DIETARY INTAKE OF FLAVONOIDS, BARRETT'S ESOPHAGUS DEVELOPMENT, AND ESOPHAGEAL AND GASTRIC CANCER INCIDENCE AND SURVIVAL

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ABSTRACT

Jessica L. Petrick: Dietary Intake of Flavonoids, Barrett's Esophagus Development, and Esophageal and Gastric Cancer Incidence and Survival
(Under the direction of Marilie D. Gammon)

Flavonoids, polyphenolic compounds concentrated in fruits and vegetables, have experimentally demonstrated chemopreventive effects against esophageal and gastric cancer and Barrett's esophagus, a precursor lesion for esophageal adenocarcinoma. Few epidemiologic studies have examined flavonoids and incidence of esophageal and gastric cancers, and none have considered flavonoids with survival. Additionally, only one epidemiologic investigation has reported an inverse association between isoflavone intake and Barrett's esophagus risk, yet no study has considered other flavonoid classes, which are more commonly consumed in the U.S. This ancillary study built upon the U.S. Multi-Center Study (esophageal adenocarcinoma cases n=274, gastric cardia adenocarcinoma cases n=248, esophageal squamous cell carcinoma cases n=191, other gastric adenocarcinoma cases n=341, and frequency-matched controls n=662) and the Study of Reflux Disease (Barrett's esophagus cases n=170 and matched controls n=183). Esophageal and gastric cancer cases were followed until 2000 for vital status. Participants completed a food frequency questionnaire, and responses were linked with USDA Flavonoid Databases and available literature for six flavonoid classes and lignans. Multivariable-adjusted odds ratios (ORs) and hazard ratios (HRs) [95% confidence intervals (CI)] were estimated, comparing highest versus lowest intake quartiles, using logistic and proportional hazards regressions, respectively. Little or no association was found for total flavonoid intake (main sources in this population: black tea, orange/grapefruit juice, and wine) and development or survival for any tumor type. Intake of

anthocyanidins, common in wine and fruit juice, was associated with a reduction in the risk of 57% for developing esophageal adenocarcinoma (OR=0.43, 95% CI: 0.29-0.66), 57% for developing squamous cell carcinoma (OR=0.43, 95% CI: 0.26-0.70), and 41% for developing Barrett's esophagus. ORs for isoflavones, for which coffee was the main source, were increased for all cancer types, except esophageal squamous cell carcinoma. A modest increased risk of Barrett's esophagus development was observed for flavones, for which the main dietary source in this population was pizza. Anthocyanidins were associated with decreased risk of mortality for gastric cardia (HR=0.63, 95% CI: 0.42-0.95) and more modestly for esophageal adenocarcinoma (HR=0.87, 95% CI: 0.60-1.26). Our findings, if confirmed, suggest that increased dietary anthocyanidin intake may reduce risk of developing these tumors and improve survival.

To Mama Dee, who lost her battle with esophageal cancer and is my muse for this work.

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LIST OF ABBREVIATIONS

AADR	Age-adjusted death rate
AAIR	Age-adjusted incidence rate
APC	Annual percentage change
ASIR	Age-standardized incidence rate
BaP	Benzo[a]pyrene
BE	Barrett's esophagus
BEACON	International Barrett's and Esophageal Adenocarcinoma Consortium
BMI	Body mass index
CI	Confidence interval
COX-2	Cyclooxygenase-2
DMNM	2,6-dimethylnitrosomorpholine
EA	Esophageal adenocarcinoma
EGA	Esophageal and gastric cardia adenocarcinoma study
ESCC	Esophageal squamous cell carcinoma
FFQ	Food frequency questionnaire
FHCRC	Fred Hutchison Cancer Research Center
GA	Gastric adenocarcinoma
GE	Gastroesophageal
GERD	Gastroesophageal reflux disease
GCA	Gastric cardia adenocarcinoma
HR	Hazard ratio
IHC	Immunohistochemical
iNOS	Nitric oxide synthase
IRR	Incidence rate ratio
LSBE	Long-segment Barrett's esophagus

MNAN	*N*-methyl-*N*-amylnitrosamine
MNNG	*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
NCGA	Non-cardia gastric adenocarcinoma
NDSR	Nutrition Data System for Research
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PH	Proportional hazards
PTK	Protein kinases
RDD	Random digit dialing
SES	Socioeconomic status
SIM	Specialized intestinal metaplasia
S-NaCl	Saturated sodium chloride
SSBE	Short-segment Barrett's esophagus
Tcf	T-cell factor
UADC	Upper aerodigestive tract cancers
USDA	United Stated Department of Agriculture
VBE	Visible Barrett's esophagus
WHR	Waist-to-hip ratio

CHAPTER 1: BACKGROUND

Introduction

Esophageal and gastric cancers have very poor survival prognoses (normally less than a year).[1] Thus, significant research efforts have focused on identifying strategies to reduce the risk of developing these cancers, which could also potentially decrease the risk of mortality among those diagnosed with these cancers. This approach is further enhanced if precancerous lesions are also considered, which facilitate determination of key windows of susceptibility. In other words, this novel approach aids in identifying optimal times along the cancer continuum (normal tissue → precancerous conditions → invasive cancer → mortality) that could be targeted for intervention with a specific chemopreventive to enhance risk reduction. The only known potential precursor of esophageal and gastric cardia adenocarcinoma is Barrett's esophagus (BE).[2] Thus, the goals of this dissertation were to examine the role of a potential risk reduction strategy – namely dietary intake of flavonoids, which are potent anti-carcinogens found in fruits, vegetables, and other dietary sources – in reducing the risk of developing or dying from esophageal or gastric tumors along the cancer continuum.

The study hypothesis was that flavonoids would reduce the risk of esophageal and gastric tumors. This study is significant because esophageal and gastric cardia adenocarcinoma (EA/GCA) have rapidly increasing incidence rates in the United States (U.S.) and other Western countries,[3-7] but incidence rates in Asian countries remain comparatively low.[8] However, incidence rates of esophageal squamous cell carcinoma (ESCC) and non-cardia gastric adenocarcinoma (NCGA) remain high in Asian countries,

and not the U.S.[5,8,9] The rates of esophageal and gastric cancer subtypes (EA, GCA, ESCC, and NCGA) vary 50- to 60-fold between high and low incidence countries.[8] These geographic differences in incidence do not appear to be only due to genetic variation between ethnic groups, as incidence rates of esophageal and gastric cancer in Asian migrants to the U.S. tend to move in the direction of European-American incidence rates.[10-12] The geographic variation in incidence rates of esophageal and gastric cancer and results of migrant studies have led to the hypothesis that the variability is in part due to differences in energy intake/composition and micronutrient intake.[5,13]

The rationale for considering flavonoid intake as a potential chemopreventive is supported by evidence from population and laboratory research. Epidemiological studies have shown that diets high in fruit and vegetable consumption are inversely associated with esophageal and gastric cancer and Barrett's esophagus.[14-28] It is hypothesized that flavonoids, which are a group of bioactive polyphenolic compounds that are naturally occurring in fruits, vegetables, and beverages of plant origin, could partially account for these risk reductions. Experimental studies have supported this hypothesis and have shown that flavonoids regulate cell cycle, proliferation, and apoptosis, which have important chemotherapeutic effects against these tumors.[29] Additionally, Phase I and II clinical trials are also using synthetic classes of flavonoids to produce cell cycle arrest and inhibit tumor growth in patients with metastatic cancer, including gastric cancer.[30-32] In sum, the purpose of this study was to identify key windows of susceptibility for the potential association between flavonoids and esophageal and gastric tumors. Specifically, aims were to: 1) examine if flavonoid intake was associated with esophageal and gastric cancer incidence, 2) examine if flavonoid intake was associated with Barrett's esophagus (BE) development, and 3) examine if flavonoid intake was associated with mortality among individuals diagnosed with esophageal and gastric cancer. Determining the association between flavonoids and risk of developing or dying

from esophageal or gastric tumors is innovative because there is potential to use flavonoids as a risk reduction strategy. This could allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as a novel chemotherapeutic.

A more thorough discussion of the scientific background, significance, and innovation that motivated the dissertation goals is presented below.

Epidemiology of Esophageal and Gastric Cancer Incidence

In 2008, esophageal cancer was the eighth most common cause of cancer morbidity worldwide, with an estimated 481,000 incident cases,[33] and gastric cancer was the fourth most common cause of cancer morbidity worldwide, with an estimated 989,000 incident cases.[34] Approximately 83% of esophageal cancers occur in developing countries (399,000 incident cases), with over 50% of all esophageal cancers occurring in China alone (258,000 incident cases). Esophageal cancer also has huge geographic variation, varying more than 15-fold in men [age-standardized incidence rate (ASIR) 22.3 per 100,000 in Southern Africa versus 1.4 in Western Africa] and close to 20-fold in women (ASIR 11.7 per 100,000 in Southern Africa versus 0.6 in Micronesia/Polynesia).[33] Almost 72% of gastric cancers occur in developing countries, with over 45% of all gastric cancers occurring in China alone (463,000 incident cases). Similar to esophageal cancer, gastric cancer has huge geographic variation, varying more than 10-fold in men (ASIR 42.4 per 100,000 in Eastern Asia versus 3.9 in Northern Africa) and over 8-fold in women (ASIR 18.3 per 100,000 in Eastern Asia versus 2.2 in Southern Africa).[34]

The majority of esophageal cancers histological types are either adenocarcinoma or squamous cell carcinoma. Over 90% of gastric cancers are adenocarcinomas.[35] Gastric adenocarcinomas are usually classified according to their anatomic location within the stomach: cardia (proximal portion of the stomach found next to the

gastroesophageal junction) or non-cardia (distal portion of the stomach).[36] Esophageal squamous cell carcinomas generally occur in the upper two-thirds of the esophagus, whereas esophageal adenocarcinomas cluster in the lower third of the esophagus at the gastroesophageal junction.[9] Esophageal squamous cell carcinoma and non-cardia gastric adenocarcinoma are the most common forms of esophageal and gastric cancer worldwide.[8,37]

Between 2003 and 2007 in the U.S., the average age of diagnosis for esophageal and gastric cancer was 68 and 70 years, respectively. During this timeframe, the age-adjusted incidence rate (AAIR) for esophageal cancer was 4.5 per 100,000 (AAIR 7.8 per 100,000 men, 1.9 per 100,000 women). For gastric cancer, the AAIR was 7.8 per 100,000 (AAIR 10.9 per 100,000 men, 5.5 per 100,000 women). Between 1975 and 2007, the annual percentage change (APC) in gastric cancer incidence was -1.6%. The APC in esophageal cancer incidence was -0.4% in the U.S. between 2001 and 2007.[38]

While overall incidence of esophageal and gastric cancers has been decreasing, esophageal adenocarcinoma and gastric cardia adenocarcinomas have been among the most rapidly increasing cancer types in the United States (U.S.) and other Western countries.[5,8,9,39] As reported in population-based cancer registries, the incidence of esophageal adenocarcinoma and gastric cardia adenocarcinoma has increased 300-575% over the last 30-40 years.[3-7] Esophageal adenocarcinoma and gastric cardia adenocarcinoma are often considered as one clinical entity because they are both cancers of the gastroesophageal junction, have similar overall 5-year survival rates of approximately 26%, and have comparable survival according to tumor stage.[40] In 2009, approximately half of the 16,470 anticipated incident esophageal cancers in the U.S. were expected to be adenocarcinomas;[5,41-43] and approximately 35% of the anticipated 21,130 incident gastric cancers were expected to be gastric cardia adenocarcinoma.[5,43]

Summary. Esophageal and gastric cancers are two of the most common causes of cancer morbidity worldwide.[33,34] In the U.S. and other Western countries, esophageal and gastric cardia adenocarcinomas have been among the most rapidly increasing cancer types.[5,8,9,39] However, esophageal squamous cell carcinoma and non-cardia gastric adenocarcinomas are the most common forms of esophageal and gastric cancer worldwide.[8,37] Therefore, it is of importance to determine the risk factors for these four types of cancers because of high morbidity worldwide and increasing incidence of EA/GCA in the U.S. Specifically, it is of interest to determine if flavonoids can be used as a risk reduction strategy for these cancer types.

Epidemiology of Esophageal and Gastric Cancer Survival

Worldwide in 2008, esophageal cancer was the sixth most common cause of cancer mortality, with an estimated 406,000 deaths,[33] and gastric cancer was the second most common cause of cancer mortality, with an estimated 737,000 deaths.[34] Approximately 83% of esophageal cancer mortality occurs in developing countries (338,000 deaths), with over 50% of all esophageal cancer mortality occurring in China alone (210,000 deaths). The highest mortality rates for esophageal cancer are found in Eastern Africa [age-standardized morality rate (ASMR) 14.3 per 100,000 in men, 6.2 per 100,000 in women], Southern Africa (ASMR 21.4 and 11.1, respectively), and Eastern Asia (ASMR 16.2 and 6.4, respectively).[33] Over 75% of gastric cancer mortality occurs in developing countries (555,000 deaths), with over 45% of all gastric cancer mortality occurring in China alone (352,000 deaths). The highest mortality rates for gastric cancer are found in Eastern Asia (ASMR 28.1 per 100,000 in men, 13.0 per 100,000 in women), and the lowest mortality rates are found in Northern America (2.8 and 1.5, respectively).[34]

Between 2003 and 2007 in the U.S., the average age of death from esophageal and gastric cancer was 69 and 73 years, respectively. During this timeframe, the age-

adjusted death rate (AADR) for esophageal cancer was 4.4 per 100,000 (AADR 7.8 per 100,000 men, 1.7 per 100,000 women). For gastric cancer, the AADR was 3.8 per 100,000 (AADR 5.3 per 100,000 men, 2.7 per 100,000 women).[38] Between 1999 and 2006, the 5-year relative survival for all stages of esophageal cancer was 17.7%. The stage distribution of esophageal cancer was 23% for localized, 31% for regional, 32% for distant, and 15% for unknown stage cancer. The respective 5-year relative survival was 37.4%, 18.8%, 3.2%, and 12.1%. The 5-year survival for all stages of gastric cancer was 26.0%. The stage distribution for gastric cancer was 23% for localized, 32% for regional, 34% for distant, and 11% for unknown stage cancer. The respective 5-year relative survival was 62.5%, 27.0%, 3.4%, and 17.3%.[38]

Studies have shown little survival difference between esophageal and gastric cardia adenocarcinomas.[40,44-48] In a study by Wijnhoven et al. of 252 patients,[40] the overall 5-year survival rates for esophageal and gastric cardia adenocarcinoma, respectively, were 26% and 27%, with a combined, overall 5-year survival rate of 26%. Tumors were staged using TNM Classification of Malignant Tumors. For patients with esophageal cancer (n=111), tumor size was 16% T_{is}/T_1, 14% T_2, 70% T_{3-4}, lymph node involvement was 44% N_0 and 56% N_{1-2}, and distant metastases were 81% M_0 and 19% M_1. For patients with gastric cardia adenocarcinoma (n=141), tumor size was 6% T_{is}/T_1, 20% T_2, 74% T_{3-4}, lymph node involvement was 38% N_0 and 62% N_{1-2}, and distant metastases were 96% M_0 and 4% M_1. Overall 5-year survival rate by TNM classification for patients was 70% for T_{is}/T_1, 37% for T_2, and 14% for T_{3-4}. The overall 5-year survival rate for patients with negative lymph nodes (N_0) was 42%, but for patients with positive lymph nodes (N_1) the survival rate was 11%. For patients without distant metastases (M_0), the overall 5-year survival rate was 27%, whereas for patients with distant metastases (M_1), the overall 5-year survival rate was 0%.[40]

Summary. Esophageal and gastric cancers are two of the most common causes

of cancer mortality worldwide,[33,34] and these cancers have very poor survival prognoses (normally less than a year).[1] Therefore, demonstration of an association between flavonoids and BE development, esophageal or gastric cancer incidence or survival among esophageal and gastric cancer cases suggests potential to use flavonoids as a risk reduction strategy for these tumors. This would offer support for use of flavonoids as a novel chemotherapeutic, which could potentially decrease the risk of mortality among those diagnosed with these cancers.

Esophageal and Gastric Cardia Adenocarcinoma

Esophageal and Gastric Cardia Adenocarcinoma Risk Factors

Medical Conditions/Clinical Characteristics. The strongest risk factors for esophageal adenocarcinoma (EA) and gastric cardia adenocarcinoma (GCA) are gastroesophageal reflux disease (GERD), esophagitis, and Barrett's esophagus (BE).[49-52] While the dominant paradigm is that the major risk factor for EA/GCA is a precursor lesion, Barrett's esophagus, that arises from GERD and esophagitis, studies have shown that GERD in the absence of BE appears to be a risk factor for EA/GCA as well.[53,54] In the U.S. Multi-Center Study, risk of EA/GCA was associated with history of GERD [odds ratio (OR)=2.1, 95% confidence interval (CI): 1.3-3.5] and esophagitis or esophageal ulcer (OR=5.2, 95% CI: 1.7-15.7).[54] In a study by Lagergren et al., among Swedish participants with symptomatic GERD at least once a week or more, compared to participants without symptoms, the odds ratios were 7.7 (95% CI: 5.3-11.4) for EA and 2.0 (95% CI: 1.4-2.9) for GCA. As frequency, severity, and duration of symptomatic GERD increased, the risk for EA/GCA increased. Among individuals with GERD symptoms more than three times a week, compared to participants without symptoms, the odds ratios were 16.7 (95% CI: 8.7-28.3) for EA and 2.3 (95% CI: 1.2-4.3) for GCA. Among individuals with greater than

20 years of GERD symptoms, compared to participants without symptoms, the odds ratios were 16.4 (95% CI: 8.3-28.4) for EA and 3.3 (95% CI: 1.8-6.3) for GCA. Among individuals with long standing symptomatic GERD (>20 years) and severe symptoms, compared to participants without symptoms, the odds ratios were 43.5 (95% CI: 18.3-103.5) for EA and 4.4 (95% CI: 1.7-11.0) for GCA. Additionally, 118 of 189 EA patients (62%) had BE, and 113 EA patients (60%) had reflux symptoms at least once a week. The association with symptomatic GERD was almost identical between EA participants who had BE versus those that did not.[53] Another study examined the GERD, esophagitis, and BE as risk factors for EA. The estimated standardized incidence ratios for EA were 3.1 (95% CI: 0.6-14.2) for GERD, 4.5 (95% CI: 1.04-19.6) for esophagitis, and 29.8 (95% CI: 9.6-106.0) for BE compared with a reference cohort.[50]

Demographic Factors. Age, Sex, and Race. The risk of developing EA/GCA has been associated with several non-modifiable risk factors, including age, race, sex, family history, and low socioeconomic status. Similar to other cancers, risk of EA/GCA increases with increasing age.[49,51,55,56] Age specific incidence of EA and GCA increases until 75-79 and 80-84 years of age, respectively, before declining in the older age groups.[56] Additionally, Caucasian males are at a higher risk of EA/GCA than African-Americans and women.[49,51,55-59] Age-adjusted incidence of EA in males is 6-8 times higher than in females and 3-4 times higher in Caucasians than African-Americans. While the increase in incidence of EA has been seen in all age and sex groups, Caucasian males comprise more than 80% of the EA cases and have experienced the largest increase in incidence rates.[56] The differences between race and sex are less pronounced in GCA. Age-adjusted incidence rates of GCA in males are 3-5 times higher than females and 1.5-2 times higher in Caucasians than African-Americans.[56]

Family History. Due to the association of EA/GCA in male gender and Caucasian race, it has been hypothesized that there is an inherited component of EA/GCA.[49] This has been supported in studies that have shown a family clustering of BE and EA/GCA.[49,60-65] In a recent study, the odds of having BE or EA/GCA in individuals with a positive family history (first or second degree relative with BE or EA/GCA) were 12.23 (95% CI: 3.34-44.76) times the odds of BE or EA/GCA in individuals without a positive family history.[65] However, the family clustering seen in BE and EA/GCA could also be due in part to shared cultural factors, especially diet.[66]

Education and Income. In the U.S. Multi-Center Study, low socioeconomic status (SES), quantified by both education and income, was a risk factor for EA/GCA. For participants that completed graduate school versus those with less than high school education, the OR was 0.7 (95% CI: 0.3-1.3) for EA and 0.8 (95% CI: 0.4-1.6) for GCA. For participants with an income greater than $75,000 compared to those with an income of less than $15,000 per year, the OR was 0.5 (95% CI: 0.3-1.0) for EA and 0.8 (95% CI: 0.4-1.6) for GCA.[67] In a more recent Swedish study comparing unskilled manual workers to professionals, the OR was 2.0 (95% CI: 0.9-4.5) for EA and 1.0 (0.6-1.8) for GCA.[68]

Epidemiologic Factors. Obesity. Modifiable risk factors for EA/GCA include obesity[49,51,69-74] and cigarette smoking.[49,51,67,72,75-81] Increasing rates of obesity in the general population have paralleled the increasing rates of EA and GCA. Elevated body mass index (BMI, defined as weight in kilograms/height in meters squared) has consistently been shown to be a significant risk factor for EA and a somewhat weaker risk factor for GCA. While the exact mechanisms are unclear, it is hypothesized that central adiposity may increase intra-abdominal pressure, thus promoting gastroesophageal reflux disease and the transition to Barrett's esophagus.[10] Two recent cohort studies examined the risk of both EA and GCA by obesity status. In the study by

Abnet et al., compared to participants with a BMI of 18.5-25 kg/m^2, participants with a BMI of 25-<30, 30-<35, and ≥35 kg/m^2 had respective hazard ratios for EA of 1.65 (95% CI: 1.26-2.18), 1.91 (95% CI: 1.38-2.66), and 2.27 (95% CI: 1.44-3.59) and for GCA of 1.06 (95% CI: 0.79-1.41), 1.70 (95% CI: 1.22-2.36), and 2.46 (95% CI: 1.60-3.80).[69] In the study by Merry et al., compared with participants with a BMI of 20.0-24.9 kg/m^2, participants with a BMI of 25.0-29.9 and ≥30.0 kg/m^2 had respective rate ratios for EA of 1.40 (95% CI: 0.95-2.04) and 3.96 (95% CI: 2.27-6.88) and for GCA of 1.32 (95% CI: 0.94-1.85) and 2.73 (95% CI: 1.56-4.79).[70] In a meta-analysis of the association between BMI and EA/GCA, the OR for EA comparing overweight (BMI 25-28 kg/m^2) and obese (BMI ≥ 28) to normal weight (18.5-25 kg/m^2) individuals was 2.2 (95% CI: 1.8-2.7; $p_{homogeneity}$=0.11) for men and 1.9 (95% CI: 1.5-2.5; $p_{homogeneity}$=0.20) for women. The OR for GCA comparing overweight and obese to normal weight was 1.5 (95% CI: 1.3-1.8; $p_{homogeneity}$=0.38) for studies conducted in the U.S. and European countries.[82]

Cigarette Smoking. Smoking is a risk factor for both types of esophageal and gastric cancers. In a pooled analysis of cigarette smoking from the International Barrett's and Esophageal Adenocarcinoma Consortium (BEACON),[81] the summary OR for ever versus never cigarette smoking for EA was 1.96 (95% CI: 1.64-2.34) and 2.18 (95% CI: 1.84-2.58) for gastroesophageal (GE) junction adenocarcinoma. Additionally, there was a trend of increasing risk of EA and GE junction adenocarcinoma by pack-years smoked. Compared to non-smokers, pack-years of smoking <15, 15-<30, 30-<45, and ≥45 had an OR for EA of 1.25 (95% CI: 1.02-1.53), 1.96 (95% CI: 1.58-2.45), 2.07 (95% CI: 1.66-2.58), and 2.71 (95% CI: 2.16-3.40) and for GE junction adenocarcinoma of 1.32 (95% CI: 0.99-1.75), 2.44 (95% CI: 1.98-3.00), 2.64 (95% CI: 2.07-3.38), and 2.68 (95% CI: 2.23-3.23), respectively.[81] Additionally, alcohol has been evaluated as a risk factor. Most studies have not shown an association between alcohol consumption and EA, however a few studies have shown a slight increase of EA risk among drinkers.[10] There is also

some evidence that alcohol may increase the risk of GCA.[10]

Asprin. Risk reduction factors for EA/GCA include non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, and other COX-2 inhibitors,[49,51,74,83-87] fruits and vegetables,[14-25] and dietary antioxidants, including vitamin C[88-91] and vitamin E.[88,89,91] In a recent meta-analysis, aspirin was inversely associated with EA (OR=0.64, 95% CI: 0.52-0.79) and GCA (OR=0.82, 95% CI: 0.65-1.04). Other NSAIDs were also inversely associated with EA (OR=0.65, 95% CI: 0.50-0.85) and GCA (OR=0.80, 95% CI: 0.67-0.95).[86] In a prospective cohort study of BE patients, the hazard ratio for EA in current NSAID users was 0.32 (95% CI: 0.14-0.76).[85]

Dietary Intake. Epidemiological studies have shown that diets high in fruit and vegetable consumption are inversely associated with EA[14-23] and GCA.[14,15,23-25] In the U.S. Multi-Center Study, the adjusted odds ratios (OR) were calculated based on increasing fruit/vegetable intake by one serving per day. Total fruit and vegetable intake were associated with decreased risk of EA (OR=0.88, 95% CI: 0.82-0.95) and potentially decreased risk of GCA (OR=0.97, 95% CI: 0.90-1.03).[15] Additionally, studies have shown that dietary antioxidants, including vitamin C[88-91] and vitamin E,[88,89,91] have risk reduction effects against EA/GCA. In the U.S. Multi-Center Study, Mayne et al. found an inverse association between vitamin C and E, comparing the highest versus lowest quartile of consumption, for EA (OR=0.45, 95% CI: 0.33-0.61 and OR=0.73, 95% CI: 0.54-1.00, respectively) and GCA (OR=0.64, 95% CI: 0.49-0.84 and OR=0.75, 95% CI: 0.55-1.02, respectively).[91]

Summary. Risk factors of EA/GCA include GERD,[49-52] esophagitis,[49-52] Barrett's esophagus,[49-52] age,[49,51,55,56] obesity,[49,51,69-74] gender, race,[49,51,55-59] cigarette smoking,[49,51,67,72,75-81] family history,[49,60-65] and low SES.[67] Risk reduction factors include non-steroidal anti-inflammatory drugs (NSAIDs) and other COX-2 inhibitors,[49,51,74,83-86] fruits and vegetables,[14-25] and dietary antioxidants, including

vitamin C[88-91] and vitamin E.[88,89,91] These risk factors were important to consider when designing the directed acyclic graph of the association between flavonoids and EA/GCA risk, as these are potential confounders or intermediates of this association.

