errors or mismatches during replication because of their repetitive nature. Instability of microsatellites is primarily due to the inability of the DNA MMR system to correct these mismatches [96]. Germline mutations in MMR genes result in HNPCC while somatic mutations in, and hypermethylation silencing of these genes accounts for about 15% of sporadic CRC [96]. CRCs with MSI features are more common in older women and are commonly proximal tumors [96].

CpG Island Methylator Phenotype or "serrated" pathway

CIMP refers to concomitant hypermethylation of multiple genes involved in colorectal carcinogenesis [97]. CIMP-high CRC accounts for 15-20% of sporadic CRC [98]. The precursor lesions for CIMP-high CRC are sessile serrated adenomas which account for 9% of colorectal polyps and are difficult to identify during colonoscopies [99, 100]. These adenomas are frequently proximal, and exhibit *BRAF* mutations and extensive DNA methylation [100].

Oxidative stress and DNA damage through reactive oxygen and nitrogen species (RONS) have been reported to be involved in or interacting with all the CRC pathways mentioned above [91, 101-103].

Oxidative Stress, Oxidative Balance and Colorectal Carcinogenesis

The concept of "oxidative stress" and its role in human diseases can be traced back to the research on ionizing radiation, free radicals, and molecular oxygen and the potential effects of such processes on human aging in the 1950s [104, 105]. Publication of important papers in the late 1960s and early 1970s reported that cells could produce superoxide free radicals through normal metabolic pathways and that enzymes such as the superoxide dismutases (SOD) had evolved to protect aerobic organisms from the adverse effects of these cellular free radicals [106, 107]. The term "oxidative stress" began to be used frequently in the 1970s, primarily to refer to the health effects of free radicals. It has since been recognized that that reactive oxygen and nitrogen species (RONS) are the primary sources of free radicals involved in human health [108-111]. Recent research has suggested that oxidative stress is not synonymous with "free radical damage" and can include mechanisms such as disruption of thiol-redox circuits (the "redox hypothesis') that can affect cell signaling pathways without involvement of free radicals [112].

Superoxide anion is considered the primary reactive oxygen species (ROS) and nitric oxide the primary reactive nitrogen species [113, 114]. RONS can be produced by both endogenous and exogenous sources. Primary endogenous sources are mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation [114]. Exogenous sources of RONS are ionizing radiation, various drugs, dietary components, and other lifestyle exposures such as smoking and alcohol intake [115].

RONS can be both beneficial and harmful to humans [116]. Beneficial effects of RONS include involvement in normal physiologic processes such as cell signaling systems and induction of antioxidant defense systems at low doses [116]. Conversely, RONS can mediate lipid, protein, and nucleic acid damage at high doses (oxidative stress) [116]. In healthy humans the harmful effects of RONS are balanced by enzymatic and non-enzymatic antioxidant defenses and oxidative DNA repair mechanisms thus leading to the state of "oxidative balance" [117, 118]. Oxidative stress is said to occur when the balance of antioxidants to pro-oxidants shifts in the favor of the latter. Oxidative stress has been reported to play a role in over 200 human diseases including chronic diseases such as diabetes, cardiovascular diseases, and CRC and other cancers [115].

Damage to DNA, lipids, and proteins through RONS in chronic oxidative stress can lead to initiation, promotion, and progression of colorectal carcinogenesis [110, 119]. RONS induced DNA damage involves single- or double stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross links [119]. DNA damage can lead to processes associated with carcinogenesis such as transcription abnormalities, induction of signal transduction pathways, and genomic instability [120, 121]. Oxidation of DNA may affect DNA methylation due to oxidation of the methylated cytosines or guanines in CpG sequences [122].

Cellular components comprised of polyunsaturated fatty acid residues of phospholipids are extremely sensitive to free radical damage and this process is known as lipid peroxidation [115]. Lipid peroxidation can lead to inflammation and DNA damage

through production of malondialdehyde (MDA), conjugated dienes, hydroperoxides, lipoperoxides, and toxic aldehydes [123]. Lipid peroxidation can change fluidity of cell membranes and lead to intracellular enzyme leakage and inflammation [124]. In addition peroxidation products such as MDA can act as signal transducers and form DNA adducts which are mutagenic and can lead to CRC [125, 126].

RONS can oxidize structural proteins and inhibit proteolytic systems leading to alteration of structural and enzymatic proteins [119]. It is believed that accumulation of damaged proteins over time contributes to various age-related diseases in humans including cancer [127-129]. In addition to cellular damage, RONS can alter proteins involved in signal transduction pathways leading to upregulation of several signaling cascades, most importantly growth factor kinase-, Src kinase-, mitogen activated protein kinase (MAPK)- and PI3-kinase-dependent signaling pathways [119]. These cascades lead to activation of several redox-regulated nuclear transcription factors, such as activator protein -1 (AP-1), nuclear factor –kappa B (NF-κB), p53, hypoxia inducible factor-1 (HIF-1), and nuclear factor of activated T cells (NFAT), that are involved in carcinogenesis [115].

Two important transcription factors involved in ROS-influenced colorectal carcinogenesis are the p53 and NF-κB pathways. Oxidative stress relevant p53 activities include transcriptional induction of redox-related genes, formation of ROS, and degradation of mitochondrial components leading to cell death [130, 131]. The oxidative stress-linked tumor suppressor p53 has also been shown to promote autophagy - a process by which cells regulate their lifecycles [132, 133]. Oxidative stress and inflammation

pathways are closely linked in carcinogenesis [134]. RONS induce and are induced by inflammatory cytokines [134]. NF-κB, induced by RONS activity, is a key link between inflammation and tumor development and acts by inducing key enzymes responsible for biosynthesis of prostaglandins such as prostaglandin E2 (PGE2) [135]. PGE2 suppresses apoptosis in human tissue by increasing levels of antiapoptotic protein bcl-2, and reducing levels of proapoptotic protein bax [136].

Oxidative stress mediated carcinogenesis is not merely the result of free radical activity but also includes the failure of the protective antioxidant defense systems to balance the increased stress. These defense systems can be broadly divided into three levels of defense: (1) the first level is represented by the organization of oxygen transport or by the proteins, which bind iron and prevent free radical formation; (2) the second level includes detoxification enzymes (metabolize harmful xenobiotics to benign end products) and antioxidant system (primary and secondary antioxidants that reduce the free radical species and maintain cellular redox balance); and (3) the third level includes repair enzymes that repair oxidative damage to lipids, proteins, and nucleic acids such as proteolytic enzymes, end- and exo-nucleases, DNA polymerases, and others [119]. For example, DNA containing oxidized bases are repaired or removed through DNA glycosylases mainly through the base excision repair pathway but also through the nucleotide excision repair or the MMR pathways [137]. Failure in any of the three levels of defenses mentioned above could be potential risk factors in colorectal carcinogenesis.

A primary mediator of the induction of many detoxification and endogenous antioxidant enzymes, mentioned above in the second level of defense, is an element

termed antioxidant response element (ARE) [138-141]. ARE induces a transcriptional cascade resulting in the induction of many protective enzymes as a response to oxidative stress [142-144]. ARE was initially found in promoters of genes encoding glutathione S-transferase (GST) and NADPH: quinone oxidoreductase 1 (NQO1) [144]. Increased production of RONS or reduced antioxidant capacity (e.g., glutathione) that alter the cellular redox balance appear to be important signals for triggering ARE mediated transcriptional response [145]. Activation of transcription through ARE is primarily mediated by nuclear factor E2-related factor 2 (Nrf2) [145, 146]. Although ARE-Nrf2 activation is triggered primarily by cellular oxidative stress, several environmental factors, such as dietary antioxidants and physical activity, have been shown to induce the ARE-Nrf2 pathway [145, 146].

Environmental Components of Oxidative Balance

Environmental factors are integral mediators of oxidative stress via exposures that might increase free radical formation and disturb the cellular redox balance (pro-oxidants), and through exposures that scavenge free radicals or contribute to the antioxidant defense systems (antioxidants). The following sections summarize the physiologic role of sixteen dietary and non-dietary lifestyle components known to affect oxidative balance in humans.

Dietary antioxidants

Carotenoids are a family of pigmented compounds that are synthesized by plants and microorganisms [147]. Fruits and vegetables are major sources of

carotenoids in the human diet [148]. More than 90% of carotenoids in humans are represented by the following [149]:

1. Pro-vitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin) are precursors of vitamin A and shown to be potent anti-oxidants in in-vitro, animal and human studies [150]. However, β-carotene has been reported as a pro-oxidant at higher doses and among smokers [150-152].

2. Lycopene is a stronger anti-oxidant that the pro-vitamin A carotenoids and is primarily found in tomatoes, watermelons, and other fruits [153]. In addition to its free radical scavenging role lycopene has been shown to affect gene regulation, gap-junction communication, hormone and immune regulation and metabolic pathways involving Phase II drug metabolizing enzymes [147, 154].

3. Lutein/zeaxanthine is commonly found in cooked spinach, collard greens, and other green vegetable [147]. It is thought to be important for lens function and is a potent anti-oxidant [147].

Other non-carotenoid dietary antioxidants are:

4. Vitamin E is a lipid-soluble antioxidant and consists of four tocopherols (α, β, γ, δ) of which α- is the most predominant form in humans [155, 156]. Although all four tocopherols have anti-oxidant activity *in vitro*, only α-tocopherol can be metabolized by the liver and meets human vitamin E requirements [157]. Although some studies report that vitamin E plays a role in cell proliferation, apoptosis, and inflammation it is not clear whether any of these effects are independent of its antioxidant actions [156].

5. Vitamin C is a water soluble vitamin and a potent antioxidant that primarily protects lipid membranes against oxidation [158]. It has been shown to have a dose-dependent effect on resistance to lipid peroxidation by heavy metals [115]. Other functions of vitamin C, mediated through antioxidant mechanisms, include regulation of gene expression and apoptosis [149].

6. ω-3 fatty acids are considered a class of polyunsaturated fatty acids (PUFA) and consist of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid (ALA) [159]. Fish and seed oils are the most common sources of these compounds in humans [159]. Although ω-3 fatty acids are believed to affect colon carcinogenesis through modulation of immunity and inflammation [160], they also have antioxidant properties. Evidence from in vitro and in vivo studies suggests that ω-3 fatty acids are potent inducers of the ARE through which they regulate Nrf2 transcription and thereby modulate cellular antioxidant response. ω-3 fatty acids have to be oxidized in the human body to induce ARE [161, 162]. It has been reported that high intake of free radical scavenging antioxidants, particularly vitamin E, can block such oxidation and decrease the antioxidant benefits of ω-3 fatty acids [162].

7. Flavonoids are a chemically defined family of plant polyphenols. There are several subclasses of flavonoids: flavan-3-ols; flavanones; flavones; isoflavones; flavonols; and anthocyanidins [163]. The sum of these different sub classes represents total flavonoid intake [163]. Evidence from *in vitro* experiments suggests that flavonoids act by donating hydrogen from their phenolic groups to free radicals thereby reducing them, conjugating heavy metals

to prevent metal-catalyzed free radical formation, and by binding to cell membranes to prevent lipid peroxidation [164]. There is less convincing evidence of their anti-oxidant action *in vivo* and concerns about poor absorption from the gastrointestinal tract [164]. However, recent studies report that high concentration of flavonoids in the gastrointestinal tract might help by binding the pro-oxidant iron and by scavenging RONS [165].

8. Glucosinolates are sulfur containing compounds responsible for the pungency and spiciness of the cruciferous vegetables. They are converted to indoles and isothiocyanates in the human body which are then metabolized by the GST enzymes. These metabolites are believed to induce ARE and phase II detoxification enzymes. They are also potent inducers of hemeoxygenase 1 (HO-1) which catalyzes heme to biliverdin and prevents heme-associated oxidative damage. [166]

9. Selenium is a trace element and one of the minerals required by the human body for healthy functioning. Selenium is an unusual antioxidant because it has its own codon in the mRNA which specifies insertion of selenium into selenoproteins and selenocysteine [167]. These are important components of the cellular antioxidant defense system. Some important selenoproteins are glutathione peroxidases (antioxidant enzymes), selenoprotein P (has antioxidant and oxygen transfer functions), and thioredoxin reductases (responsible for regeneration of antioxidant systems and maintenance of intracellular redox state) [115]. The most important sources of selenium in human diet are breads, grains, meat, poultry, fish, and eggs [167]. Amount of selenium in a given plant or

animal source is heavily dependent upon the soil in which the plant or animal feed was grown resulting wide variation by geographic location [10]. This complicates assessment of selenium from dietary questionnaire.

Dietary pro-oxidants

10. Iron, primarily heme iron from red meat intake, is reported to be a pro-oxidant in the human body and in the gut [168]. Heme iron is more easily absorbed than non-heme iron (from plant sources) [115]. The major pathway for iron induced oxidative damage includes combination with lipid hydroperoxicides to generate alkoxy and heme oxyradicals which then catalyze oxidative chain reactions resulting in oxidative damage to DNA, lipids, and proteins [115, 169].

11. Saturated fat causes oxidative damage through increased production of bile acids, deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) [170]. Both bile acids have been shown to be involved in the RONS mediated genotoxic damage to colon cells *in vitro* [170]. Mechanisms of bile acid carcinogenesis not related to oxidative stress include induction of proto-oncogenes to promote proliferation, activation of the COX-2 inflammation pathway, and modulation of apoptosis to favor selection of apoptosis-resistant cells [171].

12. ω-6 fatty acids (linoleic acid and arachidonic acid) are the other major class of PUFA in the human diet [159]. Unlike ω-3 fatty acids this class of PUFA does not induce the ARE but rather higher intakes of ω-6 fatty acids have

been shown to increase oxidative stress with increased production of free radicals primarily through their action on the inflammation pathway [172, 173].

Non-dietary antioxidants

13. Physical activity, especially regular moderate physical activity, can act as an anti-oxidant by increasing the adaptive cellular response to oxidative stress and by increased performance of the antioxidant defense systems. Regular short bouts of physical activity increase levels of ROS in the body and might be considered as short-term pro-oxidants. However, these short bursts in ROS production increase adaptation of the antioxidant defense systems to free radical damage. However, chronic high-intensity exercise or "overtraining" shifts this balance towards a pro-oxidative stress and can lead to chronic oxidative stress. [174]

Non-dietary pro-oxidants

14. Tobacco smoking has been shown to contribute to an overall internal oxidative environment in human cells [175]. Free radicals in cigarette smoke have been reported to increase blood and tissue markers of oxidative stress and decrease blood levels of antioxidants [175]. Tobacco smoking also interacts with dietary antioxidants, such as beta-carotene, to decrease the protective effect of antioxidants [151, 152].

15. Alcohol intake, especially long-term moderate to heavy alcohol intake, results in an oxidative microenvironment in the cells [176]. Alcohol

causes increased oxidative stress through a variety of mechanisms: oxidation of ethanol to acetaldehyde leads to reduction of nicotinamide adenine dinucleotide (NAD) to NADH which results in decreased xanthine dehydrogenase activity and increases in purine oxidation, microsomal oxidation, and increased ROS production; ethanol oxidation to aldehydes can lead to formation of DNA or protein aldehyde adducts some of which are known to be carcinogenic; and, alcohol intake decreases levels of GSH thus decreasing mitochondrial antioxidant activity [176, 177].

16. Obesity is an important contributor to oxidative stress. Obesity has been independently associated with increased biomarkers of oxidative stress and impaired serum redox balance leading to lowering of the antioxidant capacity [178]. Additionally, obesity results in increased concentrations of free fatty acids which increase oxidative stress through activation of cytochrome P450 2E1 which increases ROS production, mitochondrial oxidative damage, and increased production of peroxisomal hydrogen peroxide [119].

The sections above describe the biochemical and physiologic basis for the anti- or pro-oxidant effects of the major environmental oxidative balance exposures. These findings are primarily based on *in vitro* assays or animal studies. However, evidence on the effect of these compounds on colorectal cancer risk in humans currently can only be based on epidemiologic studies. None of the dietary antioxidants or pro-oxidants mentioned above have been shown to be independent risk factors for CRC or adenoma

[10, 179]. Epidemiologic evidence is conflicting and recent meta-analyses and pooled analyses confirm that current evidence does not indicate that any of the dietary components are independently associated with risk of CRC [10]. Evidence from multiple randomized controlled trials of dietary antioxidants suggests that use of traditional antioxidant vitamins, singly or in limited combinations, at pharmacologic doses, cannot be justified for CRC prevention [180-185].

The discrepancies between mechanistic experiments and human data (both observational and experimental) may be explained by the following factors: (1) lack of sufficient biological rationale for selecting specific agents for the studies; (2) use of pharmacological doses that do not reflect typical dietary intake and may be responsible for harmful effects seen in some trials; and (3) insufficient durations of intervention and follow-up [182]. In addition to the factors mentioned above, focus on a single component or limited combinations of components in observational studies and RCTs may explain the lack of strong associations seen between antioxidant components and CRC risk seen in these studies [117]. As described in the sections above, oxidative stress associated carcinogenesis is a complex, multifactorial pathway and investigation of single components in that pathway might not represent the entire pathway. Additionally, trying to measure the effect of one component against the background of average risk associated with other components on the same pathway might not be possible given the small individual associations of these components and the requirement of prohibitively large study samples to measure these small associations [117, 186]. Moreover, analyses of individual components often ignore the many potential interactions between other components on the pathway and disease risk [186]. Alternative approaches that combine

these multiple and frequently interacting components into a single exposure metric might be preferred to the traditional epidemiologic studies. Use of dietary patterns and indices in nutritional epidemiology are examples of such approaches that have been successfully applied to complex exposures [186]. We and other authors have previously proposed and published such approaches to measure oxidative balance [117, 118, 187-190].

Oxidative Balance Score (OBS)

An OBS can be conceptually visualized as a weighted combination (sum) of anti- and pro-oxidant components determined *a priori*. Previous studies on OBS (some investigators have called it the antioxidant score or oxidative stress score) have reported associations of OBS with colorectal adenoma, CRC, prostate cancer, lung cancer, esophageal cancer, and breast cancer [118, 187, 188, 191]. However, some recent studies failed to find an association between OBS and prostate cancer [190, 192]. Weightings for individual components in these studies have either used principal components / factor analysis or have assumed that all components contribute equally to the score. A limitation of the principal components analysis approach to weighting is that it is largely data driven, such that the principal components themselves are not readily interpretable, and inherent variations in exposure patterns across populations make the summary score less applicable to other studies. Similarly, a limitation of the equal weighting approach is the assumption that all components contribute equally to oxidative stress, and that their effect on the outcome is uniform across all components. An equal weighting approach does not likely represent the true biological contributions of the individual exposures contributing to oxidative balance. Another limitation of previous studies was the use of only one method to develop the OBS, which raises concern that the results might be

sensitive to the assumptions underlying the weighting of the variables in the score. For this dissertation I proposed to improve on previous research by creating three new weighting methods for OBS and comparing them to the equal weighting approach. In addition, previous studies on OBS and colorectal neoplasms have either only evaluated the dietary antioxidants without accounting for the lifestyle components or the pro-oxidants, or have not included some of the OBS components that we have described above. For this dissertation I proposed to investigate the associations of multiple comprehensive OBS with colorectal adenoma and CRC risk.