Flavonoids and Esophageal/Gastric Cardia Adenocarcinoma: Biologic Mechanisms

Flavonoids are a group of bioactive polyphenolic compounds that are naturally occurring in fruits, vegetables, and beverages of plant origin and are thought to partially account for the risk reduction of fruits and vegetables on EA and GCA.[29] Experimental studies have shown that some classes of flavonoids regulate cell cycle, proliferation, and apoptosis, and modulate carcinogen metabolism and inflammatory pathways, which have important chemopreventive effects against EA and GCA.[29] Lignans are another polyphenolic compound that has been shown to have antioxidant and anti-inflammatory effects, induce apoptosis, and promote cell cycle arrest.[92] There are six classes of flavonoids (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones), and different foods and beverages contribute to each class of flavonoid.

A recently published review[93] has outlined the different foods and beverages that contribute to the flavonoid classes and the mechanism of chemoprevention for each class and is briefly described below.

- Anthocyanidins (e.g., cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin) are found in berries, grain, grape seed extracts, wine, red cabbages, and purple sweet potatoes. The chemopreventive mechanisms for anthocyanidins are through antioxidant effects, induction of apoptosis, anti-inflammatory effects, and cell cycle arrest.[93]
- Flavan-3-ols (e.g., (+)-catechin, (+)-catechin-3-gallate, (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (+)-

gallocatechin, (+)-gallocatechin-3-gallate, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, theaflavin-3,3'-digallate, and thearubigins) are found in apple skin, celery, berries, citrus fruits, soybeans, onions, green tea, and cocoa. The chemopreventive mechanisms for flavan-3-ols are through antioxidant effects, anti-inflammatory effects, and inhibition of telomerase.[93]

- Flavanones (e.g., eriodictyol, hesperitin, and naringenin) are found in oranges and grapefruit. The chemopreventive mechanisms for flavanones are through induction of apoptosis and antiproliferative effects.[94]
- Flavones (e.g., apigenin and luteonin) are found in legumes, broccoli, parsley, thyme, olives, cherries, and tea. The chemopreventive mechanisms for flavones are through induction of apoptosis, anti-inflammatory effects, suppression of mitogen-activated protein kinase, cell cycle arrest, and suppression of NF-κB activation.[93]
- Flavonols (e.g., isorhamnetin, kaempferol, myricetin, and quercetin) are found in olive oil, red wine, tea, citrus fruits, tomato, onion, cotton seed, and yellow vegetables. The chemopreventive mechanisms for flavonols are through antioxidant effects, anti-inflammatory effects, induction of apoptosis, suppression of NF-κB activation, suppression of protein kinases (PTKs), suppression of angiogenesis, cell cycle arrest, and inhibitor of telomerase.[93]
- Isoflavones (e.g., daidzein, genistein, and glycitein) are found in soybean and citrus fruits. The chemopreventive mechanisms for isoflavones are through induction of apoptosis, anti-inflammatory effects, suppression of PTKs, cell cycle arrest, and suppression of angiogenesis.[93]
- Lignans (e.g., secoisolariciresinol and matairesinol) are found in sesame oil and sunflower oil. The chemopreventive mechanisms for lignans are through

antioxidant effects, induction of apoptosis, cell cycle arrest, and anti-inflammatory effects.[93]

Only three flavonoid classes (flavan-3-ols, flavones, and flavonols) have been studied and also directly linked to adenocarcinomas of the esophagus and gastric cardia in laboratory experiments, as discussed below.

Flavan-3-ols. The effects of flavan-3-ols on two cell lines – one a poorly differentiated esophageal adenocarcinoma and one a moderately differentiated gastric cardia/gastroesophageal junction adenocarcinoma – were investigated, and exposure of these cell lines to flavan-3-ols reduced the number of cancer cells. This was attributed to increased apoptosis and arrest of the cell cycle at G0/G1 phase, and reduction of cells in cell cycle S phase.[29] A more recent study[95] looked at human EA cells and showed that Polyphenon E, a green tea extract of Epigallocatechin gallate (which is found to a lesser extent in black tea),[96] inhibited growth, produced cell cycle arrest (G1 phase), and down-regulated the expression of cyclin D1 protein in EA cells.[95]

Another study found that cranberry proanthocyanidins inducted apoptosis and cell cycle arrest (G1 phase) and inhibited acid-induced proliferation of human EA cells.[97] Using a grape seed proanthocyanidin extract (GSPE) against human gastric adenocarcinoma cells *in vitro*, researchers found that GA cancer cells treated with 50 mg/liter GSPE had 41% inhibition of cellular growth compared with GA cancer cells treated with 25 mg/liter GSPE that had a 34% inhibition of cellular growth.[98]

Flavones. In human esophageal adenocarcinoma cells, flavones (i.e., luteolin, apigenin, and chrysin) induced cytotoxicity, which was mediated by G2/M cell cycle arrest and apoptosis. Of the flavones, luteolin had the most cytotoxic potency.[99]

One animal study has shown that a synthetic flavone, Flavopiridol, reduces the development of EA. In this study, mice were exposed to a carcinogen that is known to induce esophageal cancer. Experimental mice (n=71) were given Flavopiridol and control mice (n=50) received diluent. Prevalence of EA in experimental mice was 11% compared to 32% in control mice (p=0.001).[100]

Flavonols. In a study mentioned above, researchers also found that flavonols (i.e., quercetin, kaempferol, and myricetin) induced cytotoxicity in human esophageal adenocarcinoma cells, which was mediated by G2/M cell cycle arrest and apoptosis. Of the flavonols, quercetin had the most cytotoxic potency.[99]

Summary. Experimental studies have shown that some classes of flavonoids have important chemopreventive effects against EA and GCA.[29] This gives biological plausibility to the hypothesis of an inverse association between flavonoid consumption and EA/GCA incidence and survival among EA/GCA cases. Demonstration of an association between flavonoids and incidence of EA/GCA or survival among EA/GCA cases suggests potential to use flavonoids as a risk reduction strategy for these cancers.

Epidemiology of Flavonoids and Esophageal/Gastric Cardia Adenocarcinoma

Four previous epidemiological studies[101-104] have analyzed the association between esophageal adenocarcinoma (EA) or gastric adenocarcinoma (GA) and total flavonoid consumption. These studies found no association with total flavonoid consumption, but two studies[101,102] did find positive associations with specific classes of flavonoids. In a U.S.-based study of EA, the odds ratio for EA comparing individuals in the highest quartile of consumption of one class of flavonoid (anthocyanidins) to participants in the lowest quartile of consumption was 0.47 (95% CI: 0.24-0.91). This study was limited by small sample size (161 cases of EA), which

can result in unstable effect estimates.[101] In a Greece-based GA study, the odds ratio (OR) per one standard deviation increase in intake of one class of flavonoid (flavanones) was 0.55 (95% CI: 0.31-0.96). However, the study examined all adenocarcinoma of the stomach and did not consider anatomical subsites (i.e., cardia versus non-cardia), and it was also limited by small sample size (100 cases of GA).[102]

The European Prospective Investigation into Cancer and Nutrition examined the association between flavonoid intake and esophageal and gastric cancer, stratifying by histology (i.e., adenocarcinoma versus squamous cell carcinoma for esophageal cancer) and anatomic subsite (i.e., cardia versus non-cardia for gastric cancer).[103,104] No associations were found in the main analyses. There was suggestion of total flavonoids being associated with decreased risk of gastric cancer in women and esophageal cancer in current smokers. When results were stratified by histology or anatomic subsite, risk reductions were not detected for esophageal or gastric cardia adenocarcinoma possibly due to small sample size (EA n=142, GCA n=201).[103,104]

One other study, which was conducted in Sweden, examined the association between a specific flavonoid, quercetin of the flavonol class, and gastric adenocarcinoma risk, both by cardia and non-cardia type. The study showed a non-significant decrease for the association between quercetin and gastric cardia adenocarcinoma (GCA) (highest versus lowest quintile OR=0.76, 95% CI: 0.40-1.44). This study included 81 cases of GCA.[105]

Two Swedish studies, by the same authors, have examined the association between dietary lignan intake and esophageal or GE junction adenocarcinoma.[106,107] In the case-control study, which included 181 EA cases and 255 GE junction adenocarcinoma cases, decreased risk of EA (OR=0.65, 95 % CI: 0.38-1.12) and GE

junction adenocarcinoma (OR=0.37, 95 % CI: 0.23-0.58) was found for the highest quartile of lignan intake compared to the lowest.[107] However, in a cohort study there was no association found between lignan intake and EA or GE junction adenocarcinoma. This study was limited by small sample size of 83 cases of EA or GE junction adenocarcinoma.[106]

Summary. The association between flavonoids and EA/GCA risk has been studied previously.[101-105] However, the sample sizes have been small; this can result in unstable effect estimates. No previous studies have been conducted examining survival among EA/GCA cases in relation to flavonoid intake.

Role of Cell Cycle-Related Genes: Cyclin D1 and p53

There is evidence that cyclin D1 (which regulates cellular proliferation, differentiation, and carcinogenesis)[108] and p53 (which regulates cell cycle control, DNA repair, and apoptosis)[109,110] mutations may reflect exposure to etiologic factors.[84,108,111,112] Cyclin D1 regulates cell cycle in the mid to late G_1 phase by phosphorylating retinoblastoma protein, which releases E2F and transitions to S-phase.[113] Cyclin D1 has been implicated in EA and may be involved in the development of BE by predisposing the epithelium to malignant transformation.[114] In the U.S. Multi-Center Study, 80 of 159 (50.3%) esophageal adenocarcinomas (EA) and 55 of 149 (36.9%) gastric cardia adenocarcinomas (GCA) were positive for cyclin D1.[108]

P53 is a tumor suppressor that halts the cell cycle in the G_1 and G_2 phases to assess DNA damage. If damage has occurred, p53 determines if the damage is repairable or not. If the damage can be repaired, p53 triggers cell cycle arrest until reparation. If not, p53 triggers apoptosis. Mutations in this gene can result in check-point errors.[110] In the U.S. Multi-Center Study, p53 overexpression was detected in 122 of 170 (71.8%) of EA and 102 of 147 (69.4%) of GCA.[84]

An *in vivo* experiment has suggested that overexpression of cyclin D1 may be an early event in the tumorgenesis process for esophageal cancer in rats induced by the carcinogen 2,6-dimethylnitrosomorpholine (DMNM), by causing an increase in proliferation of esophageal stem cells. In this study, increased p53 expression began at the dysplastic stage of carcinogenesis.[115] It was hypothesized that cyclin D1 is an initiating event during Barrett's metaplasia, and abnormal p53 is required during dysplasia to promote the development of EA.[113,115]

Summary. Cyclin D1 and p53 are involved in tumor differentiation, proliferation and apoptosis,[84,108,109,111,112] and mutations may reflect exposure to etiologic factors.[84,108,111,112] We will examine if the association between flavonoids and EA/GCA varies by p53 or cyclin D1 overexpression. Categorizing cases by these markers was conducted to examine potentially etiologically distinct subgroups.

Flavonoid use During Cancer Progression and Chemotherapy

Carcinogenesis is characterized by multistage genetic and cellular changes, and this sequence of events has many points for intervention, with the purpose of preventing, slowing down, or reversing the process.[116] Therefore, targets for chemoprevention could be multiple and could vary by stage – from initiation to promotion to progression.[117] In a normal cell, targets for chemoprevention include scavenging reactive oxygen species and altering carcinogen metabolism. In an initiated cell, targets for chemoprevention include preventing further DNA damage and inducing apoptosis. In a preneoplasic cell, targets for chemoprevention include preventing further DNA damage, inducing cell-cycle arrest, inducing apoptosis, and inhibiting angiogenesis. In a tumor cell, targets for chemoprevention include preventing further DNA damage, inhibiting angiogenesis, and inhibiting invasion.[116,117]

Flavonoids have been shown to have a wide array of cellular effects and can

influence the carcinogenic process during initiation, progression, and promotion.[116] However, some concerns have been raised about using antioxidants, such as flavonoids, for chemoprevention or chemotherapy. Some scientists have expressed concerns that antioxidants may not differentiate between healthy cells and tumor cells and may protect tumors from cytotoxic cancer treatments.[118-120] In a breast cancer study where participants were given mega-doses of antioxidants, including beta-carotene, vitamin C, niacin, selenium, coenzyme Q10, and zinc, risk of breast cancer-specific mortality and breast cancer recurrence was increased for the group given the antioxidant vitamins and minerals compared to a group given standard therapy [hazard ratio (HR)=1.75, 95% CI: 0.83-2.69; 1.55, 95% CI: 0.94-2.54, respectively].[121]

In addition to mortality, there are potential adverse effects from antioxidants in high-risk patients. In both the alpha-tocopherol, beta-carotene trial (ATBC) and β-carotene and retinol efficacy trial (CARET), participants were at high-risk for lung cancer (i.e., participants in both trials were current or former heavy smokers and participants in CARET were also exposed to asbestos). Vitamins A and E were given to participants as a chemopreventive, but the chemoprevention had unintended consequences. Participants in both ATBC and CARET that were given vitamins A or E had higher risk of incident of lung cancer (HR=1.18, 95% CI: 1.03-1.36; HR=1.28, 95% CI: 1.04-1.57, respectively) and higher risk of mortality (HR=1.08, 95% CI: 1.01-1.16; HR=1.17, 95% CI: 1.03-1.33, respectively).[122] While it is of interest to determine if there is potential to use flavonoids as a risk reduction or chemotherapy of these tumors, flavonoids will need to be carefully evaluated in the future for these therapy options.

Summary. The specific aims were to determine the association between flavonoids and incidence of esophageal and gastric tumors or survival among esophageal and gastric cancer cases. There could be potential to use flavonoids as a risk reduction strategy or chemotherapy of these tumors. This could allow some

esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs. While this study allowed investigation of these aims by utilizing data on dietary intake of flavonoids, care needs to be taken when thinking of utilizing flavonoids as a risk reduction or chemotherapy of these tumors in the future. There is potential, as evidenced by previous research with other antioxidants, that the use of flavonoids as a chemopreventive or chemotherapy could have unintended consequences.

Esophageal and Gastric Cardia Adenocarcinoma Prognostic Factors

As discussed above, EA and GCA are extremely lethal cancers, and there are very few factors that contribute to a better prognosis and survival benefit. However, several factors that have been explored include age, sex, BE, GERD, tumor location, stage, grade, dysphagia at presentation, weight loss, obesity, education, income, cigarette consumption, alcohol use, non-steroidal anti-inflammatory drugs (NSAIDs), type of surgical operation, and surgical complications.[1,123]

Demographic Factors. Non-modifiable factors of age, sex, and education have not been shown to have a prognostic impact on EA and GCA.[1] In the U.S. Multi-Center Study,[1] income was an important prognostic factor. The adjusted hazard ratio (HR) comparing individuals with ≥$15,000 income to those with <$15,000 income was 0.64 (95% CI: 0.48-0.87) for EA and 0.62 (95% CI: 0.43-0.88) for GCA. It was suggested that decreased survival among low income cases may indicate lack of access to medical care or death from other comorbidities.[1]

Epidemiologic Factors. Modifiable factors of cigarette consumption, alcohol use, and NSAIDs use also did not have a prognostic impact on EA and GCA.[1] However, adiposity and weight loss have been shown to be prognostic factors. The survival benefit from adiposity is complex, with overweight individuals having the

better overall survival compared to normal weight and obese individuals. This relationship between weight and survival was more defined for EA patients. Compared to normal weight participants (BMI <25 kg/m^2), the adjusted HR for EA was 0.67 (95% CI: 0.51-0.88) for overweight participants (BMI 25-29.9 kg/m^2) and 0.78 (95%CI: 0.55-1.12) for obese participants (BMI 30+ kg/m^2). Compared to normal weight participants, the unadjusted HR for GCA was 0.90 (95% CI: 0.67-1.20) for overweight participants and 0.98 (95% CI: 0.67-1.43) for obese participants.[1] Pre-treatment weight loss is thought to indicate advanced disease. Patients, including both EA and esophageal squamous cell carcinoma, with pre-treatment weight loss of >10% body weight had a worse prognosis than those without weight loss (HR=0.63, 95% CI: 0.26-1.00).[124]

Medical Conditions/Clinical Characteristics. The presence of precursor lesions or dysphagia at diagnosis, as well as characteristics of the first primary such as tumor location, stage, and grade, have prognostic importance for patients with adenocarcinomas of the GE junction. Patients presenting with dysphagia have worse prognosis, likely reflecting an advanced disease.[123] In a study of esophageal adenocarcinoma, tumors arising in BE were associated with better survival than tumors not arising in BE (overall 5-year survival 64% and 32%, respectively).[125] Tumors associated with BE are detected earlier, with smaller diameter and more differentiation. This suggests that tumors not associated with BE more advanced tumors that may have overgrown the BE.[123] This is supported by a study where 79 patients underwent endoscopy, biopsy, and neoadjuvant chemotherapy. Prior to chemotherapy, 59 patients (74%) had associated BE, and the other 20 patients exhibited no evidence of BE. After chemotherapy, BE was “unmasked” through biopsy or histopathological assessment in 18 of the 20 patients without signs of BE pre-treatment. This resulted in 97% of EA associated with BE.[126]

Gastroesophageal reflux disease (GERD) has also been found to be associated with survival among patients diagnosed with GE junction cancers. Unadjusted HR comparing those with GERD to those without were 0.80 (95% CI: 0.63-1.03) for EA and 0.74 (95% CI: 0.56-0.98) for GCA.[1]

Tumors located in the esophagus have better prognoses than tumors in the gastric cardia. This is thought to be because tumors might be detected in the esophagus due to screening or possibly because adenocarcinoma in the esophagus presents with dysphagia earlier than adenocarcinoma arising in the gastric cardia.[123]

As expected, stage is an important prognostic factor. Compared with distant stage, the adjusted HRs for regionalized, localized, and unknown stage, respectively, are 0.32 (95% CI: 0.23-0.45), 0.22 (95% CI: 0.15-0.31), and 0.42 (95% CI: 0.30-0.60) for EA and 0.48 (95% CI: 0.35-0.65), 0.18 (95% CI: 0.11-0.31), and 0.54 (95% CI: 0.35-0.83) for GCA.[1]

Grade of the tumor did not appear to be as important. Compared to poor/undifferentiated tumors, the unadjusted HR for well/moderately differentiated tumors was 0.85 (95% CI: 0.65-1.11) for EA and 0.83 (95% CI: 0.62-1.10) for GCA.[1]

Summary. Prognostic factors of EA/GCA include income,[1] adiposity,[1] weight loss,[124] BE,[125] GERD,[1] dysphagia,[123] tumor location,[123] stage,[1] and grade.[1] These prognostic factors were important to consider when designing the directed acyclic graph of the association between flavonoids and survival among EA/GCA cases, as these are potential confounders or intermediates of this association. Flavonoids may also reflect the presence of a tumor at a particular stage. The association between flavonoids and stage of EA/GCA was explored to examine potentially etiologically distinct subgroups.

Precursor Lesion of Esophageal/Gastric Cardia Adenocarcinoma: Barrett's Esophagus

The only known potential precursor of esophageal adenocarcinoma (EA) and gastric cardia adenocarcinoma (GCA) is Barrett's esophagus (BE).[2] EA appears to develop from normal mucosal lining through a sequence of pathologic events.[42] Animal studies have shown that the squamous mucosa must be destroyed to allow for reepithelialization of the esophagus.[127] The normal squamous mucosa is believed to be destroyed by chronic gastroesophageal reflux disease (GERD), which is an extremely common condition that affects 10-20% of individuals in Western countries. GERD is a digestive disease that is characterized by reflux of gastric contents, including gastric acid and potentially bile, into the esophagus.[128] This reflux can cause ulceration of the esophagus, known as esophagitis,[129] followed by development of Barrett epithelium, commonly referred to as BE.[130] The important metaplastic change from GERD to BE is intestinal metaplasia, which can progress from low-grade to high-grade dysplasia. It is not known if BE is a necessary precursor of EA,[42] but high-grade BE dysplasia will be detected with simultaneous EA approximately 25% of the time.[128]

It has been postulated that gastric cardia adenocarcinoma (GCA) arises from a similar process where there is intestinal metaplasia of the gastric cardia;[44,131-133] however, this is still controversial.[128] In a study focused on early-stage cancers in an attempt to avoid concealment of underlying intestinal metaplasia, intestinal metaplasia was detected in 96% (25 of 26 patients) of esophageal adenocarcinomas and 69% (11 of 16 patients) of gastric cardia adenocarcinomas.[131]

Studying precursor lesions, such as BE, provides insight into the etiology of cancer by elucidating key risk factors that act early in disease onset. However, not all precursor lesions develop into cancer. Therefore, understanding the mechanisms by which precursor lesions do result in cancerous tumors can offer an opportunity to

intervene and prevent disease progression. Further, studying the etiology of cancer closer to the time of exposure can allow for more accurate effect measure estimates. When outcomes are assessed further from the exposure, it is more difficult to measure associations because effect measure estimates tend to be diluted over time. In other words, lengthy follow-up, as is often the case with latency periods for cancers, can lead to attenuated estimates. Therefore, studying the time period closer to disease onset, such as with precursor lesions, mitigates recall error and loss to follow-up.[134] However, by studying the precursor lesion of BE versus EA/GCA, a different stage in the cancer process from initiation to promotion to progression could be examined. Risk factors for BE could be involved in initiation or promotion, while risk factors for EA/GCA could also be involved in progression of cancer. This could affect the interpretation of results if an association is seen between flavonoids and BE but not EA/GCA, or vice versa, as flavonoids may have an effect at different stages of the cancer process.[135]

Summary. EA appears to develop from normal mucosal lining through a sequence of pathologic events.[42] Normal squamous mucosa is thought to be destroyed by chronic GERD, which can cause esophageal ulceration followed by BE development.[130] It is postulated that GCA arises from similar processes,[131] but this is controversial.[128] By studying the precursor lesion of BE, the association between flavonoids and the EA/GCA cancer continuum can be examined. Studying BE will also help mitigate recall error and loss to follow-up by studying the time period closer to disease onset.[134]

Epidemiology of Barrett's Esophagus Prevalence

The incidence and prevalence of Barrett's esophagus is not known with precision, partially because BE can be asymptomatic. As a nonmalignant tumor, BE is considered a prevalent condition because individuals can live with BE for years and not be

diagnosed. The average age of diagnosis is 63 years, but the estimated median age of onset is 40 years.[51] However, it is estimated that there are approximately 86,000 new cases of BE diagnosed each year.[136]

Between 1977 and 1996, newly diagnosed cases of BE increased 800-3000%.[137-139] This drastic increase was attributed to a real increase in incidence, increased knowledge of the condition by endoscopists, or both.[137-139] Since 1996, newly diagnosed cases of BE has increased 150-200%.[140,141] This increase is independent of the number of gastrointestinal endoscopies that are being performed.[107,108] However, older studies show that BE was found when endoscopists looked for it. In a Swiss study of 4,950 endoscopies conducted between 1963 and 1971, 62 patients (1.25%) had BE.[142] This is similar to the percentage of new cases of BE diagnosed during routine clinical endoscopies (1.5%), suggesting that the incidence of BE has not increased over the past 40 years.[143]

Several studies have estimated the population prevalence of BE.[144-154] However, one of the best estimates comes from a study conducted in two Swedish communities of 3,000 randomly selected adults who underwent endoscopy and biopsy. BE was present in 1.6% of the subjects (95% CI: 0.8-2.4). Notably, the prevalence of BE was very similar between individuals who reported reflux symptoms and those who did not (2.3 v. 1.2%, respectively).[144] Using this prevalence estimate, it is estimated that 3.3 million adults over 18 years of age in the U.S. have prevalent BE.[155] The estimate of BE prevalence from the Swedish study is similar to a study of two Italian villages of 1,033 people where 1.3% were found to have BE. Reflux symptoms were a poor predictor of BE in this study as well, as only 53.8% of participants found to have BE reported reflux symptoms.[154] However, a recent study estimated prevalence using modeling techniques to determine the prevalence of BE needed to simulate SEER incidence rates for EA. From this model, the estimated prevalence for BE in the general U.S. population was 5.6% (95% CI: 5.5-

5.7).[156] Using this prevalence estimate with the 2000 U.S. Census estimates,[157] it is estimated that 11.7 million adults over 18 years of age in the U.S. have prevalent BE.

Summary. BE is the only known precursor of EA/GCA,[2] whose incidence in the U.S. and other Western countries is increasing rapidly.[5,8,9,39] Therefore, it is of importance to determine the risk factors for BE because of the increasing incidence of EA/GCA in the U.S. Specifically, it is of interest to determine if flavonoids can be used as a risk reduction strategy for BE.