Biomarkers of oxidative stress

Accurate measurement of oxidative stress *in vivo* requires identification of sensitive, specific, and validated markers. Several markers of lipid, protein, and DNA oxidation have been proposed in the last two decades. Lipid hydroperoxides in plasma, MDA in plasma and urine, and 8-iso-prostaglandin-F2α (F2-isoprostanes) in plasma and urine have been proposed as markers of lipid peroxidation [193-195]. Protein carbonyls, methionine sulfoxidation, and tyrosine products (e.g., dityrosine and nitrotyrosine) are markers of protein oxidation that have been measured in plasma and urine [196, 197]. Markers of DNA oxidation include the semi-quantitative Comet assay, measurement of MDA-DNA adducts in blood, and measurement of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine [198-201]. However, issues such as lack of validation, instability in stored specimens, and confounding factors associated with measurement limits the use of some of these markers [202]. A multi-laboratory validation study by the National Institute of the Environmental Health Sciences suggested that F2-isoprostane and MDA measurements in plasma are highly reproducible, sensitive, and specific measures of lipid

peroxidation and suitable for use in stored specimens [202]. Urinary isoprostanes were also reported to be promising markers of oxidative damage [202]. However, protein and DNA oxidation markers in plasma were reported to be unreliable markers of oxidative stress [202]. Recently, biomarkers of nonradical oxidative stress mechanisms have been developed and found to be associated with several known oxidative stress-associated exposures and diseases [203-206]. The redox states of glutathione/glutathione disulfide (GSH/GSSG) and cysteine/cysteine (Cys/CySS) are examples of such markers [203-206].

Evidence from studies in colorectal tumor tissue suggests that oxidative stress is associated with colorectal neoplasia [119]. Increased levels of ROS (measured by chemiluminescence), 8-OHdG, nitric oxide, F2-isoprostanes, glutathione peroxidase, and catalase have been reported in colorectal adenomas and carcinomas [207-209]. Studies among CRC patients have also reported higher levels ROS in whole blood and of 8-OHdG in DNA in leukocytes and serum of such patients compared to those of healthy controls [207, 210]. However, studies on the levels of F2-isoprostanes among adenoma patients and association of F2-isoprostanes with dietary antioxidants are limited (described in detail in Chapter 3 of this dissertation). In addition, it is unclear whether OBS is associated with markers of oxidative stress. This dissertation contributes to knowledge on associations of F2-isprostanes with colorectal adenoma risk, dietary intakes of antioxidant nutrients, and antioxidant nutrient biomarkers in plasma.

Objectives

My primary objective in this dissertation was to develop, compare and evaluate comprehensive "oxidative balance scores (OBS)", comprised of individual dietary, and environmental exposures that are known to affect physiologic oxidative processes, and use these OBS to investigate the role of oxidative balance in colorectal adenoma and carcinoma risk. In addition, I also proposed to investigate associations of the OBS and its components with markers of oxidative stress to assess the validity of OBS as a tool to measure oxidative balance.

Specific Aims

Aim #1: Develop four different OBS and investigate whether high OBS reduce risk of colorectal adenomas in a pooled analysis of three case-control studies of incident, sporadic colorectal adenomas. *[Addressed in Chapter 2]*

Aim #2: Using data from a large, prospective US cohort investigate whether high levels of four different OBS are associated with lower risk of incident colorectal cancer in a prospective cohort study. *[Addressed in Chapter 3]*

Aim #3: Evaluate associations of F2-isoprostanes, a sensitive and specific marker of oxidative stress *in vivo*, with four different OBS and individual components of the OBS using pooled data from two case-control studies of incident, sporadic colorectal adenomas. *[Addressed in Chapter 4]*

CHAPTER 2. USING PATHWAY-SPECIFIC COMPREHENSIVE EXPOSURE SCORES IN EPIDEMIOLOGY: APPLICATION TO OXIDATIVE BALANCE IN A POOLED CASE-CONTROL STUDY OF INCIDENT, SPORADIC COLORECTAL ADENOMAS

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Abstract

Identifying associations of risk factors sharing the same pathway with disease risk is complicated by small individual effects and inter-correlated components; this can be addressed by creating comprehensive exposure scores. The authors developed and validated three novel weighting methods (literature review derived, study data-based, and a Bayesian method that combines prior knowledge with study data) to incorporate components into a pathway score for oxidative balance in addition to a commonly used method that assumes all components contribute equally to the score. They illustrate their method using pooled data from three U.S. case-control studies of sporadic colorectal adenomas. Four oxidative balance scores (OBS) were created to reflect combined summary measures of dietary and non-dietary anti- and pro-oxidant exposures. A higher score represents a predominance of anti- over pro-oxidant exposures. In the pooled data the odds ratios comparing the highest tertile of OBS to the lowest for adenoma risk ranged from 0.38 to 0.54 for the four measures; all statistically significant. These findings suggest that: 1) OBS are indicators of oxidative balance and may be inversely associated with colorectal adenoma risk, and 2) using comprehensive exposure scores may be preferable to investigating individual component-disease associations for complex exposures, such as oxidative balance.

Keywords: Case Control Studies; Colorectal Tumors; Oxidative Stress; Methodological Study; Weighting

Introduction

Published studies of diet-disease associations usually focused on investigating one food or nutrient at a time. However, most foods / nutrients have small effects and are inter-correlated, either by intake or by contributing similarly to a biologic pathway, which complicates attempts to analyze their individual effects[186]. Advantages of combining these dietary exposures into a comprehensive variable were previously summarized[186, 211], contributing to the development and application of dietary patterns in observational epidemiology. Dietary patterns derived from *a priori* diet-quality scores[212-214] or the more exploratory principal components analysis or factor analysis methods[215-217] do not necessarily relate to specific biologic pathways. Moreover, by definition, they do not include non-dietary lifestyle factors – factors that might be correlated with dietary behaviors and act on the same pathway.

The rationale and method for combining multiple dietary and non-dietary lifestyle exposures to create comprehensive scores for oxidative balance (balance of anti- to pro-oxidants *in vivo* that modulate levels of potentially harmful reactive oxygen species) have been published[117, 187]. Oxidative balance scores (OBS) were reported to be statistically significantly associated with decreased risk for incident colorectal adenoma and prostate cancer, but the individual components of the score were weakly or not associated with either disease[117, 187]. Other investigators, using slightly different methods to create oxidative balance scores, mostly reported similar results for other cancers and cancer mortality[118, 188, 218, 219].

In these studies only one method for developing an OBS was used, the most common being a simple summation and equal weighting of the selected components. Because results could be unduly influenced by the weighting assumptions, and components do not contribute equally to the pathway under consideration, we present four different methods to construct comprehensive exposure scores, and illustrate the utility of this approach to investigate the association of oxidative balance with risk for incident, sporadic colorectal adenomas. In addition, since it is unknown whether these scores actually measure oxidative balance, we present data on the association of OBS with F_2-isoprostanes – considered to be the most reliable marker of oxidative stress *in vivo*[202].

Materials and Methods

Study population

Data from three, methodologically similar, endoscopy-based case-control studies of incident, sporadic colorectal adenomas conducted by the same principal investigator were pooled. The first study (the Cancer Prevention Research Unit study, CPRU) was conducted in Minnesota from 1991-1994, the second (Markers of Adenomatous Polyps study, MAPI) was conducted from 1994-1997 in North Carolina, and the third (MAPII) was conducted in 2002 in South Carolina. Participants in all three studies were recruited from patients with no history of colorectal neoplasms who were scheduled for outpatient, elective endoscopy for screening or gastrointestinal symptoms in large, community-based gastroenterology practices. Participants aged 30-74 years, English speaking, without contraindications to colonoscopy, and no known genetic syndromes associated with

colonic neoplasia or history of inflammatory bowel diseases, colorectal adenomas, or cancer (except non-melanoma skin cancer) were eligible to participate. The participation rate was similar in all three studies (68% to 76%).

We combined data from the MAPI and MAPII (hereafter referred to as MAP) studies because the selection criteria, study protocols, and questionnaires were identical for these studies. Details of the study protocols for the CPRU[220], MAPI[202], and MAPII[221] studies were previously reported. The final sample size for the pooled data analyses was 789 incident adenoma cases and 1,500 polyp-free controls. All participants, prior to undergoing endoscopy, completed questionnaires on demographics, medical and family history, lifestyle, anthropometrics, diet (using a semi-quantitative Willett food frequency questionnaire (FFQ)[222, 223]), and, in women, hormonal and reproductive history.

The studies were approved by the Institutional Review Boards of the institutions at which they were conducted, and all participants provided written informed consent.

OBS components and their assessment

The 15 components included in the OBS (Table 2.1) were determined *a priori* based on their expected physiologic effects on oxidative processes. The dietary components were derived from the FFQs; nutrient values included dietary and supplemental sources. Supplemental selenium was not included in the OBS because <5% of the participants reported regular use. All nutrient values were energy adjusted according to the residual regression method and analyzed as continuous variables[224].

Non-dietary lifestyle variables included in the OBS were smoking (current, former, or never smoker), alcohol intake (<1, 1-6, or ≥7 drinks/week), obesity (BMI <30 kg/m^2 *and* waist-to-hip ratio (WHR) <1.0 in men or <0.8 in women, *either* BMI ≥30 kg/m^2 *or* WHR ≥1.0 in men or ≥0.8 in women, BMI ≥30 kg/m^2 *and* WHR ≥1.0 in men or ≥0.8 in women), and physical activity (in metabolic equivalents).

Colorectal adenoma

Participants with an adenoma removed during colonoscopy, and verified by an index study pathologist using diagnostic criteria established by the National Polyp Study[194], were considered cases. Participants who had no adenomatous or hyperplastic polyps on colonoscopy were considered controls. All controls in the MAP studies underwent colonoscopy, but in the CPRU study 518 (43%) participants were polyp-free on sigmoidoscopic assessment and were not referred for a colonoscopy.

Assessment of F_2-isoprostanes

Plasma F_2-isoprostanes were assessed in a validation sub-sample from the MAP studies (157 cases and 184 controls). Fasting peripheral venous blood samples were drawn into red-coated, pre-chilled vacutainer tubes and then immediately placed on ice and shielded from light. Blood fractions were aliquotted into amber-colored cryopreservation tubes, the air displaced with argon gas, and the aliquots then immediately placed in a -80^o C freezer until analysis. Plasma F_2-isoprostanes were measured using a gas chromatography-mass spectrometry-based (GC-MS) method[194].

Statistical methods

We used four methods of weighting the 15 components (Table 2.1) to create the respective OBS:

1. OBS-equal weight (an *a priori* method): For OBS-equal weight, we assumed that all components are equally important and should contribute similar weights. Antioxidants and pro-oxidants identified *a priori* were assigned arbitrary weights of 1 and -1, respectively. All components, including the categorical variables, were transformed to a standard normal distribution. We then multiplied the transformed variables by the respective weights (1 for antioxidants and -1 for pro-oxidants), and summed the weighted components to generate the OBS-equal weight.

2. OBS-lit. review (an *a priori* method): Weights for OBS-lit. review were derived from literature reviews (Table 2.1). Coefficient estimates were calculated using pooled adjusted risk estimates derived from published reviews/meta analyses of individual CRC risk factors, where available. Pooled effect estimates for Ω-3 and Ω-6 fatty acids, flavonoids, glucosinolates, and iron were not readily available and are based on reviews done by one of the authors (CD). For continuous components, reported effect estimates commonly compare the highest to the lowest quantile of intake. For weighting, we calculated the effect estimate for one standard unit increase in the continuous variable based on the highest category risk estimate (*e.g.*, 4^{th} quartile vs. 1^{st} quartile) reported in the literature. Our calculations assumed a log-linear dose response between the OBS

component and CRC risk in the published estimates. Based on a previously described method[225], we calculated the mid-points of the highest and lowest categories using the category boundaries of a standard normal distribution, and used the following formula to calculate the coefficient estimate for a particular component[225, 226]:

[ln(1/effect estimate)] / [(midpoint of high category – midpoint of low category)]

The inverse of the effect estimate was used so that components inversely associated with CRC had a positive weight and those with higher risk a negative weight.

OBS-lit. review was calculated for each study participant by weighting each standardized component based on the weights derived from the literature reviews, and then summing the weighted components.

3. *OBS-a posteriori* (an *a posteriori* method): Weights were derived from the CPRU study and applied to the MAP study and pooled data. We used multivariable logistic regression to estimate the odds ratio of colorectal adenoma for each OBS component after adjusting for other components and additional covariates. The coefficient estimates for each of the components obtained from the regression model were used to calculate weights for OBS-*a posteriori*. Coefficients were multiplied by -1 (natural log of the inverse of the odds ratio) so that components inversely associated with adenoma risk had a positive weight, and vice versa. OBS-*a posteriori* was then calculated as a weighted sum of the 15 components.

4. OBS-Bayesian (combination of *a priori* and data-based methods): We conceptualized OBS-Bayesian as a combination of the weighting schemes in OBS-lit. review and OBS-*a posteriori*. We used a hierarchical modeling approach, utilizing a logistic regression model with informative priors within the Bayesian framework, to derive weights for OBS-Bayesian. Details of the Bayesian approach are discussed in greater detail elsewhere[227-229]. The priors for the OBS components were defined as normally distributed with mean and variance as determined for OBS-lit. review. The covariates (see statistical analysis section below) in the model were assigned non-informative normal priors with mean zero, and large standard deviations (10^6). The components were transformed to a standard normal distribution prior to analysis. We used the BAYES statement in PROC GENMOD in SAS v 9.2 for the Bayesian analysis[230]. Convergence of the Markov chain was determined by visual analysis of trace plots, and by two (Gelman-Rubin and Geweke) diagnostic tests[231, 232]. No departures from convergence were found for any of the components in the model. The first 2,000 burn-in sampling iterations were not used for determining the posterior summaries. The posterior summary estimates were multiplied by -1 and used as weights for OBS-Bayesian. Similar to the other OBS, OBS-Bayesian was then calculated as a weighted sum of the 15 components. Similar to the OBS-*a posteriori*, the weights for OBS-Bayesian were developed in the CPRU data and applied to the MAP study and pooled data.

Non-dietary lifestyle variables such as physical activity are considered stronger risk factors for colorectal neoplasia than are dietary antioxidants and pro-oxidants[10].

To examine whether dietary factors meaningfully contribute to the OBS-colorectal adenoma association, we created a dietary OBS by excluding smoking, alcohol intake, obesity, and physical activity from the OBS measures described above. We also created a lifestyle OBS variable that only included the four non-dietary lifestyle variables.

We used multivariable logistic regression to estimate the odds ratio (OR) and corresponding 95% confidence interval (95% CI) for incident colorectal adenoma in relation to each OBS, adjusted for age, sex, education, family history of colorectal cancer (CRC) in a first degree relative, regular use (≥ once/week) of aspirin, regular use (≥ once/week) of other nonsteroidal anti-inflammatory drugs (NSAIDs), calcium, vitamin D, folate, fiber, total energy intake, cumulative estrogen exposure excluding oral contraceptive use (in women), and use of menopausal hormone therapy (in women). These covariates were selected *a priori* as potential confounders based on being established risk factors for colorectal adenomas and a potential for association with OBS, or its components. Stratified analyses were conducted to examine the association of colorectal adenoma with dietary OBS stratified by tertiles of lifestyle OBS and vice-versa. Effect-measure modification by the covariates was determined by comparing stratum-specific ORs, and by the model-based log-likelihood ratio. We also examined whether the association between OBS and adenoma risk varied by tumor site (distal to the splenic flexure versus proximal versus rectal) or advanced adenoma status (defined as size ≥ 1 cm, adenoma with any villous component, or high-grade dysplasia). Prior to analyses, each OBS was categorized into tertiles based on the study-specific distribution in the controls. To test for linear trend we created a continuous variable using the median OBS value within each tertile.

We used general linear models (GLM) to evaluate the association of OBS measures with F_2-isoprostane levels adjusted for age, race, and study. F_2-isoprostane values were log transformed prior to analysis. Separate analyses were performed for men and women because mean F_2-isoprostanes are reported to be higher and have more variability in women than men[214, 220].

All statistical tests were two-sided, and P <0.05 was considered statistically significant. All analyses were conducted in SAS version 9.2 (SAS institute).

Results

Selected characteristics of the study participants are summarized in Table 2.2. Cases were more likely than controls to be older, male, not taking aspirin or NSAIDs regularly, and to report lower calcium, vitamin D, and folate intakes and higher energy intake. Among women, cases were less likely to report using postmenopausal hormone therapy.

The weights for the individual OBS components differed among the four methods and are shown in Table 2.1, and the OBS medians and interquartile ranges in Table 2.2.

The results of logistic regression modeling of the associations of the various OBS with colorectal adenoma are shown in Table 2.3. For both studies, participants in the highest relative to the lowest tertile of the "OBS" were, on average, 50% less likely to have colorectal adenomas. In the pooled analyses the ORs were around 0.50 (range: 0.38-0.54) for the four different OBS, and all 95% CIs excluded 1.0. The tests for trend for all four OBS were statistically significant, consistent with a dose-response association

of decreasing adenoma risk with increasing OBS. Overall, the findings for OBS-lit. review, OBS-*a posteriori*, and OBS-Bayesian were more similar to each other than to those from OBS-equal weights.

Associations of dietary OBS with adenoma stratified by tertiles of lifestyle OBS are presented in Table 2.4. For all OBS measures except OBS-equal weight, the inverse association between dietary OBS and adenoma risk was stronger (and statistically significant) among participants in the lowest tertile of lifestyle OBS (i.e., those with more pro-oxidant lifestyle exposures) than among those with higher lifestyle OBS.

"OBS", "dietary OBS", and "lifestyle OBS" were more strongly associated with lower risk of advanced than with non-advanced adenomas (Table 2.5). This finding was especially true for the "dietary OBS" variables; the average ORs for adenoma risk comparing the highest tertile with the lowest were 0.88 (all 95% CIs included 1.0) for non-advanced adenomas, and 0.55 (statistically significant) for advanced adenomas. The tests for trend were also statistically significant for the advanced adenoma outcome but not for the non-advanced adenomas. ORs for "lifestyle OBS" were stronger than "dietary OBS" for all adenomas, but the associations were more comparable between advanced and non-advanced adenomas. Associations between the OBS and adenoma were similar for proximal colon, distal colon, and rectal sites (data not shown).

OBS-F_2-isoprostanes associations are presented in Table 2.6. F_2-isoprostanes were lower, indicating lower systemic oxidative stress, with increasing OBS in both men and women, but the results for OBS-equal weights and OBS-*a posteriori* were not statistically significant among men (Table 2.5). Increasing tertiles of dietary OBS were

also inversely associated with F_2-isoprostanes after adjusting for lifestyle OBS components. Although F_2-isoprostane levels were lower in participants in the highest tertile of lifestyle OBS compared to the lowest after adjusting for dietary OBS, the results were not statistically significant.

Discussion

We developed three novel weighting schemes (OBS-lit. review, OBS-*a posteriori*, and OBS-Bayesian) and compared them to a previously used weighting scheme (OBS-equal weights) for combining dietary and non-dietary exposures associated with oxidative balance. Using data from the pooled study, we found a substantial inverse association between OBS and risk for incident, sporadic colorectal adenomas. Our approach is robust as evidenced by the similarity of the conclusions from the different weighting methods suggesting that the observed associations are unlikely to be artifacts of weighting assumptions. Our results also suggest a dose dependent decrease in F_2-isoprostane levels with increasing levels of OBS, providing support for OBS as a valid measure of oxidative balance.