Barrett's Esophagus Risk Factors

Medical Conditions/Clinical Characteristics. GERD. The main risk factor of BE is GERD symptoms.[51,158-160] Liberman et al. studied the risk of BE with a history of reflux symptoms. Individuals with a longer history of reflux symptoms, compared to individuals without a history of reflux symptoms, had increased odds of having BE: odds ratio (OR) of 3.0 (95% CI, 1.2–8.0) for a 1- to 5-year history, OR of 5.1 (95% CI, 1.7–14.7) for 5- to 10-year history, and OR of 6.4 (95% CI, 2.4–17.1) for more than 10 years.[160]

Hiatal Hernia. BE occurs more frequently among individuals with a hiatal hernia, which is a condition where the stomach protrudes through an opening in the diaphragm into the chest cavity.[161,162] A recent study of 48 BE patients and 103 controls found 2 cm or longer hiatal hernia in 96% of BE patients and 42% of controls, and the authors concluded that hiatal hernia likely contributes to the development of BE.[162]

Demographic Factors. Non-modifiable risk factors for BE include age,[51,146,163] race,[51,164,165] gender,[51,146] and low socioeconomic status.[166] Age of more than 40 years is an independent predictor of BE,[163] and the prevalence of BE increases with age until it plateaus in 60-69 year olds.[146] As with EA and GCA, BE is more common in Caucasians and males. In patients undergoing endoscopy in the Clinical Outcomes Research Initiative database, suspected BE was detected in 7.8% of Caucasians, 4.8% of

Hispanics, 1.3% of Asian-Americans, and 1.1% of African-Americans.[155] The male:female sex ratio for BE ranges from 1.7:1 to 4:1.[51] In a study by Kubo et al., they showed that individuals with at least a college education compared to individuals with high school or less education had a decreased risk of BE (OR=0.47, 95% CI: 0.27-0.82) and individuals with an income of >$75,000 compared to individuals with an income of <$50,000 were at a decreased risk of BE (OR=0.68, 95% CI: 0.42-1.11).[166]

Epidemiologic Factors. Obesity. Obesity is another risk factor for BE that has received attention.[51,167,168] In the Study of Reflux Disease, Edelstein et al. showed that overweight and obese individuals, compared to normal weight, were at an increased risk of BE (adjusted OR=1.6, 95% CI: 0.9-2.8 and 2.6, 95% CI: 1.5-4.4, respectively) and individuals with a high, compared to low, waist-to-hip ratio (WHR) were also at an increased risk of BE (adjusted OR=2.4, 95% CI: 1.4-3.9). When BMI and waist-to-hip ratio were adjusted for each other, the estimate for BMI was more attenuated than the estimate for WHR.[169] This suggests that central obesity could be more important to the development of BE, potentially through hiatal hernia, intragastric pressure promoting reflux, or high levels of insulin.[170]

Cigarette Smoking. Although cigarette smoking is a risk factor for esophageal adenocarcinoma, the association between cigarette smoking and BE is not clear, but there is a suggested trend of increasing risk with increasing pack-years of smoking and increased risk of BE for current smokers, compared to never smokers (adjusted OR=1.27, 95% CI: 0.74-2.17).[171]

Aspirin. Risk reduction factors against these tumors include non-steroidal anti-inflammatory drugs (NSAIDs) and other COX-2 inhibitors,[172] fruits and vegetables,[21,26,27] and dietary antioxidants, including vitamin C and vitamin E.[173] A case-control study conducted in Ireland found that use of NSAIDs or aspirin, at least 1 year prior to interview, was associated with a decreased risk of BE (OR=0.40, 95%

CI: 0.19-0.81 and 0.53, 95% CI: 0.31-0.90, respectively).[172]

Dietary Intake. In the Study of Reflux Disease, researchers showed that individuals in the second and third tertile of fruit and vegetable intake had a lower risk of BE than individuals in the lowest tertile of intake (adjusted OR=0.40, 95% CI: 0.23-0.71 and 0.33, 95% CI: 0.17-0.63, respectively).[26] In a case-control study conducted at Kaiser Permanente Northern California, individuals with high intakes of vitamin C or E were found to have a decreased risk of BE (4th versus 1st quartile, adjusted OR=0.48, 95% CI: 0.26-0.90 and 0.25, 95% CI: 0.11-0.59, respectively).[27]

Summary. Risk factors for BE include GERD,[51,158-160] hiatal hernia,[161,162] age,[51,146,163] race,[51,164,165] gender,[51,146] low socioeconomic status,[166] obesity,[51,167,168] and potentially cigarette smoking.[171] Risk reduction factors against these tumors include non-steroidal anti-inflammatory drugs (NSAIDs) and other COX-2 inhibitors,[172] fruits and vegetables,[21,26,27] and dietary antioxidants, including vitamin C and vitamin E.[173] These risk factors were important to consider when designing the directed acyclic graph of the association between flavonoids and BE risk, as these are potential confounders or intermediates of this association.

Flavonoids and Barrett's Esophagus: Biologic Mechanisms

Few studies have looked at the biological mechanisms of how flavonoids affect Barrett's esophagus. In an *in vitro* study, mentioned above, researchers looked at BE cells and found that Polyphenon E, a flavan-3-ol derived from green tea, inhibited growth and down-regulated the expression of cyclin D1 protein in BE cells.[95] One animal study, also mentioned above, has shown that a synthetic flavone, Flavopiridol, reduces the development of BE. In this study, mice were exposed to a carcinogen that is known to induce esophageal cancer. Prevalence of BE in experimental mice was 7% compared to 26% in control mice (p=0.008).[100]

Summary. Experimental studies have shown that some classes of flavonoids have important chemopreventive effects against BE.[95,100] This gives biological plausibility to the hypothesis of an inverse association between flavonoid intake and BE risk. Demonstration of an association between flavonoids and BE development suggests potential to use flavonoids as a risk reduction strategy.

Epidemiology of Flavonoids and Barrett's Esophagus

One epidemiological investigation to date has examined the association between dietary flavonoid intake and risk of Barrett's esophagus.[174] This case-control study of 151 Barrett's esophagus cases from the Veterans Affairs Medical Center in Houston, Texas, considered one flavonoid class, isoflavones, and found an inverse association (highest versus lowest tertile OR=0.45, 95% CI: 0.25-0.81).[174]

Summary. One previous study[174] has reported on the association between isoflavone intake and BE. However, intake of isoflavone-containing foods in the United States is limited. The other five flavonoid classes are found in foods more commonly consumed by Americans,[175] yet their associations with BE have not been considered.

Barrett's Esophagus: Natural History

Although BE patients have a 125-fold greater risk of EA/GCA, the probability that BE will develop into adenocarcinoma is still rare – incidence of cancer is 1 out of 227 BE patient-years of follow-up.[176] Therefore, it is of interest to determine which BE patients will progress to EA/GCA. A recent review examined this question and discussed the use of chromosome abnormalities as markers of BE that will progress.[177] One study of note was a cohort of BE patients that were followed for the outcome of EA.[83] The baseline biopsies were examined for 9p loss of heterozygosity (LOH), 17p LOH, DNA content abnormalities (teteraploidy and aneuploidy), TP53

mutation, and CDKN2A mutation and methylation. While at 10-years follow-up everything except CDKN2A mutation and methylation contributed to EA risk in univariate analyses, the chromosome instability panel of 9p LOH, 17p LOH, DNA content abnormalities was found to be the best predictor of EA (HR=38.7, 95% CI: 10.8-138.5) compared to participants with no baseline abnormalities. At 10-years of follow-up, 3 of 85 (3.5%) participants without baseline abnormalities developed incident EA and 14 of 22 (63.6%) participants with the chromosome instability panel of three baseline abnormalities developed EA.[83]

Another characteristic of BE that is related to the risk of developing EA/GCA is BE segment length.[10] Long-segment BE (LSBE) is defined as specialized intestinal metaplasia (SIM) and visible column epithelium greater than or equal to 2 or 3 cm (definitions vary).[169,178] Similarly, short-segment BE (SSBE) is defined as SIM and visible column epithelium less than 2 or 3 cm (definitions vary).[169,178] In a study of 889 patients utilizing the LSBE definition of ≥3 cm, dysplasia or EA/GCA was noted in 31% of LSBE and only 10% of SSBE.[178]

Summary. Unfortunately, categorization of BE participants by chromosome abnormalities was not possible, as these were not determined for the parent BE study. This will be an important categorization for future studies. However, categorization of individuals by BE segment length was conducted and allowed examination of potentially etiologically distinct subgroups.

Esophageal Squamous Cell Carcinoma and Non-cardia Gastric Adenocarcinoma

Esophageal and gastric cardia adenocarcinoma is of particular interest due to rapidly increasing incidence in the U.S. and other Western countries[3-7] and a highly prevalent precursor lesion,[144-154] which aids in identifying optimal times along the

cancer continuum to target for intervention with specific chemopreventives to enhance risk reduction. However, esophageal squamous cell carcinoma (ESCC) and non-cardia gastric adenocarcinoma (NCGA) are significant to study because they are the most common forms of esophageal and gastric cancer worldwide.[8,37]

Esophageal Squamous Cell Carcinoma/Non-cardia Gastric Adenocarcinoma Risk Factors

ESCC: Medical Conditions/Clinical Characteristics. Squamous Dysplasia. Similarly to EA, ESCC has a precursor lesion, squamous dysplasia, which increases individuals' risk of ESCC.[179,180] In a study by Wang et al. in a high-risk Chinese population with 13 years of follow-up, the risk of ESCC increased along the squamous dysplasia continuum, from mild dysplasia [adjusted hazard ratio (HR)=2.9, 95% CI: 1.6-5.2] to moderate dysplasia (HR=9.8, 95% CI: 5.3-18.3) to severe dysplasia (HR=28.3, 95% CI: 15.3-52.3) to carcinoma in situ (HR=34.4, 95% CI: 16.6-71.4).[181]

Human Papillomavirus (HPV). There is some evidence that HPV may be involved in the etiology of ESCC. A review reported that 22.9% and 15.2% of ESCC cases were positive for HPV when tested by in situ hybridization and polymerase chain reaction, respectively.[182] A more recent meta-analysis reported the average HPV prevalence in ESCC cases of 27.7% (95% CI: 23.4-32.0%) by polymerase chain reaction; 24.3% (95% CI: 15.9-32.6%) by in situ hybridization; 30.4 (95% CI: 18.5-42.3%) by immunohistochemistry; 32.2% (95% CI: 15.4-49.0%) by L1 serology; and 17.6% (95% CI: 6.1-29.2%) by Southern/slot/dot blot.[183]

ESCC: Epidemiologic Factors. Tobacco and Alcohol. The primary modifiable risk factors for ESCC are tobacco and alcohol use. Cigarette use is more strongly associated with ESCC than EA.[28] The NIH–AARP Diet and Health Study investigated the association between cigarette use and ESCC. The risk of ESCC

among current smokers was increased almost 10-fold compared to non-smokers (HR=9.27, 95% CI: 4.04-21.29).[184] In the U.S. Multi-Center Study, researchers found a 5-fold increase of ESCC (OR=5.1, 95% CI: 2.8-9.2) for current smokers compared to never smokers.[67] This study also estimated the risk of ESCC in alcohol users, considered as consuming at least one drink per month, more than a year prior to diagnosis. Ever drinkers were at a higher risk of ESCC than never drinkers (OR=3.5, 95% CI: 1.9-6.2).[67] It has also been shown that tobacco and alcohol act synergistically.[28]

Aspirin. Two risk reduction factors for ESCC that have been consistent in the literature are fruit and vegetable intake and non-steroidal anti-inflammatory drug (NSAID) use.[28] A meta-analysis of the association between NSAID use and ESCC found that any use of NSAIDs compared to no use reduced risk of ESCC (OR=0.58, 95% CI: 0.43-0.78).[185]

Dietary Intake. Most studies conducted have found an inverse association between high intake of fruits and vegetables and ESCC.[28] The U.S. Multi-Center Study showed that increasing total fruit and vegetable intake by one serving per day resulted in an OR of 0.90 (95% CI: 0.82-0.99) for ESCC.[15]

ESCC: Demographic Factors. Non-modifiable risk factors for ESCC include low socioeconomic status (SES), age, race, and sex.[28] In a 1939 case series of ESCC, it was noted that ESCC was primarily a cancer found among lower SES.[28,186] For participants in the U.S. Multi-Center Study with an income greater than $75,000 compared to those with an income of less than $15,000 per year, the OR was 0.2 (95% CI: 0.1-0.6).[67] Age over 60 years is a risk factor for ESCC.[187] Unlike EA, ESCC has the highest rates among African-American males. Among African-Americans, ESCC accounts for approximately 87% of esophageal cancer but only 45% in Caucasians.[188] Between 1992 and 2005 in SEER 13 data, the male-to-female

incidence rate ratio was about 2 in Caucasians and 3 in African-Americans. The rates for ESCC are over four-fold higher among African-American men compared to Caucasian men (rate per 100,000: 9.4 versus 2.1).[188]

NCGA: Medical Conditions/Clinical Characteristics. *Helicobacter pylori* and Gastric Ulcers. NCGA has been associated with *H. pylori* infection and gastric ulcers.[189] A recent nested case-control study conducted in Norway found that for NCGA the odds ratio was 4.75 (95% CI: 2.56-8.81) for *H. pylori* positive compared to negative.[190] The U.S. Multi-Center Study found that gastric ulcers were associated with an increased risk of NCGA (OR=2.1, 95% CI 1.4-3.2).[191]

NCGA: Demographic Factors. Non-modifiable risk factors associated with non-cardia gastric adenocarcinoma include low socioeconomic status, age, race, and sex. Low socioeconomic status has been associated with gastric adenocarcinoma in many studies.[189] For participants in the U.S. Multi-Center Study with an income greater than $75,000 compared to those with an income of less than $15,000 per year, the adjusted OR for NCGA was 0.5 (95% CI: 0.2-0.9).[67] Risk of NCGA increases with age; risk of NCGA is 6-7 times higher among 60-84 years old than among 40-59 year olds.[192] Similarly to ESCC, the rates of NCGA are highest for African-American males. Incidence rates of NCGA are approximately twice as high in African-Americans compared to Caucasians [incidence rate ratio (IRR)=2.35, 95% CI: 2.25-2.45] and men compared to women (IRR for women versus men=0.56, 95% CI: 0.54-0.57).[193]

NCGA: Epidemiologic Factors. Tobacco and Alcohol. While the literature has been somewhat inconsistent on the association between NCGA and tobacco use,[189] the U.S. Multi-Center Study found that current cigarette smokers have an increased risk of NCGA compared to non-smokers (OR=1.8, 95% CI: 1.2-2.7).[67] Alcohol consumption is primarily associated with GCA, and there has not been

convincing evidence of an association between alcohol and NCGA.[67,189,194]

Aspirin. In a recent meta-analysis of the association between NSAIDs and NCGA, researchers found both aspirin (OR=0.64, 95% CI: 0.52-0.80) and other NSAID use (OR=0.80, 95% CI: 0.67-0.95) to be risk reduction factors for NCGA.[86]

Dietary Intake. Modifiable dietary risk reduction factors for NCGA include fruit and vegetable intake and vitamin C intake; dietary risk factors include salted foods and nitrates/nitrosamines. While the literature on gastric cancer has consistently shown a risk reduction with higher levels of fruit and vegetable intake,[189] recent studies differentiating between GCA and NCGA have not found strong risk reduction by fruits and vegetables for NCGA.[14,15] Thus far, vitamin C has been the micronutrient most consistently associated with a reduction in risk of gastric cancer.[189] In the U.S. Multi-Center Study, vitamin C, both from food sources and supplements, was found to decrease the risk of NCGA (highest versus lowest quartile OR=0.59, 95% CI: 0.45-0.76 and 0.60, 95%CI: 0.41-0.88, respectively).[91]

Salted Foods. Salt and salted foods have long been associated with gastric cancer.[189] The U.S. Multi-Center Study found that high sodium intake in the diet increased risk for NCGA (highest versus lowest quartile OR=1.46, 95% CI: 1.00–2.15).[91] Nitrites and *N*-nitroso compounds have been found to carcinogenic in animal models.[189,195] In the U.S. Multi-Center Study, both meats high in nitrite (adjusted OR for increase of 1 serving/day=1.88, 95% CI: 1.24-2.84)[15] and diets high in nitrite (highest versus lowest quartile OR=1.64, 95% CI: 1.30-2.07) were associated with an increased risk of NCGA.[91]

Summary. Risk factors of ESCC include squamous dysplasia,[179,180] tobacco and alcohol use,[67] low SES,[28] age,[28] race,[28] and sex.[28] Risk reduction factors for ESCC are fruit and vegetable intake and non-steroidal anti-inflammatory drug (NSAID) use.[28] Risk factors of NCGA include *H. pylori* and gastric ulcers,[189] low

SES,[67] age,[192] race,[193] sex,[193] cigarette smoking,[67] and salted foods.[91] Risk reduction factors for NCGA include NSAIDs[86] and vitamin C intake.[189] These risk factors were important to consider when designing the directed acyclic graph of the association between flavonoids and ESCC and NCGA risk, as these are potential confounders or intermediates of this association.

Flavonoids and Esophageal Squamous Cell Carcinoma/Non-cardia Gastric Adenocarcinoma: Biologic Mechanisms

All flavonoid classes (anthocyanidins**,** flavan-3-ols, flavanones, flavones, flavonols, and isoflavones) have been directly linked to ESCC or NCGA in laboratory experiments, as discussed below.

Anthocyanidins. In ESCC research, a group from the Ohio State University demonstrated that freeze-dried berries (black and red raspberries, blackberries, strawberries, blueberries, noni, açaí and wolfberry) inhibited 24-56% of the formation of esophageal tumors in rats caused by N-nitrosomethylbenzylamine (NMBA),[196,197] which has long been long been used to model ESCC in rats. Then they also evaluated two anthocyanidin compounds and found that black raspberries inhibited growth, induced apoptosis, and controlled gene expression in rat esophageal epithelial cells. Researchers found that the anthocyanidins inhibited proliferation, induced apoptosis, and down-regulated the expression of cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS) genes.[198] Finally, the group tested various diets in rats, including one with black raspberry powder and one rich in anthocyanidins. The diet of black raspberry powder and anthocyanidin-rich diet were almost equally effective in preventing NMBA-induced esophageal cancer in rats, suggesting that anthocyanidins are the main chemopreventive in the black raspberry powder.[199]

In gastric cancer research, an *in vitro* study of gastric cancer cells found that

berry juice (mixture of strawberry, raspberry, black currant, red currant, white currant, gooseberry, high-bush blueberry, low-bush blueberry, velvet leaf blueberry, serviceberry, blackberry, black chokeberry, sea buckthorn, and cranberry) produced cell-cycle arrest through down-regulation of cdk4, cdk6, cyclin D1 and cyclin D3 expression and inhibited TNF-induced activation of COX-2 expression.[200] Similarly, Shih et al. in an *in vitro* study of human gastric cells found that anthocyanidins, specifically malvidin, inhibited proliferation and induced apoptosis.[201]

Flavan-3-ols. A study using human gastric cancer cells demonstrated that six active flavan-3-ols [epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCg), gallocatechin (GC), epicatechin gallate (ECg), gallocatechin gallate (GCg)] had inhibited proliferation of gastric cancer cells. EGCg and GCg had the highest levels of antiproliferative activity.[202]

Flavanones. In ESCC studies, another model for ESCC in rats is using *N*-methyl-*N*-amylnitrosamine (MNAN) to produce esophageal tumors. Rats given the flavanone hesperidin had a 40% decrease in incident ESCC, compared to the control group (45% versus 75%).[203]

In an *in vitro* gastric cancer study, flavanones down-regulate b-catenin/T-cell factor (Tcf) transcriptional target gene cyclin D1 through modulation of Tcf activity.[204] The group that conducted this study from Seoul National University also looked at a specific flavanone, naringenin, and found that it inhibited b-catenin/Tcf signaling in a gastric cancer cell line.[205] Another study group also examined naringenin in gastric cancer induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and saturated sodium chloride (S-NaCl) in rats. The researchers found that naringenin has chemopreventive action against gastric cancer in rats,[206] naringenin could potentially prevents damage of cell membranes and gastric mucosa by oxidative damage,[207] and up-regulation of antioxidants by naringenin might be responsible for the chemopreventive effects seen in

rats.[208]

Flavones. In a study of ESCC induced by benzo[a]pyrene (BaP) in human esophageal epithelial cells, researchers found that flavones inhibited BaP-induced CYP1B1 (an enzyme important in smoking-induced esophageal cancer) transcription, showing that flavones might be a potential chemopreventive for ESCC.[209] The same group that investigated the cytotoxic effects of flavones and flavonols in EA also examined these for ESCC. In human ESCC cells, flavones, specifically luteolin, apigenin, and chrysin, induced cytotoxicity, which was mediated by G(2)/M cell cycle arrest and apoptosis. Of the flavones, luteolin had the most cytotoxic potency.[210] In a study mentioned above, researchers also tested the flavone diosmin and found that it decreased incident MNAN associated ESCC by 46% (75% versus 29%).[203] Another study also examined specific types of flavones, including baicalein, baicalin, and luteolin, and found that baicalein and luteolin had the strongest anti-proliferative effects against gastric carcinoma.[211]

In gastric cancer research, a study of the flavone nobiletin found that nobiletin prevented the dissemination of gastric adenocarcinoma to the peritoneum, which happens in the final stages of gastric adenocarcinoma, in mice.[212] Another study of nobiletin *in vitro* found that nobiletin had antiproliferative effects, induced apoptosis, and down-regulation of the cell cycle in human gastric adenocarcinoma cells.[213] The flavone acacetin inhibited cellular proliferation and induced apoptosis characterized with changes in nuclear morphology, DNA fragmentation, and cell morphology in human gastric carcinoma cells.[214] The flavone apigenin inhibited growth, clone formation, and proliferation of human gastric carcinoma cells.[215] Luteolin, a flavone, inhibited the growth of human gastric cancer cells.[216] Similarly, luteolin and daidzein inhibited cell cycle progression at G1 in human gastric carcinoma cells.[217]

Flavonols. In a ESCC study mentioned above, researchers found that

flavonols, specifically quercetin, kaempferol, and myricetin, induced cytotoxicity in human ESCC cells, which was mediated by G(2)/M cell cycle arrest and apoptosis. Of the flavonols, quercetin had the most cytotoxic potency.[210] Additionally, isorhamnetin, which is a metabolite of quercetin, has been found to inhibit proliferation and induce apoptosis in a human ESCC cell line.[218]

Quercetin has been shown to inhibit the growth of malignant human gastric cancer cells, suppress DNA synthesis, and block cell cycle progression.[219] Another study also showed that quercetin inhibited growth and induced apoptosis of human gastric carcinoma cells. The mechanism for this is thought to be down-regulation of p53 and C-myc protein expression and the up-regulation of p16 expression by quercetin.[220]

Isoflavones. In esophageal squamous cell lines, soybean isoflavone was shown to induce apoptosis, possibly mediated by down-expression of gene bcl-2 and up-expression of gene bax.[221] A study of human gastric adenocarcinoma and ESCC cells *in vitro* tested seven isoflavones (biochanin A, daidzein, genistein, genistin, prunectin, puerarin, and pseudobaptigenin) for their chemopreventive effects. Gastric adenocarcinoma proliferation was strongly inhibited by biochanin A and genistein, and ESCC proliferation was moderately suppressed by these isoflavones.[222]

Genistein has been shown to have chemopreventive effects in human gastric carcinoma cells; genistein inhibited cell cycle progression at G2-M,[217,223] induced apoptosis,[224,225] inhibited growth[223,225] and proliferation,[223] decreased cyclin D1 protein expression,[223] and enhanced cyclin B1 and p21(waf/cip1) protein expression.[223] One study showed that in seven days with the highest dose (20.0 microg/mL), the growth inhibitory rate was 84.7%.[223] In rats with gastric carcinogenesis induced by MNNG, genistein increased apoptosis and lowered cellular proliferation for gastric cancer.[226]

Summary. Experimental studies have shown that all classes of flavonoids have important chemopreventive effects against ESCC or NCGA. This gives biological plausibility to the hypothesis of an inverse association between flavonoid intake and ESCC and NCGA incidence and survival among ESCC and NCGA cases. Demonstration of an association between flavonoids and incidence of ESCC and NCGA or survival among ESCC and NCGA cases suggests potential to use flavonoids as a risk reduction strategy for these cancers.