Other epidemiologic studies reported inverse associations of summary oxidative balance/stress scores with colorectal adenoma, lung cancer, esophageal cancer, prostate cancer, and total cancer mortality[117, 118, 187, 188, 218]. However, Agalliu *et al.* recently reported a null association between OBS and prostate cancer[219]. These studies used only one method to develop the summary score variable, which raises concern that the results might be sensitive to the assumptions underlying the weighting of the variables in the score. Another limitation of previous studies was the assumption that

all components contribute equally to oxidative stress[117, 118]. Since an equal weighting approach (OBS-equal weights) unlikely represents the true biological contributions of individual contributors to oxidative balance, we tested multiple approaches to weight the OBS. Also, in contrast to previous studies, we created three OBS measures that are specific for colorectal neoplasms. Although each approach has certain limitations (discussed below), the conclusions from the results were generally consistent across the weighting methods. The use of multiple approaches can be viewed as sensitivity analyses for weighting OBS components.

The similarity of the conclusions obtained for the adenoma-OBS and the OBS-F_2-isoprostanes associations suggests that all four scoring methods may be valid. Although the OBS-equal weights method is the easiest to use, concerns still remain about its biological appropriateness, and it is possible that this approach might not perform as well in designing exposure scores for pathways other than oxidative stress. The weighting approaches proposed as alternatives to OBS-equal weights also have limitations. Weights for OBS-*a posteriori* are based on data from one study and might not be applicable to other studies. An obvious improvement on this weighting scheme is to derive weights from multiple studies rather than just one. This led us to develop OBS-lit. review. However, estimates obtained from pooling prior studies may be imprecise because of the lack of uniformity in the exposure measurement and covariate selection across studies. Additionally, weighting based on epidemiologic studies considers the effect of each component on disease risk possibly without accounting for other factors. This weighting approach may not be the most suitable given our main premise that combined effects of components are more important than their individual effects.

Therefore, *a priori* weighting schemes, based on the association of OBS components with a panel of oxidative stress biomarkers that best represent systemic oxidative stress, may need to be developed. The OBS-Bayesian approach combines elements from the "lit. review" and "*a posteriori*" weighting schemes, and aims to strike a balance between using available study data and published information from prior studies. This approach may be preferred not only for creating an OBS, but for determining the weights for other comprehensive pathway scores.

Our results suggest that increasing dietary OBS among those with predominantly pro-oxidant lifestyle exposures, such as those in the lowest tertile of lifestyle OBS (Table 2.4), might be a promising approach for adenoma prevention. Overall, lifestyle OBS was more strongly associated with adenoma incidence than was dietary OBS; however, the dietary OBS was more strongly associated with isoprostanes than was the lifestyle OBS (Table 2.6). This paradoxical observation could be because the non-dietary lifestyle components, especially compared to the dietary components, also act through pathways in addition to oxidative stress[233].

This study had several limitations. Although the OBS presented is the most comprehensive reported to date, we might have missed potential components because of a lack of published evidence of their effects on oxidative processes. The OBS components do not include endogenous factors that modify oxidative stress, such as DNA damage repair genes or genes responsible for cellular response against oxidative stress[234, 235]. The OBS dietary components are based on self-report from FFQs and are subject to measurement error and biases[236], even when adjusted for total energy

intake. Using nutrient biomarkers as dietary OBS components should be evaluated in future studies. Study participants were predominantly white, and our results might not be generalizable to non-white populations. In the CPRU study, some controls did not have a colonoscopy, raising concerns about missed proximal tumors and possible outcome misclassification. However, such misclassification would be expected to attenuate the results. Most participants underwent colonoscopy for indications other than routine screening, such as gastrointestinal bleeding and other symptoms that might be related to increased oxidative stress. Although unlikely, it is also possible that participants with symptoms had recently changed their behaviors (e.g., diet) to more healthy patterns. Data on F_2-isoprostanes were not available for the CPRU study and were only available for a sub-sample from the MAP studies. Additionally, F_2-isoprostanes are indicators of lipid peroxidation and do not represent the entire spectrum of *in vivo* oxidative stress biomarkers which includes oxidation products of proteins and nucleic acids.

Strengths of our study include histologically verified adenoma cases, thus reducing outcome misclassification; community-based control selection; assessment of exposure and covariate information prior to endoscopy, thus reducing recall bias; and low likelihood of unmeasured confounding because of collection of detailed information on covariates. Finally, ours is the first study to investigate the validity of OBS using biomarkers of oxidative stress.

In summary, we developed three novel weighting methods to create disease-specific exposure scores for oxidative balance, and demonstrated their application to data from a large pooled case-control study of incident, sporadic colorectal adenomas. We

compared the performance and validity of the different weighting schemes and concluded that all four methods perform equally well for OBS. However, given the potential limitations of the other methods we recommend the use of a Bayesian approach to generate weights for multi-component exposure scores. This method appears potentially useful for exposures, such as diet, where small individual effects contributing to a larger pathway and the inter-correlations among the exposures limit our ability to evaluate exposure-disease associations. Finally, in contrast to the conclusions from analyses that evaluated individual anti-/pro-oxidants, our approach suggests that oxidative balance may be associated with risk for incident, sporadic colorectal adenomas.

Tables and Figures

Table 2.1. Oxidative Balance Score (OBS) Components, Rationale for Their Inclusion in the OBS, and Weights Given to Them in Different Measures of the OBS*

OBS components	Rationale for inclusion	OBS weights‡			
		OBS-equal weights	**OBS-lit. review¶**	**OBS-*a posteriori***	**OBS-Bayesian**
Dietary antioxidants					
Pro-vitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin)	Precursors to vitamin A, potent antioxidants [147]	+1	0.0039	-0.0230	0.0048
Lutein	Antioxidant [147]	+1	0.0325	0.0803	0.0193
Lycopene	Antioxidant [153]	+1	-0.0153	0.0149	-0.0212
Vitamin C	Prevents lipid peroxidation, helps regenerate α-tocopherol [158]	+1	0.0810	0.0541	0.0510
Vitamin E	Membrane bound antioxidant, protects against lipid peroxidation [155]	+1	0.1368	0.1247	0.1052
Ω-3 fatty acids (marine)	Induce electrophile-responsive element (EpRE) regulated genes responsible for transcription regulation of antioxidant enzymes [161, 162]	+1	0.0044	-0.0184	0.0309
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 [164, 165]	+1	-0.0043	0.1451	0.0060
Glucosinolates	Sulfur-containing plant compounds with antioxidant functions: induce EpRE as Ω-3 fatty acids, induce hemoxygenase-1 which catalyzes heme to biliverdin, induce glutathione peroxidase [166]	+1	0.0411	-0.0344	0.0290
Dietary pro-oxidants					
Dietary iron	Primarily available from red meat, preferentially	-1	-0.0744	-0.0089	-0.0756

	catalyzes oxidative reactions through production of free radicals resulting in lipid, protein, and DNA and other nucleic acid damage [168, 169]				
Ω-6 fatty acids	Higher intakes associated with increased oxidative stress through increased free-radical production; unlike Ω-3 fatty acids, does not induce EpRE [162, 172, 173]	-1	0.0410	-0.1214	0.0031
Saturated fat	Oxidative DNA damage through increased production of known pro-oxidant bile acids in the colon [170, 171]	-1	-0.0153	-0.1024	-0.0393
Non-dietary lifestyle antioxidants					
Physical activity	Although acute bouts of exercise increase RONS production, regular exercise results in increase in adaptive response to oxidative stress by activating cellular antioxidant signaling systems and enhancing expression of antioxidant enzymes through a process termed "hormesis" [174]	+1	0.1080	0.0043	0.0976
Non-dietary lifestyle pro-oxidants					
Smoking	Potent producer of free radicals, associated with increase in blood/tissue markers of oxidative stress [175, 237]	-1	-0.7031 (current smokers) -0.0953 (former smokers)	-0.7503 (current smokers) -0.2620 (former smokers)	-0.7764 (current smokers) -0.2426 (former smokers)
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 [176, 177]	-1	-0.2390 (heavy drinkers) -0.0676 (moderate drinkers)	-0.5633 (heavy drinkers) -0.2108 (moderate drinkers)	-0.4854 (heavy drinkers) -0.0707 (moderate drinkers)
Obesity	Independently associated with increased oxidative stress markers, impaired serum redox balance, and	-1	-0.0770 (obese)	-0.2683 (obese)	-0.3507 (obese)

	increased lipid peroxidation; source of free fatty acids which can lead to oxidative stress through increased RONS production [178]	-0.0295 (overweight)	-0.0596 (overweight)	-0.1766 (overweight)

* For each participant, OBS was calculated as a weighted sum of the components listed in the table

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review; OBS-*a posteriori*: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data.

¶ Weights derived from published reviews/meta-analysis for all components except Ω-3 fatty acids, Ω-6 fatty acids, flavonoids, glucosinolates, and iron where one of the authors (CD) conducted the meta-analyses.

Table 2.2. Selected Characteristics and OBS Components in Cases and Controls in Three Case-Control Studies of Incident Sporadic Colorectal Adenoma

	CPRU		MAPI		MAPII	
Selected characteristics	***Cases (n=564)***	***Controls (n=1,202)***	***Cases (n=177)***	***Controls (n=179)***	***Cases (n=48)***	***Controls (n=119)***
Age, Mean (y)	58 (10)	53 (11)[a]	58 (8)	55 (9)[a]	58 (9)	53 (11)[a]
Male, (%)	62	39[a]	60	37[a]	62	38[a]
College education or higher (%)	30	28	22	31	28	29
Family history of colon or rectal cancer in first degree relative (%)	14	17	20	36[a]	15	19
Regular (≥once/week) NSAID use (%)	9	19[a]	24	35[b]	13	22[a]
Regular (≥once/week) aspirin use (%)	20	26[b]	35	34	24	28
Mean years of total estrogen exposure, (y)	14 (19)	21 (18)[a]	16 (21)	24 (20)[a]	15 (19)	22 (19)[a]
Current hormone therapy use (%)	22	40[a]	65	72	35	47[a]
Total energy intake, Mean (kcal/day)	2,091 (775)	2,003 (718)[b]	2,003 (758)	1795 (677)[a]	2,065 (771)	1,961 (715)[a]
Total calcium intake, Mean (mg/day) [§]	952 (446)	990 (458)	789 (380)	859 (445)	905 (434)	964 (458)[a]
Total vitamin D intake, Mean (IU/day) [§]	325 (245)	350 (252)[b]	321 (257)	355 (306)	324 (249)	351 (263)[b]
Total folate intake, Mean (µg/day) [§]	398 (219)	442 (234)[a]	435 (230)	466 (251)	409 (222)	447 (238)[a]
Dietary fiber intake, Mean (gm/day)	22 (7)	22 (8)	21 (8)	20 (8)	22 (7)	22 (8)
OBS components						
Pro-vitamin A carotenoids intake, Mean (IU/day)	9,822 (9,067)	10,861 (10,330)[b]	5,186 (4,203)	5433 (4228)	8,501 (8,255)	9,779 (9,679)[a]
Lutein intake*, Mean (µg/day)	6.9 (6)	7.5 (6)[b]	3,669 (2,882)	3211 (2817)	-	-
Lycopene intake*, Mean (µg/day)	2.2 (2.3)	2.2 (2.5)	4,307 (3,817)	4,507 (4,075)	-	-
Vitamin C intake, Mean (mg/day)	246 (293)	299 (312)[a]	277 (346)	275 (303)	255 (310)	294 (310)[a]
Vitamin E intake, Mean (mg – TE/day)	62 (143)	83 (170)[a]	74 (164)	73 (147)	66 (149)	81 (166)[a]
Ω-3 fatty acid intake (marine), Mean (gm/day)	1.85 (1.48)	1.88 (1.62)	0.22 (0.20)	0.22 (0.25)	1.39 (1.45)	1.55 (1.60)[b]
Flavonoid intake, Mean (mg/day)	228 (194)	261 (250)[a]	399 (355)	388 (349)	277 (262)	286 (277)
Glucosinolate intake, Mean (mg/day)	14.9 (14.9)	15.6 (16.2)	20.5 (28.7)	17.4 (14.2)	16.5 (20.0)	16.0 (15.8)
Ω-6 fatty acid intake, Mean (gm/day)	11.5 (3.6)	10.8 (3.5)[a]	11.9 (3.8)	11.4 (4.8)	11.6 (3.7)	10.9 (3.8)[a]
Saturated fat intake, Mean (gm/day)	11.9 (3.2)	11.4 (3.1)[a]	11.6 (3.1)	11.5 (3.0)	11.8 (3.2)	11.4 (3.1)[a]
Dietary iron intake, Mean (mg/day)	18 (14)	20 (16)[b]	19 (17)	22 (21)	19 (15)	21 (17)[a]
Current smoker, (%)	21	13[a]	34	15[a]	25	14[a]
Former smoker (%)	47	40	40	37	45	40

1-6 alcoholic drinks/week, (%)	17	18	35	36	22	21
≥7 alcoholic drinks/week, (%)	40	26[a]	23	14[a]	35	23[a]
BMI, Mean	27.4 (4.7)	26.6 (4.9)[a]	27.9 (6.3)	27.8 (6.0)	27.6 (5.2)	26.8 (5.1)[a]
Waist-to-hip ratio, Mean	0.93 (0.13)	0.88 (0.12)[a]	0.94 (0.12)	0.89 (0.14)[a]	0.93 (0.13)	0.88 (0.12)[a]
Physical activity, Mean (MET-hrs/week)	37 (39)	35 (33)	27 (19)	28 (19)	34 (35)	33 (31)
OBS measures‡						
OBS-equal weight, Median (IQR¶)	-1.66 (-4.93, 1.94)	0.06 (-3.27, 3.35)	-0.85 (-4.45, 1.97)	0.07 (-2.38, 3.57)	-1.12 (-3.85, 1.34)	-0.39 (-3.01, 2.66)
OBS-lit. review, Median (IQR¶)	-0.51 (-0.92, -0.18)	-0.25 (-0.61, -0.06)	-0.62 (-0.97, -0.23)	-0.23 (-0.55, -0.08)	-0.55 (-0.77, -0.16)	-0.25 (-0.56, -0.09)
OBS-*a posteriori*, Median (IQR¶)	-0.66 (-1.05, -0.28)	-0.37 (-0.75, -0.08)	-0.69 (-1.12, -0.27)	-0.34 (-0.71, -0.05)	-0.65 (-1.02, -0.25)	-0.39 (-0.74, -0.16)
OBS-Bayesian, Median (IQR¶)	-0.52 (-0.93, -0.24)	-0.29 (-0.61, -0.11)	-0.46 (-0.83, -0.05)	-0.09 (-0.34, 0.11)	-0.43 (-0.80, -0.09)	-0.25 (-0.55, -0.06)

Note: All nutrients adjusted for total energy intake. Abbreviations: NSAID, non-steroidal anti-inflammatory drug; BMI, body mass index; MET, metabolic equivalents; IQR, interquartile range

a P <0.01 based on t-test or chi-square test

b P <0.05 based on t-test or chi-square test

*****For CPRU, lutein and lycopene intake was available as servings of lutein- and lycopene-rich fruits and vegetables

§ Diet plus supplements

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review; OBS-a posteriori: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data. Tertiles for OBS are sex-specific, and the dietary components adjusted for total energy intake.

¶ Interquartile range represented as (25^{th} percentile (Q1), 75^{Th} percentile (Q3))

Table 2.3. Associations of OBS Measures With Incident, Sporadic, Colorectal Adenoma in the Pooled Case-Control Studies

	CPRU			MAP			POOLED		
Tertiles	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**
OBS[‡]									
OBS-equal weight									
1	258	1.00	<0.0001	102	1.00	0.09	360	1.00	<0.0001
2	182	0.68 (0.53-0.87)		65	0.67 (0.43-1.07)		247	0.67 (0.54-0.83)	
3	124	0.51 (0.39-0.68)		58	0.67 (0.41-1.09)		182	0.54 (0.43-0.69)	
OBS-lit. review									
1	301	1.00	<0.0001	141	1.00	<0.001	442	1.00	<0.0001
2	150	0.62 (0.47-0.81)		36	0.32 (0.19-0.53)		186	0.53 (0.42-0.67)	
3	113	0.47 (0.35-0.64)		48	0.44 (0.27-0.73)		161	0.45 (0.35-0.58)	
OBS-*a posteriori*									
1	299	1.00	<0.0001	122	1.00	<0.0001	421	1.00	<0.0001
2	164	0.57 (0.44-0.74)		66	0.56 (0.36-0.88)		230	0.57 (0.46-0.71)	
3	101	0.40 (0.30-0.53)		37	0.34 (0.21-0.57)		138	0.38 (0.29-0.49)	
OBS-Bayesian									
1	305	1.00	<0.0001	139	1.00	<0.001	444	1.00	<0.0001
2	150	0.57 (0.43-0.73)		35	0.27 (0.16-0.46)		185	0.47 (0.38-0.60)	
3	109	0.45 (0.34-0.60)		51	0.47 (0.29-0.76)		160	0.45 (0.35-0.58)	

Adjusted for age, sex, education, family history of colorectal cancer in first degree relative, regular aspirin use, regular NSAID use, total calcium intake, total vitamin D intake, total energy intake, total folate intake, dietary fiber intake, and hormone therapy (among women).

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review; OBS-a posteriori: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data. Tertiles for OBS are sex-specific, and the dietary components adjusted for total energy intake.