Epidemiology of Flavonoids and Esophageal Squamous Cell Carcinoma/Non-cardia Gastric Adenocarcinoma

The European Prospective Investigation into Cancer and Nutrition examined the association between flavonoid intake and esophageal and gastric cancer, stratifying by histology (i.e., adenocarcinoma versus squamous cell carcinoma for esophageal cancer) and anatomic subsite (i.e., cardia versus non-cardia for gastric cancer).[103,104] No associations were found in the main analyses. There was suggestion of total flavonoids being associated with decreased risk of gastric cancer in women and esophageal cancer in current smokers. When results were stratified by histology or anatomic subsite, risk reductions were not detected for ESCC or NCGA possibly due to small sample size (ESCC n=176, NCGA n=323).[99,100]

Another recent study to examine the association between ESCC and flavonoid intake was by Bobe et al.[101] This study examined the effect of total flavonoid intake separately for Caucasians and African-Americans (highest versus lowest quartile OR=1.19, 95% CI: 0.50-2.81; OR=0.72, 95% CI: 0.35-1.46, respectively). The authors found an inverse association among whites between ESCC and isoflavones (highest versus lowest quartile OR=0.43, 95% CI: 0.20-0.93). This study had a limited sample size (114 Caucasian cases and 218 African-

American cases of ESCC) to examine this association, though.[101] One other study of the association between ESCC and total dietary flavonoid intake has been conducted in Italy and did not show an association between total flavonoids and ESCC (highest versus lowest quintile OR=0.99, 95% CI: 0.55-1.79), but it did shown an inverse association between flavanones and ESCC (highest versus lowest quintile OR=0.38, 95% CI: 0.23-0.66). This study had a sample size of 304 cases and 743 controls.[227]

Another study examined all upper aerodigestive tract cancers (UADC) in Uruguay, including esophageal (n=66), oral cavity and pharyngeal (n=33), and larynx cancer cases (n=34), and found an association between total flavonoids and UADC (highest versus lowest tertile OR=0.3, 95% CI: 0.1-0.5).[228] Additionally, a study in Shanghai, China, used a nested case-control study to determine the association between prediagnostic urinary tea polyphenol markers and risk of esophageal (42 cases) and gastric cancer (190 cases). Among esophageal and gastric cancers that developed four years or more after enrollment, there was an inverse association between epigallocatechin positivity, a flavan-3-ol, and esophageal/gastric cancer risk (OR=0.58, 95% CI: 0.34-0.98).[229]

The only other study of total dietary flavonoids and gastric adenocarcinoma (GA) was conducted by Lagiou et al., mentioned above. This study did not find an association between total flavonoids and GA risk, but did find an inverse association between one class of flavonoids, flavanones, and GA risk (per one standard deviation increase in intake OR=0.55, 95% CI: 0.31-0.96). However, the study examined all adenocarcinoma of the stomach and did not consider anatomic subsites (i.e., cardia or non-cardia), and it was also limited by small sample size (100 cases of GA).[102] Two other studies examined the association between flavonoids or lignans and overall gastric cancer risk.[105,230] A study in Mexico City did not find an

association between total flavonoids or lignans and gastric cancer risk, but the authors did find an inverse association between one type of lignan, secoisolariciresinol, and gastric cancer risk (highest versus lowest tertile OR=0.42, 95% CI: 0.27-0.65).[230] The other study was conducted in Sweden and looked at the association between quercetin, a flavonol, and gastric adenocarcinoma risk, both by cardia and non-cardia type. The study reported an inverse association between quercetin and NCGA (highest versus lowest quintile OR=0.57, 95% CI: 0.40-0.83). This study included a large sample size (420 cases of NCGA).[105]

Summary. There have been several epidemiologic studies to examine the association ESCC or NCGA and flavonoids. With two notable exceptions,[105,227] the majority of these studies have been limited by small sample size, which can result in unstable effect estimates. No previous studies have been conducted examining survival among ESCC or NCGA cases in relation to flavonoid intake.

Role of Cell Cycle-Related Genes: Cyclin D1 and P53

Both cyclin D1 and p53 have roles controlling cell cycle and cancer progression. Multiple studies indicate that cyclin D1 and p53 overexpression predict poor ESCC prognosis and some studies additionally show that cyclin D1 and p53 could be used as independent prognostic factors.[231] The U.S. Multi-Center Study found that 91 of 117 (77.8%) ESCC and 59 of 205 (28.8%) NCGA were positive for cyclin D1.[108] In this same study, the authors reported 75 of 112 (67.0%) ESCC and 114 of 220 (51.8%) NCGA overexpressed p53.[84]

An *in vivo* experiment has suggested that overexpression of cyclin D1 may be an early event in the tumorgenesis process for esophageal cancer in rats induced by the carcinogen 2,6-dimethylnitrosomorpholine (DMNM), by causing an increase in proliferation of esophageal stem cells. In this study, increased p53 expression began

at the dysplastic stage of carcinogenesis. It was hypothesized that cyclin D1 is an initiating event during metaplasia, and abnormal p53 is required during dysplasia to promote the development of esophageal cancer.[115]

Summary. Cyclin D1 and p53 are involved in tumor differentiation, proliferation and apoptosis,[84,108,109,111,112] and mutations may reflect exposure to etiologic factors.[84,108,111,112] The association between flavonoids and ESCC or NCGA was examined by p53 and cyclin D1 status. Categorizing cases by these markers was conducted to examine potentially etiologically distinct subgroups.

Esophageal Squamous Cell Carcinoma/Non-cardia Gastric Adenocarcinoma Prognostic Factors

Similar to EA/GCA, ESCC and NCGA are extremely lethal cancers, and there are very few factors that contribute to a better prognosis and survival benefit. However, several factors that have been explored include age, sex, stage, grade, obesity, education, income, cigarette smoking, alcohol use, non-steroidal anti-inflammatory drugs (NSAIDs), esophageal cancer type, and perioperative chemotherapy.[1,232]

Demographic Factors. Age has not been shown to have a prognostic impact on ESCC or NCGA.[1] In the U.S. Multi-Center Study,[1] sex, education, and income were important non-modifiable prognostic factors for ESCC, but these were not shown to have a prognostic impact on NCGA. Women with ESCC had a better prognosis than men (adjusted HR=0.61, 95% CI: 0.42-0.88), and individuals with higher education (more than high school versus less than high school adjusted HR=0.79, 95% CI: 0.59-1.05) and higher income (≥$15,000 versus <$15,000 adjusted HR=0.74, 95% CI: 0.55-0.99) had better prognosis. It was suggested that decreased survival among low income cases may indicate lack of access to medical care or death from other comorbidities.[1]

Epidemiologic Factors. Cigarette smoking did not have a prognostic impact on ESCC or NCGA.[1] The U.S. Multi-Center Study reported that modifiable factors of alcohol consumption and NSAID use had a prognostic impact on ESCC but not NCGA.[1] Adiposity and weight loss have also been shown to be prognostic factors for both ESCC and NCGA.[1,124] For ESCC, ever having consumed alcohol (defined as ≥1 alcoholic drink per month for ≥6 months), compared with never, was associated with poorer prognosis (adjusted HR=1.77, 95% CI: 0.93-3.35), and ever using NSAIDs (defined as ≥1 tablet of aspirin or non-aspirin NSAIDs per week for ≥6 months), compared with never, was associated with better prognosis (adjusted HR=0.79, 95% CI: 0.57-1.11).[1]

The survival benefit from adiposity for ESCC and NCGA is complex, with overweight individuals generally having the better overall survival compared to normal weight and obese individuals. This relationship between weight and survival is more complex for ESCC patients. Compared to normal weight participants (BMI <25 kg/m^2), the adjusted HR for ESCC was 0.90 (95% CI: 0.67-1.20) for overweight participants (BMI 25-29.9 kg/m^2) and 1.24 (95%CI: 0.70-2.20) for obese participants (BMI 30+ kg/m^2). Compared to normal weight participants, the unadjusted HR for NCGA was 0.68 (95% CI: 0.53-0.87) for overweight participants and 0.77 (95% CI: 0.53-1.11) for obese participants.[1] Pre-treatment weight loss is thought to indicate advanced disease. Patients, including both EA and ESCC, with pre-treatment weight loss of >10% body weight has been shown to have worse prognosis than those without weight loss (HR=0.63, 95% CI: 0.26-1.00).[124]

Medical Conditions/Clinical Characteristics. Histologic type, stage, and grade have prognostic importance. Compared with EA, ESCC has a worse prognosis (EA versus ESCC HR=0.44, 95% CI: 0.23-0.83).[232] As expected, stage is an important prognostic factor. Compared with distant stage, the adjusted HRs for

regionalized, localized, and unknown stage, respectively, are 0.52 (95% CI: 0.35-0.77), 0.41 (95% CI: 0.26-0.64), and 0.47 (95% CI: 0.30-0.74) for ESCC and 0.37 (95% CI: 0.29-0.49), 0.12 (95% CI: 0.08-0.19), and 0.47 (95% CI: 0.29-0.74) for NCGA.[1] Grade of the tumor did not appear to be as important. Compared to poor/undifferentiated tumors, the unadjusted HR for well/moderately differentiated tumors was 1.16 (95% CI: 0.85-1.58) for ESCC and 0.68 (95% CI: 0.51-0.89) for NCGA.[1] Additionally perioperative chemotherapy was associated with better prognosis (no chemotherapy versus chemotherapy HR=0.43, 95% CI: 0.24-0.77).[232] History of gastroesophageal reflux disease (GERD; defined as at least once per week or 2 years of weekly antacid use) was also associated with poorer prognosis of ESCC (adjusted HR=1.41, 95% CI: 1.00-1.99) but not NCGA.[1]

Summary. Prognostic factors of ESCC include sex,[1] education,[1] income,[1] GERD,[1] and histology.[232] Prognostic factors for both ESCC and NCGA include alcohol consumption,[1] NSAID use,[1] adiposity,[1] weight loss,[124] stage,[1] and grade.[1] These prognostic factors were important to consider when designing the directed acyclic graph of the association between flavonoids and survival among ESCC and NCGA cases, as these are potential confounders or intermediates of this association. Flavonoids may also reflect the presence of a tumor at a particular stage. The association between flavonoids and stage of ESCC or NCGA was conducted to examine potentially etiologically distinct subgroups.

Specific Aims

This dissertation examined the association between flavonoid intake and esophageal and gastric tumors along the cancer continuum (normal tissue → precancerous conditions → invasive cancer → mortality). This novel approach aided in identifying key windows of susceptibility that signified when along the cancer continuum this

potential intervention strategy could be implemented in an effort to reduce the high disease burden associated with these cancers. Specific aims are as follows.

Specific Aim 1: Determine if flavonoid intake is associated with esophageal and gastric cancer incidence.

Hypothesis: There will be an inverse association between flavonoid intake and esophageal/gastric cancer incidence. The association may vary by: (a) the six flavonoid classes (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones) and lignans, because of different foods that contribute to each flavonoid class; and (b) tumor subtype.

Rationale: The incidence of esophageal adenocarcinoma (EA) and gastric cardia adenocarcinoma (GCA) has increased 300-575% over the last 30-40 years.[3-7] EA/GCA also have a highly prevalent precursor lesion (BE),[144-154] which will aid in identifying optimal times along the EA/GCA cancer continuum to target for intervention with specific chemopreventives to enhance risk reduction. However, esophageal squamous cell carcinoma (ESCC) and non-cardia gastric adenocarcinoma (NCGA) are significant to study because they are the most common forms of esophageal and gastric cancer worldwide.[8,37]

Few epidemiologic studies to date have analyzed the association between esophageal or gastric cancer risk and flavonoid intake. None of these studies found associations between total flavonoid or lignan intake and esophageal or gastric cancer. However, several studies have found associations between specific flavonoid or lignan types, including anthocyanidins,[101] flavanones,[101,102,227] flavones,[102] flavonols (overall[102,227,233] and quercetin[105]), isoflavones,[101] and lignans (secoisolariciresinol),[230] and esophageal or gastric cancer risk. Experimental studies have supported this hypothesis and have shown that flavonoids regulate cell cycle, proliferation, and

apoptosis, which have important chemotherapeutic effects against these tumors.[29] It will be important to examine the associations by anatomic subsite and histologic type of cancer (i.e., esophageal adenocarcinoma, gastric cardia adenocarcinoma, esophageal squamous cell carcinoma, or non-cardia gastric adenocarcinoma), as these cancer types have distinct etiologies.[234,235] Cyclin D1 and p53 are involved in tumor differentiation, proliferation and apoptosis.[84,108,109,111,112] Categorizing cases by these markers, and by stage, will be conducted to examine potentially etiologically distinct subgroups

Specific Aim 2: Determine if flavonoid intake is associated with Barrett's esophagus (BE) development.

Hypothesis: There will be an inverse association between total flavonoid intake and BE. It will also be important to consider: (a) the six classes of flavonoids and lignans; and (b) BE segment length.

Rationale: BE is the only known potential precursor of EA/GCA.[2] Studying precursor lesions, such as BE, can elucidate key risk factors that act early in disease onset. Understanding the mechanisms by which precursor lesions develop into cancer can offer opportunities to intervene and prevent disease progression.[134] This dissertation examines the association between flavonoids and BE and esophageal/gastric cancer risk, which was innovative because it will be the first study to look at flavonoid intake and both precursor and invasive tumors in the continuum of EA/GCA development.

Laboratory studies report reduced risk of BE when using synthetic flavones.[100,236] In these studies, flavonoids regulated cell cycle, proliferation, and apoptosis. BE segment length is a common marker for tumor stage.[237,238] Categorizing cases by this marker may elucidate the underlying association.

Specific Aim 3: Determine if flavonoid intake is associated with survival among cases of

esophageal and gastric cancer.

Hypothesis: There will be an inverse association between total flavonoid intake and survival among cases of esophageal/gastric cancer. It will also be important to consider: (a) the six classes of flavonoids and lignans; and (b) tumor subtype.

Rationale: Esophageal and gastric cancers are two of the most common causes of cancer mortality worldwide,[33,34] and these cancers have very poor survival prognoses (normally less than a year).[1] Phase I and II clinical trials are also using synthetic flavonoids as chemotherapy drugs, including flavone (Flavopiridol)[31] and isoflav-3-ene (Phenoxodial),[32] to produce cell cycle arrest and inhibit tumor growth in patients with metastatic cancer.

No epidemiologic studies have been conducted to determine the association between flavonoid intake and survival in esophageal/gastric cancer cases. However, *in vitro* and *in vivo* studies have shown flavones induce cytotoxicity and reduce dissemination of cancer,[210-217] flavonols induce cytotoxicity and inhibit growth and progression,[210,218-220] and isoflavones induce apoptosis and inhibit proliferation.[221-226]

Demonstration of an association between flavonoids and BE development, esophageal or gastric cancer incidence or survival among esophageal and gastric cancer cases suggests potential to use flavonoids as a risk reduction strategy for these tumors. The current study provides important preliminary data for future intervention studies (i.e., by determining the most effective class of flavonoids for esophageal and gastric cancer risk reduction). This could allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs.

Summary

Esophageal and gastric cancer have very poor survival prognoses (normally less than a year),[1] and the only known potential precursor of esophageal and gastric cardia adenocarcinoma is Barrett's esophagus (BE).[2] Studying precursor lesions, such as BE, provides insight into the etiology of cancer by elucidating key risk factors that act early in disease onset. However, not all precursor lesions develop into cancer. Therefore, understanding the mechanisms by which precursor lesions do result in cancerous tumors can offer an opportunity to intervene and prevent disease progression.[134] Epidemiological studies have shown that diets high in fruit and vegetable intake are inversely associated with esophageal and gastric cancer and BE.[14-28] Flavonoids naturally occur in fruits and vegetables and are thought to partially account for the decreased risk of esophageal/gastric cancer and BE in individuals with higher intakes of fruits and vegetables.[29] Therefore, since an association between fruits and vegetables and esophageal/gastric cancer and BE has been reported, it is plausible to evaluate the association between flavonoids, esophageal and gastric cancer, and BE to determine if flavonoids are associated with risk reduction for these tumors. This dissertation investigated the association between flavonoid intake and the esophageal/gastric cancer continuum. Demonstration of an association between flavonoids and BE development, esophageal or gastric cancer incidence, or survival among esophageal and gastric cancer cases suggests potential to use flavonoids as a risk reduction strategy for these tumors. This could allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs.

REFERENCES

1. Trivers KF, De Roos AJ, Gammon MD, Vaughan TL, Risch HA, Olshan AF, Schoenberg JB, Mayne ST, Dubrow R, Stanford JL, Abrahamson P, Rotterdam H, West AB, Fraumeni JF, Chow WH. Demographic and lifestyle predictors of survival in patients with esophageal or gastric cancers. *Clin Gastroenterol Hepatol* 2005;3(3):225-30.

2. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, Cameron A, Corley D, Falk G, Goldblum J, Hunter J, Jankowski J, Lundell L, Reid B, Shaheen NJ, Sonnenberg A, Wang K, Weinstein W. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. *Gastroenterology* 2004;127(1):310-30.

3. Daly JM, Karnell LH, Menck HR. National Cancer Data Base report on esophageal carcinoma. *Cancer* 1996;78(8):1820-8.

4. Bytzer P, Christensen PB, Damkier P, Vinding K, Seersholm N. Adenocarcinoma of the esophagus and Barrett's esophagus: a population-based study. *Am J Gastroenterol* 1999;94(1):86-91.

5. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-53.

6. Pohl H, Welch HG. The role of overdiagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst* 2005;97(2):142-6.

7. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. *J Natl Cancer Inst* 2008;100(16):1184-7.

8. Corley DA, Buffler PA. Oesophageal and gastric cardia adenocarcinomas: analysis of regional variation using the Cancer Incidence in Five Continents database. *International journal of epidemiology* 2001;30(6):1415-25.

9. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF, Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *Jama* 1991;265(10):1287-9.

10. Schottenfeld D, Fraumeni JF. *Cancer epidemiology and prevention*. 3rd ed. Oxford ; New York: Oxford University Press, 2006.

11. Kamineni A, Williams MA, Schwartz SM, Cook LS, Weiss NS. The incidence of gastric carcinoma in Asian migrants to the United States and their descendants. *Cancer causes & control : CCC* 1999;10(1):77-83.

12. Lee J, Demissie K, Lu SE, Rhoads GG. Cancer incidence among Korean-American immigrants in the United States and native Koreans in South Korea. *Cancer control : journal of the Moffitt Cancer Center* 2007;14(1):78-85.

13. Tzonou A, Lipworth L, Garidou A, Signorello LB, Lagiou P, Hsieh C, Trichopoulos D. Diet and risk of esophageal cancer by histologic type in a low-risk population. *International journal of cancer. Journal international du cancer* 1996;68(3):300-4.

14. Gonzalez CA, Pera G, Agudo A, Bueno-de-Mesquita HB, Ceroti M, Boeing H, Schulz M, Del Giudice G, Plebani M, Carneiro F, Berrino F, Sacerdote C, Tumino R, Panico S, Berglund G, Siman H, Hallmans G, Stenling R, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quiros JR, Allen N, Key TJ, Bingham S, Day NE, Linseisen J, Nagel G, Overvad K, Jensen MK, Olsen A, Tjonneland A, Buchner FL, Peeters PH, Numans ME, Clavel-Chapelon F, Boutron-Ruault MC, Roukos D, Trichopoulou A, Psaltopoulou T, Lund E, Casagrande C, Slimani N, Jenab M, Riboli E. Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). *Int J Cancer* 2006;118(10):2559-66.

15. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer* 2008;123(4):852-60.

16. Chen H, Ward MH, Graubard BI, Heineman EF, Markin RM, Potischman NA, Russell RM, Weisenburger DD, Tucker KL. Dietary patterns and adenocarcinoma of the esophagus and distal stomach. *Am J Clin Nutr* 2002;75(1):137-44.

17. Brown LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, Silverman DT, Pottern LM, Hayes RB, Schwartz AG, et al. Adenocarcinoma of the esophagus: role of obesity and diet. *J Natl Cancer Inst* 1995;87(2):104-9.

18. Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargon N, Karpeh M, Jr., Fein JS, Harlap S. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. *Nutr Cancer* 1997;27(3):298-309.

19. Terry P, Lagergren J, Hansen H, Wolk A, Nyren O. Fruit and vegetable consumption in the prevention of oesophageal and cardia cancers. *Eur J Cancer Prev* 2001;10(4):365-9.

20. Tzonou A, Lipworth L, Garidou A, Signorello LB, Lagiou P, Hsieh C, Trichopoulos D. Diet and risk of esophageal cancer by histologic type in a low-risk population. *Int J Cancer* 1996;68(3):300-4.

21. Anderson LA, Watson RG, Murphy SJ, Johnston BT, Comber H, Mc Guigan J, Reynolds JV, Murray LJ. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J Gastroenterol* 2007;13(10):1585-94.

22. Cheng KK, Sharp L, McKinney PA, Logan RF, Chilvers CE, Cook-Mozaffari P, Ahmed A, Day NE. A case-control study of oesophageal adenocarcinoma in women: a preventable disease. *Br J Cancer* 2000;83(1):127-32.

23. Bahmanyar S, Ye W. Dietary patterns and risk of squamous-cell carcinoma and adenocarcinoma of the esophagus and adenocarcinoma of the gastric cardia: a population-based case-control study in Sweden. *Nutr Cancer* 2006;54(2):171-8.

24. Chen MJ, Wu DC, Lin JM, Wu MT, Sung FC. Etiologic factors of gastric cardiac adenocarcinoma among men in Taiwan. *World J Gastroenterol* 2009;15(43):5472-80.

25. Bastos J, Lunet N, Peleteiro B, Lopes C, Barros H. Dietary patterns and gastric cancer in a Portuguese urban population. *Int J Cancer* 2010;127(2):433-41.

26. Thompson OM, Beresford SA, Kirk EA, Vaughan TL. Vegetable and fruit intakes and risk of Barrett's esophagus in men and women. *Am J Clin Nutr* 2009;89(3):890-6.

27. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am J Gastroenterol* 2008;103(7):1614-23; quiz 1624.

28. Kamangar F, Chow WH, Abnet CC, Dawsey SM. Environmental causes of esophageal cancer. *Gastroenterol Clin North Am* 2009;38(1):27-57, vii.

29. Pierini R, Kroon PA, Guyot S, Ivory K, Johnson IT, Belshaw NJ. Procyanidin effects on oesophageal adenocarcinoma cells strongly depend on flavan-3-ol degree of polymerization. *Mol Nutr Food Res* 2008;52(12):1399-407.

30. Schwartz GK, Ilson D, Saltz L, O'Reilly E, Tong W, Maslak P, Werner J, Perkins P, Stoltz M, Kelsen D. Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2001;19(7):1985-92.

31. Carvajal RD, Tse A, Shah MA, Lefkowitz RA, Gonen M, Gilman-Rosen L, Kortmansky J, Kelsen DP, Schwartz GK, O'Reilly EM. A phase II study of flavopiridol (Alvocidib) in combination with docetaxel in refractory, metastatic pancreatic cancer. *Pancreatology* 2009;9(4):404-9.

32. de Souza PL, Liauw W, Links M, Pirabhahar S, Kelly G, Howes LG. Phase I and pharmacokinetic study of weekly NV06 (Phenoxodiol), a novel isoflav-3-ene, in patients with advanced cancer. *Cancer Chemother Pharmacol* 2006;58(4):427-33.

33. Ferlay J SH, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN Cancer Fact Sheet 2008: Oesophageal Cancer Incidence and Mortality Worldwide in 2008 Summary. http://globocan.iarc.fr/factsheets/cancers/oesophagus.asp Accessed August 31, 2010.

34. Ferlay J SH, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN Cancer Fact Sheet 2008: Stomach Cancer Incidence and Mortality Worldwide in 2008 Summary. http://globocan.iarc.fr/factsheets/cancers/stomach.asp Accessed August 31, 2010.

35. Lingen MW. The gastrointestinal tract. In: Kumar V, Abbas AK, Fausto N, Aster J, eds. *Robbins & Cotran Pathologic Basis of Disease.* 8 ed. Philadelphia, PA: SAUNDERS ELSEVIER., 2010;1450.

36. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001;49(3):347-53.

37. Roder DM. The epidemiology of gastric cancer. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association* 2002;5 Suppl 1:5-11.

38. Altekruse SF KC, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK (eds). *SEER Cancer Statistics Review, 1975-2007*. Bethesda, MD: National Cancer Institute, 2009.

39. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. *CA: a cancer journal for clinicians* 2012.

40. Wijnhoven BP, Siersema PD, Hop WC, van Dekken H, Tilanus HW. Adenocarcinomas of the distal oesophagus and gastric cardia are one clinical entity. Rotterdam Oesophageal Tumour Study Group. *Br J Surg* 1999;86(4):529-35.

41. Spechler SJ. Barrett's esophagus: an overrated cancer risk factor. *Gastroenterology* 2000;119(2):587-9.

42. Shaheen N, Ransohoff DF. Gastroesophageal reflux, barrett esophagus, and esophageal cancer: scientific review. *Jama* 2002;287(15):1972-81.

43. Horner MJ RL, Krapcho M, Neyman N, Aminou R, Howlader N, Altekruse SF, Feuer EJ, Huang L, Mariotto A, Miller BA, Lewis DR, Eisner MP, Stinchcomb DG, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2006, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2006/, based on November 2008 SEER data submission, posted to the SEER web site, 2009. 2009.

44. Clark GW, Smyrk TC, Burdiles P, Hoeft SF, Peters JH, Kiyabu M, Hinder RA, Bremner CG, DeMeester TR. Is Barrett's metaplasia the source of adenocarcinomas of the cardia? *Arch Surg* 1994;129(6):609-14.

45. Steup WH, De Leyn P, Deneffe G, Van Raemdonck D, Coosemans W, Lerut T. Tumors of the esophagogastric junction. Long-term survival in relation to the pattern of lymph node metastasis and a critical analysis of the accuracy or

inaccuracy of pTNM classification. *J Thorac Cardiovasc Surg* 1996;111(1):85-94; discussion 94-5.

46. Ellis FH, Jr., Gibb SP. Esophagogastrectomy for carcinoma: current hospital mortality and morbidity rates. *Ann Surg* 1979;190(6):699-705.

47. Ellis FH, Jr., Gibb SP, Watkins E, Jr. Limited esophagogastrectomy for carcinoma of the cardia. Indications, technique, and results. *Ann Surg* 1988;208(3):354-61.

48. Lerut T, De Leyn P, Coosemans W, Van Raemdonck D, Scheys I, LeSaffre E. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. *Ann Surg* 1992;216(5):583-90.