Table 2.4. Associations of Dietary OBS Measures With Incident, Sporadic, Colorectal Adenoma Stratified by Lifestyle OBS and Vice-versa in the Pooled Case-Control Data

	LIFESTYLE OBS								
	Tertile 1			**Tertile 2**			**Tertile 3**		
Tertiles	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**
DIETARY OBS[‡]									
OBS-equal weight									
1	160	1.00	0.51	93	1.00	0.66	67	1.00	0.02
2	117	0.78 (0.55-1.10)		69	0.92 (0.61-1.36)		50	0.47 (0.30-0.76)	
3	99	0.90 (0.62-1.32)		86	0.91 (0.62-1.39)		48	0.56 (0.35-0.91)	
OBS-lit. review									
1	180	1.00	<0.001	57	1.00	0.63	46	1.00	0.49
2	156	0.69 (0.50-0.96)		77	1.16 (0.76-1.77)		50	1.10 (0.68-1.80)	
3	108	0.51 (0.35-0.73)		69	1.12 (0.72-1.72)		46	0.83 (0.50-1.38)	
OBS-*a posteriori*									
1	188	1.00	0.02	93	1.00	0.99	52	1.00	0.04
2	121	0.82 (0.58-1.15)		72	0.81 (0.54-1.22)		52	0.72 (0.44-1.17)	
3	95	0.65 (0.45-0.93)		74	1.01 (0.66-1.54)		42	0.58 (0.35-0.98)	
OBS-Bayesian									
1	197	1.00	0.01	72	1.00	0.87	50	1.00	0.49
2	133	0.89 (0.63-1.24)		57	0.70 (0.45-1.07)		56	1.06 (0.66-1.70)	
3	108	0.63 (0.45-0.89)		76	0.96 (0.63-1.45)		40	0.83 (0.50-1.39)	

	DIETARY OBS								
	Tertile 1			**Tertile 2**			**Tertile 3**		
	Cases	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR[#] (95% CI)**	***P* trend**
LIFESTYLE OBS[‡]									
OBS-equal weight									
1	160	1.00	0.004	117	1.00	<0.0001	99	1.00	<0.0001
2	93	0.63 (0.44-0.91)		69	0.74 (0.50-1.11)		86	0.62 (0.42-0.91)	

3	67	0.60 (0.40-0.88)		50	0.43 (0.28-0.66)		48	0.40 (0.26-0.62)	
OBS-lit. review									
1	180	1.00	<0.0001	156	1.00	<0.001	108	1.00	<0.01
2	57	0.37 (0.24-0.58)		77	0.60 (0.41-0.90)		69	0.72 (0.47-1.10)	
3	46	0.30 (0.19-0.47)		50	0.49 (0.31-0.75)		46	0.50 (0.31-0.80)	
OBS-*a posteriori*									
1	188	1.00	<0.0001	121	1.00	<0.0001	95	1.00	<0.0001
2	93	0.58 (0.41-0.82)		72	0.57 (0.38-0.86)		74	0.75 (0.50-1.12)	
3	52	0.48 (0.32-0.71)		52	0.43 (0.28-0.65)		42	0.41 (0.26-0.64)	
OBS-Bayesian									
1	197	1.00	<0.0001	133	1.00	<0.001	108	1.00	<0.001
2	72	0.58 (0.39-0.86)		57	0.40 (0.26-0.60)		76	0.67 (0.45-1.01)	
3	50	0.44 (0.29-0.68)		56	0.46 (0.30-0.71)		40	0.41 (0.26-0.66)	

Adjusted for age, sex, education, family history of colorectal cancer in first degree relative, regular aspirin use, regular NSAID use, total calcium intake, total vitamin D intake, total energy intake, total folate intake, dietary fiber intake, and hormone therapy (among women).

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review; OBS-a posteriori: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data. Tertiles for OBS are sex-specific, and the dietary components adjusted for total energy intake.

Table 2.5. Associations of OBS Measures With Incident, Sporadic, Colorectal Adenoma Stratified by Advanced Adenoma Status in the Pooled Case-Control Study

	NON-ADVANCED ADENOMA			ADVANCED ADENOMA		
Tertiles	**Cases**	**Multivariate RR [#] (95% CI)**	***P* trend**	**Cases**	**Multivariate RR [#] (95% CI)**	***P* trend**
OBS[‡]						
OBS-equal weight						
1	246	1.00	<0.001	114	1.00	<0.0001
2	189	0.76 (0.59-0.96)		58	0.47 (0.33-0.68)	
3	139	0.60 (0.46-0.79)		43	0.39 (0.26-0.58)	
OBS-lit. review						
1	313	1.00	<0.0001	129	1.00	<0.0001
2	141	0.56 (0.43-0.72)		45	0.44 (0.30-0.65)	
3	120	0.48 (0.36-0.63)		41	0.41 (0.27-0.62)	
OBS-*a posteriori*						
1	290	1.00	<0.0001	131	1.00	<0.0001
2	177	0.65 (0.51-0.83)		53	0.40 (0.28-0.58)	
3	107	0.43 (0.33-0.57)		31	0.27 (0.17-0.43)	
OBS-Bayesian						
1	315	1.00	<0.0001	129	1.00	<0.0001
2	140	0.51 (0.39-0.65)		45	0.38 (0.26-0.56)	
3	119	0.47 (0.36-0.62)		41	0.41 (0.27-0.62)	
DIETARY OBS						
OBS-equal weight						
1	217	1.00	0.57	103	1.00	0.003
2	180	0.86 (0.67-1.11)		56	0.53 (0.36-0.77)	
3	177	0.93 (0.71-1.21)		56	0.57 (0.39-0.85)	
OBS-lit. review						
1	189	1.00	0.22	94	1.00	<0.001
2	219	1.08 (0.84-1.39)		64	0.54 (0.37-0.79)	
3	166	0.84 (0.64-1.10)		57	0.53 (0.36-0.78)	
OBS-*a posteriori*						
1	230	1.00	0.37	103	1.00	<0.001
2	178	0.87 (0.68-1.12)		67	0.70 (0.49-1.01)	
3	166	0.89 (0.68-1.16)		45	0.49 (0.32-0.75)	
OBS-Bayesian						
1	231	1.00	0.23	123	1.00	0.01
2	169	0.85 (0.66-1.10)		59	1.07 (0.75-1.54)	
3	174	0.85 (0.66-1.11)		33	0.59 (0.39-0.88)	
LIFESTYLE OBS						
OBS-equal weight						
1	266	1.00	<0.0001	110	1.00	<0.0001
2	185	0.71 (0.56-0.90)		63	0.59 (0.41-0.84)	
3	123	0.51 (0.39-0.66)		42	0.40 (0.27-0.60)	
OBS-lit. review						
1	316	1.00	<0.0001	128	1.00	<0.0001
2	155	0.58 (0.45-0.76)		48	0.45 (0.30-0.68)	
3	103	0.43 (0.32-0.58)		39	0.41 (0.27-0.64)	

OBS-*a posteriori*						
1	291	1.00	<0.0001	113	1.00	<0.0001
2	173	0.64 (0.50-0.81)		66	0.65 (0.46-0.93)	
3	110	0.46 (0.36-0.61)		36	0.38 (0.25-0.58)	
OBS-Bayesian						
1	314	1.00	<0.0001	124	1.00	<0.0001
2	150	0.54 (0.42-0.69)		55	0.51 (0.35-0.75)	
3	110	0.46 (0.35-0.61)		36	0.39 (0.25-0.60)	

Adjusted for age, sex, education, family history of colorectal cancer in first degree relative, regular aspirin use, regular NSAID use, total calcium intake, total vitamin D intake, total energy intake, total folate intake, dietary fiber intake, and hormone therapy (among women). In addition, *dietary OBS* adjusted for smoking, alcohol intake, obesity, and physical activity, and *lifestyle OBS* adjusted for *dietary OBS*.

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review; OBS-a posteriori: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data. Tertiles for OBS are sex-specific, and the dietary components adjusted for total energy intake.

Table 2.6. Association of F_2-isoprostanes with OBS measures in a validation sample of the pooled MAP case-control study

Tertiles	**F2-isoprostanes (mean, nmol/L)**			
	Men	***Proportional difference*¶ (%)***	**Women**	***Proportional difference*¶ (%)***
OBS‡				
OBS-equal weights				
1	78	Ref.	122	Ref.
2	72	-7.69	89	-27.05[c]
3	66	-15.38[b]	80	-34.43[c]
OBS-lit. review				
1	76	Ref.	107	Ref.
2	77	1.32	102	-4.67
3	61	-19.74[b]	83	-22.43[b]
OBS-*a posteriori*				
1	78	Ref.	107	Ref.
2	70	-10.26	102	-4.67
3	66	-15.38[a]	81	-24.30[c]
OBS-Bayesian				
1	75	Ref.	108	Ref.
2	77	2.67	96	-11.11
3	64	-14.67[a]	89	-17.59[b]
DIETARY OBS				
OBS-equal weights				
1	78	Ref.	117	Ref.
2	73	-6.41	90	-23.08[c]
3	67	-14.10[b]	86	-26.50[c]
OBS-lit. review				
1	79	Ref.	112	Ref.
2	76	-3.80	92	-17.86
3	67	-15.19[b]	84	-25.00[b]
OBS-*a posteriori*				
1	78	Ref.	111	Ref.
2	72	-7.69	95	-14.41
3	68	-12.82[a]	86	-22.52[c]
OBS-Bayesian				
1	77	Ref.	106	Ref.
2	73	-5.19	101	-4.72
3	67	-12.99[a]	88	-16.98
LIFESTYLE OBS				
OBS-equal weights				
1	73	Ref.	105	Ref.

2	77	5.48	95	-9.52
3	66	-9.59	86	-18.10[b]
OBS-lit. review				
1	75	Ref.	101	Ref.
2	69	-8.00	100	-0.99
3	68	-9.33	88	-12.87
OBS-*a posteriori*				
1	74	Ref.	101	Ref.
2	75	1.35	99	-1.98
3	68	-8.11	88	-12.87
OBS-Bayesian				
1	74	Ref.	102	Ref.
2	76	2.70	99	-2.94
3	67	-9.46	88	-13.73

* Proportional difference in mean isoprostane levels = (tertile 2- tertile 1) / tertile 1, expressed as a percentage for the comparison of tertile 2 with tertile 1 (referent). Similarly, proportional difference for the comparison between tertile 3 and tertile 1 = (Tertile 3- Tertile 1) / Tertile 1, expressed as a percentage.

¶ *P* values for the differences based on t-test of difference between the tertiles of $\log_e(F_2$-isoprostanes). All analyses are adjusted for age, race, and study. Dietary OBS additionally adjusted for smoking, alcohol, obesity and physical activity. Lifestyle OBS additionally adjusted for the dietary OBS variable.

[a] $P < 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$

‡ OBS – equal weight: all OBS components received equal weights; OBS-lit. review: weights for OBS components based on effect estimates derived from literature review

OBS-a posteriori: weights for OBS components based on CPRU data; OBS-Bayesian: weights for OBS components based on Bayesian analysis of case-control data. Tertiles for OBS are sex-specific, and the dietary components adjusted for total energy intake.

CHAPTER 3. OXIDATIVE BALANCE SCORES AND RISK OF INCIDENT COLORECTAL CANCER IN A U.S. PROSPECTIVE COHORT STUDY

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Abstract

Although oxidative stress is implicated in colorectal carcinogenesis, human studies that evaluated associations of individual pro- and antioxidants with colorectal cancer (CRC) have been inconclusive. The authors incorporated individual environmental factors known to affect oxidative stress into four Oxidative Balance Scores (OBS) and investigated their associations with CRC in a large US cohort.

During 1999-2009, 1,109 incident CRC cases were identified among 80,202 participants in the Cancer Prevention Study-II Nutrition Cohort who had completed detailed questionnaires. Four OBS with different weighting methods (equal weights, literature review based, *a posteriori* data-based, and weights based on Bayesian analysis) were created by combining 16 dietary and non-dietary lifestyle factors. Higher values of all four OBS were associated with 41%-53% lower risk of CRC; e.g., the risk ratio for the highest to lowest OBS quartile in the Bayesian analysis was 0.50 (95% confidence interval 0.41-0.61; $P_{trend} < 0.001$). The associations were more modest when the OBS was restricted to either dietary or non-dietary components.

Results using comprehensive summary measures of oxidative balance—especially considering the similarity of the findings using the different weighting methods—support the hypothesis that a predominance of antioxidant over pro-oxidant lifestyle exposures (both dietary and non-dietary) reduces risk of colorectal cancer.

Introduction

Colorectal cancer (CRC), a multifactorial disease, is the second leading cause of cancer deaths in the US, and despite advances in screening, prevention, and treatment, mortality due to CRC remains high [238]. Preventive approaches aimed at specific, known pathways of CRC causation might be effective in reducing CRC morbidity and mortality. Oxidative stress, defined as a disturbance in the balance of pro- to antioxidants in favor of the former, is the primary cause of reactive oxygen and nitrogen species (RONS)-induced cellular injury, and is considered to be involved in the pathogenesis of CRC [239].

There is substantial basic science evidence for the role of RONS in the initiation and promotion of colorectal carcinogenesis [110, 240]. At moderate concentrations, RONS protect cells against the adverse effects of oxidative stress thereby maintaining "redox homeostasis", and play an important role as signaling molecules for numerous physiological processes [241]. Excessive and sustained increases in RONS levels, however, may overwhelm antioxidant defense mechanisms can lead to oxidative imbalance and mutagenesis and result in tumor initiation and progression through activation of redox-responsive signaling cascades involved in cell growth promotion [242-244].

Diet and other modifiable lifestyle factors such as smoking affect RONS production and oxidative balance, and are valid targets for reducing oxidative stress *in vivo*. Despite the strong mechanistic evidence linking oxidative balance to colon carcinogenesis, epidemiologic investigations on the role of specific dietary contributors

(e.g., individual antioxidant vitamins) to oxidative balance in CRC causation have been inconclusive [10]. For example, results from multiple chemoprevention trials of antioxidant supplements do not support routine use of such supplements for CRC prevention [245]. Methodological issues, such as uncontrolled and/or residual confounding, inappropriate range of intakes, and measurement error may contribute to conflicting results from observational studies. Other potential explanations include small individual effects of specific antioxidants, short follow-up periods in the supplement trials, the dose and formulation of antioxidants supplements not reflecting dietary intakes, and the lack of measures for overall oxidative exposure [182].

To account for the generally small anti-/pro-oxidant effects of, and the interactions among, dietary and lifestyle factors that act along the same pathway, we previously developed summary measures of oxidative balance, referred to as Oxidative Balance Scores (OBS) [246]. Individual environmental components known to affect oxidative processes were combined into scores using four weighting methods. The OBS were associated with oxidative stress biomarkers and were risk factors for incident, sporadic colorectal adenoma [246]. OBS have also been reported to be associated with CRC in a case-control study [189]. Herein we report findings of an investigation of associations of OBS with risk for incident CRC in a large U.S. cohort. To our knowledge, this is the first prospective study to examine CRC incidence with respect to a summary OBS.

Materials and Methods

Study Cohort

Participants were drawn from the Cancer Prevention Study (CPS) - II Nutrition Cohort, a prospective study of cancer incidence and mortality in the United States established in 1992 and described in detail elsewhere [247]. Participants completed a mailed self-administered questionnaire on demographic, medical, diet, and lifestyle factors at enrollment. Follow-up questionnaires to update exposure information and ascertain newly diagnosed cancers were sent in 1997, 1999, 2001, 2003, 2005, 2007, and 2009. The Emory University Institutional Review Board approves all aspects of the CPS-II Nutrition Cohort.

Follow-up for this analysis began on the date of completion of the 1999 follow-up questionnaire which included a 152-item food frequency questionnaire (FFQ), first administered in 1999, to provide a more comprehensive assessment of dietary exposures than the 68-item FFQ administered at enrollment in 1992 [247]. The response rate for the 1999 follow-up was 90% (n = 151,345). A total of 19,151 (13%) participants completed a shorter follow-up questionnaire with no dietary information and were excluded. After excluding participants who were lost to follow-up (n = 11,564), had a history of CRC (n = 2,964) or cancer other than non-melanoma skin cancer (n = 35,705) at baseline, had unverified self-reported CRC with the 1999 survey being their last cancer free survey (n = 32), had non-adenocarcinomatous (mostly lymphomas and carcinoids) tumors in the colon or rectum (n = 37), had incomplete or improbable FFQ data at baseline (n = 1,587) as indicated by implausibly high (men: >4,200 kcal; women: >3,500 kcal) or low (men: <800 kcal; women: <600 kcal) total energy intake, or who did not report data on lifestyle variables required for calculating an OBS (n = 242), a total of 80,063 participants (33,354 men and 46,709 women) comprised the analytic cohort.

Follow-up for each participant began on the date of the returned 1999 survey and continued until the date of colorectal cancer diagnosis, the date of censoring due to loss to follow-up, death, report of a cancer other than non-melanoma skin cancer, or June 30, 2009, whichever came first. Individuals who reported CRC diagnosis that could not be verified were censored at the last cancer-free survey. A total of 703,862 person-years were accrued during the 10 years of follow-up.

Incident CRC

We identified and verified a total of 1,107 incident cases (528 in men and 579 in women) of CRC (International Classification of Diseases Oncology codes C18.0, C18.2-C18.9, C19.9, C20.9) in the analytic cohort. Reported cancers were verified through medical records, registry linkage, or death certificates. Of the 1,107 total cases, we identified 889 incident cancers of the colon (419 proximal, 186 distal, 278 unspecified, and 6 overlapping), and 194 cancers of the rectosigmoid junction or rectum. The sub-site of 24 additional cases was unknown.

OBS components and their assessment

The components included in the OBS were determined *a priori* based on their expected physiologic effects on oxidative processes. The 16 components in the OBS and the rationale behind their inclusion are listed in Table 3.1. The dietary components were derived from the previously validated 152-item food frequency questionnaire administered in 1999 [222, 223]. Nutrient values included those derived from dietary intake as well as supplement use, where available (see footnotes to Table 3.1). All

nutrient values derived from the FFQ were energy adjusted according to the residual regression method [224]. Because measurement of dietary selenium is unreliable, only supplemental selenium was used for the OBS [248-251]. Supplemental selenium intake was categorized as: intake < the adult recommended dietary allowance of 55μg/day, intake ≥ 55μg/day but <100μg/day, and intake ≥100μg/day [251]. All energy-adjusted dietary variables, except selenium, were used as continuous variables in the OBS calculation.

Non-dietary lifestyle variables included in the OBS were smoking (current smoker, former smoker, never smoker), alcohol intake (<1 drink/week, 1-6 drinks/week, ≥7 drinks/week), obesity (BMI <30 kg/m^2 *and* waist circumference <102 cm in men or <88 cm in women, *either* BMI ≥30 kg/m^2 *or* waist circumference ≥102 cm in men or ≥88 cm in women, BMI ≥30 kg/m^2 *and* waist circumference ≥102 cm in men or ≥88 cm in women), and recreational physical activity (moderate to vigorous in metabolic equivalents). All non-dietary lifestyle variables were based on self-report on questionnaires administered in 1999, except for waist circumference, which was reported in 1997.

Creation and weighting of OBS

Details of the methods and assumptions used in creating the multiple OBS have been previously published [246]. The OBS presented in this paper include supplemental selenium, a known antioxidant, which was not included in the original OBS methods published previously. Briefly, the OBS for each participant in the study was calculated as a continuously distributed weighted score combining 16 components selected *a priori*,

with higher scores representing oxidative balance in favor of antioxidants than pro-oxidants (Table 3.1). All continuous variables were transformed to standard normal distributions for OBS calculations. All OBS components except selenium intake, smoking, alcohol intake, and obesity were treated as continuous variables to create the OBS. We used four different weighting schemes for the OBS:

1. OBS-equal weight (an *a priori* method): For OBS-equal weight, we assumed that all components are equally important and should contribute a similar weight towards the score. We multiplied the OBS components by the respective weights (1 for antioxidants and -1 for pro-oxidants), and summed the weighted components to generate the OBS-equal weight for each participant.

2. OBS-lit. review (an *a priori* method): Weights for OBS-lit. review were derived from literature reviews and were published previously [246]. Coefficient estimates were calculated using pooled adjusted risk estimates derived from reviews/meta analyses of associations of individual OBS components with colorectal cancer risk. The inverse of the effect estimate was used so that components inversely associated with colorectal cancer had a positive weight and those with higher risk a negative weight.