49. Falk GW. Risk factors for esophageal cancer development. *Surg Oncol Clin N Am* 2009;18(3):469-85.

50. Solaymani-Dodaran M, Logan RF, West J, Card T, Coupland C. Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut* 2004;53(8):1070-4.

51. Pondugula K, Wani S, Sharma P. Barrett's esophagus and esophageal adenocarcinoma in adults: long-term GERD or something else? *Curr Gastroenterol Rep* 2007;9(6):468-74.

52. Chang JT, Katzka DA. Gastroesophageal reflux disease, Barrett esophagus, and esophageal adenocarcinoma. *Arch Intern Med* 2004;164(14):1482-8.

53. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 1999;340(11):825-31.

54. Chow WH, Finkle WD, McLaughlin JK, Frankl H, Ziel HK, Fraumeni JF, Jr. The relation of gastroesophageal reflux disease and its treatment to adenocarcinomas of the esophagus and gastric cardia. *Jama* 1995;274(6):474-7.

55. van Blankenstein M, Looman CW, Hop WC, Bytzer P. The incidence of adenocarcinoma and squamous cell carcinoma of the esophagus: Barrett's esophagus makes a difference. *Am J Gastroenterol* 2005;100(4):766-74.

56. El-Serag HB, Mason AC, Petersen N, Key CR. Epidemiological differences between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia in the USA. *Gut* 2002;50(3):368-72.

57. Rogers EL, Goldkind SF, Iseri OA, Bustin M, Goldkind L, Hamilton SR, Smith RL. Adenocarcinoma of the lower esophagus. A disease primarily of white men with Barrett's esophagus. *J Clin Gastroenterol* 1986;8(6):613-8.

58. Chalasani N, Wo JM, Waring JP. Racial differences in the histology, location, and risk factors of esophageal cancer. *J Clin Gastroenterol* 1998;26(1):11-3.

59. Younes M, Henson DE, Ertan A, Miller CC. Incidence and survival trends of esophageal carcinoma in the United States: racial and gender differences by histological type. *Scand J Gastroenterol* 2002;37(12):1359-65.

60. Crabb DW, Berk MA, Hall TR, Conneally PM, Biegel AA, Lehman GA. Familial gastroesophageal reflux and development of Barrett's esophagus. *Ann Intern Med* 1985;103(1):52-4.

61. Jochem VJ, Fuerst PA, Fromkes JJ. Familial Barrett's esophagus associated with adenocarcinoma. *Gastroenterology* 1992;102(4 Pt 1):1400-2.

62. Fahmy N, King JF. Barrett's esophagus: an acquired condition with genetic predisposition. *Am J Gastroenterol* 1993;88(8):1262-5.

63. Eng C, Spechler SJ, Ruben R, Li FP. Familial Barrett esophagus and adenocarcinoma of the gastroesophageal junction. *Cancer Epidemiol Biomarkers Prev* 1993;2(4):397-9.

64. Poynton AR, Walsh TN, O'Sullivan G, Hennessy TP. Carcinoma arising in familial Barrett's esophagus. *Am J Gastroenterol* 1996;91(9):1855-6.

65. Chak A, Lee T, Kinnard MF, Brock W, Faulx A, Willis J, Cooper GS, Sivak MV, Jr., Goddard KA. Familial aggregation of Barrett's oesophagus, oesophageal adenocarcinoma, and oesophagogastric junctional adenocarcinoma in Caucasian adults. *Gut* 2002;51(3):323-8.

66. Hopper JL, Carlin JB. Familial aggregation of a disease consequent upon correlation between relatives in a risk factor measured on a continuous scale. *American journal of epidemiology* 1992;136(9):1138-47.

67. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ, Fraumeni JF, Jr. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1997;89(17):1277-84.

68. Jansson C, Johansson AL, Nyren O, Lagergren J. Socioeconomic factors and risk of esophageal adenocarcinoma: a nationwide Swedish case-control study. *Cancer Epidemiol Biomarkers Prev* 2005;14(7):1754-61.

69. Abnet CC, Freedman ND, Hollenbeck AR, Fraumeni JF, Jr., Leitzmann M, Schatzkin A. A prospective study of BMI and risk of oesophageal and gastric adenocarcinoma. *Eur J Cancer* 2008;44(3):465-71.

70. Merry AH, Schouten LJ, Goldbohm RA, van den Brandt PA. Body mass index, height and risk of adenocarcinoma of the oesophagus and gastric cardia: a prospective cohort study. *Gut* 2007;56(11):1503-11.

71. Engeland A, Tretli S, Bjorge T. Height and body mass index in relation to esophageal cancer; 23-year follow-up of two million Norwegian men and women. *Cancer Causes Control* 2004;15(8):837-43.

72. Veugelers PJ, Porter GA, Guernsey DL, Casson AG. Obesity and lifestyle risk factors for gastroesophageal reflux disease, Barrett esophagus and esophageal adenocarcinoma. *Dis Esophagus* 2006;19(5):321-8.

73. Lagergren J, Bergstrom R, Nyren O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 1999;130(11):883-90.

74. Chow WH, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S, Fraumeni JF, Jr. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1998;90(2):150-5.

75. de Jonge PJ, Steyerberg EW, Kuipers EJ, Honkoop P, Wolters LM, Kerkhof M, van Dekken H, Siersema PD. Risk factors for the development of esophageal adenocarcinoma in Barrett's esophagus. *Am J Gastroenterol* 2006;101(7):1421-9.

76. Lindblad M, Rodriguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes Control* 2005;16(3):285-94.

77. Wu AH, Wan P, Bernstein L. A multiethnic population-based study of smoking, alcohol and body size and risk of adenocarcinomas of the stomach and esophagus (United States). *Cancer Causes Control* 2001;12(8):721-32.

78. Vaughan TL, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 1995;4(2):85-92.

79. Brown LM, Silverman DT, Pottern LM, Schoenberg JB, Greenberg RS, Swanson GM, Liff JM, Schwartz AG, Hayes RB, Blot WJ, et al. Adenocarcinoma of the esophagus and esophagogastric junction in white men in the United States: alcohol, tobacco, and socioeconomic factors. *Cancer Causes Control* 1994;5(4):333-40.

80. Kabat GC, Ng SK, Wynder EL. Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. *Cancer Causes Control* 1993;4(2):123-32.

81. Cook MB, Kamangar F, Whiteman DC, Freedman ND, Gammon MD, Bernstein L, Brown LM, Risch HA, Ye W, Sharp L, Pandeya N, Webb PM, Wu AH, Ward MH, Giffen C, Casson AG, Abnet CC, Murray LJ, Corley DA, Nyren O, Vaughan TL, Chow WH. Cigarette smoking and adenocarcinomas of the esophagus and esophagogastric junction: a pooled analysis from the international BEACON consortium. *J Natl Cancer Inst* 2010;102(17):1344-53.

82. Kubo A, Corley DA. Body mass index and adenocarcinomas of the esophagus or gastric cardia: a systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006;15(5):872-8.

83. Galipeau PC, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007;4(2):e67.

84. Figueroa JD, Terry MB, Gammon MD, Vaughan TL, Risch HA, Zhang FF, Kleiner DE, Bennett WP, Howe CL, Dubrow R, Mayne ST, Fraumeni JF, Jr., Chow WH. Cigarette smoking, body mass index, gastro-esophageal reflux disease, and non-steroidal anti-inflammatory drug use and risk of subtypes of esophageal and gastric cancers by P53 overexpression. *Cancer Causes Control* 2009;20(3):361-8.

85. Vaughan TL, Dong LM, Blount PL, Ayub K, Odze RD, Sanchez CA, Rabinovitch PS, Reid BJ. Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. *Lancet Oncol* 2005;6(12):945-52.

86. Abnet CC, Freedman ND, Kamangar F, Leitzmann MF, Hollenbeck AR, Schatzkin A. Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. *Br J Cancer* 2009;100(3):551-7.

87. Farrow DC, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF, Jr., Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1998;7(2):97-102.

88. Bollschweiler E, Wolfgarten E, Nowroth T, Rosendahl U, Monig SP, Holscher AH. Vitamin intake and risk of subtypes of esophageal cancer in Germany. *J Cancer Res Clin Oncol* 2002;128(10):575-80.

89. Dong LM, Kristal AR, Peters U, Schenk JM, Sanchez CA, Rabinovitch PS, Blount PL, Odze RD, Ayub K, Reid BJ, Vaughan TL. Dietary supplement use and risk of neoplastic progression in esophageal adenocarcinoma: a prospective study. *Nutr Cancer* 2008;60(1):39-48.

90. Terry P, Lagergren J, Ye W, Nyren O, Wolk A. Antioxidants and cancers of the esophagus and gastric cardia. *Int J Cancer* 2000;87(5):750-4.

91. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10(10):1055-62.

92. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer* 2010;62(1):1-20.

93. Fresco P, Borges F, Diniz C, Marques MP. New insights on the anticancer properties of dietary polyphenols. *Med Res Rev* 2006;26(6):747-66.

94. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. *Med Res Rev* 2003;23(4):519-34.

95. Song S, Krishnan K, Liu K, Bresalier RS. Polyphenon E inhibits the growth of human Barrett's and aerodigestive adenocarcinoma cells by suppressing cyclin D1 expression. *Clin Cancer Res* 2009;15(2):622-31.

96. Hamilton-Miller JM. Antimicrobial properties of tea (Camellia sinensis L.). *Antimicrobial agents and chemotherapy* 1995;39(11):2375-7.

97. Kresty LA, Howell AB, Baird M. Cranberry proanthocyanidins induce apoptosis and inhibit acid-induced proliferation of human esophageal adenocarcinoma cells. *J Agric Food Chem* 2008;56(3):676-80.

98. Ye X, Krohn RL, Liu W, Joshi SS, Kuszynski CA, McGinn TR, Bagchi M, Preuss HG, Stohs SJ, Bagchi D. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultured human cancer cells. *Mol Cell Biochem* 1999;196(1-2):99-108.

99. Zhang Q, Zhao XH, Wang ZJ. Flavones and flavonols exert cytotoxic effects on a human oesophageal adenocarcinoma cell line (OE33) by causing G2/M arrest and inducing apoptosis. *Food Chem Toxicol* 2008;46(6):2042-53.

100. Lechpammer M, Xu X, Ellis FH, Bhattacharaya N, Shapiro GI, Loda M. Flavopiridol reduces malignant transformation of the esophageal mucosa in p27 knockout mice. *Oncogene* 2005;24(10):1683-8.

101. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int J Cancer* 2009;125(5):1147-54.

102. Lagiou P, Samoli E, Lagiou A, Peterson J, Tzonou A, Dwyer J, Trichopoulos D. Flavonoids, vitamin C and adenocarcinoma of the stomach. *Cancer causes & control : CCC* 2004;15(1):67-72.

103. Zamora-Ros R, Agudo A, Lujan-Barroso L, Romieu I, Ferrari P, Knaze V, Bueno-de-Mesquita HB, Leenders M, Travis RC, Navarro C, Sanchez-Cantalejo E, Slimani N, Scalbert A, Fedirko V, Hjartaker A, Engeset D, Skeie G, Boeing H, Forster J, Li K, Teucher B, Agnoli C, Tumino R, Mattiello A, Saieva C, Johansson I, Stenling R, Redondo ML, Wallstrom P, Ericson U, Khaw KT, Mulligan AA, Trichopoulou A, Dilis V, Katsoulis M, Peeters PH, Igali L, Tjonneland A, Halkjaer J, Touillaud M, Perquier F, Fagherazzi G, Amiano P, Ardanaz E, Bredsdorff L, Overvad K, Ricceri F, Riboli E, Gonzalez CA. Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into

Cancer and Nutrition (EPIC) study. *The American journal of clinical nutrition* 2012;96(6):1398-408.

104. Vermeulen E, Zamora-Ros R, Duell EJ, Lujan-Barroso L, Boeing H, Aleksandrova K, Bueno-de-Mesquita HB, Scalbert A, Romieu I, Fedirko V, Touillaud M, Fagherazzi G, Perquier F, Molina-Montes E, Chirlaque MD, Vicente Arguelles M, Amiano P, Barricarte A, Pala V, Mattiello A, Saieva C, Tumino R, Ricceri F, Trichopoulou A, Vasilopoulou E, Ziara G, Crowe FL, Khaw KT, Wareham NJ, Lukanova A, Grote VA, Tjonneland A, Halkjaer J, Bredsdorff L, Overvad K, Siersema PD, Peeters PH, May AM, Weiderpass E, Skeie G, Hjartaker A, Landberg R, Johansson I, Sonestedt E, Ericson U, Riboli E, Gonzalez CA. Dietary flavonoid intake and esophageal cancer risk in the European prospective investigation into cancer and nutrition cohort. *American journal of epidemiology* 2013;178(4):570-81.

105. Ekstrom AM, Serafini M, Nyren O, Wolk A, Bosetti C, Bellocco R. Dietary quercetin intake and risk of gastric cancer: results from a population-based study in Sweden. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2011;22(2):438-43.

106. Lin Y, Wolk A, Hakansson N, Lagergren J, Lu Y. Dietary intake of lignans and risk of esophageal and gastric adenocarcinoma: a cohort study in Sweden. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2013;22(2):308-12.

107. Lin Y, Yngve A, Lagergren J, Lu Y. Dietary intake of lignans and risk of adenocarcinoma of the esophagus and gastroesophageal junction. *Cancer causes & control : CCC* 2012;23(6):837-44.

108. Gammon MD, Terry MB, Arber N, Chow WH, Risch HA, Vaughan TL, Schoenberg JB, Mayne ST, Stanford JL, Dubrow R, Rotterdam H, West AB, Fraumeni JF, Jr., Weinstein IB, Hibshoosh H. Nonsteroidal anti-inflammatory drug use associated with reduced incidence of adenocarcinomas of the esophagus and gastric cardia that overexpress cyclin D1: a population-based study. *Cancer Epidemiol Biomarkers Prev* 2004;13(1):34-9.

109. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991;351(6326):453-6.

110. Zhao Y, Wang R, Fan D. Review Article: The molecular mechanisms of esophageal cancer. *EXCLI Journal* 2006;5:79-92.

111. Bennett WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC. Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in human lung cancer. *J Pathol* 1999;187(1):8-18.

112. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat* 2002;19(6):607-14.

113. Lin J, Beerm DG. Molecular biology of upper gastrointestinal malignancies. *Seminars in oncology* 2004;31(4):476-86.

114. Arber N, Lightdale C, Rotterdam H, Han KH, Sgambato A, Yap E, Ahsan H, Finegold J, Stevens PD, Green PH, Hibshoosh H, Neugut AI, Holt PR, Weinstein IB. Increased expression of the cyclin D1 gene in Barrett's esophagus. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1996;5(6):457-9.

115. Pera M, Fernandez PL, Palacin A, Cardesa A, Dasenbrock C, Tillman T, Mohr U. Expression of cyclin D1 and p53 and its correlation with proliferative activity in the spectrum of esophageal carcinomas induced after duodenal content reflux and 2,6-dimethylnitrosomorpholine administration in rats. *Carcinogenesis* 2001;22(2):271-7.

116. Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J Nutr Biochem* 2007;18(7):427-42.

117. Manson MM. Cancer prevention -- the potential for diet to modulate molecular signalling. *Trends Mol Med* 2003;9(1):11-8.

118. D'Andrea GM. Use of antioxidants during chemotherapy and radiotherapy should be avoided. *CA: a cancer journal for clinicians* 2005;55(5):319-21.

119. Labriola D, Livingston R. Possible interactions between dietary antioxidants and chemotherapy. *Oncology* 1999;13(7):1003-8; discussion 1008, 1011-2.

120. Greenlee H, Hershman DL, Jacobson JS. Use of antioxidant supplements during breast cancer treatment: a comprehensive review. *Breast cancer research and treatment* 2009;115(3):437-52.

121. Lesperance ML, Olivotto IA, Forde N, Zhao Y, Speers C, Foster H, Tsao M, MacPherson N, Hoffer A. Mega-dose vitamins and minerals in the treatment of non-metastatic breast cancer: an historical cohort study. *Breast cancer research and treatment* 2002;76(2):137-43.

122. Omenn GS. Chemoprevention of lung cancers: lessons from CARET, the beta-carotene and retinol efficacy trial, and prospects for the future. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation* 2007;16(3):184-91.

123. Lagarde SM, ten Kate FJ, Reitsma JB, Busch OR, van Lanschot JJ. Prognostic factors in adenocarcinoma of the esophagus or gastroesophageal junction. *J Clin Oncol* 2006;24(26):4347-55.

124. Stahl M, Wilke H, Stuschke M, Walz MK, Fink U, Molls M, Siewert JR, Schroeder M, Makoski HB, Schmidt U, Seeber S, Vanhoefer U. Clinical response to induction chemotherapy predicts local control and long-term survival in multimodal treatment of patients with locally advanced esophageal cancer. *J Cancer Res Clin Oncol* 2005;131(1):67-72.

125. Hoff SJ, Sawyers JL, Blanke CD, Choy H, Stewart JR. Prognosis of adenocarcinoma arising in Barrett's esophagus. *Ann Thorac Surg* 1998;65(1):176-80; discussion 180-1.

126. Theisen J, Stein HJ, Dittler HJ, Feith M, Moebius C, Kauer WK, Werner M, Siewert JR. Preoperative chemotherapy unmasks underlying Barrett's mucosa in patients with adenocarcinoma of the distal esophagus. *Surg Endosc* 2002;16(4):671-3.

127. Bremner CG, Lynch VP, Ellis FH, Jr. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. *Surgery* 1970;68(1):209-16.

128. Clouston AD. Timely topic: Premalignant lesions associated with adenocarcinoma of the upper gastrointestinal tract. *Pathology* 2001;33(3):271-7.

129. Vaira D, Gatta L, Ricci C, Castelli V, Fiorini G, Kajo E, Lanzini A. Gastroesophageal reflux disease and Barrett's esophagus. *Intern Emerg Med* 2010.

130. Riddell RH. The genesis of Barrett esophagus: has a histologic transition from gastroesophageal reflux disease-damaged epithelium to columnar metaplasia ever been seen in humans? *Arch Pathol Lab Med* 2005;129(2):164-9.

131. Ruol A, Parenti A, Zaninotto G, Merigliano S, Costantini M, Cagol M, Alfieri R, Bonavina L, Peracchia A, Ancona E. Intestinal metaplasia is the probable common precursor of adenocarcinoma in barrett esophagus and adenocarcinoma of the gastric cardia. *Cancer* 2000;88(11):2520-8.

132. Cameron AJ, Lomboy CT, Pera M, Carpenter HA. Adenocarcinoma of the esophagogastric junction and Barrett's esophagus. *Gastroenterology* 1995;109(5):1541-6.

133. van Sandick JW, van Lanschot JJ, ten Kate FJ, Offerhaus GJ, Fockens P, Tytgat GN, Obertop H. Pathology of early invasive adenocarcinoma of the esophagus or esophagogastric junction: implications for therapeutic decision making. *Cancer* 2000;88(11):2429-37.

134. Morrison AS. Sequential pathogenic components of rates. *Am J Epidemiol* 1979;109(6):709-18.

135. Farber E. Cancer development and its natural history. A cancer prevention perspective. *Cancer* 1988;62(8 Suppl):1676-9.

136. Skelly L. Emory Surgeons First in Georgia to Treat Barrett's Esophagus with Ablation System http://whsc.emory.edu/press_releases2.cfm?announcement_id_seq=3959 Accessed 26 September 2011.

137. Caygill CP, Reed PI, Johnston BJ, Hill MJ, Ali MH, Levi S. A single centre's 20 years' experience of columnar-lined (Barrett's) oesophagus diagnosis. *Eur J Gastroenterol Hepatol* 1999;11(12):1355-8.

138. Todd JA, Johnston DA, Dillon JF. The changing spectrum of gastroesophageal reflux disease. *Eur J Cancer Prev* 2002;11(3):215-9.

139. Prach AT, MacDonald TA, Hopwood DA, Johnston DA. Increasing incidence of Barrett's oesophagus: education, enthusiasm, or epidemiology? *Lancet* 1997;350(9082):933.

140. van Soest EM, Dieleman JP, Siersema PD, Sturkenboom MC, Kuipers EJ. Increasing incidence of Barrett's oesophagus in the general population. *Gut* 2005;54(8):1062-6.

141. Corley DA, Kubo A, Levin TR, Block G, Habel L, Rumore G, Quesenberry C, Buffler P. Race, ethnicity, sex and temporal differences in Barrett's oesophagus diagnosis: a large community-based study, 1994-2006. *Gut* 2009;58(2):182-8.

142. Naef AP, Savary M. Conservative operations for peptic esophagitis with stenosis in columnar-lined lower esophagus. *Ann Thorac Surg* 1972;13(6):543-51.

143. Sharma P, Sampliner RE. *Barrett's esophagus and esophageal adenocarcinoma*. 2nd ed. Malden, Mass.: Blackwell Pub., 2006.

144. Ronkainen J, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Talley NJ, Agreus L. Prevalence of Barrett's esophagus in the general population: an endoscopic study. *Gastroenterology* 2005;129(6):1825-31.

145. Rex DK, Cummings OW, Shaw M, Cumings MD, Wong RK, Vasudeva RS, Dunne D, Rahmani EY, Helper DJ. Screening for Barrett's esophagus in colonoscopy patients with and without heartburn. *Gastroenterology* 2003;125(6):1670-7.

146. Cameron AJ, Lomboy CT. Barrett's esophagus: age, prevalence, and extent of columnar epithelium. *Gastroenterology* 1992;103(4):1241-5.

147. Clark GW, Ireland AP, Peters JH, Chandrasoma P, DeMeester TR, Bremner CG. Short-segment Barrett's esophagus: A prevalent complication of gastroesophageal reflux disease with malignant potential. *J Gastrointest Surg* 1997;1(2):113-22.

148. O'Connor JB, Falk GW, Richter JE. The incidence of adenocarcinoma and dysplasia in Barrett's esophagus: report on the Cleveland Clinic Barrett's Esophagus Registry. *Am J Gastroenterol* 1999;94(8):2037-42.

149. Corley DA, Levin TR, Habel LA, Weiss NS, Buffler PA. Surveillance and survival in Barrett's adenocarcinomas: a population-based study. *Gastroenterology* 2002;122(3):633-40.

150. Corey KE, Schmitz SM, Shaheen NJ. Does a surgical antireflux procedure decrease the incidence of esophageal adenocarcinoma in Barrett's esophagus? A meta-analysis. *Am J Gastroenterol* 2003;98(11):2390-4.

151. Westhoff B, Brotze S, Weston A, McElhinney C, Cherian R, Mayo MS, Smith HJ, Sharma P. The frequency of Barrett's esophagus in high-risk patients with chronic GERD. *Gastrointest Endosc* 2005;61(2):226-31.

152. Gerson LB, Shetler K, Triadafilopoulos G. Prevalence of Barrett's esophagus in asymptomatic individuals. *Gastroenterology* 2002;123(2):461-7.

153. Pera M. Trends in incidence and prevalence of specialized intestinal metaplasia, barrett's esophagus, and adenocarcinoma of the gastroesophageal junction. *World J Surg* 2003;27(9):999-1008; discussion 1006-8.

154. Zagari RM, Fuccio L, Wallander MA, Johansson S, Fiocca R, Casanova S, Farahmand BY, Winchester CC, Roda E, Bazzoli F. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. *Gut* 2008;57(10):1354-9.

155. Sampliner RE. A population prevalence of Barrett's esophagus--finally. *Gastroenterology* 2005;129(6):2101-3.

156. Hayeck TJ, Kong CY, Spechler SJ, Gazelle GS, Hur C. The prevalence of Barrett's esophagus in the US: estimates from a simulation model confirmed by SEER data. *Dis Esophagus* 2010;23(6):451-7.

157. U.S. Summary: 2000. U.S. Census Bureau, 2002.

158. Buttar NS, Wang KK, Leontovich O, Westcott JY, Pacifico RJ, Anderson MA, Krishnadath KK, Lutzke LS, Burgart LJ. Chemoprevention of esophageal adenocarcinoma by COX-2 inhibitors in an animal model of Barrett's esophagus. *Gastroenterology* 2002;122(4):1101-12.

159. Li H, Walsh TN, O'Dowd G, Gillen P, Byrne PJ, Hennessy TP. Mechanisms of columnar metaplasia and squamous regeneration in experimental Barrett's esophagus. *Surgery* 1994;115(2):176-81.

160. Lieberman DA, Oehlke M, Helfand M. Risk factors for Barrett's esophagus in community-based practice. GORGE consortium. Gastroenterology Outcomes Research Group in Endoscopy. *Am J Gastroenterol* 1997;92(8):1293-7.

161. Avidan B, Sonnenberg A, Schnell TG, Sontag SJ. Hiatal hernia and acid reflux frequency predict presence and length of Barrett's esophagus. *Digestive diseases and sciences* 2002;47(2):256-64.

162. Cameron AJ. Barrett's esophagus: prevalence and size of hiatal hernia. *The American journal of gastroenterology* 1999;94(8):2054-9.

163. Eloubeidi MA, Provenzale D. Clinical and demographic predictors of Barrett's esophagus among patients with gastroesophageal reflux disease: a multivariable analysis in veterans. *J Clin Gastroenterol* 2001;33(4):306-9.

164. Sampliner R, Eisen G, De Garmo P. Ethnicity and gender of patients with suspected Barrett's esophagus in a large endoscopy cohort (CORI) [abstract]. *Gastrointest Endosc* 2001;53(AB151).

165. Wani S, Rastogi A, Bansal A. Symptom profiles and endoscopic findings in African-American patients with gastroesophageal reflux disease (GERD). *Gastroenterology* 2007;132(Suppl 2):A161.

166. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Alcohol types and sociodemographic characteristics as risk factors for Barrett's esophagus. *Gastroenterology* 2009;136(3):806-15.

167. Stein DJ, El-Serag HB, Kuczynski J, Kramer JR, Sampliner RE. The association of body mass index with Barrett's oesophagus. *Aliment Pharmacol Ther* 2005;22(10):1005-10.

168. Smith KJ, O'Brien SM, Smithers BM, Gotley DC, Webb PM, Green AC, Whiteman DC. Interactions among smoking, obesity, and symptoms of acid reflux in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2005;14(11 Pt 1):2481-6.

169. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007;133(2):403-11.

170. Shaheen NJ, Richter JE. Barrett's oesophagus. *Lancet* 2009;373(9666):850-61.

171. Anderson LA, Watson RG, Murphy SJ, Johnston BT, Comber H, Mc Guigan J, Reynolds JV, Murray LJ. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World journal of gastroenterology : WJG* 2007;13(10):1585-94.

172. Anderson LA, Johnston BT, Watson RG, Murphy SJ, Ferguson HR, Comber H, McGuigan J, Reynolds JV, Murray LJ. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. *Cancer Res* 2006;66(9):4975-82.

173. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Dietary patterns and the risk of Barrett's esophagus. *Am J Epidemiol* 2008;167(7):839-46.

174. Jiao L, Kramer JR, Rugge M, Parente P, Verstovsek G, Alsarraj A, El-Serag HB. Dietary intake of vegetables, folate, and antioxidants and the risk of Barrett's esophagus. *Cancer causes & control : CCC* 2013;24(5):1005-14.

175. Fink BN, Steck SE, Wolff MS, Kabat GC, Gammon MD. Construction of a flavonoid database for assessing intake in a population-based sample of women on Long Island, New York. *Nutr Cancer* 2006;56(1):57-66.

176. Cameron AJ. Barrett's esophagus: does the incidence of adenocarcinoma matter? *Am J Gastroenterol* 1997;92(2):193-4.

177. Reid BJ, Li X, Galipeau PC, Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. *Nat Rev Cancer* 2010;10(2):87-101.

178. Hirota WK, Loughney TM, Lazas DJ, Maydonovitch CL, Rholl V, Wong RK. Specialized intestinal metaplasia, dysplasia, and cancer of the esophagus and esophagogastric junction: prevalence and clinical data. *Gastroenterology* 1999;116(2):277-85.

179. Shimizu M, Ban S, Odze RD. Squamous dysplasia and other precursor lesions related to esophageal squamous cell carcinoma. *Gastroenterol Clin North Am* 2007;36(4):797-811, v-vi.

180. Shimizu M, Ban S, Odze RD. Squamous dysplasia and other precursor lesions related to esophageal squamous cell carcinoma. *Gastroenterology clinics of North America* 2007;36(4):797-811, v-vi.

181. Wang GQ, Abnet CC, Shen Q, Lewin KJ, Sun XD, Roth MJ, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Histological precursors of oesophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. *Gut* 2005;54(2):187-92.

182. Syrjanen KJ. HPV infections and oesophageal cancer. *Journal of clinical pathology* 2002;55(10):721-8.

183. Petrick JL, Wyss AB, Butler AM, Cummings C, Sun X, Poole C, Smith JS, Olshan AF. Prevalence of human papillomavirus among oesophageal squamous cell carcinoma cases: systematic review and meta-analysis. *British journal of cancer* 2014.

184. Freedman ND, Abnet CC, Leitzmann MF, Mouw T, Subar AF, Hollenbeck AR, Schatzkin A. A prospective study of tobacco, alcohol, and the risk of esophageal and gastric cancer subtypes. *Am J Epidemiol* 2007;165(12):1424-33.

185. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology* 2003;124(1):47-56.

186. Watson W. Cancer of the esophagus: some etiological considerations. *Am J Roentgenol* 1939;41(3):420-424.

187. Layke JC, Lopez PP. Esophageal cancer: a review and update. *Am Fam Physician* 2006;73(12):2187-94.

188. Cook MB, Chow WH, Devesa SS. Oesophageal cancer incidence in the United States by race, sex, and histologic type, 1977-2005. *Br J Cancer* 2009;101(5):855-9.

189. Terry MB, Gaudet MM, Gammon MD. The epidemiology of gastric cancer. *Semin Radiat Oncol* 2002;12(2):111-27.

190. Hansen S, Vollset SE, Derakhshan MH, Fyfe V, Melby KK, Aase S, Jellum E, McColl KE. Two distinct aetiologies of cardia cancer; evidence from premorbid serological markers of gastric atrophy and Helicobacter pylori status. *Gut* 2007;56(7):918-25.

191. Farrow DC, Vaughan TL, Sweeney C, Gammon MD, Chow WH, Risch HA, Stanford JL, Hansten PD, Mayne ST, Schoenberg JB, Rotterdam H, Ahsan H, West AB, Dubrow R, Fraumeni JF, Jr., Blot WJ. Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. *Cancer Causes Control* 2000;11(3):231-8.

192. Anderson WF, Camargo MC, Fraumeni JF, Jr., Correa P, Rosenberg PS, Rabkin CS. Age-specific trends in incidence of noncardia gastric cancer in US adults. *Jama* 2010;303(17):1723-8.

193. Lau M, Le A, El-Serag HB. Noncardia gastric adenocarcinoma remains an important and deadly cancer in the United States: secular trends in incidence and survival. *Am J Gastroenterol* 2006;101(11):2485-92.

194. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Alcohol consumption, cigarette smoking and risk of subtypes of oesophageal and gastric cancer: a prospective cohort study. *Gut* 2010;59(1):39-48.

195. Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* 2003;56(1):1-9.

196. Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. *Nutr Cancer* 2006;54(1):33-46.

197. Stoner GD, Wang LS, Seguin C, Rocha C, Stoner K, Chiu S, Kinghorn AD. Multiple berry types prevent N-nitrosomethylbenzylamine-induced esophageal cancer in rats. *Pharm Res* 2010;27(6):1138-45.

198. Zikri NN, Riedl KM, Wang LS, Lechner J, Schwartz SJ, Stoner GD. Black raspberry components inhibit proliferation, induce apoptosis, and modulate gene expression in rat esophageal epithelial cells. *Nutr Cancer* 2009;61(6):816-26.

199. Wang LS, Hecht SS, Carmella SG, Yu N, Larue B, Henry C, McIntyre C, Rocha C, Lechner JF, Stoner GD. Anthocyanins in black raspberries prevent esophageal tumors in rats. *Cancer Prev Res (Phila)* 2009;2(1):84-93.

200. Boivin D, Blanchette M, Barrette S, Moghrabi A, Beliveau R. Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFkappaB by edible berry juice. *Anticancer Res* 2007;27(2):937-48.

201. Shih PH, Yeh CT, Yen GC. Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells. *Food Chem Toxicol* 2005;43(10):1557-66.

202. Kinjo J, Nagao T, Tanaka T, Nonaka G, Okawa M, Nohara T, Okabe H. Activity-guided fractionation of green tea extract with antiproliferative activity against human stomach cancer cells. *Biol Pharm Bull* 2002;25(9):1238-40.

203. Tanaka T, Makita H, Kawabata K, Mori H, Kakumoto M, Satoh K, Hara A, Sumida T, Fukutani K, Ogawa H. Modulation of N-methyl-N-amylnitrosamine-induced rat oesophageal tumourigenesis by dietary feeding of diosmin and hesperidin, both alone and in combination. *Carcinogenesis* 1997;18(4):761-9.

204. Park CH, Hahm ER, Lee JH, Jung KC, Yang CH. Inhibition of beta-catenin-mediated transactivation by flavanone in AGS gastric cancer cells. *Biochem Biophys Res Commun* 2005;331(4):1222-8.

205. Lee JH, Park CH, Jung KC, Rhee HS, Yang CH. Negative regulation of beta-catenin/Tcf signaling by naringenin in AGS gastric cancer cell. *Biochem Biophys Res Commun* 2005;335(3):771-6.

206. Ganapathy E, Peramaiyan R, Rajasekaran D, Venkataraman M, Dhanapal S. Modulatory effect of naringenin on N-methyl-N'-nitro-N-nitrosoguanidine- and saturated sodium chloride-induced gastric carcinogenesis in male Wistar rats. *Clin Exp Pharmacol Physiol* 2008;35(10):1190-6.

207. Ekambaram G, Rajendran P, Devaraja R, Muthuvel R, Sakthisekaran D. Impact of naringenin on glycoprotein levels in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in rats. *Anticancer Drugs* 2008;19(9):885-90.

208. Ekambaram G, Rajendran P, Magesh V, Sakthisekaran D. Naringenin reduces tumor size and weight lost in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis in rats. *Nutr Res* 2008;28(2):106-12.

209. Wen X, Walle T. Cytochrome P450 1B1, a novel chemopreventive target for benzo[a]pyrene-initiated human esophageal cancer. *Cancer Lett* 2007;246(1-2):109-14.

210. Zhang Q, Zhao XH, Wang ZJ. Cytotoxicity of flavones and flavonols to a human esophageal squamous cell carcinoma cell line (KYSE-510) by induction of G2/M arrest and apoptosis. *Toxicol In Vitro* 2009;23(5):797-807.

211. Cherng JM, Shieh DE, Chiang W, Chang MY, Chiang LC. Chemopreventive effects of minor dietary constituents in common foods on human cancer cells. *Biosci Biotechnol Biochem* 2007;71(6):1500-4.

212. Minagawa A, Otani Y, Kubota T, Wada N, Furukawa T, Kumai K, Kameyama K, Okada Y, Fujii M, Yano M, Sato T, Ito A, Kitajima M. The citrus flavonoid, nobiletin, inhibits peritoneal dissemination of human gastric carcinoma in SCID mice. *Jpn J Cancer Res* 2001;92(12):1322-8.

213. Yoshimizu N, Otani Y, Saikawa Y, Kubota T, Yoshida M, Furukawa T, Kumai K, Kameyama K, Fujii M, Yano M, Sato T, Ito A, Kitajima M. Anti-tumour effects of nobiletin, a citrus flavonoid, on gastric cancer include: antiproliferative effects, induction of apoptosis and cell cycle deregulation. *Aliment Pharmacol Ther* 2004;20 Suppl 1:95-101.

214. Pan MH, Lai CS, Hsu PC, Wang YJ. Acacetin induces apoptosis in human gastric carcinoma cells accompanied by activation of caspase cascades and production of reactive oxygen species. *J Agric Food Chem* 2005;53(3):620-30.

215. Wu K, Yuan LH, Xia W. Inhibitory effects of apigenin on the growth of gastric carcinoma SGC-7901 cells. *World J Gastroenterol* 2005;11(29):4461-4.

216. Wu B, Zhang Q, Shen W, Zhu J. Anti-proliferative and chemosensitizing effects of luteolin on human gastric cancer AGS cell line. *Mol Cell Biochem* 2008;313(1-2):125-32.

217. Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, Nishino H, Aoike A. Genistein arrests cell cycle progression at G2-M. *Cancer Res* 1993;53(6):1328-31.

218. Ma G, Yang C, Qu Y, Wei H, Zhang T, Zhang N. The flavonoid component isorhamnetin in vitro inhibits proliferation and induces apoptosis in Eca-109 cells. *Chem Biol Interact* 2007;167(2):153-60.

219. Yoshida M, Sakai T, Hosokawa N, Marui N, Matsumoto K, Fujioka A, Nishino H, Aoike A. The effect of quercetin on cell cycle progression and growth of human gastric cancer cells. *FEBS Lett* 1990;260(1):10-3.

220. Wang HY, Guo LM, Chen Y, Zhao XH, Cheng CL, Wu MY, He LY. [Quercetin inhibits growth and induces apoptosis of human gastric carcinoma cells]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2006;22(5):585-7.

221. Ma JX, Su JY, Ma JS, Li HQ, Yan Y. [Study on the molecular mechanism of apoptosis in esophageal cancer cells induced by soybean isoflavone]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2003;24(11):1040-3.

222. Yanagihara K, Ito A, Toge T, Numoto M. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res* 1993;53(23):5815-21.

223. Cui HB, Na XL, Song DF, Liu Y. Blocking effects of genistein on cell proliferation and possible mechanism in human gastric carcinoma. *World J Gastroenterol* 2005;11(1):69-72.

224. Song D, Na X, Liu Y, Chi X. [Study on mechanisms of human gastric carcinoma cells apoptosis induced by genistein]. *Wei Sheng Yan Jiu* 2003;32(2):128-30.

225. Zhou HB, Chen JJ, Wang WX, Cai JT, Du Q. Apoptosis of human primary gastric carcinoma cells induced by genistein. *World J Gastroenterol* 2004;10(12):1822-5.

226. Tatsuta M, Iishi H, Baba M, Yano H, Uehara H, Nakaizumi A. Attenuation by genistein of sodium-chloride-enhanced gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Int J Cancer* 1999;80(3):396-9.

227. Rossi M, Garavello W, Talamini R, La Vecchia C, Franceschi S, Lagiou P, Zambon P, Dal Maso L, Bosetti C, Negri E. Flavonoids and risk of squamous cell esophageal cancer. *International journal of cancer. Journal international du cancer* 2007;120(7):1560-4.

228. De Stefani E, Ronco A, Mendilaharsu M, Deneo-Pellegrini H. Diet and risk of cancer of the upper aerodigestive tract--II. Nutrients. *Oral oncology* 1999;35(1):22-6.

229. Sun CL, Yuan JM, Lee MJ, Yang CS, Gao YT, Ross RK, Yu MC. Urinary tea polyphenols in relation to gastric and esophageal cancers: a prospective study of men in Shanghai, China. *Carcinogenesis* 2002;23(9):1497-503.

230. Hernandez-Ramirez RU, Galvan-Portillo MV, Ward MH, Agudo A, Gonzalez CA, Onate-Ocana LF, Herrera-Goepfert R, Palma-Coca O, Lopez-Carrillo L. Dietary intake of polyphenols, nitrate and nitrite and gastric cancer risk in Mexico City. *International journal of cancer. Journal international du cancer* 2009;125(6):1424-30.

231. Lin DC, Du XL, Wang MR. Protein alterations in ESCC and clinical implications: a review. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus / I.S.D.E* 2009;22(1):9-20.

232. Lee PC, Port JL, Paul S, Stiles BM, Altorki NK. Predictors of long-term survival after resection of esophageal carcinoma with nonregional nodal metastases. *Ann Thorac Surg* 2009;88(1):186-92; discussion 192-3.

233. Rossi M, Rosato V, Bosetti C, Lagiou P, Parpinel M, Bertuccio P, Negri E, La Vecchia C. Flavonoids, proanthocyanidins, and the risk of stomach cancer. *Cancer causes & control : CCC* 2010;21(10):1597-604.

234. Hongo M, Nagasaki Y, Shoji T. Epidemiology of esophageal cancer: Orient to Occident. Effects of chronology, geography and ethnicity. *Journal of gastroenterology and hepatology* 2009;24(5):729-35.

235. de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. *Gastroenterology clinics of North America* 2013;42(2):219-40.

236. Schrump DS, Nguyen DM. Strategies for molecular intervention in esophageal cancers and their precursor lesions. *Dis Esophagus* 1999;12(3):181-5.

237. Fass R, Hell RW, Garewal HS, Martinez P, Pulliam G, Wendel C, Sampliner RE. Correlation of oesophageal acid exposure with Barrett's oesophagus length. *Gut* 2001;48(3):310-3.

238. Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. *Am J Gastroenterol* 2002;97(8):1888-95.

CHAPTER 2: METHODS

Overview of Study Methods

The goals of this dissertation were to determine the associations between intake of dietary flavonoids and lignans and the risk of developing Barrett's esophagus (BE) and esophageal and gastric cancer and survival among esophageal and gastric cancer cases. To address these goals, existing data from two parent studies – one study of esophageal/gastric cancer and one of BE – was used to complete three steps. Step 1: Development of two study-specific flavonoid databases, one for each parent study [Esophageal and Gastric Cardia Adenocarcinoma (EGA) Study and BE Study]. The two parent study-specific food frequency questionnaires (FFQs) were linked with the existing flavonoid and lignan databases, utilizing weights from the FFQ, to determine flavonoid content of each FFQ line item. Step 2: Utilization of the two study-specific flavonoid/lignan databases to estimate flavonoid intake for the corresponding participants in the two studies, by using reported serving sizes and frequency of intake. Step 3: Estimation of (i) odds ratios for the associations between flavonoid/lignan intake and the risk of developing esophageal/gastric cancer and BE and (ii) hazard ratios for the association between flavonoid/lignan intake and risk of mortality in esophageal/gastric cancer cases. Whether risk varies by flavonoid/lignan type or stage/differentiation of the case tumor was also explored. Details of the study methods are presented below.

Study Populations

Existing data was used from two parent studies of esophageal/gastric cancer and BE to

determine the association between flavonoids/lignans and the risk of developing esophageal and gastric cancer and BE and survival among esophageal and gastric cancer cases.

The parent EGA study, as known as the U.S. Multi-Center Study, was a multi-center, case-control study conducted in the state of Connecticut, a 15-county area of New Jersey, and a three-county area of western Washington state.[1] Each of these geographic areas had a population-based cancer registry, which were used to identify the cases using rapid reporting methods.[1] Eligible cases were English-speaking men and women between the ages of 30 and 79, who were diagnosed with a primary invasive cancer of the esophagus or stomach between the years of 1993 and 1995 [February 1, 1993-January 31, 1995 in Connecticut (CT); April 1, 1993-November 30, 1994 in New Jersey (NJ); and March 1, 1993-February 28, 1995 in Washington state (WA)]. All cases of esophageal or gastric cardia adenocarcinoma were recruited (target cases of primary interest). Esophageal squamous cell carcinoma and non-cardia gastric adenocarcinoma cases were frequency matched to the target cases by geographic location and 5-year age group (CT, NJ, WA), sex (NJ, WA), and race (white or other, NJ).[1]

Population-based controls were frequency matched by 5-year age group and sex. Control participants 30-64 years of age were identified through random digit dialing (RDD) techniques.[2] Control participants 65-79 years of age were identified by random sampling of Health Care Financing Administration rosters. Current use of RDD may be problematic, but the parent EGA study was conducted in 1993-1995. Two recent studies demonstrated that RDD contact, cooperation, and response rates were still high until after the mid-1990s.[3,4]

The parent study of BE, also known as the Study of Reflux Disease, was a case-control study conducted in western Washington state.[5] Eligible cases were men and women between the ages of 20 and 80 without previously diagnosed BE who were undergoing an upper endoscopy for gastroesophageal reflux disease symptoms between the years of 1997 and 2000. Potentially eligible cases were identified through community gastroenterology

clinics. Controls were identified through RDD techniques. The parent BE study was conducted between 1997 and 2000, after the response rates began to decline, but the investigators concluded that RDD was still a viable option for control recruitment until 2000.[5]

For both parent studies, information on potential risk factors was obtained by a structured questionnaire administered face-to-face by a trained interviewer. Information was collected on demographics, tobacco and alcohol use, medical conditions, and medications. Dietary information was collected via face-to-face interview for the EGA study but by self-report for the BE study. Written informed consent was obtained for all participants prior to the interview.[1,5] For the EGA study, the average time between cancer diagnosis and interview was 3.7 months when the participant was interviewed and 8.5 months when a proxy was interviewed. Average time for interview completion was 130 minutes.[1] The time between endoscopy and interview for the BE cases was 1-2 months, with the interview averaging 45 minutes.[5]

In the EGA study, initial participant selection was based on the state tumor registry reports, which were obtained for potentially eligible participants that were diagnosed with esophageal adenocarcinoma (EA), gastric cardia adenocarcinoma (GCA), esophageal squamous cell carcinoma (ESCC), or non-cardia gastric adenocarcinoma (NCGA). Additional potential participants with diagnosed esophageal and gastric cancer classified as not otherwise specified (NOS), mixed, undifferentiated, uncertain histologic type, and unspecified subsite of the stomach were approached for participation in the study.

At the time of the interview, signed medical record release forms were obtained from the case participants, in order to retrieve relevant medical records and corresponding pathologic specimens for the first primary diagnosis of esophageal or gastric cancer. Histologic slides of the diagnostic biopsy were reviewed for more than 99% of cases.[1] After a systematic review of these materials, the study pathologists (Drs. Heidi Rotterdam for NJ and A. Brian West for CT and WA) made the final determination of case eligibility. Cancers

were classified using ICD-O codes and histology codes. Eligible EA cases were classified as ICD-O[6] codes C150.0-150.9 and histologic codes 8140-8381. Eligible GCA cases were classified as ICD-O code C151.0 and histologic codes 8140-8381. Eligible ESCC cases were classified as ICD-O codes C150.0-150.9 and histologic codes 8050-8082. Eligible NCGA cases were classified as ICD-O codes C151.1-151.9 and histologic codes 8140-8381.

Although pathologic specimens were available for review, tumors that involve the distal esophagus and gastric cardia (i.e., proximal stomach) are difficult to classify.[7] Therefore, the site of origin was determined by estimating the tumor's center. For tumors where the site of origin could not be determined by H. Rotterdam, A.B. West reviewed the records, and vice versa. Disagreements were resolved by consensus. To ensure consistency of the diagnoses between pathologists throughout the study period, randomly chosen pathology materials were reviewed by both pathologists on a regular basis; the rare inconsistencies were resolved by consensus.

In the BE study, participants were recruited from individuals undergoing an upper endoscopy for gastroesophageal reflux disease (GERD) symptoms. Consenting participants had a four-quadrant biopsy specimen collected. Biopsy specimens were evaluated by one of three university-based pathologists, who were blinded to the endoscopy findings. Cases were defined as those with specialized intestinal metaplasia (SIM) on at least one of the four biopsy specimens.[5]

Demographics of Study Participants

Demographic characteristics of the parent studies of EGA and BE have been previously published[1,5] and are summarized in **Table 2.1**. For the EGA parent study, the median age at diagnosis was 66 years, and the median overall survival times were 9.6 months for EA, 12.8 months for GCA, 10.7 months for ESCC, and 12.9 months for NCGA. The majority of the participants were diagnosed with regional or distant stage tumors. In the BE parent study,

the mean age at diagnosis was 55 years. In both parent studies, the majority of participants were Caucasian males and cases were less likely to be well educated than controls.

Exposure Assessment

Dietary information for the EGA study was collected by trained research interviewers during structured interviews, utilizing a food frequency questionnaire (FFQ). The FFQ was modified from the FFQ developed by investigators at the Fred Hutchinson Cancer Research Center (FHCRC)[8] and included 104 items.[9] Participants in the EGA study were asked to report their usual dietary intake in the 3-5 years prior to diagnosis (cases) or interview (controls). Completed FFQs were not obtained for 33 cases who had abbreviated interviews because of serious illness.[9] Face-to-face interviews were conducted for 80.6% (n=554) of eligible EA and GCA cases, 74.1% (n=589) of eligible ESCC and NCGA cases, and 70.2% (n=695) of eligible controls in the EGA study. The overall response rate for the control participants was 70.2%, when accounting for the telephone screener response rate of 90.8% for the 51.8% of control participants identified through RDD. The primary two reasons for non-participation were participant refusal (12% of EA and GCA cases, 17% of ESCC and NCGA cases, and 23% of controls) and physician refusal for case participants (4% for each group).[1]

The BE study used the standard self-administered FHCRC FFQ, which included 131 items.[8] Participants were asked to report their dietary intake for the year prior to interview. The response rates for the parent BE study were 92.8% for eligible cases and 68.7% for eligible controls. Completed FFQs were obtained for 88.1% (n=170) of cases and 86.3% (n=182) of controls. While there is expected to be some level of reporting error in an FFQ, the FHCRC FFQ has been validated by using 4-day food records[8] and four 24-hour recalls[10] as criterion instruments.