3. *OBS-a posteriori* (an *a posteriori* method): Data from the CPS-II nutrition cohort were used to derive weights for this OBS. We used a Cox proportional hazards model to estimate the relative risk of CRC for each OBS component after adjusting for other OBS components and confounders (see statistical analysis section below for a list of confounders). None of the 16 components violated the

Cox proportional hazards assumption, as judged by the likelihood ratio test [252]. The coefficient estimates (natural log of the relative risk) for each of the components obtained from the Cox model were used to calculate weights for OBS-*a posteriori*, using methods described previously [246]. Since this OBS was evaluated in the same dataset used for its development, over-fitting of the data is an issue. To address this concern we conducted a 10-fold cross validation; however, the results based on cross-validation weights were not meaningfully different from those presented [253].

4. OBS-Bayesian (combination of *a priori* and data-based methods): We conceptualized OBS-Bayesian as a combination of the weighting schemes in OBS-lit. review and OBS-*a posteriori*. We used a hierarchical modeling approach, utilizing a Cox proportional hazards model with informative priors within the Bayesian framework, to derive weights for OBS-Bayesian. The priors for the OBS components were defined as normally distributed with mean and variance as determined for OBS–lit. review. The covariates in the model were assigned noninformative normal priors with a mean of zero and large standard deviations (10^6). We used the BAYES statement in PROC PHREG in SAS v 9.2 for the Bayesian analysis [230]. Convergence of the Markov chain was determined through visual analysis of trace plots and by means of two diagnostic tests (Gelman-Rubin and Geweke) [231, 232]. No departures from convergence were found for any of the components in the model. Details of this weighting approach are discussed in greater detail elsewhere [246].

Non-dietary lifestyle variables such as smoking, alcohol intake, obesity, and physical activity are considered stronger risk factors for CRC than are individual dietary antioxidants and pro-oxidants [10]. To examine whether dietary factors meaningfully contribute to the association between OBS and CRC risk, we created dietary OBS by excluding the non-dietary variables from the OBS measures described above. A high dietary OBS represents increased intake of different fruits and vegetables, a higher omega-3 to omega-6 intake ratio, and lower intakes of red meat and dairy (thereby lowering iron and saturated fat intake). We also created a lifestyle OBS variable that only included the four non-dietary variables.

Statistical Analysis

We used Cox proportional hazards models to estimate the relative risk (RR) (95% CI confidence interval [95% CI]) for incident CRC in relation to each OBS, after adjusting for age, sex, education, family history of CRC in a first degree relative, CRC screening (defined as ever had a colonoscopy or sigmoidoscopy), nonsteroidal anti-inflammatory drug (NSAID) use including aspirin, total (dietary and supplemental) calcium intake, total vitamin D intake, total energy intake, and hormone replacement therapy (HRT) among women. These covariates were selected *a priori* as established CRC risk factors, and therefore, potential confounders. In addition to the covariates mentioned above, models with dietary OBS as the main exposure variable also adjusted for smoking, alcohol intake, obesity, and physical activity (i.e., individual non-dietary components of OBS), and models with lifestyle OBS adjusted for the dietary OBS variable. Cox proportional hazards assumption and statistical interactions were assessed

using the likelihood ratio tests [252]. Separate models were fit to analyze OBS as a continuous variable (effect estimates presented are based on 1 standard deviation increase in OBS) and as a categorical variable (in quartiles with the first quartile being the referent group). To test for linear trend we created a continuous variable using the median OBS value within each quartile. We also examined whether the association between OBS and CRC incidence varied by sex, age (<65 versus ≥65 years), NSAID use (no regular use, 1-29 pills /month, ≥30 pills/month), CRC screening history (never screened versus ever screened), and colorectal tumor site (distal to the splenic flexure versus proximal versus rectal).

All statistical tests were two-sided, and P <0.05 was considered statistically significant. All analyses were conducted in SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

Results

The descriptive statistics of the four OBS are presented in Table 3.2 and the component weights are presented in Table 3.3. The distributions of baseline characteristics and OBS components according to quartile of OBS-equal weight are shown in Table 3.4. Mean age and the proportion of participants with a family history of CRC in a first degree relative were similar between those in the highest and lowest quartile of OBS-equal weight in both men and women. In general, study participants in the highest quartile of OBS-equal weight were more likely to have been screened for CRC, and had lower total energy intakes, and higher reported intakes of total calcium and vitamin D than those in the lowest quartile. They were also more likely to have higher

intakes of antioxidant nutrients and lower intakes of pro-oxidant nutrients than those in the lowest quartile. There was a strong correlation between the OBS-lit. review and OBS-Bayesian (92%). The other correlations ranged from 56% (between OBS-lit. review and OBS- *a posteriori*) to 71% (between OBS- *a posteriori* and OBS-Bayesian). OBS-equal weight had similar correlations (~60%) with the other three scores.

The associations between the different OBS measures and risk of CRC among men and women separately and combined, are provided in Table 3.5. Irrespective of the weighting scheme used, 1 standard deviation increase in OBS was associated with about a 20% lower risk of CRC among study participants (men and women combined) after adjustment for covariates. For categorical analyses, participants in the highest quartile of all four OBS measures were less likely to be at risk for CRC than those in the lowest quartile, with statistically significant linear trends for the inverse association. The RRs comparing the highest to the lowest OBS quartiles were consistent and statistically significant for the different measures (41%-53% lower risk of incident CRC). As expected, the inverse association between OBS and CRC risk was stronger for the OBS measures that utilized CPS-II data to derive weights (OBS-*a posteriori* and OBS-Bayesian) compared to the completely *a priori* weighting schemes (OBS-equal weight and OBS-lit. review). The RRs comparing CRC risk among those in the highest to those in the lowest quartile of all four OBS were somewhat lower in men than in women ($p_{interaction} \geq 0.29$).

Dietary OBS (smoking, alcohol intake, obesity and physical activity not included in the scores) and non-dietary lifestyle OBS (dietary variables not included in the scores)

were also independently associated with lower risk of CRC among those in the highest quartile compared to those in the lowest quartile (Table 3.6). The RRs for both the dietary and lifestyle OBS were more modest than those for the combined OBS. The association between all four dietary and lifestyle OBS and CRC risk was not different between men and women ($p_{interaction} \geq 0.12$ and ≥ 0.36 for dietary OBS and lifestyle OBS, respectively; data not shown).

No statistically significant interactions were detected for age, NSAID use, or CRC screening status (data not shown). The OBS-CRC association did not differ by tumor location (distal colon, proximal colon, or rectum, data not shown).

Discussion

The findings from this large prospective cohort study using comprehensive summary measures of oxidative balance suggest that a predominance of antioxidant over pro-oxidant lifestyle exposures (both dietary and non-dietary) may reduce risk of colorectal cancer.

Multiple reviews and meta-analyses of observational studies and trials that investigated associations/effects of individual (or combinations of selected) antioxidants have concluded that there is no evidence supporting a causal role of such antioxidants for primary or secondary prevention of CRC [10, 254, 255]. However, strong mechanistic evidence and the multitude of factors that affect the oxidative stress pathway suggest otherwise [110, 111, 116, 240, 256]. Consequently, approaches (such as ours) that combine the major antioxidants and pro-oxidants into a single score may be more powerful measures of oxidative stress than single antioxidants [117, 187, 189, 246].

Since an assumption of equal weights is unlikely to reflect the real biological contributions of the individual exposures affecting oxidative balance, we used multiple approaches to weight the OBS. Each of the three additional OBS measures we created (OBS-lit. review, OBS-*a posteriori*, and OBS-Bayesian) are colorectal cancer-specific. Although each measure has limitations [246], the results were generally consistent across the weighting methods and support the use of comprehensive measures of oxidative balance in studies of CRC risk.

Our results suggest that both dietary and non-dietary lifestyle factors contribute to oxidative balance and CRC risk. Irrespective of the weighting method, dietary OBS was statistically significantly associated with decreased risk of CRC, indicating that it contributed importantly to the inverse associations observed. Our results for CRC are in agreement with the findings of Slattery *et al.* [189] and those observed for the association of dietary OBS with advanced colorectal adenomas [246]. In contrast to our findings, Mekary, *et al*. suggested that total antioxidant capacity (TAC) of foods consumed was not associated with colorectal cancer risk [257]. TACs of the foods reported in the dietary questionnaire were derived using the ferric-reducing ability of plasma (FRAP) assay [258, 259]. Use of TAC is different from the current analysis in that it measures the intrinsic antioxidant capacity of foods *in vitro*, and does not include measures of pro-oxidants. Another factor that was different between our study and that of Mekary, *et al*. is the length of follow up (10 vs. 18 years, respectively). It is possible that the OBS affects tumor promotion rather than initiation.

The dietary OBS evaluated in this analysis differ from most other published studies of diet patterns (or scores) and colorectal cancer risk [219, 260] in two important ways. The first is the use of different weighting approaches for the OBS. The second is our use of a pathway-based approach, whereas other scores reflect either general dietary guidance or hypothesized ideal diets (e.g., Mediterranean diet [261, 262]). While these patterns likely represent multiple preventive pathways, the OBS, by design, is specific to oxidative balance. Higher OBS have been reported to be associated with lower levels of F_2-isoprostanes (a sensitive and specific marker of oxidative stress *in vivo*) in a dose-dependent manner, thus demonstrating the specificity of the OBS in capturing oxidative stress associated exposures [246, 263].

Our study had certain limitations. Although our OBS is comprehensive, we could have missed potential components because of a lack of published evidence of their effects on oxidative processes. The OBS components do not include endogenous factors that modify oxidative stress, such as DNA damage repair genes or genes encoding enzymes that regulate the cellular response against oxidative stress [234, 235]. The importance of these factors was highlighted by Slattery *et al.*, who reported significant interactions between OBS and a polygenic summary score, comprised of markers for four genes that regulate endogenous antioxidant mechanisms, in a case-control study [189]. Each of the components included in our study, especially the non-dietary lifestyle components, also act through pathways other than oxidative stress, and it is likely that some of the observed inverse association of OBS with CRC is a result of this [233]. Finally, use of CRC risk estimates to weight OBS components might limit its applicability to other oxidative stress-associated diseases such as cardiovascular, metabolic, and neurological diseases.

Use of endogenous biomarkers of lipid, protein, and DNA/RNA oxidation to weight dietary and lifestyle OBS components would be an ideal alternative.

Our study also had several strengths. The prospective design of data collection, large sample size, and availability of data on all OBS components are strengths inherent in the CPS-II nutrition cohort. The similarity of results across the four different OBS weighting methods indicates the robustness of our findings and can be viewed as sensitivity analyses for weighting OBS components.

In conclusion, the findings from this large prospective cohort study using four different weighting methods for constructing comprehensive summary measures of oxidative balance—especially considering the similarity of the results using the different weighting methods—support the hypothesis that a predominance of antioxidant over pro-oxidant lifestyle exposures (both dietary and non-dietary) reduces risk for colorectal cancer. Future directions include validation of these scores in other populations/datasets using weights reported in the current study, extension of the OBS to incorporate endogenous factors affecting oxidative balance, and development of biomarker-based OBS weights.

Tables and Figures

Table 3.1. Oxidative Balance Score (OBS) components and rationale for inclusion in the OBS

OBS components	Rationale for inclusion
Dietary antioxidants	
Pro-vitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin)	Precursors to vitamin A, potent antioxidants [147]
Lutein	Antioxidant [147]
Lycopene	Antioxidant [153]
Vitamin C	Prevents lipid peroxidation, helps regenerate α-tocopherol [158]
Vitamin E	Membrane bound antioxidant, protects against lipid peroxidation [155]
Ω-3 fatty acids (marine)	Induce electrophile-responsive element (EpRE) regulated genes responsible for transcription regulation of antioxidant enzymes [161, 162]
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 [164, 165]
Glucosinolates	Sulfur-containing plant compounds with antioxidant functions: induce EpRE as Ω-3 fatty acids, induce hemoxygenase-1 which catalyzes heme to biliverdin, induce glutathione peroxidase [166]
Selenium	Trace element that is part of important antioxidant selenoproteins, such as glutathione peroxidase, selenoprotein P, and thioredoxin reductases [167]
Dietary pro-oxidants	
Dietary iron	Primarily available from red meat, preferentially catalyzes oxidative reactions through production of free radicals resulting in lipid, protein, and DNA and other nucleic acid damage [168, 169]
Ω-6 fatty acids	Higher intakes associated with increased oxidative stress through increased free-radical production; unlike Ω-3 fatty acids, does not induce EpRE [162, 172, 173]
Saturated fat	Oxidative DNA damage through increased production of known pro-oxidant bile acids in the colon [170, 171]
Non-dietary lifestyle antioxidants	
Physical activity	Although acute bouts of exercise increase RONS production, regular exercise results in increase in adaptive response to oxidative stress by activating cellular antioxidant signaling systems and enhancing expression of antioxidant enzymes through a process termed “hormesis” [174]
Non-dietary lifestyle pro-	

oxidants	
Smoking	Potent producer of free radicals, associated with increase in blood/tissue markers of oxidative stress [175, 237]
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 [176, 177]
Obesity	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 [178]

Table 3.2. Descriptive statistics of the OBS

	Mean (SD)	**Range**	**25th percentile**	**50th percentile**	**75th percentile**
OBS-equal weight	0.001 (5.53)	-17.38, 48.03	-3.83	-0.67	3.07
OBS-lit. review	-0.26 (0.38)	-1.53, 3.88	-0.49	-0.26	-0.01
OBS-*a posteriori*	-0.16 (0.30)	-1.87, 2.84	-0.37	-0.17	0.03
OBS-Bayesian	-0.21 (0.29)	-1.28, 1.71	-0.38	-0.19	-0.02

Table 3.3. Components of 4 Different Oxidative Balance Scores (OBS) and Weights Given to Them in Different Measures of the OBS[a]

OBS Component	OBS Weight[b] - MEN				OBS Weight[b] - WOMEN			
	OBS–Equal Weights	OBS–Lit. Review[c]	OBS–*a posteriori*	OBS–Bayesian	OBS–Equal Weights	OBS–Lit. Review[c]	OBS– *a posteriori*	OBS–Bayesian
Dietary antioxidants								
Provitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin)	+1	0.0039	-0.1004	0.00927	+1	0.0039	-0.0070	0.00567
Lutein	+1	0.0325	0.0989	0.0287	+1	0.0325	0.0338	0.0315
Lycopene	+1	−0.0153	-0.0009	-0.0199	+1	-0.0153	0.0514	-0.00933
Vitamin C	+1	0.0810	0.1089	0.0485	+1	0.0810	-0.0460	0.0203
Vitamin E	+1	0.1368	0.0185	0.0876	+1	0.1550	0.0757	0.0815
ω-3 fatty acids (marine)	+1	0.0044	-0.0660	0.00824	+1	-0.0454	-0.0453	-0.0372
Flavonoids	+1	−0.0043	-0.0164	-0.00396	+1	-0.0043	0.0262	0.00844
Glucosinolates	+1	0.0411	0.1305	0.0379	+1	0.0411	0.0864	0.0503
Selenium	+1	0.0512[j] 0.1053[k]	0.0559[j] 0.0670[k]	0.1024[j] 0.1122[k]	+1	0.0512[j] 0.1053[k]	0.1529[j] -0.0822[k]	0.1144[j] -0.0528[k]
Dietary prooxidants								
Dietary iron	−1	−0.0744	0.0409	-0.0522	−1	-0.0744	-0.0394	-0.0698
ω-6 fatty acids	−1	0.0410	0.1223	0.0480	−1	-0.0454	-0.0301	-0.0531
Saturated fat	−1	−0.0153	-0.0543	-0.0472	−1	-0.0153	-0.0417	-0.0481
Nondietary lifestyle antioxidants								
Physical activity	+1	0.1080	0.0758	0.1040	+1	0.1080	-0.0343	0.0937
Nondietary lifestyle prooxidants								
Smoking	−1	−0.7031[d] −0.0953[e]	−0.2774[d] −0.1066[e]	−0.1480[d] −0.1027[e]	−1	-0.1398[d] -0.1823[e]	-0.4265[d] -0.3695[e]	-0.1957[d] -0.1344[e]
Alcohol intake	−1	−0.2390[f] −0.0676[g]	−0.0511[f] −0.0934[g]	−0.3869[f] −0.0704[g]	−1	-0.2390[f] -0.0676[g]	0.0151[f] 0.2141[g]	-0.0610[f] -0.1576[g]
Obesity	−1	−0.0770[h] −0.0295[i]	−0.3070[h] −0.0833[i]	−0.3350[h] −0.1026[i]	−1	-0.0770[h] -0.0295[i]	-0.1834[h] -0.1372[i]	-0.0360[h] -0.0976[i]

[a] For each participant, OBS was calculated as a weighted sum of the components listed in the table.
[b] OBS–equal weight: all OBS components received equal weights; OBS–lit. review: weights for OBS components were based on effect estimates derived from literature review; OBS–a posteriori: weights for OBS components were based on CPRU Study data; OBS–Bayesian: weights for OBS components were based on Bayesian analysis of case-control data.

[c] Weights were derived from published reviews/meta-analysis for all components except ω-3 fatty acids, ω-6 fatty acids, flavonoids, glucosinolates, and iron, where one of the authors (C.D.) conducted the meta-analyses.
[d] Current smokers.
[e] Former smokers.
[f] Heavy alcohol drinkers.
[g] Moderate alcohol drinkers.
[h] Obese persons.
[i] Overweight persons.
[j] Persons with selenium intake between 55-100 μg/day
[k] Persons with supplemental selenium intake ≥100 μg/day

Table 3.4. Baseline characteristics of CPS-II men and women by extreme quartiles of Oxidative Balance Score (OBS), 1999

	MEN		WOMEN	
	OBS-equal weight[1]		OBS-equal weight[1]	
	Q1	*Q4*	*Q1*	*Q4*
Age at 1999 interview, Mean (y)	69.9	70.2	68.2	68.1
Caucasian (%)	98	97	98	97
College education or higher (%)	39	63	26	41
Family history of colon or rectal cancer in a first degree relative (%)	12	12	13	14
Current ERT/CHRT use (%)	-	-	40	49
Colonoscopy / sigmoidoscopy screening (%)	60	72	54	63
NSAID use 1-29 pills/month (%)	33	42	35	41
NSAID use ≥30 pills/month (%)	30	29	27	24
Total energy intake, Mean (kcal/day)	1,962.4	1,842.0	1,642.5	1,561.0
Total calcium intake, Mean (mg/day)[2]	778.3	1,093.6	1,030.9	1,517.6
Total vitamin D intake, Mean (IU/day)[2]	304.3	476.3	328.1	481.2
OBS components				
Total pro-vitamin A carotenoids intake, Mean (IU/day)[2]	2,870.0	8,314.9	3,398.7	9,355.2
Total lutein intake, Mean (µg/day)[2]	1,202.0	3,140.3	1,408.6	3,556.3
Total lycopene intake, Mean (µg/day)[2]	3,826.6	6,984.5	3,788.7	6,664.1
Total vitamin C intake, Mean (mg/day)[2]	188.2	726.6	223.9	778.6
Total vitamin E intake, Mean (mg – TE/day)[2]	36.1	152.0	47.1	161.3
Marine Ω-3 fatty acid intake, Mean (gm/day)[3]	1.2	1.4	1.2	1.3
Dietary flavonoid intake, Mean (mg/day)	174.4	393.4	174.4	387.7
Dietary glucosinolate intake, Mean (mg/day)	6.8	20.8	7.0	19.7
Selenium supplements, Mean (µg/day)	0.2	0.8	0.3	0.8
Total Ω-6 fatty acid intake, Mean (gm/day)[4]	12.7	11.3	11.3	9.9
Saturated fat intake, Mean (gm/day)	1.3	0.9	1.3	0.9
Dietary iron intake, Mean (mg/day)	17.1	21.2	17.5	20.4
Current smoker, (%)	11	1	11	1
Former smoker (%)	69	42	44	27
1-6 alcoholic drinks/week, (%)	27	29	27	27
≥7 alcoholic drinks/week, (%)	38	25	21	11
BMI (kg/m^2), Mean	27.9	25.3	27.3	24.3
Waist circumference (inches), Mean	40.2	37.2	35.7	31.8
Physical activity, Mean (MET-hrs/week)	11.4	24.7	9.2	21.5

Note: All nutrients adjusted for total energy intake. Abbreviations: ERT or CHRT, estrogen or combined hormone replacement therapy; NSAID, non-steroidal anti-inflammatory drug; BMI, body mass index; MET, metabolic equivalents.

[1] OBS – equal weight: all OBS components received equal weights

[2] Diet plus supplements
[3] Sum of docosahexaenoic acid, eicosapentaenoic acid, and docosapentaenoic acid
[4] Sum of linoleic acid, arachidonic acid, γ-linoleic acid, and other minor Ω-6 acids

Table 3.5. Associations of Oxidative Balance Score (OBS) measures with incident colorectal cancer among men and women in the CPS-II Nutrition Cohort (1999-2009)

OBS	MEN (N=33,354) # Cases	MEN (N=33,354) Multivariate RR [1] (95% CI)	WOMEN (N=46,709) # Cases	WOMEN (N=46,709) Multivariate RR [1] (95% CI)	ALL PARTICIPANTS (N=80,063) # Cases	ALL PARTICIPANTS (N=80,063) Multivariate RR [1] (95% CI)
OBS-equal weight [2]						
1 SD increase[3]	-	0.81 (0.73, 0.90)	-	0.85 (0.78, 0.93)	-	0.83 (0.77, 0.89)
Quartiles[4]						
1	163	1.00	182	1.00	352	1.00
2	141	0.87 (0.69, 1.09)	150	0.82 (0.66, 1.02)	283	0.79 (0.67, 0.92)
3	136	0.85 (0.68, 1.08)	135	0.76 (0.61, 0.96)	272	0.78 (0.66, 0.92)
4	88	0.57 (0.43, 0.75)	112	0.65 (0.50, 0.83)	200	0.59 (0.49, 0.70)
P_{trend}[5]		*<0.001*		*<0.001*		*<0.001*
OBS-lit. review [2]						
1 SD increase[3]	-	0.82 (0.74, 0.89)	-	0.86 (0.79, 0.95)	-	0.82 (0.76, 0.88)
Quartiles[4]						
1	152	1.00	179	1.00	371	1.00
2	154	1.01 (0.80, 1.26)	158	0.89 (0.72, 1.10)	299	0.81 (0.69, 0.95)
3	135	0.88 (0.70, 1.11)	120	0.71 (0.56, 0.89)	230	0.64 (0.54, 0.76)
4	87	0.58 (0.44, 0.76)	122	0.75 (0.59, 0.95)	207	0.60 (0.50, 0.73)
P_{trend}[5]		*<0.001*		*0.004*		*<0.001*
OBS-*a posteriori* [2]						
1 SD increase[3]	-	0.73 (0.66, 0.80)	-	0.76 (0.70, 0.82)	-	0.74 (0.69, 0.78)
Quartiles[4]						
1	184	1.00	186	1.00	379	1.00
2	145	0.78 (0.63, 0.98)	168	0.88 (0.71, 1.08)	307	0.80 (0.69, 0.93)
3	118	0.64 (0.51, 0.81)	128	0.67 (0.53, 0.84)	245	0.64 (0.55, 0.76)
4	81	0.45 (0.35, 0.59)	97	0.53 (0.41, 0.67)	176	0.47 (0.39, 0.57)
P_{trend}[5]		*<0.001*		*<0.001*		*<0.001*
OBS-Bayesian [2]						
1 SD increase[3]	-	0.79 (0.72, 0.87)	-	0.82 (0.75, 0.89)	-	0.79 (0.74, 0.84)

Quartiles[4]						
1	170	1.00	178	1.00	376	1.00
2	137	0.80 (0.64, 1.00)	161	0.90 (0.73, 1.11)	292	0.79 (0.67, 0.93)
3	139	0.81 (0.65, 1.02)	130	0.73 (0.58, 0.92)	258	0.70 (0.59, 0.83)
4	82	0.48 (0.37, 0.63)	110	0.63 (0.50, 0.81)	181	0.50 (0.41, 0.61)
P_{trend}[5]		*<0.001*		*<0.001*		*<0.001*

Abbreviations: CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug; HRT, hormone replacement therapy; RR, relative risk; CI, confidence interval

[1] Adjusted for age, sex, education, family history of CRC in a first degree relative, CRC screening by colonoscopy/sigmoidoscopy, NSAID use, total calcium intake, total vitamin D intake, total energy intake, and HRT (among women)

[2] OBS – equal weight: all OBS components received equal weights.
OBS-lit. review: weights for OBS components based on effect estimates derived from literature review
OBS-*a posteriori*: weights for OBS components based on CPS-II Nutrition Cohort data
OBS-Bayesian: weights for OBS components based on Bayesian analysis of CPS-II Nutrition Cohort data

[3] Based on a Cox proportional hazards model with OBS modeled as a continuous variable

[4] Based on a Cox proportional hazards model with OBS modeled as a categorical (quartiles) variable

[5] P_{trend} assessed by χ^2 test for linear trend

Table 3.6. Associations of DIETARY Oxidative Balance Score (Dietary OBS) and LIFESTYLE Oxidative Balance Score (Lifestyle OBS) with incident colorectal cancer among study participants (N=80,063) in the CPS-II Nutrition Cohort (1999-2009)

	DIETARY OBS		**LIFESTYLE OBS**	
OBS	**Cases**	**Multivariate RR [1] (95% CI)**	**Cases**	**Multivariate RR [2] (95% CI)**
OBS-equal weight [3]				
1 SD increase[4]	-	0.89 (0.83, 0.95)	-	0.87 (0.82, 0.92)
Quartiles[5]				
1	334	1.00	331	1.00
2	307	0.95 (0.81, 1.11)	284	0.84 (0.71, 0.98)
3	246	0.79 (0.67, 0.94)	251	0.74 (0.63, 0.88)
4	220	0.75 (0.62, 0.89)	241	0.71 (0.60, 0.84)
P_{trend}[6]		*<0.001*		*<0.001*
OBS-lit. review [3]				
1 SD increase[4]	-	0.89 (0.83, 0.95)	-	0.86 (0.81, 0.92)
Quartiles[5]				
1	342	1.00	347	1.00
2	277	0.81 (0.69, 0.95)	282	0.83 (0.70, 0.99)
3	265	0.84 (0.71, 0.98)	256	0.74 (0.61, 0.89)
4	223	0.73 (0.62, 0.89)	222	0.66 (0.54, 0.80)
P_{trend}[6]		*0.002*		*<0.001*
OBS-*a posteriori* [3]				
1 SD increase[4]	-	0.81 (0.76, 0.86)	-	0.81 (0.77, 0.86)
Quartiles[5]				
1	375	1.00	350	1.00
2	293	0.83 (0.71, 0.97)	306	0.85 (0.73, 0.99)
3	225	0.67 (0.56, 0.79)	255	0.67 (0.57, 0.79)
4	214	0.65 (0.54, 0.77)	196	0.56 (0.47, 0.66)
P_{trend}[6]		*<0.001*		*<0.001*
OBS-Bayesian [3]				
1 SD increase[4]	-	0.85 (0.79, 0.91)	-	0.86 (0.81, 0.92)
Quartiles[5]				
1	376	1.00	359	1.00
2	267	0.74 (0.63, 0.87)	271	0.79 (0.67, 0.93)
3	243	0.71 (0.60, 0.84)	244	0.68 (0.57, 0.81)
4	221	0.66 (0.55, 0.78)	233	0.68 (0.56, 0.81)
P_{trend}[6]		*<0.001*		*<0.001*

Abbreviations: CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug; HRT, hormone replacement therapy; RR, relative risk; CI, confidence interval

[1] Adjusted for age, sex, education, family history of CRC in a first degree relative, CRC screening by colonoscopy/sigmoidoscopy, NSAID use, total calcium intake, total vitamin D intake, total energy intake, HRT (among women), smoking, alcohol intake, obesity, and physical activity.

[2] Adjusted for age, sex, education, family history of CRC in a first degree relative, CRC

screening by colonoscopy/sigmoidoscopy, NSAID use, total calcium intake, total vitamin D intake, total energy intake, HRT (among women), and dietary OBS.

[3] OBS – equal weight: all OBS components received equal weights
OBS-lit. review: weights for OBS components based on effect estimates derived from literature review
OBS-*a posteriori*: weights for OBS components based on CPS-II Nutrition Cohort data
OBS-Bayesian: weights for OBS components based on Bayesian analysis of CPS-II Nutrition Cohort data

[4] Based on a Cox proportional hazards model with OBS modeled as a continuous variable

[5] Based on a Cox proportional hazards model with OBS as a categorical (quartiles) variable.

[6] P_{trend} assessed by χ^2 test for linear trend

CHAPTER 4. ASSOCIATIONS OF PLASMA F_2-ISOPROSTANES WITH ANTIOXIDANT MICRONUTRIENT LEVELS IN A CASE-CONTROL STUDY OF INCIDENT, SPORADIC COLORECTAL POLYPS

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Abstract

Evidence on associations between measures of oxidative stress and risk of colorectal neoplasms is limited. We investigated associations of colorectal adenomas with antioxidant micronutrient dietary intakes and circulating levels of plasma F_2-isoprostanes, a reliable marker of lipid peroxidation *in vivo*, in a colonoscopy-based, pooled case-control study of incident, sporadic colorectal adenomas (n = 157 cases, 184 controls). We measured antioxidant intakes using food frequency questionnaires, and plasma levels of antioxidants by high-performance liquid chromatography and F_2-isoprostanes by gas chromatography-mass spectrometry. Plasma F_2-isoprostanes were 20.7% and 33.7% higher in adenoma and hyperplastic polyp cases, respectively, than in controls (P=0.04) among women, but differed minimally among men, and decreased with increasing plasma α-carotene, β-carotene, and α-tocopherol levels in women, but not men. Increasing plasma levels of γ-tocopherol were associated with increasing F_2-isoprostanes in both women (P<0.01) and men (P<0.01). Our findings suggest that lipid peroxidation, as indicated by circulating F_2-isoprostanes, may be 1) positively associated with risk for incident, sporadic colorectal adenoma in women, but not men, and 2) inversely associated with antioxidant micronutrient exposures, with some differences according to sex.

Keywords: isoprostanes, antioxidant, biomarkers, oxidative stress, colorectal adenoma

Introduction

Most colorectal cancers, the third most common cancer in the US, develop in pre-cancerous adenomatous polyps [11, 264]. Evidence suggests that oxidative stress, thought to be acting through reactive oxygen and nitrogen species, plays an important role in colorectal neoplasia [265]. Although these free radicals can affect almost all cellular components, including proteins and DNA, lipids are most susceptible to free radical damage. Lipid peroxidation is believed to be one of the major determinants of oxidative stress-related colorectal carcinogenesis, and elevated levels of lipid peroxidation metabolites have been reported in human colorectal cancer tissue [266, 267].

8-epi-prostaglandin $F_{2\alpha}$, also known as F_2-isoprostanes, are almost exclusively formed by free-radical oxidation of arachidonic acid [268], and have been shown to be the most reliable non-invasive marker of lipid peroxidation *in vivo* [202]. F_2-isoprostanes in human plasma/urine/tissues have been directly associated with several oxidative stress-related chronic diseases (e.g., diabetes, atherosclerosis, and Alzheimer's disease) [269-271], and cancers of the prostate, lung, and breast [272-274]. Despite the strong mechanistic evidence suggesting a role of oxidative stress in colorectal carcinogenesis, only one study, which reported null findings, on the association of F_2-isoprostanes with colorectal polyps has been published [275]. Diet and other modifiable lifestyle factors, most of which contribute to inflammation and oxidative stress pathways, have been thought to account for a large proportion of colorectal cancers in the US [276]. However, supplemental antioxidants, alone or in limited combinations, did not reduce risk of colorectal neoplasms in randomized clinical trials [277]. These conflicting results

indicate that the complex processes of anti-/pro-oxidant regulation and oxidative stress in colorectal neoplasia are poorly understood. Investigating associations of dietary and non-dietary anti-/pro-oxidants with valid oxidative stress biomarkers *in vivo* may clarify this issue. Additionally, it has been suggested that nutrient biomarkers are better measures of micronutrient exposure than are dietary questionnaires because they are more accurately measured and indicate intake, absorption, and metabolism rather than intake alone [278]. Comparing the association of biomarker-based versus questionnaire-derived antioxidant micronutrients with F_2-isoprostanes may clarify their role as indicators of oxidative stress *in vivo*.

Herein we report on associations of plasma F_2-isprostane levels with risk of incident, sporadic colorectal adenoma, antioxidant micronutrient exposures estimated via a semi-quantitative food frequency questionnaire and measurements of plasma antioxidant micronutrient levels, and other risk factors in a pooled case-control study.

Materials and Methods

Study population

Data from two, methodologically similar, colonoscopy-based case-control studies of incident, sporadic colorectal adenomas conducted by the same principal investigator (RMB) were pooled. The first study (Markers of Adenomatous Polyps study, MAPI) was conducted from 1994-1997 in Winston-Salem and Charlotte, North Carolina, and the second (MAPII) was conducted in 2002 in Columbia, South Carolina. Participants in both studies were recruited from patients without a prior history of colorectal neoplasms scheduled for outpatient, elective endoscopy in large, community-based gastroenterology

practices. Participants aged 30-74 years, English speaking, without contraindications to colonoscopy, and no known genetic syndromes associated with colonic neoplasia or history of inflammatory bowel diseases, colorectal adenomas, or cancer (except non-melanoma skin cancer) were eligible to participate.

In the MAPI study, 669 (30%) of the 2,246 colonoscopy patients identified over 12 months were eligible to participate. Of these, 617 (92%) were contacted, and 417 (68%) consented to participate. A total of 177 adenoma cases, 47 hyperplastic polyp cases, and 179 polyp free controls were identified. In the MAPII study, 351 colonoscopy patients were identified over five months. Of these, 305 (87%) were eligible on initial screening, and 232 (76%) were contacted and agreed to participate. Among participants who met final eligibility criteria, 48 adenoma cases, 28 hyperplastic polyp cases, and 119 polyp free controls were identified. We combined the data from the two studies (hereafter referred to as MAP) since their selection criteria, study protocols, and questionnaires were identical. All participants completed questionnaires prior to colonoscopy on demographics, medical and family history, lifestyle, body size characteristics, diet, and, in women, hormonal and reproductive history. Details of the study protocols for MAPI [279], and MAPII [280] studies were previously reported.

Fasting peripheral venous blood samples were drawn into red-coated, pre-chilled vacutainer tubes and then immediately placed on ice and shielded from light. Blood fractions were aliquotted into amber-colored cryopreservation tubes, the air was displaced with an inert gas (nitrogen in MAPI and argon in MAPII) and the cryopreservation tubes sealed with O-ring screw caps, and then the aliquots were

immediately placed in a -80º C freezer until analysis. The present study was conducted after most of the stored plasma samples were exhausted in previous studies; remaining samples were available for 69% of the adenoma cases (n=157), 80% of the hyperplastic polyp cases (n=60), and 62% of the controls (n=184), and the analyses reported herein are based on these sample sizes.

The study protocols for both studies were approved by the respective Institutional Review Boards of the corresponding institutions, and all participants were willing to participate and able to understand and provide informed consent.

Case-control status assignment

Participants with an incident, colorectal adenoma or hyperplastic polyp detected and removed during colonoscopy and verified by an index study pathologist using diagnostic criteria established by the National Polyp Study [281] were identified as adenoma and hyperplastic polyp cases, respectively. Participants who had no adenomatous or hyperplastic polyps on colonoscopy were considered controls.

Assessment of antioxidant micronutrients

Dietary intakes of antioxidant micronutrients (α-carotene, β-carotene, β-cryptoxanthin, lutein plus zeaxanthin, lycopene, vitamin E, and vitamin C) were based on participant self-report using a previously validated, semi-quantitative 153-item Willett food frequency questionnaire (FFQ) [222, 223].

Stored plasma was used to assay the carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein plus zeaxanthin, and lycopene) and α- and γ-tocopherols using high-performance liquid chromatography-based assays. Details of the original method [282], calibration [283], sample handling [284], and modifications to the original method [285] were previously reported. Calibration was performed with pure compounds (Hoffman-La Roche; Sigma Chemical Co.). Quality control of control pools revealed coefficients of variation of <11% for all analytes.

Assessment of F_2-isoprostanes

A highly specific and quantitative gas chromatography-mass spectrometry-based method was used to measure plasma F_2-isoprostanes [286]. An internal standard, [$^2H_4$]8-iso-$PGF_{2\alpha}$ (>98% pure; Cayman Chemical), was added to plasma prior to analysis. Quality control procedures included analysis of two control pools which had varying concentration ranges of F_2-isoprostanes. The coefficients of variation for the two pools were 9.5% and 11%.

All laboratory assays were performed at the Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota Medical Center, Fairview.

Assessment of covariates

Covariates for analysis, derived from participant-reported questionnaires, included regular (at least once/week) use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAID), cigarette smoking (current, former, and never smoker), alcohol intake (nondrinker, 1-6 drinks/week, and ≥7 drinks/week), physical activity

(MET-hrs./week), and menopausal hormone therapy use in women. Body mass index (BMI) and waist-to-hip ratio were included as anthropometric covariates. Dietary factors of interest were total energy, fat (total fat, saturated fat, and Ω-6 polyunsaturated fatty acids), calcium, folate, dietary fiber, iron, red meat, and serum 25-OH-vitamin D_3.

Statistical analyses

Cases and controls were compared with respect to selected characteristics using the *t*-test and chi square test for continuous and categorical variables, respectively. All nutrient variables were adjusted for total energy intake using the residual regression method prior to analyses [224].

F_2-isoprostane values were log transformed to normalize their skewed distribution. We used general linear models to i) compare F_2-isoprostane (dependent variable) levels among adenoma cases, hyperplastic polyp cases, and controls adjusted for age, study, sex, and family history of colorectal cancer; and ii) evaluate associations of dietary (FFQ-derived) antioxidant intakes and plasma antioxidant levels with F_2-isoprostanes, adjusted for age and study. Analyses involving the plasma antioxidants were additionally adjusted for plasma total cholesterol. Because F_2-isoprostane levels vary substantially by sex [214, 220], we report results separately for males and females. The continuous dietary and plasma antioxidant variables were categorized into tertiles prior to analysis.