Rapid case ascertainment was conducted in the EGA study. However proxies, usually spouses, were interviewed when the cases were too ill or died prior to being

interviewed. One-year overall survival is approximately 40% for EA and GCA,[11] and overall five-year survival is 12.2% and 21.1% for esophageal and gastric cancer diagnosed between 1993 and 1995, respectively.[12] Next-of-kin proxies were interviewed for 30.9% (n=354) of cases and 3.4% (n=24) of controls.[1]

While reliability and validity of proxy versus self-report have not been investigated in the parent EGA and BE studies, other studies have examined this issue for a number of factors, including dietary intake. For example, next-of-kin proxy recall for fruits and vegetables has been shown to be similar to self-report.[13,14] For a 5-year recall period, self-respondents had fair agreement for fruits (κ=0.35) and vegetables (κ=0.31). Any next-of-kin respondent had a fair agreement for fruits (κ=0.37) and vegetables (κ=0.20). Spouses had a slightly lower correlation for fruits (κ=0.29) but better reporting for vegetables (κ=0.33) than overall next-of-kin proxies.[13] Another study showed that agreement between next-of-kin and colon cancer cases was fair-to-moderate for fruits (κ=0.42) and vegetables (κ=0.45); agreement between controls and next-of-kin was similar. The study also found that next-of-kin response was more highly correlated with self-report when the questionnaire was interviewer administered (as was the case in the parent EGA study).[14] While the kappa coefficients may appear to show low agreement, this is common in nutritional epidemiology. For example, a study of the correlation between the FHCRC FFQ and the mean of two, four-day diet records found Pearson correlation coefficients of 0.31-0.64.[15]

From these previous studies,[13,14] it was expected that self-report and proxy-report would be similar. However, there was still potential for the agreement between self-report and proxy to be dissimilar in this ancillary study. However, in a study of the association between micronutrients and esophageal and gastric cancer in the parent EGA study, results from analyses excluding versus including proxy data were almost identical.[9] Proxies may even provide better information than the participants, as the majority of participants were older males, and their wives – who were the most likely source of the proxy responses –

may have prepared most meals.[16,17]

To develop an expanded flavonoid database, each FFQ was linked on frequency of dietary intake and portion size with existing databases of flavonoid intake from the U.S. Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods[18] and the USDA-Iowa State University Database on the Isoflavone Content of Selected Foods.[19,20] While these are useful in estimating flavonoid intake, none of the USDA databases contain content values for lignans. Therefore, these USDA databases were supplemented with available literature on the lignan content of foods.[20,21]

While the USDA databases for flavonoid content were utilized in this ancillary study, there is also a European database that includes both flavonoids and lignans, known as the Phenol-Explorer. This database was explored for quality by examining: 1) correspondence of FFQ line items and foods included in Phenol-Explorer, 2) completeness of flavonoids included in the database, and 3) readiness of Phenol-explorer for use. It was determined that, at the time of data linkage, the Phenol-Explorer was less complete than the USDA databases. Additionally, flavonoid content determined by chromatography and chromatography after hydrolysis was not combined in this database. Thus, it was decided not to utilize the Phenol-Explorer.[22,23]

There were 91 items from the standard 131-item FHCRC FFQ that contain at least one class of flavonoid or lignan (**Table 2.2**). Some FFQ items represent multiple foods or beverages, such as “apples and pears”, rather than a single food or beverage. Therefore, the individual foods and beverages from the FFQ item were weighted according to weights used by the FHCRC for computation of total energy and nutrient intake. For the parent EGA study, the FHCRC was unable to locate records of the weighting scheme that was utilized for computation of total energy and nutrient intake from the FFQ. Thus, verification of the weighting scheme was done by recreating total energy intake estimates. This was accomplished by using a weighting scheme utilized on a similar FHCRC FFQ and examining

the correlations between the calculated total energy and the FHCRC computed total energy. The Pearson correlation coefficient between the FHCRC total energy variable and the calculated total energy variable, without including alcohol, was 0.97. Thus, the weights that were used were reasonably close to how the FHCRC originally weighted the FFQ items.

To estimate the mean intake of flavonoids among participants in the parent EGA and BE studies, the frequency of dietary intake and portion size from the FHCRC FFQ was used. For both studies, participants were asked "how often did you eat/drink these foods." For the EGA parent study, participants gave the number of times the food item was consumed per day, week, month, or year. Participants were not asked the portion size, so portion size was assumed to be a medium serving, which is consistent with the FHCRC procedures. For the BE parent study, frequency of dietary intake was categorized as never or less than once per month, 1 per month, 2-3 per month, 1 per week, 2 per week, 3-4 per week, 5-6 per week, 1 per day, or 2+ per day. Then participants were given a definition of medium serving size (e.g., for "apples and pears" medium serving size was defined as "1 medium or ½ cup") and asked to report the serving size of the dietary line item they usually ate – small, medium, or large.[8]

For example, the FFQ line item of "apples and pears" was assigned a weight of 0.75 for "apples" and 0.25 for "pears".[24] To calculate the flavonoid intake, the weight assigned to each food in the FFQ item was multiplied by the flavonoid content of that food, summed across all foods in the FFQ line item, and then multiplied by the number of times consumed per day and serving size.[20] In this example, 100 grams of apple contains 1.29 mg and pear contains 12.18 mg of anthocyanidins.[18] A medium serving size of apples or pears was estimated as 145 grams. Therefore, if an individual reported consuming one medium serving of apples or pears per day, the individual's daily intake of anthocyanidins from apples and pears was calculated as follows:

Daily intake of anthocyanidins from apples or pears = 145g apple or pear/day * [(0.75 apple weight * 1.29 mg/100g apples) + (0.25 pear weight * 12.18 mg/100g pears)] = 5.82 mg anthocyanidins/day

Recall error may be of concern, given that participants were asked to recall past behavior, including usual dietary intake. However, moderate agreement has been shown between baseline food intakes and recall of these intakes assessed by a food frequency questionnaire 3-10 years later for total energy (r=0.54,[25] r_s=0.62,[26] and K=0.32[27]) vegetables (K=0.31),[27] and fruits and vegetables (r=0.41).[28]

An additional potential source of error in estimating flavonoid content in foods, especially fruits and vegetables, may be associated with the variability of environmental conditions, horticultural practices, degree of ripeness, plant variety, storage conditions, cooking methods, and industrial processing, which may vary regionally and over time.[20,29-33] To estimate the impact of these potential influences on the flavonoid content of select foods, the USDA Food Composition and Nutrient Data Laboratories determined the flavonoid content for over 60 fruits, vegetables, and nuts by sampling foods from four United States regions at two times of the year. While this study found variation in flavonoid content within and between foods was high, the average flavonoid content was similar to values reported in the USDA Database for the Flavonoid Content of Selected Foods.[34]

Outcome Assessment: Cyclin D1 and P53 Status

Archived Tumor Block Collection. Tumor blocks with sufficient tissue for immunohistochemical (IHC) analyses were collected for 630 (55.1%) and 649 (56.8%) of the 1130 cancer cases for cyclin D1[35] and p53,[36] respectively. As reported in the parent EGA study,[35] availability of tumor tissue varied little by tumor subsite, histology, stage at diagnosis, or suspected risk factors for these tumor types (age, sex, race, cigarette smoking, alcohol intake, BMI, and NSAID use). Additionally, tumor availability did not vary by proxy

versus self-report status.

Processing and Staining. Cyclin D1. Immunohistochemical techniques were used to determine cyclin D1 status, utilizing previously published methods.[37,38] In this method, 5-µm sections from the formalin-fixed, paraffin-embedded tumor sample were deparaffinized, hydrated, and placed in a 10 mM citrate buffer (pH 6) and microwaved for antigen retrieval for a total of 10 minutes. Blocking serum (horse serum), primary antibody (mouse monoclonal IgG2a), and the antihuman cyclin D1 (1:20; Immunotech) were used in the IHC analysis. The detection method for cyclin D1 was the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA); chromogen diaminobenzidine was used and sections counterstained with methyl green.[35]

P53. The methods utilized for p53 protein analyses were based on previously published methods.[39] In this method, dewaxed sections from the formalin-fixed, paraffin-embedded tumor sample were microwaved for antigen retrieval, utilizing the p53 DO-7 monoclonal antibody (1:50; DAKO Corp., Santa Barbara, CA).[40] Using a 32-minute incubation of the primary antibody and avidin-biotin complex immunoperoxidase technique, IHC protocol was performed by a Ventana Automated Immunostainer (Ventana Corp., Tucson, AZ).[41] Slides were stained in multiple batches by the same lab, using positive and negative controls to ensure that results were consistent across batches.[36]

Interpretation. Cyclin D1. The study pathologist (Dr. Hanina Hibshoosh) was responsible for interpretation of the IHC results, utilizing previously published methodology.[37,42] A semiquantitative scoring system was used to categorize the nuclear staining in esophageal and gastric cancers by considering both staining intensity and percentage of positive nuclei. In this system, nuclear staining intensity is a four-level ordered categorical variable (0=none, 1=mild, 2=moderate, 3=strong), and the percentage of positive cells is a dichotomous variable (negative=none or rare, defined as ≤5% positive cells; positive=more than rare, defined as >5% positive cells). The cutoff value for rare cells

reflects the level of background staining seen in adjacent normal cells. Cases were classified as cyclin D1 positive only if: 1) the staining number was moderate or strong and 2) the percentage of positive cells was classified as positive (>5%). If a case did not meet these criteria, they were classified as cyclin D1 negative.[35]

P53. Nuclear p53 protein staining was assessed by a study pathologist. Nuclear staining percentage was classified using a five-level ordered categorical variable (0=negative, 1=<10%, 2=10–50%, 3=51–90%, and 4=>90% cells stained). Cases were classified as p53 positive only if: 1) the staining number was moderate or strong and 2) the percentage of positive cells was ≥10%. If cases did not meet these criteria, they were classified as p53 negative.[36]

Outcome Assessment: Segment Length

Tumor Biopsy Collection, Processing, and Staining. Consenting participants in the BE study had a four-quadrant biopsy specimen taken from the tubular esophagus just distal to the squamocolumnar junction during an endoscopy visit. Tumor biopsies were fixed in zinc formaldehyde. Alcain blue stain (pH 2.5) was utilized to assist in establishing the presence of specialized intestinal metaplasia (SIM).[5]

Interpretation. Biopsy specimens were evaluated by one of three university-based pathologists, who were blinded to the endoscopy findings. Cases were defined as those with SIM on at least one of four standard biopsy specimens.[5]

During the endoscopy procedure, physicians recorded the presence (and length) or absence of visible columnar epithelium, or visible BE (VBE). This allowed the cases to be classified into one of three progressively exclusive groups: 1) SIM (i.e. all cases), 2) SIM and VBE (VBE cases), and 3) SIM and VBE greater than 2 centimeters in length [long-segment BE (LSBE) cases].[5] The first and most inclusive category of all SIM cases adheres to the concept of “ultra-short segment BE”.[5,43-45] The latter two categories adhere to the BE case

definition as put forth by the American College of Gastroenterology.[5,46]

Outcome Assessment: Vital Status

In a follow-up of the EGA study, vital status and date of death were determined by the state tumor registries, which linked the tumor registry data with the National Death Index.[47] Overall survival time (in months) was calculated from the date of diagnosis until death or last follow-up. The maximum follow-up was 90 months. Follow-up ended for western Washington state in July 2000, for New Jersey on September 15, 2000, and for Connecticut on October 28, 2000. At the end of the follow-up period, participants that were still alive were censored. The outcome of death was ascertained from all-cause mortality.[47]

Covariate Assessment

Covariate information for both parent studies of EGA and BE was collected during the structured interviews, which were conducted by trained research interviewers. Directed acyclic graphs (DAGs, **Figures 2.1-2.5**) were utilized as conceptual models to determine which variables were evaluated as potential confounders. Variables that were evaluated as potential confounders in this ancillary study for risk of developing esophageal/gastric cancer include proxy status, income, education, cigarette smoking, alcohol consumption, and body mass index (BMI). Variables that were evaluated as potential confounders in this ancillary study for esophageal/gastric cancer survival include income, education, cigarette smoking, alcohol consumption, BMI, tumor stage, tumor grade, dysphagia, study site, sex, and self-reported race. The predetermined covariates evaluated as effect measure modifiers were cigarette smoking, BMI, and GERD (**Tables 2.9-2.11**). While the generally accepted model for EA is that it develops through GERD and BE,[48] not all EA cases report having GERD or BE.[49] Therefore, there are potentially other pathways to development of EA/GCA that are not through GERD or BE. As stated earlier, it is of concern that proxy responses may

misclassify exposure or covariate information. However, most validation studies that have compared self-report to proxy report have found good concordance for cigarette intake,[50,51] weight and height,[50] and dietary recall.[13,14]

Variable assessment and corresponding definitions of covariates for the EGA study are as follows.[1,9] To assess yearly household income in the parent EGA study, participants were asked to look at card showing six levels of income (A. less than $15,000; B. $15,000-$29,999; C. $30,000-49,999; D. $50,000-74,999; E. $75,000-$99,999; F. $100,000 or more) and state the letter that best describes the total household income during the last calendar year. Education was categorized as <12 years, 12 years, vocational school, some college, college graduate, and graduate school. To be considered a cigarette smoker, participants had to have smoked at least 100 cigarettes or more in his/her lifetime. Participants who were considered smokers were then asked the intensity of use (number of cigarettes/day) and duration. Alcohol consumption was assessed by asking the participant to report consumption patterns for beer, wine, and liquor separately for anytime one year or more prior to the interview. A never drinker was defined as having less than one drink per month. Beer, wine, and liquor were combined to form an overall consumption variable for alcohol. BMI (weight in kg/height in m^2) was assessed by asking the participant what his/her height and their usual adult weight (i.e., the most common weight during adulthood) one year prior to diagnosis (cases) or interview (controls). Energy intake (kcal/day) was assessed using the Fred Hutchinson Cancer Research Center (FHCRC) food frequency questionnaire (FFQ), with kilocalories from alcohol included. The data from the FFQ was double-entered at the study site and then sent to FHCRC to be analyzed for energy and nutrient intake. Energy intake was calculated by summing the kilocalories for each food item consumed during the reported year and then the kilocalories were averaged over the year. Self-reported race was categorized as white and other.

Variables[5,52] evaluated as potential confounders in this ancillary study of BE

development include income, education, BMI, self-reported race, cigarette smoking, and total energy intake. Income (less than $45,000, 45,000-74,999, 75,000 or more), cigarette smoking, and total energy intake (kcal/day) were assessed in a similar manner to the parent EGA study. Education was categorized as high school or less, technical school, and college or more. To calculate BMI (kg/m^2) in the parent BE study, interviewers measured participant's height and weight at the time of the participant interview using established protocols.[53] As with the EGA study, cigarette smoking and BMI were also be evaluated as a potential effect measure modifiers. Participants were additionally asked to self-report their race (white, black or African, Asian, American Indian/Eskimo, other/unknown).

Study Design

Barrett's esophagus and esophageal and gastric cancer are rare diseases; therefore a case-control study is the most efficient study design. Two possible alternative designs for this study would be to 1) conduct a cohort study of Barrett's esophagus patients to analyze the exposure of dietary flavonoids and the outcome of esophageal or gastric cardia adenocarcinoma (EA/GCA) or 2) conduct a cohort study of gastroesophageal reflux disease (GERD) patients to analyze the exposure of dietary flavonoids and the outcome of BE. However, these study designs are inefficient. Although BE patients have a 125-fold greater risk of EA/GCA, the probability that BE will develop into adenocarcinoma is still rare – incidence of cancer is 1 out of 227 BE patient-years of follow-up.[54] Similarly, only 10-15% of GERD patients develop BE.[55] Therefore, using a cohort design to study these aims would be an incredibly time-intensive and expensive method to study esophageal and gastric cancer and BE. This ancillary study is a practical and cost-efficient use of resources because the dietary and confounder data has been previously collected.

Results from Previous Analyses

Published results using the dietary data from both parent studies have shown that diets high in fruit and vegetable intake are inversely associated with esophageal/gastric cancer and Barrett's esophagus (BE).[52,56] In the EGA parent study, adjusted odds ratios (OR) were calculated based on increasing fruit/vegetable intake by one serving per day. Total fruit and vegetable intake was associated with decreased risk of incident esophageal adenocarcinoma (OR=0.88, 95% CI: 0.82-0.95) and esophageal squamous cell carcinoma (OR=0.90, 95% CI: 0.82-0.99). Results were near null for gastric cardia adenocarcinoma (OR=0.97, 95% CI: 0.90-1.03) and non-cardia gastric adenocarcinoma (OR=0.99, 95% CI: 0.93-1.05).[56] In the parent BE study, adjusted ORs were calculated based on tertiles of fruit/vegetable intake. Participants in the highest tertile of combined fruit and vegetable intake had lower risk of developing BE (OR=0.39, 95% CI: 0.21-0.75) as compared with participants in the lowest tertile of fruit/vegetable intake.[52]

Statistical Analysis

The first aim of this ancillary study was to determine if flavonoid/lignan intake is associated with esophageal/gastric cancer incidence, utilizing existing data from the EGA study collected as part of a multi-center, population-based case-control study conducted in Connecticut, New Jersey, and western Washington state.[1] The second aim was to determine if flavonoid/lignan intake is associated with Barrett's esophagus (BE) development, using existing data from the BE study, a community-based case-control study conducted in western Washington state.[5] The third aim was to determine if flavonoid/lignan intake is associated with survival among esophageal/gastric cancer cases, utilizing existing follow-up data from the EGA study. For all three aims, the effects for total flavonoid intake were estimated, as well as the effects for each of the six classes of flavonoids (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones). Lignans were considered separately for all three aims due to the substantial differences in the foods and beverages that contribute to lignans as compared with each class of flavonoid.

Demonstration of an association between flavonoids and BE development, esophageal or gastric cancer incidence or survival among esophageal and gastric cancer cases suggests potential to use flavonoids as a risk reduction strategy for these tumors. This could allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs.

Details of the statistical analyses are discussed below.

Data Management/Quality Control

The EGA parent study was a multi-site study in Connecticut, New Jersey, and western Washington state. Since esophageal and gastric cancers remain relatively rare, the study included data collection efforts from multiple centers to ensure an adequate sample size within the given time frame for the study (1993-1995). The principal investigators [PIs; Drs. Marilie Gammon (NJ), Harvey Risch (CT), and Thomas Vaughan (WA)] of this study collaborated closely together to ensure that the study methods and the same questionnaire were implemented at each study site so the data could be compiled for the planned analyses. The BE parent study was conducted in western Washington state by a PI (T. Vaughan) from the EGA parent study. Therefore, data collection and management have been performed in similar ways for both studies.[57]

Data for both parent studies were collected during a structured paper-based interview conducted by trained research interviewers at the participant's home or another requested location. Participant responses were double entered into a computerized database to ensure that there were minimal entry errors. Discrepancies in data entry were resolved by referencing the original interview document. After the data from the food frequency questionnaire was entered, it was sent to the Fred Hutchinson Cancer Research Center to be analyzed for micro- and macronutrient intake, along with total energy intake.[57]

Descriptive Analysis for the Ancillary Study

Data analysis began with an examination of the data distributions of the exposure, outcome, and covariates, using tables and histograms. For the EGA ancillary study analysis, the frequency (n) and relative frequency (%) of case-control status, flavonoid intake, proxy status, race, age, gender, body mass index (BMI), cigarette smoking, alcohol consumption, socioeconomic status (SES), total energy intake, and geographic site, were assessed using tabular methods. In the BE ancillary study analysis, the frequency and relative frequency was assessed for the following covariates: case-control status, flavonoid intake, race, age, gender, BMI, SES, and total energy intake. For both analyses, age, BMI, cigarette smoking, alcohol consumption, flavonoid intake, and total energy intake were assessed as continuous measures using histograms and descriptive statistics including the mean, median, standard deviation, range, and skewness.

Exposure Variable Construction

After linking the flavonoid and lignan databases with the FFQ, the flavonoid and lignan intakes were continuous variables. Flavonoid intake was categorized as deciles, quintiles, and quartiles, based on the distribution of overall and sex-specific intake (**Tables 2.3 and 2.4**) among the controls (logistic regression) or all cases (survival analysis).[17] Results were similar; therefore, only the overall quartiles are presented. By using multiple categories, no assumptions are made about the dose-response relationship. However, finer divisions of extreme categories were also considered to extend the examination of a dose-response relationship. Categorical analysis also constrained any undue influence from outlying observations. However, a crudely categorized variable may not account for the full effect of the variable, leading to residual confounding. Thus, the exposure variable was examined as a continuous variable and a quartiled categorical variable.[17] In addition to examining total flavonoid intake, continuous and categorical forms of the six flavonoid classes (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and

isoflavones) and lignans were constructed for analysis. Examination of flavonoid intake utilizing restricted quadratic splines was also performed (**Figures 2.6 and 4.1**). Both categorization and spline models produced consistent results for the main analysis; however, the categorized results for quartiles were more stable. Therefore, only results for categorized quartiles are reported. Tests for linear trends utilized the original continuous flavonoid values in mg/day. All p-values were 2-sided.

Analysis to Address Specific Aim 1

To determine if flavonoid intake was associated with risk of esophageal and gastric cancer incidence, unordered polytomous unconditional logistic regression was used to calculate odds ratios (ORs), as an estimate of relative risk, and 95% confidence intervals (CIs) for esophageal adenocarcinoma (EA), gastric cardia adenocarcinoma (GCA), esophageal squamous cell carcinoma (ESCC), and non-cardia gastric adenocarcinoma (NCGA) as distinct outcomes (**Table 3.3**). Polytomous logistic regression allows simultaneous estimation of risk. All models for the outcomes of esophageal and gastric cancer included the following matching variables: geographic site (CT, NJ, WA), age (continuous), sex (male, female), and race (white, other). Confounders and effect measure modifiers were chosen using *a priori* knowledge of the relationships between the exposure, outcome, and potential confounders (**Figure 2.1-2.2**):[58] proxy status (proxy, non-proxy), income (evaluated as <$15,000, $15,000-29,999, $30,000-49,999, $50,000-74,999, or ≥$75,000 and <$15,000, ≥$15,000), education (evaluated as <high school, high school, technical school/some college, >college and <high school, >high school), cigarette smoking (evaluated as ever/never, continuous pack-years, and continuous cigarettes/day), alcohol consumption (evaluated as ever/never and continuous for beer, wine, and liquor), and BMI (evaluated as continuous and as categorized, <25, 25-29.9, or ≥30 kg/m^2).

To further determine if there is an association between flavonoid intake and

esophageal and gastric cancer subtypes, cyclin D1 and p53 status and tumor stage were used. Unordered polytomous unconditional logistic regression was used for each tumor (EA, GCA, ESCC, and NCGA) to calculate odds ratios and 95% confidence intervals for the association between flavonoids and cyclin D1+ tumors (compared to controls) and cyclin D1- tumors (compared to controls), adjusting for matching factors and potential confounders. This analysis was repeated to determine the association between flavonoids and p53 status (p53+/p53-) and tumor stage (localized, regional, and distant). The case-only odds ratio, or ratio of the odds ratios, was calculated to assess etiologic heterogeneity. The case-only OR equals 1 if there are homogeneous subgroups.[58] These analyses are shown in **Tables 2.5-2.7**.

Analysis to Address Specific Aim 2

To determine if flavonoid intake is associated with BE development, conditional and unconditional logistic regression was used to calculate odds ratios and 95% confidence intervals (**Tables 2.8 and 4.3**). All models for the outcome of BE included the matching variables age (continuous) and sex (male, female). Potential covariates included[58] BMI (evaluated as continuous and as dichotomous, <25 or ≥25 kg/m^2), race (white, other), income (<$45,000, ≥$45,000-74,999, ≥$75,000), education (≤high school, technical school, ≥college), and cigarette smoking (ever/never).

To further determine if there was an association between BE and flavonoids, segment length was used. Unconditional logistic regression was used to calculate ORs and 95% CIs for progressively more exclusive groups of SIM, VBE, and LSBE cases, adjusting for matching factors and potential confounders (**Table 4.4**). Additionally, an analysis was conducted examining exclusive groups of SIM (i.e., no visible columnar epithelium on endoscopy), VBE (i.e., visible columnar epithelium ≤2 cm), and LSBE (i.e., visible columnar epithelium >2 cm). The results utilizing exclusive groups and progressively more exclusive

groups were similar; however, the ORs for the exclusive groups were unstable due to small sample sizes (data not shown).

Logisitic Regression Analyses for Specific Aims 1 and 2

Since the outcomes for both analyses in specific aims 1 and 2 were dichotomous and the relationship between the outcome and predictor was non-linear, logistic regression was appropriate. Assumptions were made that the outcomes were statistically independent because there were no repeated events and the residual variation is binomially distributed; this assumption of logistic regression is rarely violated. To model continuous or ordinal variables, such as flavonoids, the first check was to ensure that they have a linear relationship with the logit form of the outcome. As they do not satisfy this assumption, these predictors were modeled with indicator variables.[59]

Covariates considered as potential confounders or effect measure modifiers for this analysis were chosen using *a priori* knowledge of the relationships between the exposure, outcome, and potential confounders (**Figure 2.1-2.3**).[58] First, to ensure that the assumption of multiplicativity was satisfied, effect measure modification was assessed between flavonoids and predetermined covariates (i.e., cigarette smoking and BMI, for both analyses, and GERD, for the EGA analysis; **Tables 2.9-2.13**) using likelihood ratio tests to compare regression models that included a multiplicative term to models without that term.[59] Then, potential confounders were assessed using backward elimination with multivariate models. If the elimination of a variable changed the log odds ratio by more than 10%, the variable was considered a confounder.[58]

Analysis to Address Specific Aim 3

To determine if flavonoid intake is associated with survival among esophageal and gastric cancer cases, Kaplan-Meier curves were constructed to examine flavonoids as a univariate

predictor of survival and Cox proportional hazard regression analysis was used to calculate multivariable hazard ratios (HRs) and 95% confidence intervals for EA, GCA, ESCC, and NCGA as distinct outcomes. The proportional hazards (PH) assumption was evaluated through use of Kaplan-Meier curves and an interaction term between exposure and follow-up time. The PH assumption was not observed to be violated. Potential confounders[58] for the survival analysis included the same variables listed above in the logistic regression analysis as well as study site (WA, NJ, CT), age (continuous), sex (male/female), tumor stage (localized, regional, distant, and unknown), tumor grade (well/moderate, poor/undifferentiated, and not determined), and dysphagia (yes/no) (**Figures 2.4 and 2.5**).[58]

Total Energy Intake

Total energy intake was included in all models for specific aims 1-3.[17] Generally, total energy intake is adjusted for in nutrient models because absolute intake of a specific nutrient is almost always positively correlated with total energy intake. Additionally, energy intake may confound relationships between specific nutrients and the outcome of interest.[17] However, some nutrients may not be positively correlated with total energy and total energy may not be associated with disease. Individuals with implausible reporting of total energy intake were explored in the main analyses in two ways: 1) an *a priori* implausible definition of <500 or >4,000 kcal/day for women and <800 or >5,000 kcal/day for men and 2) an empirical approach of excluding individuals with reported total energy intake in the upper and lower 1% and 2.5%.[17] As the results from exclusion of participants based on implausible energy intake were similar between the *a priori* definition and the empirical approach, the *a priori* definition was utilized for excluding participants. The standard multivariate model, which includes a term for nutrient and total energy intake, was used for energy adjustment. This approach models nutrient intake in relation to the outcome when energy is held constant.