We also used logistic regression models to further evaluate these associations. Models to evaluate associations of F_2-isoprostanes (in tertiles) with adenoma and hyperplastic polyp risk were adjusted for age, sex, study, and family history of colorectal

cancer. F_2-isoprostanes were then dichotomized based on the sex-specific median levels in the controls, and we modeled the risk of a participant being in the higher category (above median, indicative of higher oxidative stress) relative to the lower category across categories of self-reported and circulating antioxidant micronutrients. All models were adjusted for age, study, non-dietary covariates mentioned above, and selected dietary variables that were associated with F_2-isoprostanes in bivariate analyses. Tests for linear trend were conducted by creating a continuous variable using the median value within tertiles for each antioxidant variable and using that variable as the predictor of interest in the logistic models.

All statistical tests were two-sided and considered statistically significant at $P<0.05$. All analyses were conducted in SAS version 9.2 (SAS institute).

Results

Selected characteristics of the cases and controls are summarized in Table 1. Adenoma cases were more likely than controls to be male, be current smokers, and drink ≥7 alcoholic drinks/week, and, on average, have greater energy intakes and a higher waist-to-hip ratio. Compared to adenoma cases, controls were more likely to regularly take NSAIDs and have a family history of colorectal cancer in a first degree relative. Hyperplastic polyp cases were more likely to be male, be current smokers, and drink ≥7 alcoholic drinks/week, and, on average, have a higher waist-to-hip ratio than controls.

Overall, circulating F_2-isoprostane levels were 14.8% higher in adenoma cases, and 24.7% higher in hyperplastic polyp cases than in controls (Figure 1); however, this difference was observed primarily in women ($P_{interaction\ for\ sex}$=0.30). Among women, F_2-

isoprostanes were 20.7% and 33.7% higher in the adenoma and hyperplastic polyp cases, respectively, compared to controls (*P*=0.04), whereas among men they were, respectively, only 4.2% and 5.6% higher (*P*=0.80). We also modeled the association of isoprostane levels (in tertiles based on the distribution among controls) with adenoma and hyperplastic polyp risk using logistic regression (Table 2). In multivariable models, higher isoprostane levels were associated with hyperplastic polyp risk [OR (95% CI) comparing the 3rd to the 1st tertile: 2.39 (1.06, 5.38)] but not risk of adenomas. Although the interaction between sex and isoprostane levels in either adenoma or hyperplastic polyp risk was not statistically significant, the results were stronger among women than men (Table 2).

Associations of F_2-isoprostanes with dietary antioxidant intakes derived from self-reported FFQs are presented in Table 3. The findings were similar in cases and controls (data not shown) so we combined cases and controls for these analyses. After adjusting for multiple covariates (footnote, Table 3), male participants in the highest tertile of vitamin C intake were less likely to have high (above median) circulating F_2-isoprostanes than those in the lowest tertile (OR: 0.25, 95% CI: 0.08, 0.81). Among men, F_2-isoprostane levels tended to be lower among those with higher reported intakes of all antioxidant micronutrients except lycopene; however, the findings were not statistically significant. Among women, lutein/zeaxanthin and vitamin E were the only dietary antioxidants statistically significantly associated with lower F_2-isoprostane levels [OR (95% CI) comparing the 3rd to the 1st tertile: 0.33 (0.14, 0.77) and 0.27 (0.11-0.65) for lutein/zeaxanthin and vitamin E, respectively]. Other antioxidant micronutrients (except

dietary β-cryptoxanthin) were also associated with lower F_2-isoprostanes in women, but the findings were not statistically significant (Table 3).

Although plasma β-carotene, β-cryptoxanthin, and α-tocopherol levels were inversely associated with isoprostane levels among men, none of the associations was statistically significant (Table 4) after adjustment for multiple covariates (Table 4 footnote), including plasma total cholesterol. Increasing plasma γ-tocopherol levels were associated with higher isoprostane levels, but the multivariable-adjusted results were not statistically significant. In contrast, concentrations of all the plasma antioxidants (except γ-tocopherol) were inversely associated with F_2-isoprostanes among women, but the results for β-cryptoxanthin and lutein/zeaxanthin were not statistically significant. Women in the highest relative to the lowest tertile of α-carotene, β-carotene, lycopene, and α-tocopherol were 44-73% less likely to have plasma F_2-isoprostane concentrations above the median level. γ-tocopherol was strongly directly associated with F_2-isoprostanes in women—OR (95% CI) comparing the highest to the lowest tertile: 5.82 (2.36-14.31).

Discussion

Our results suggest that F_2-isoprostanes—a highly reliable and valid biomarker of oxidative stress [202]—may be directly associated with risk for colorectal adenomatous and hyperplastic polyps in women, but not men. Our results also suggest that self-reported dietary and supplemental intakes of vitamin C in men, and vitamin E and lutein/zeaxanthin in women may be inversely associated with lipid peroxidation levels. After multivariable adjustment, plasma α-tocopherol and carotenoids were inversely associated with F_2-isoprostanes among women, but not men, and plasma concentrations of γ-tocopherol were directly associated with F_2-isoprostane levels in men and women.

Oxidative stress is one of the proposed pathways in colorectal carcinogenesis [265]. However, evidence of an association between lipid peroxidation biomarkers and colorectal neoplasia risk is conflicting and primarily based on studies using unreliably measured non-specific peroxidation products, such as malondialdehyde [210, 275, 287, 288]. To our knowledge, this is the first study to report an association between circulating F_2-isoprostanes and colorectal adenoma and hyperplastic polyp risk, and to suggest that the association may only be substantial in women.

Our results confirm findings from previous studies that found higher isoprostane levels and more variability in women than in men [214, 220]. The reasons for this observation are unclear. Recent studies found that estradiol in premenopausal women is associated with higher F_2-isoprostanes, and the commonly held view of estrogens as natural antioxidants may be untrue [289, 290]. Although women had higher F_2-isoprostanes than did men in our study, they had lower risk of colorectal adenoma. It has

been suggested that there might be a differential response to F_2-isoprostane formation based on sex [220], and it is possible that lipid peroxidation level changes may impact colorectal carcinogenesis more in women than in men. Given the lower circulating levels of F_2-isoprostanes in men, it is also possible that compared to women, relatively smaller differences in isoprostane levels may indicate important differences in systemic oxidative stress in men.

We further explored sex differences in lipid peroxidation and dietary factors by investigating associations of isoprostanes with individual antioxidants in men and women. A notable aspect of the current study was the use of both dietary intake estimates (FFQ-derived) and biomarkers to estimate antioxidant exposures. Plasma biomarkers reflect dietary intake over a relatively short time, but better measure systemic antioxidant exposure after absorption, metabolism, and tissue distribution [291]. FFQ-derived estimates, in contrast, reflect regular intake over a longer period of time and might be more representative of a person's usual diet. Correlations of FFQ-derived dietary intakes of antioxidants with plasma antioxidants levels in our study were modest (0.30-0.50) and similar to estimates reported previously [292].

Although higher levels of most FFQ-derived and plasma carotenoids were associated with lower F_2-isoprostane levels in men, none of the multivariable-adjusted results was statistically significant. Few studies have reported sex-specific data on the association of carotenoids with isoprostanes, and none reported results for plasma carotenoids and isoprostanes among men. Among women in our study, plasma carotenoids were more strongly (and statistically significantly) inversely associated with

plasma F_2-isoprostane levels than were FFQ-derived carotenoids. Other studies found associations similar to ours between carotenoids and isoprostanes in women. Block et al. reported lower levels of plasma F_2-isoprostanes with all carotenoids among 298 healthy men and women [214]. In our study, FFQ-based total vitamin E intake and plasma α-tocopherol levels were inversely associated with F_2-isoprostanes primarily among women. Previous studies generally found null results for the association between tocopherols and isoprostanes. In the only previously reported investigation of plasma α-tocopherol and F_2-isoprostanes, in 298 healthy US men and women, no association was found [214]. However, in a 6-month trial among 80 overweight/obese adults (60 women, 20 men) vitamin E supplementation statistically significantly decreased plasma F_2-isoprostanes at 6, but not 3, months [293].

In contrast to our α-tocopherol results, we observed a higher level of lipid peroxidation in men and women with higher plasma γ-tocopherol concentrations. In a cross-sectional study of 298 healthy US adults, Block et al. reported findings similar to ours [214]. γ-tocopherol may have antioxidant and anti-inflammatory properties, and its antioxidant potential may exceed that of α-tocopherol *in vitro* [294, 295]. However, plasma levels of γ-tocopherol correlate weakly with dietary intake of γ-tocopherol [296, 297]. Furthermore, α-tocopherol supplementation reduced plasma γ-tocopherol levels in clinical trials [298-300]. Most commercially available supplements include α-tocopherol as the primary form of vitamin E and the high γ-tocopherol levels among participants may indicate low α-tocopherol / antioxidant supplement intake.

Vitamin C, in our study, was associated with lower oxidative stress in a dose-dependent manner in both men and women. In a cohort study of Swedish men, vitamin C intakes were inversely associated with F_2-isoprostanes [301]. In a small trial in male smokers, short-term vitamin C supplementation reduced F_2-isoprostanes [302]. Other studies of both men and women also reported vitamin C to be inversely associated with F_2-isoprostanes [214, 303]. Plasma ascorbate (vitamin C biomarker) estimation requires addition of a specific preservative to prevent degradation of ascorbate during storage, and is accurate over a narrow range of low intakes (50-90 mg/day) but not at higher intakes [304, 305]. We therefore did not measure plasma ascorbate in this study.

Our study had certain limitations. Because we had depleted our plasma and serum samples in previous studies, data on F_2-isoprostanes for 31% of cases and 38% of controls were unavailable. However, we found no differences in the demographic and dietary characteristics between participants with and without isoprostane measurements (data not shown). Only about 25% of the participants who underwent colonoscopy in the MAP study were asymptomatic screenees, and more than 50% had GI symptoms. Oxidative stress may be related to the pathogenesis of conditions associated with such symptoms, and lipid peroxidation levels may have been similar between cases and controls, thus attenuating the observed association for adenoma risk. Also, lipid peroxidation is only part of the oxidative stress spectrum, and since we had no biomarkers of DNA or protein oxidation, our results should not be extrapolated to those mechanisms. Finally, because this is a case-control study, the temporality of increased F_2-isoprostanes with respect to adenoma incidence cannot be established.

Strengths of our study include the extensive information on dietary and non-dietary factors, and the availability of properly handled plasma for biomarker analyses. F_2-isoprostanes have been shown to be the most accurate indicators of lipid peroxidation *in vivo*, and GC-MS, as we used, is the preferred quantification method. Additionally, we assessed antioxidant micronutrients by two methods, each with certain strengths and limitations.

In conclusion, we found strong, direct associations of circulating F_2-isoprostanes with adenomatous and hyperplastic polyps in women. We also found that associations of antioxidant micronutrients with lipid peroxidation, although similar in direction for both sexes and exposure assessment methods, were stronger in women than in men, especially for the plasma micronutrients. Given that the isoprostanes and plasma antioxidants were measured in the same blood samples, it is possible that this association may better reflect a biological association than the one with diet history determined from FFQs. However, that the results are so similar supports the use of FFQs for estimating antioxidant micronutrients in relation to their associations on oxidative balance. Our observation of a direct association of plasma γ-tocopherol with F_2-isoprostanes emphasizes the complexity of vitamin E metabolism and function *in vivo* and the need for more basic science and epidemiologic research in this area.

Tables and Figures

Table 4.1. Selected Characteristics of Cases and Controls in the Pooled Markers of Adenomatous Polyps (MAP) Case-Control Studies of Incident, Sporadic Colorectal Adenoma

Selected characteristics[c]	***Cases***		***Controls (n = 184)***
	Adenoma (N = 157)	***Hyperplastic polyps (N = 60)***	
Age (y)	57 (8)	58 (8)	56 (10)
Male (%)	59[a]	53[a]	33
Caucasian race (%)	90	98	90
College education or higher (%)	23	25	30
Family history of colon or rectal cancer in first degree relative (%)	19[a]	33	31
Regular (≥ once/week) NSAID use (%)	20[b]	25	33
Regular (≥ once/week) aspirin use (%)	35	37	33
Current hormone therapy use among women (%)	63	61	72
Energy intake (kcal/day)	2,058 (793)[a]	1,934 (588)	1,779 (680)
Total[d] calcium intake (mg/day)	781 (361)	774 (424)	854 (419)
Serum 25-OH-vitamin D_3 (nmol/L)	25 (12)	24 (10)	26 (12)
Total[d] folate intake (µg/day)	443 (244)	473 (276)	471 (254)
Dietary fiber intake (gm/day)	22 (8)	20 (7)	20 (7)
Current smoker (%)	36[a]	33[a]	14
Former smoker (%)	39	37	36
1 - 6 alcoholic drinks/week (%)	36	36	36
≥ 7 alcoholic drinks/week (%)	23[a]	23[a]	11
BMI (kg/m^2)	27.5 (6.3)	29.2 (6.4)	27.6 (5.7)
Waist-to-hip ratio	0.94 (0.12)[a]	0.94 (0.12)[a]	0.88 (0.15)
Physical activity (MET-hrs./week)	27 (19)	30 (17)	28 (19)
Total[d] antioxidant intake (FFQ-derived)			
α-carotene (µg/day)	719 (1,099)	568 (686)	648 (607)
β-carotene (µg/day)	4,630 (3,666)	4,127 (2,993)	5,055 (4,130)
β-cryptoxanthin (µg/day)	49.6 (54.9)[a]	66.8 (90.9)	65.9 (59.1)
Lutein/zeaxanthin (µg/day)	3,813 (3,058)	3,245 (2,773)	3,278 (3,067)
Lycopene (µg/day)	4,078 (3,529)	4,786 (3,270)	4,622 (4,160)
Vitamin C (mg/day)	271 (290)	214 (196)	297 (321)
Vitamin E (mg-TE/day)	81.3 (175)	58.3 (147)	78.3 (151)
Plasma antioxidants (µmol/L)			
α-carotene	2.9 (2.8)[b]	2.6 (2.6)[b]	3.9 (4.4)
β-carotene	13.7 (11.7)[b]	13.2 (14.9)	17.5 (15.8)
β-cryptoxanthin	6.2 (5.3)	6.1 (4.3)	7.5 (6.9)
Lutein/zeaxanthin	16.8 (6.8)	17.9 (8.6)	16.8 (8.7)
Lycopene	25.9 (23.5)	25.6 (16.2)	25.6 (23.7)
α-tocopherol	1.15 (0.52)	1.28 (0.78)	1.18 (0.49)
γ-tocopherol	0.23 (0.11)	0.22 (0.11)	0.21 (0.11)

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; 25-OH-vitamin D_3, 25-hydroxyvitamin D_3; BMI, body mass index; MET, metabolic equivalents; FFQ, food frequency questionnaire; TE, tocopherol equivalents

Note: All nutrients adjusted for total energy intake
[a] $P < 0.01$ based on t-test or chi-square test
[b] $P < 0.05$ based on t-test or chi-square test
[c] Mean (standard deviation) presented unless otherwise specified
[d] Diet plus supplements

Table 4.2. Associations of F_2-Isoprostane Levels with Adenoma and Hyperplastic Polyp Risk in the Pooled MAP Study

Outcome	***F_2-isoprostane levels***	***ALL PARTICIPANTS***		***MEN***		***WOMEN***	
		Cases	***OR[a] (95% CI)***	***Cases***	***OR[b] (95% CI)***	***Cases***	***OR[b] (95% CI)***
Adenoma							
	Tertile 1	43	1.00 (Ref)	25	1.00 (Ref)	18	1.00 (Ref)
	Tertile 2	50	0.99 (0.56, 1.78)	34	1.16 (0.49, 2.75)	16	0.86 (0.38, 1.97)
	Tertile 3	64	1.35 (0.77, 2.38)	33	1.09 (0.47, 2.57)	31	1.60 (0.75, 3.38)
	P-value[c]		*0.28*		*0.85*		*0.19*
Hyperplastic polyps							
	Tertile 1	11	1.00 (Ref)	8	1.00 (Ref)	3	1.00 (Ref)
	Tertile 2	23	2.08 (0.91, 4.73)	12	1.28 (0.42, 3.97)	11	3.21 (0.82, 12.55)
	Tertile 3	26	2.39 (1.06, 5.38)	12	1.28 (0.42, 3.89)	14	4.49 (1.18, 17.03)
	P-value[c]		*0.04*		*0.68*		*0.03*

Abbreviations: OR, odds ratio; 95% CI, 95% confidence intervals

[a] OR adjusted for age, study, sex, and family history of colorectal cancer in first-degree relative

[b] OR adjusted for age, study, and family history of colorectal cancer in first-degree relative

[c] *P*-value of the test of linear trend across tertiles

Table 4.3. Associations of Plasma F_2-Isoprostanes with FFQ-Derived Antioxidant Micronutrients among Participants[a] in the Pooled MAP Study

Selected nutrient levels from FFQ (tertiles)	***MEN***			***WOMEN***		
	Tertile range	***F_2-isoprostanes Mean (ng/L)****	***OR[b] (95% CI)***	***Tertile value***	***F_2-isoprostanes Mean (ng/L)****	***OR[c] (95% CI)***
CAROTENOIDS						
Total α-carotene intake[d] (μg/day)						
T1	*≤ 320*	77.43	1.00 (Referent)	*≤ 366*	115.39	1.00 (Referent)
T2	*321 - 588*	74.68	1.24 (0.54, 2.88)	*367 - 712*	99.86	1.47 (0.66, 3.26)
T3	*≥ 589*	68.59	0.54 (0.22, 1.30)	*≥ 713*	87.00	0.77 (0.34, 1.76)
P-value		*0.07*	*0.09*		*0.02*	*0.33*
Total β-carotene intake[d] (μg/day)						
T1	*≤ 2,238*	81.50	1.00 (Referent)	*≤ 3,098*	116.35	1.00 (Referent)
T2	*2,239 - 4,735*	69.73	0.36 (0.15, 0.88)	*3,099 - 5,524*	100.05	0.61 (0.28, 1.33)
T3	*≥ 4,736*	68.12	0.40 (0.15, 1.09)	*≥ 5,525*	85.76	0.83 (0.35, 1.95)
P-value		*<0.01*	*0.14*		*<0.01*	*0.80*
Total β-cryptoxanthin intake[d] (μg/day)						
T1	*≤ 38.6*	77.48	1.00 (Referent)	*≤ 31.2*	100.00	1.00 (Referent)
T2	*38.7 - 67.9*	73.99	1.03 (0.46, 2.33)	*31.3 - 77.0*	99.23	1.09 (0.51, 2.31)
T3	*≥ 68.0*	67.19	0.71 (0.30, 1.72)	*≥ 77.1*	108.13	1.33 (0.56, 3.15)
P-value		*0.02*	*0.43*		*0.73*	*0.50*
Total lutein/zeaxanthin intake[d] (μg/day)						
T1	*≤ 1,509*	77.43	1.00 (Referent)	*≤ 2,068*	106.67	1.00 (Referent)
T2	*1,510 - 3,088*	73.64	0.53 (0.22, 1.29)	*2,069 - 3,715*	118.89	0.94 (0.40, 2.17)
T3	*≥ 3089*	69.69	0.56 (0.23, 1.33)	*≥ 3,716*	83.38	0.33 (0.14, 0.77)
P-value		*0.19*	*0.29*		*<0.01*	*<0.01*
Total lycopene intake[d] (μg/day)						
T1	*≤ 2,506*	75.23	1.00 (Referent)	*≤ 2,879*	98.57	1.00 (Referent)
T2	*2,507 - 4,919*	73.02	1.18 (0.52, 2.64)	*2,880 - 5,031*	111.91	0.84 (0.39, 1.80)

T3	*≥ 4,920*	73.08	1.28 (0.52, 3.18)	*≥ 5,032*	93.67	0.76 (0.35, 1.65)
P-value		*0.84*	*0.64*		*0.31*	*0.51*
Total vitamin E[d] **(mg-TE/day)**						
T1	*≤ 8.8*	76.31	1.00 (Referent)	*≤ 8.9*	113.37	1.00 (Referent)
T2	*8.9 - 21.3*	78.20	1.90 (0.78, 4.62)	*9.0 - 26.0*	99.30	0.63 (0.27, 1.44)
T3	*≥ 21.4*	64.20	0.70 (0.23, 2.13)	*≥ 26.1*	89.76	0.27 (0.11, 0.65)
P-value		*<0.01*	*0.08*		*<0.01*	*<0.01*
Total vitamin C[d] **(mg/day)**						
T1	*≤ 96*	83.29	1.00 (Referent)	*≤ 128*	115.30	1.00 (Referent)
T2	*97 - 204*	73.72	0.53 (0.20, 1.40)	*129 - 255*	96.18	0.92 (0.41, 2.09)
T3	*≥ 205*	65.24	0.25 (0.08, 0.81)	*≥ 256*	92.53	0.65 (0.28, 1.48)
P-value		*<0.01*	*0.03*		*<0.01*	*0.27*

Abbreviations: OR, odds ratio; 95% CI, 95% confidence intervals

[¶] Cases and controls are combined since the findings by case/control status were similar (data not shown)

Note: All nutrients adjusted for total energy intake

[a] *P*-values based on F-test of difference in log(F_2-isoprostanes) between tertiles, adjusted for age and study

[b] OR for risk of being in the higher category (above median) of F2-isoprostanes among men, adjusted for age, study, regular use of aspirin, cigarette smoking, BMI, physical activity, total energy intake, total folate intake, dietary fiber intake, and total iron intake. *P*-value of the test of linear trend across tertiles.

[c] OR for risk of being in the higher category (above median) of F2-isoprostanes among women, adjusted for age, study, regular use of NSAID/aspirin, cigarette smoking, BMI, physical activity, total energy intake, total folate intake, dietary fiber intake, saturated fat intake, and total iron intake. *P*-value of the test of linear trend across tertiles.

[d] Diet plus supplements

Table 4.4. Associations of F_2-Isoprostanes with Plasma Levels of Antioxidant Micronutrients among Participants[a] in the Pooled MAP Study

Selected nutrient biomarker (tertiles)	***MEN***			***WOMEN***		
	Tertile range	***F_2-isoprostanes Mean (ng/L)[b]***	***OR[c] (95% CI)***	***Tertile value***	***F_2-isoprostanes Mean (ng/L)[b]***	***OR[d] (95% CI)***
CAROTENOIDS						
α-carotene						
T1	*≤ 1.54*	77.52	1.00 (Referent)	*≤ 2.16*	114.47	1.00 (Referent)
T2	*1.55 - 3.50*	73.44	0.84 (0.33, 2.14)	*2.17 - 4.06*	96.52	0.73 (0.33, 1.63)
T3	*≥ 3.51*	71.05	1.01 (0.40, 2.55)	*≥ 4.07*	90.98	0.41 (0.17, 0.99)
P-value[e]		*0.18*	*0.91*		*<0.01*	*0.05*
β-carotene						
T1	*≤ 7.45*	78.08	1.00 (Referent)	*≤ 9.10*	118.96	1.00 (Referent)
T2	*7.46 - 18.00*	75.06	1.44 (0.59, 3.51)	*9.11 - 19.29*	102.62	0.69 (0.30, 1.57)
T3	*≥ 18.01*	67.95	0.67 (0.24, 1.88)	*≥ 19.30*	78.30	0.27 (0.11, 0.64)
P-value[e]		*0.07*	*0.26*		*<0.01*	*<0.01*
β-cryptoxanthin						
T1	*≤ 4.50*	78.19	1.00 (Referent)	*≤ 4.02*	110.09	1.00 (Referent)
T2	*4.51 - 7.83*	77.15	0.75 (0.30, 1.86)	*4.03 - 7.18*	98.63	0.76 (0.34, 1.68)
T3	*≥ 7.84*	64.72	0.48 (0.18, 1.26)	*≥ 7.19*	97.11	0.56 (0.24, 1.28)
P-value[e]		*<0.01*	*0.13*		*0.07*	*0.18*
Lutein / zeaxanthin						
T1	*≤ 13.22*	74.46	1.00 (Referent)	*≤ 11.94*	113.92	1.00 (Referent)
T2	*13.23 - 17.74*	75.75	1.06 (0.42, 2.69)	*11.95 - 18.86*	98.38	0.90 (0.40, 2.04)
T3	*≥ 17.75*	72.59	1.17 (0.49, 2.78)	*≥ 18.87*	94.87	0.51 (0.23, 1.15)
P-value[e]		*0.69*	*0.73*		*0.03*	*0.08*
Lycopene						
T1	*≤ 19.35*	77.69	1.00 (Referent)	*≤ 19.45*	109.09	1.00 (Referent)
T2	*19.36 - 31.04*	72.80	0.96 (0.39, 2.33)	*19.46 - 29.29*	104.03	0.42 (0.17, 1.08)
T3	*≥ 31.05*	71.76	1.10 (0.42, 2.90)	*≥ 29.30*	92.39	0.36 (0.15, 0.83)
P-value[e]		*0.41*	*0.84*		*0.24*	*0.13*
TOCOPHEROLS						
α-tocopherol						
T1	*≤ 0.85*	80.24	1.00 (Referent)	*≤ 0.94*	113.97	1.00 (Referent)
T2	*0.86 - 1.18*	73.61	1.46 (0.56, 3.84)	*0.95 - 1.34*	94.61	0.42 (0.18, 1.00)

T3	*≥ 1.19*	69.99	0.70 (0.23, 2.13)	*≥ 1.35*	99.43	0.36 (0.13, 0.98)
P-value[e]		*0.16*	*0.26*		*0.01*	*0.09*
γ-tocopherol						
T1	*≤ 0.14*	65.98	1.00 (Referent)	*≤ 0.15*	80.46	1.00 (Referent)
T2	*0.15 - 0.22*	73.51	1.94 (0.76, 4.95)	*0.16 - 0.25*	99.97	2.63 (1.16, 5.99)
T3	*≥ 0.23*	81.68	2.79 (0.95, 8.20)	*≥ 0.26*	122.92	5.82 (2.36, 14.31)
P-value[e]		*<0.01*	*0.07*		*<0.01*	*<0.01*

Abbreviations: OR, odds ratio; 95% CI, 95% confidence intervals

[a] Cases and controls are combined since the findings by case/control status were similar (data not shown)

Note: All nutrients adjusted for total energy intake

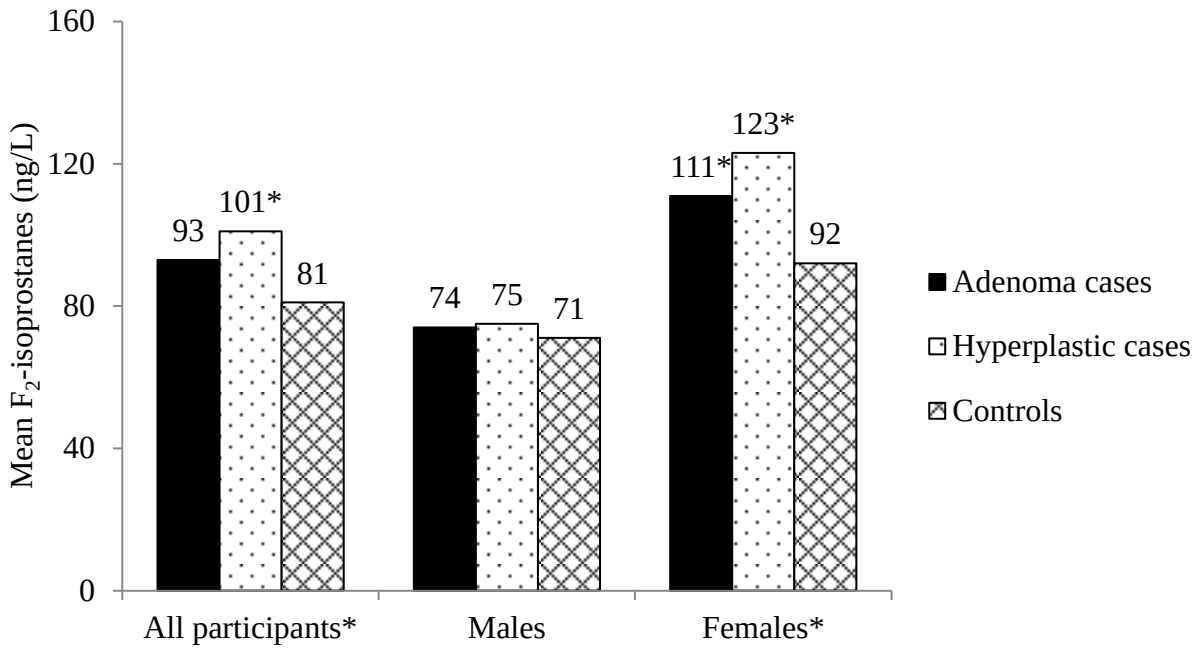
[b] *P*-values based on F-test of difference in log(F_2-isoprostanes) between tertiles, adjusted for age, study, and plasma total cholesterol

[c] OR for risk of being in the higher category (above median) of F2-isoprostanes among men, adjusted for age, study, regular use of aspirin, cigarette smoking, BMI, physical activity, total energy intake, total folate intake, dietary fiber intake, total iron intake, and plasma total cholesterol. *P*-value is of the test of linear trend across tertiles.

[d] OR for risk of being in the higher category (above median) of F2-isoprostanes among women, adjusted for age, study, regular use of NSAID/aspirin, cigarette smoking, BMI, physical activity, total energy intake, total folate intake, dietary fiber intake, saturated fat intake, total iron intake, and plasma total cholesterol. [e]*P*-value of the test of linear trend across tertiles.

FIGURE LEGENDS

Figure 4.1. Mean plasma F_2-isoprostane levels (ng/L) in cases and controls in the pooled MAP study



Footnote to figure 1: * $P < 0.05$ based on F-test of difference in log(F_2-isoprostane) between cases and controls. All comparisons adjusted for age, sex, family history of colorectal cancer, and study.

CONCLUSIONS AND PUBLIC HEALTH IMPLICATIONS

In this dissertation, we developed new methodologies to construct comprehensive oxidative balance scores (OBS) that combine multiple antioxidant and pro-oxidant exposures in a single score and investigated the role of oxidative stress as represented by OBS in reducing risk for colorectal adenomas and cancer. We further examined the association of the OBS and its components with F_2-isoprostanes, a sensitive and specific biomarker of oxidative stress *in vivo*.

In the first project (Study #1, Chapter 2) we developed three novel weighting schemes (OBS-lit. review, OBS-*a posteriori*, and OBS-Bayesian) and compared them to a previously used weighting scheme (OBS-equal weights) for combining dietary and non-dietary exposures associated with oxidative balance. Using data from a pooled colonoscopy-based case-control study, we found a substantial inverse association between OBS and risk for incident, sporadic colorectal adenomas (Study #1).

The methods developed in Study #1 were used to construct OBS and relate them to risk for incident colorectal cancer in the Cancer Prevention Study II (CPS-II) cohort (Study #2, Chapter 3). The findings from this large prospective cohort study supported the hypothesis that a predominance of antioxidant over pro-oxidant lifestyle exposures (both dietary and non-dietary), represented by higher OBS, reduces risk for colorectal cancer (Study #2).

Finally, we investigated associations of OBS and its individual components with F_2-isoprostanes (oxidative lipid peroxidation marker) using data from a pooled case-

control study of adenomas (Studies #1 and #3, Chapters 2 and 4). We observed a dose dependent decrease in F_2-isoprostane levels with increasing levels of OBS, providing support for OBS as a valid measure of oxidative balance (Study #1). However, individual components of the OBS (measured by self-report and plasma biomarkers) were not as strongly associated with F_2-isoprostanes as the summary scores and there were sex-based differences in the associations of the individual components with the F_2-isoprostane levels (Study #3). Our results suggested that self-reported dietary and supplemental intakes of vitamin C in men, and vitamin E and lutein/zeaxanthin in women were inversely associated with lipid peroxidation levels (Study #3). In addition, plasma α-tocopherol and carotenoids were inversely associated with F_2-isoprostanes among women, but not men, and plasma concentrations of γ-tocopherol were directly associated with F_2-isoprostane levels in men and women (Study #3).

Overall, in this dissertation I introduced three novel methods for constructing OBS that use literature-based, data-based, and a Bayesian approach (a combination of the literature- and data-based approaches) to assign weights to the different antioxidant and pro-oxidant components of the OBS and compare them to the commonly used approach of assigning similar weights to all OBS components. The results of this dissertation support the hypotheses that higher OBS, representing a predominance of antioxidant over pro-oxidant lifestyle exposures, is associated with lower risk of incident, sporadic colorectal adenomas and colorectal cancer, irrespective of the weighting method used. This has public health significance because it suggests that oxidative balance modulation may be an effective pathway-driven approach for preventing colorectal neoplasms.

The results from our studies support the role of both dietary and non-dietary lifestyle contributors to oxidative balance in reducing risk of colorectal neoplasms. The dietary part of OBS was significantly associated with lower risk of advanced colorectal adenomas and colorectal cancer. The similarity in the strengths of association for dietary and non-dietary OBS with risk of colorectal cancer in our study suggested that both sources of oxidative balance exposures are important in colorectal cancer causation and prevention.

We also provide evidence to suggest that the different OBS are valid measures of oxidative balance. All four OBS were strongly associated with plasma F_2-isoprostanes, a sensitive and specific marker of lipid peroxidation [202]. The strong dose-dependent association between OBS and F_2-isoprostanes in our results supports the use of OBS in future studies of oxidative balance.

Our results are also significant because they provide a possible explanation for the equivocal associations of single antioxidants with colorectal adenoma and cancer risk in previous epidemiologic studies and the failure of traditional antioxidant vitamin trials of single agents or a limited combination of agents to reduce risk of cancer [180, 245, 255]. The oxidative balance pathway is a complex pathway with numerous pro-oxidant and antioxidant components that are related to diet, other lifestyle factors such as smoking and physical activity, body composition, and endogenous antioxidant defense systems. It is impossible to accurately characterize these complex exposures for a diverse group of study participants by measuring only one or a few components. Our approach provides a blueprint for constructing complex weighted exposures that include a

comprehensive list of components involved in the oxidative balance pathway. The list of components included in our OBS is limited by the state of the available knowledge and it is possible that future strategies may include even more components using methods described in this dissertation. Additionally, our methods for constructing complex exposure scores are not limited to the oxidative balance pathway but can be used for other pathways such as inflammation and insulin resistance.

In summary, this dissertation supports the use of pathway exposure scores to measure complex multicomponent exposures, provides evidence to suggest that oxidative balance is strongly associated with colorectal adenoma and cancer incidence, and supports further investigations of oxidative balance with other chronic diseases. This dissertation also provides a framework for the development of oxidative balance-based interventions to reduce colorectal cancer risk.

FUTURE DIRECTIONS

We developed novel methods of weighting oxidative balance components to construct three new OBS in the first study of this dissertation. Previous studies on the association of OBS with chronic disease risk have used an equal weighting method (OBS-equal weight) whereby all OBS components are assumed to contribute equally to the score [117, 118, 187-190, 192, 218]. Our proposed new methods do not make this biologically implausible assumption. However, the prior literature-based (OBS-lit. review), data-based (OBS- *a posteriori*), and Bayesian (OBS-Bayesian) weighting methods are disease-specific, i.e., the weights developed are applicable only to the disease outcome under study. The first and second study of this dissertation present colorectal adenoma-specific and colorectal cancer-specific weights, respectively. Although the weights for the OBS are mostly similar between colorectal adenoma and colorectal cancer they are not exactly alike. Additionally, it is unknown whether the weights for other oxidative balance associated cancers (e.g., prostate cancer) and chronic diseases (e.g., diabetes) might be different from the weights we calculated for colorectal neoplasms in the current dissertation. The methods we present have thus far not been adapted for diseases other than colorectal neoplasms. Therefore, I propose to extend this methodology to construct OBS specific for breast cancer, prostate cancer, cardiovascular disease, and diabetes. The extension of our methodology to other cancers and chronic diseases is important because OBS has either not been investigated as a risk factor (e.g., diabetes and cardiovascular disease) or only OBS-equal weight and OBS-lit. review have been investigated (e.g., prostate cancer) [190, 192].

The first and second studies of this dissertation report colorectal adenoma- and colorectal cancer-specific OBS weights, respectively. Although our weights, especially for OBS-*a posteriori* and OBS-Bayesian, are based on large well-designed studies, they have yet to be validated in other samples and for populations other than the predominantly White populations we examined. I propose to use the weights derived in this dissertation to investigate OBS-colorectal adenoma and OBS-colorectal cancer associations in study samples with diverse and minority populations, such as the Black Women's Health Study and the Women's Health Initiative. Reproduction of our results that suggest OBS as a strong risk factor for colorectal neoplasms in other racially/ethnically diverse study samples would add to the evidence on the role of OBS in colorectal cancer risk.

Results from this dissertation also suggest that OBS is associated with F_2-isoprostanes, a marker of lipid peroxidation *in vivo* [202], in a dose-dependent manner. Other studies support this observation and have reported OBS to be associated with markers of inflammation (C-reactive protein) [263]. However, lipid peroxidation is only part of the oxidative stress spectrum, and studies on the association of OBS with markers of DNA and protein oxidation have been inconclusive [263, 306]. I propose to extend the third study in this dissertation to investigate the association of OBS with DNA oxidation markers such as 8-hydroxy-2'-deoxyguanosine and markers of protein oxidation such as, protein carbonyl, nitrotyrosine, and dityrosine formation [307].

In this dissertation I present weighting methods that are predominantly based on known or data-based associations of OBS components with the specific disease outcome

(e.g., colorectal cancer). As stated previously, these disease-specific weights might or might not be applicable to other cancers and chronic diseases. One approach to create a more "universal" OBS that might be applicable to all oxidative stress-associated health conditions is to derive component weights using biomarkers of oxidative stress. I propose to use markers of lipid peroxidation, and DNA and protein oxidation to derive OBS component weights.

In study 3 of this dissertation we present the association of F_2-isoprostanes with individual OBS components. For some dietary components, such as carotenoids and vitamin E, we had data on self-reported intakes elicited via food frequency questionnaires and on circulating biomarker levels in the blood. The OBS we presented in Studies 1 and 2 were based on self-reported dietary data. It is unknown whether OBS based on antioxidant and pro-oxidant biomarker levels would be similar to those based on self-reported data. Therefore, I propose to use antioxidant biomarker data to create biomarker-based OBS and test the hypothesis that the associations of self-report-based OBS and biomarker-based OBS with colorectal neoplasms will be similar.

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