Sensitivity Analysis

The main exposure of interest (flavonoids) was calculated from a food frequency questionnaire (FFQ), and some degree of misclassification due to recall error and proxy response was expected. Therefore, care was taken when interpreting the study results, and several analyses were conducted to determine the robustness of the study results. For the EGA study, an analysis restricting the data to participants who were interviewed directly (excluding all of the dietary data obtained in proxy interviews) was undertaken (**Tables 2.14 and 2.15**). The effect estimates were consistent with the overall analysis, which included proxy-report and self-report; therefore, proxy responses were included to increase power.

Collinearity was assessed using the residual model, where the main exposure variable in the logistic regression models is energy-adjusted flavonoid intake.[17] A few challenges of the residual model include negative residuals, which can be difficult to interpret.[17] As the results obtained from the standard multivariate and residual models were similar, only the results from the standard multivariate model were reported to ease interpretation of the study results (**Tables 2.16 and 2.17**).

The multivariate nutrient density model, where the main exposure variable in the logistic regression models is flavonoid intake per 1000 kilocalories, was also utilized as a sensitivity analysis. This model is often highly correlated with the residual model. If energy intake is associated with the outcome, an association could be introduced by using the nutrient density model. The multivariate nutrient density model addresses the problem of confounding by total energy by adding total energy intake as additional covariate in the model.[60] This model may be particularly advantageous if body size (and therefore total energy intake) varies greatly among study participants, as the standard multivariate and residual models imply that nutrient intake increments have the same effect in a small participant (with low levels of energy intake) and a large participant (with high levels of energy intake) (**Tables 2.16 and 2.17**).[17] However, results were similar between the multivariate nutrient density and standard multivariate models. Thus,

only the standard multivariate model is reported.

Another sensitivity analysis was conducted excluding the anthocyanidin value for bananas. The USDA Flavonoid Database assigns a value of 7.39 mg/100 g of banana for anthocyanidins,[18] which is controversial.[61,62]

All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

Statistical Power

Power calculations are shown in **Table 2.18** for risk of developing esophageal adenocarcinoma (EA), gastric cardia adenocarcinoma (GCA), esophageal squamous cell carcinoma (ESCC) and non-cardia gastric adenocarcinoma (NCGA) as separate outcomes and Barrett's esophagus (BE). The sample size used for power calculations was based on the number of individuals with a food frequency questionnaire (FFQ) available. For the parent EGA study, participants with a FFQ available were: 282 EA cases, 255 GCA cases, 206 ESCC cases, 352 NCGA cases, and 687 controls. For the parent BE study, participants with a FFQ available were: 170 BE cases and 182 controls. Power was computed by overall sample size and non-proxy sample size for the EGA study. In this study, proxies were utilized for 30.2% of case interviews (30.9% of EA, 25.9% of GCA, 34.5% of ESCC, and 30.1% of NCGA) and 3.4% of control interviews. In **Table 2.18**, power calculations are shown for survival among cases of EA, GCA, ESCC, and NCGA as separate outcomes.

The goal of specific aim 1 was to determine if flavonoid intake was associated with risk of esophageal and gastric cancer incidence. Power to detect an association between flavonoids (dichotomous or quartiled) and esophageal or gastric cancer types as separate outcomes was determined using a 2-sided likelihood ratio chi-square test with a sample size of 255 cases and 687 controls and α=0.05. This study had 96% power to detect an OR of 0.45 for the association between quartiled flavonoids and esophageal or gastric cancer. This effect estimate was similar to the association between anthocyanidins and EA incidence reported by Bobe et al. (OR=0.47).

As shown in **Table 2.18**, excluding proxies did not substantially alter power.

The goal of specific aim 2 was to determine if flavonoid intake was associated with Barrett's esophagus development. Power to detect an association between flavonoids (dichotomous or tertiled) and BE was determined using a 2-sided likelihood ratio chi-square test with sample size of 170 BE cases and 182 controls and α=0.05. This study had 84% power to detect an OR of 0.45 for the association between tertiled flavonoids and BE. The expected OR for the association between flavonoids and BE was anticipated to be stronger than the OR for flavonoids and EA or GCA[63] or fruit/vegetable intake and BE (OR=0.39).[52,64,65]

The goal of specific aim 3 was to determine if flavonoid intake was associated with survival among esophageal and gastric cancer cases. Power to detect an association between flavonoids (dichotomous or quartiles) and esophageal or gastric cancer types as separate outcomes was determined using a 2-sided log-rank test with a sample size of 255 cases, α=0.05, 7.5 years of follow-up, 86% events, and 5% loss. This study had 96% power to detect an OR of 0.45 for the association between quartiled flavonoids and survival among esophageal or gastric cancer cases. Excluding proxies did not substantially alter power, as shown in **Table 2.18**.

All power analyses were conducted in SAS version 9.2 (SAS Institute, Cary, NC).

Strengths and Weaknesses

The study approach was carefully developed to enhance study interpretation and mitigate the negative impact of any study limitations, which have been discussed throughout the study methods. Below, a brief review of the study approach is presented.

The major advantage of this study design is consideration of flavonoid intake across the cancer continuum – from precursor lesion, to invasive cancer, and finally mortality among cancer cases. This approach is novel – no other epidemiologic studies have considered the cancer continuum in relation to flavonoid intake. It is also biologically

plausible to consider this approach, because experimental data indicate that flavonoids may influence cancer development differently along the cancer continuum.[66,67] Finally this study is significant because these cancers have a high fatality rate,[47] and thus identification of a risk reduction strategy is a priority.

Data interpretation was enhanced because the study draws upon data from two parent studies of EGA and BE that are population- or community-based. This type of design enhances external validity.

Limitations of the study are that both study populations are primarily Caucasian and male, which limits external validity. However, rates of EA and GCA are highest among white males.[68-71] Therefore, the study results are still generalizable to the majority of the population who are at higher risk for developing EA and GCA.

There is also possible selection bias in both parent studies from refusal to participate, potentially due to race or socioeconomic status (SES). However, in the parent EGA study, New Jersey was the only center to recruit African-Americans and the refusal rates were equivalent between races.[57] In both parent studies, the distribution of SES included both high-income and low-income individuals.

In the parent EGA study, there was additional selection bias of those individuals who died before they could be recruited. When this happened, the next-of-kin (usually a spouse), was recruited when possible. Two studies on the reliability of dietary recall among self-respondents and next-of-kin respondents showed that recall of dietary information was similar in both self-respondents and next-of-kin respondents.[13,14]

Another important consideration is that this study utilized dietary intake data that was collected as part of a FFQ. While some level of reporting error in a FFQ is expected, the FHCRC FFQ has been validated using 4-day food records[8] and four 24-hour recalls[10] as criterion instruments. Further, to date, no other studies of BE[52,72,73] or EA/GCA[56,72,74-81] have used an alternative to the FFQ to assess dietary intake. Thus, to overcome the limitations of

the FFQ, we would have to conduct a new study, probably a cohort study, which would be a far less cost effective strategy with which to test our hypothesis by employment of multiple alternative dietary assessment methods repeatedly over time. Additionally, despite rapidly increasing incidence rates in Western countries, esophageal and gastric tumors remain relatively rare. Thus a case-control study remains the best strategy for identifying risk factors for these tumors. In a case-control approach, the goal is to assess usual adult exposures prior to diagnosis, which are likely to better reflect the etiologic window of exposure for cancer. However, the use of currently measured flavonoid biomarkers is of limited usefulness in epidemiologic studies, because of the variation in absorption profiles, with maximum concentrations reached between 0.5-9 hours after dietary intake.[18,82-85] To capture exposures over time, these alternatives would have to be repeated, which is not feasible in a cohort study.

Finally, multiple comparisons are a potential problem in this study looking at eight exposures (total flavonoids, six flavonoid classes, and lignans) and nine outcomes (development of BE, EA, GCA, ESCC, and NCGA, and survival among cases of EA, GCA, ESCC, and NCGA). Qualitative comparisons were made and care was taken in the interpretation of the results to not over interpret a result that could be due to multiple comparisons.

In sum, the strength and innovation of the study is that it is the first study to examine dietary flavonoid intake along the continuum of EA/GCA development. This approach is enhanced by utilizing population-based and community-based data. The major limitations that complicated data interpretation include the reliance on dietary data that was collected using a FFQ and the use of proxy data. However, for the latter issue, sensitivity analyses were conducted to assess the influence of this concern on the study results. Finally, this study is significant because there is potential to use flavonoids as a risk reduction strategy for these tumors.

Summary

In the United States and many other Western countries, incidence of esophageal and gastric cardia adenocarcinoma (EA/GCA) is rapidly increasing, and Barrett's esophagus (BE) is the only known potential precursor.[86] Studying precursor lesions, such as Barrett's esophagus, provides insight into the etiology of cancer by elucidating key risk factors that act early in disease onset. However, not all precursor lesions develop into cancer. Therefore, understanding the mechanisms by which precursor lesions do result in cancerous tumors can offer an opportunity to intervene and reduce the risk of disease progression. The goals of this dissertation were to investigate the potential association between flavonoid intake and esophageal and gastric cancer and BE as an ancillary study of two parent studies. The main exposure of interest (flavonoids) was calculated from a food frequency questionnaire, and some degree of misclassification due to recall error and proxy responses was possible. Therefore, care was taken when interpreting the study results; several analyses were conducted to explore the robustness of the results. Demonstration of an association between flavonoids and BE development, esophageal and gastric cancer incidence or survival among esophageal and gastric cancer cases suggests potential to use flavonoids as a risk reduction strategy for these tumors. This could allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs.

Table 2.1. Distribution of Demographic Characteristics Among Case and Control Participants in the Parent EGA and BE Studies.

	Esophageal and Gastric Cardia Adenocarcinoma Study						Barrett's Esophagus Study	
Characteristic	Control Participants n=695	Esophageal Adeno-carcinoma n=293	Gastric Cardia Adeno-carcinoma n=261	Esophageal Squamous Cell Carcinoma n=221	Non-cardia Gastric Adeno-carcinoma n=368	Characteristic	Control Participants n=211	Barrett's Esophagus n=193
Age						Age		
<57	179 (25.8)	76 (25.9)	65 (24.9)	34 (15.4)	65 (17.7)	20-39	31 (14.7)	27 (14.0)
57-64	178 (25.6)	48 (16.4)	56 (21.5)	53 (24.0)	61 (16.6)	40-49	53 (25.1)	49 (25.4)
65-71	176 (25.3)	79 (27.0)	71 (27.2)	74 (33.5)	93 (25.3)	50-59	64 (30.3)	57 (29.5)
>71	162 (23.3)	90 (30.7)	69 (26.4)	60 (27.2)	149 (40.5)	60-80	63 (29.9)	60 (31.1)
Sex						Sex		
Male	555 (79.9)	245 (83.6)	223 (85.4)	176 (79.6)	254 (69.0)	Male	133 (63.0)	118 (61.1)
Female	140 (20.1)	48 (16.4)	38 (14.6)	45 (20.4)	114 (31.0)	Female	78 (37.0)	75 (38.8)
Race						Race		
White	646 (93.0)	289 (98.6)	252 (96.6)	168 (76.0)	307 (83.4)	White	192 (91.0)	172 (89.1)
Black	34 (4.9)	2 (0.7)	4 (1.5)	48 (21.7)	36 (9.8)	Black	5 (2.4)	3 (1.6)
Other	15 (2.2)	2 (0.7)	5 (1.9)	5 (2.3)	25 (6.8)	Other	14 (6.6)	18 (9.3)
Education						Education		
≤ High School	308 (44.3)	158 (53.9)	141 (54.0)	155 (70.1)	225 (61.1)	≤ High School	42 (19.9)	49 (25.4)
Technical	175 (25.2)	78 (26.6)	61 (23.4)	38 (17.2)	81 (22.0)	Technical	9 (4.2)	9 (4.7)
College+	212 (30.5)	57 (19.5)	59 (22.6)	28 (12.7)	61 (16.6)	College+	160 (75.8)	135 (70.0)
Income, US $						Income, US $		
<15,000	93 (13.4)	60 (20.5)	41 (15.7)	71 (32.1)	87 (23.6)	< 45,000	61 (28.9)	57 (29.5)
15,000-29,999	177 (25.5)	87 (29.7)	81 (31.0)	75 (33.9)	15 (4.1)	45,000-74,999	68 (27.0)	56 (29.0)
30,000-49,999	175 (25.2)	69 (23.5)	65 (24.9)	56 (25.3)	100 (27.2)	75,000+	77 (36.5)	61 (31.6)
50,000-74,000	126 (18.1)	42 (14.3)	37 (14.2)	9 (4.1)	46 (12.5)	Unknown	16 (7.6)	19 (9.8)
>75,000	124 (17.8)	35 (11.9)	37 (14.2)	10 (4.5)	20 (5.4)			
Geographic Center						Geographic Center		
Connecticut	206 (29.6)	80 (27.3)	82 (31.4)	83 (37.6)	117 (31.8)	Washington	211 (100.0)	193 (100.0)
New Jersey	333 (47.9)	138 (47.1)	113 (43.3)	99 (44.8)	172 (46.7)			
Washington	156 (22.5)	75 (25.6)	66 (25.3)	39 (17.7)	79 (21.5)			
Tumor Stage								
Localized		76 (25.9)	34 (13.0)	50 (22.6)	70 (19.1)	Tumor Length*		
Regional		72 (24.6)	120 (46.0)	82 (37.1)	161 (43.9)	SIM		193 (100.0)
Distant		83 (28.3)	70 (26.8)	40 (18.1)	104 (28.3)	VBE		97 (50.3)
Unknown		62 (21.2)	37 (14.2)	49 (22.2)	29 (7.9)	LSBE		54 (28.0)
Missing		0	0	0	3			

Table 2.2. Fred Hutchinson Cancer Research Center Food Frequency Questionnaire Items That Contain at Least One Class of Flavonoid.

Apples and Pears
Apricots (fresh, canned, dried)
Avocado and guacamole, including added to mixed dishes
Bacon, breakfast sausage, and scrapple
Bananas
All other beans, such as baked, lima beans, black-eyed peas and chili without meat
Bean soups, such as pea, lentil, black bean, potajes
Beer
Black tea
Broccoli
Regular burrito and enchilada
Doughnuts, cakes, pastries, Pop-Tarts®, and pan dulce
Cantaloupe, orange melon, muskmelon, mango and papaya
Carrots, including mixed dishes with carrots
Cauliflower, cabbage, sauerkraut and Brussels sprouts
Cold cereal
Milk on cereal (cold and cooked)
Chili with meat and beans
Chocolate candy and candy bars
Coffee
Milk, cream, or creamer in coffee and tea
Coleslaw
Cooked greens, such as spinach, mustard greens, turnip greens, collards
Cookies
Tomatoes cooked, tomato sauce, salsa and salsa picante
Corn and hominy
Cream soups such as chowders, potato, tomato, cheese, ajiaco
Crispy quesadilla
Dark breads, including dark bagels, rolls, pita bread, and English muffins
Other dried fruit, such as raisins or prunes
Eggs
Flauta and crispy rolled tacos
Butter, margarine or oil, on bread or tortillas
Fat added when cooking beans, rice, vegetables, and potatoes
Fat used to deep-fry, pan fry, or sauté
Butter, margarine, sour cream, oils, or other fat added to vegetables, beans, rice, and potatoes, after cooking
Fried chicken
French fries, fried potatoes, fried rice, fried cassava and fritters
Orange juice and grapefruit juice
Tomatoes, fresh or juice
Gravies made with meat drippings and white sauce
Green tea
Green peppers, green chilies, jalapenos, and green chili salsa
Lettuce and plain lettuce salad
Hot dogs, chorizo, and other sausage such as bratwurst
Hard candy, jam, jelly, honey, or syrup

(Table 2.2. continued)

Ice cream
Low-fat or non-fat frozen desserts, such as frozen yogurt, sherbet, ice milk, and low-fat milkshakes
Low-fat pizza
Macaroni and cheese, lasagna, or noodles with a cream sauce
All other melon, such as honeydew
Menudo and tortilla soup
Milk, all types (including canned and soy) not on cereal
Mixed lettuce or spinach salad with vegetables such as carrots or tomatoes
Cooked cereal and grits
Onions and leeks, including in cooking
Oranges, grapefruit and tangerines (not juice)
Any other fruit, such as fruit cocktail, berries, grapes, applesauce, pineapple
Other soups such as chicken noodle
Other fruit juices such as apple grape
Other potatoes, cassava, and yucca (boiled, baked, or mashed)
Pancakes and waffles
Peaches, nectarines and plums (fresh or canned)
Peanut butter, peanuts, other nuts and seeds
Green or English Peas
All other pies, fried pastries, pastelitos and fruit empanadas
Pizza
Potato, macaroni, or pasta salads made with mayonnaise or oil
Pudding, custard, and flan
Pumpkin and sweet potato pie
Red peppers and red chilies
Rice, grains and plain noodles
Refried beans
Soft quesadilla
Soft taco and enchilada baked without oil
Spaghetti or other noodles with tomato sauce (and no meat)
Spaghetti or other noodles with meat sauce
Stew, pot pie and casseroles with meat or chicken
Green or string beans
Strawberries and kiwi
Summer squash, zucchini, nopales, and okra
Sweet potatoes and yams
Taco and tostada
Tamales, with or without meat
Tofu and textured vegetable products
Canned tuna, tuna salad, and tuna casserole
Vegetables soups
Watermelon and red melon
White breads, including bagels, rolls, pita bread, and English muffins
Wine
Winter squash, such as acorn, butternut, pumpkin

Figure 2.1. Directed Acyclic Graph (DAG) of Potential Confounders of the Association Between Dietary Flavonoid Intake and Esophageal/Gastric Cardia Adenocarcinoma Development Risk. - - - Denotes a pathway that may not be present for all EA/GCA cases.

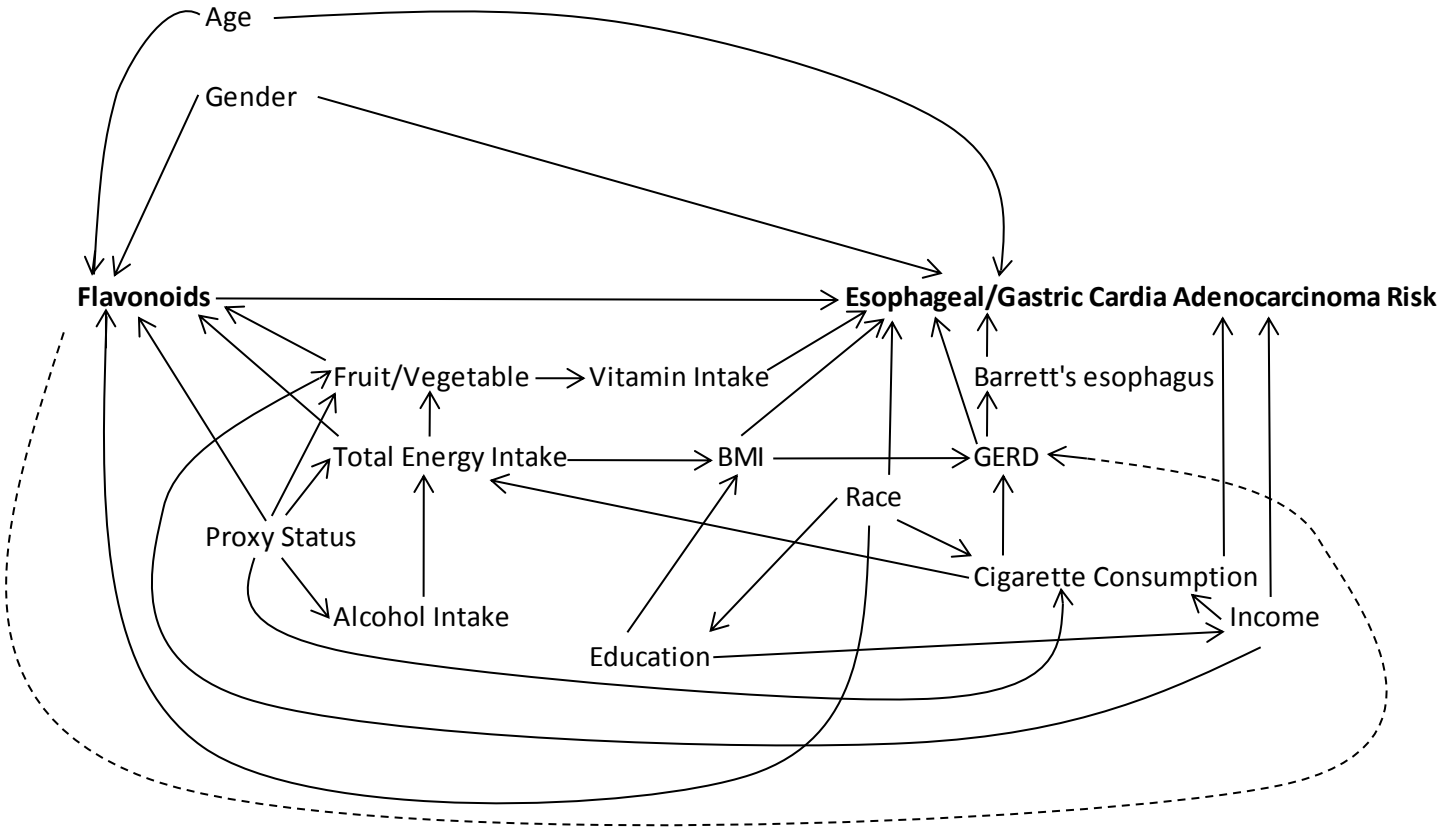


Figure 2.2. Directed Acyclic Graph (DAG) of Potential Confounders of the Association Between Dietary Flavonoid Intake and Esophageal Squamous Cell Carcinoma and Non-cardia Gastric Adenocarcinoma Development Risk.

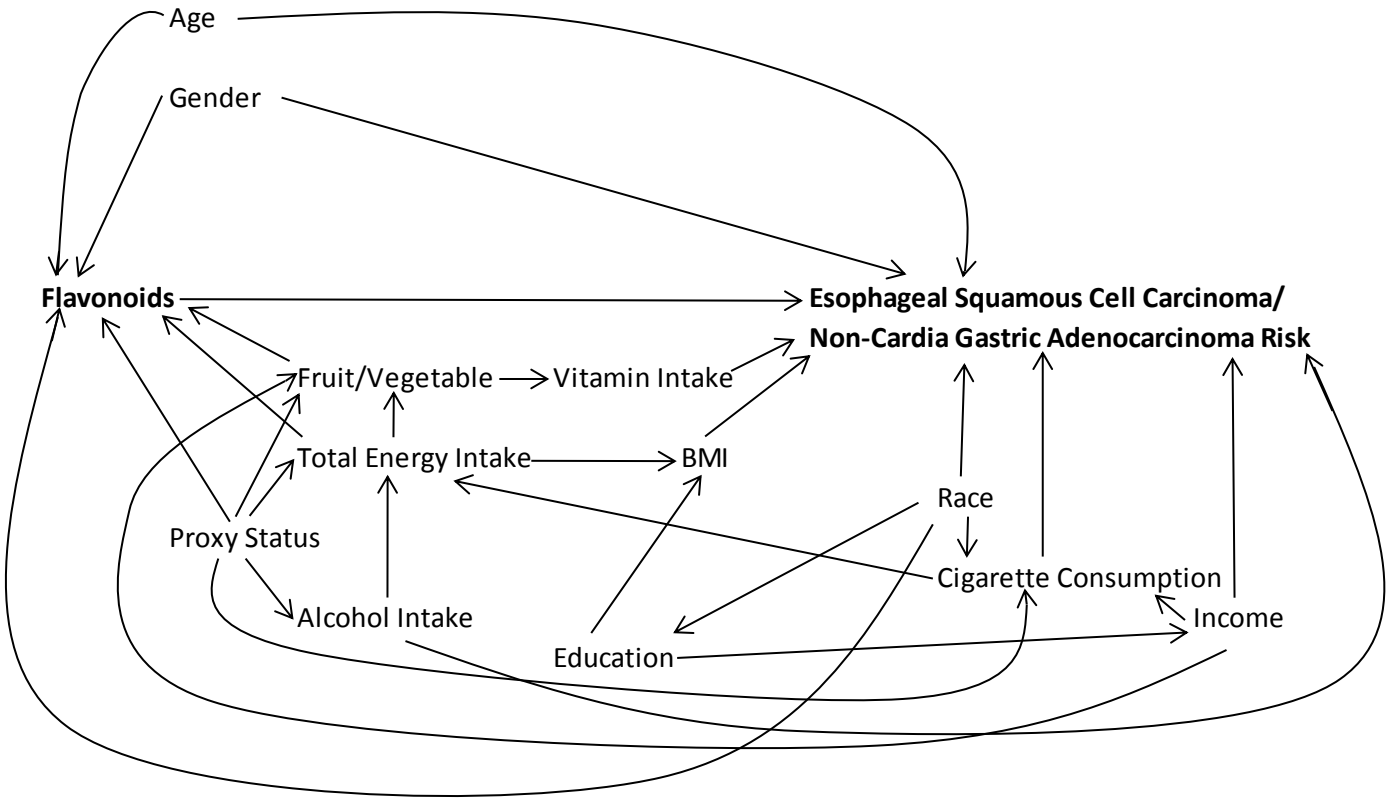


Figure 2.3. Directed Acyclic Graph (DAG) of Potential Confounders of the Association Between Dietary Flavonoid Intake and Barrett's Esophagus Development Risk. - - - Denotes a pathway that may not be present for all BE cases.

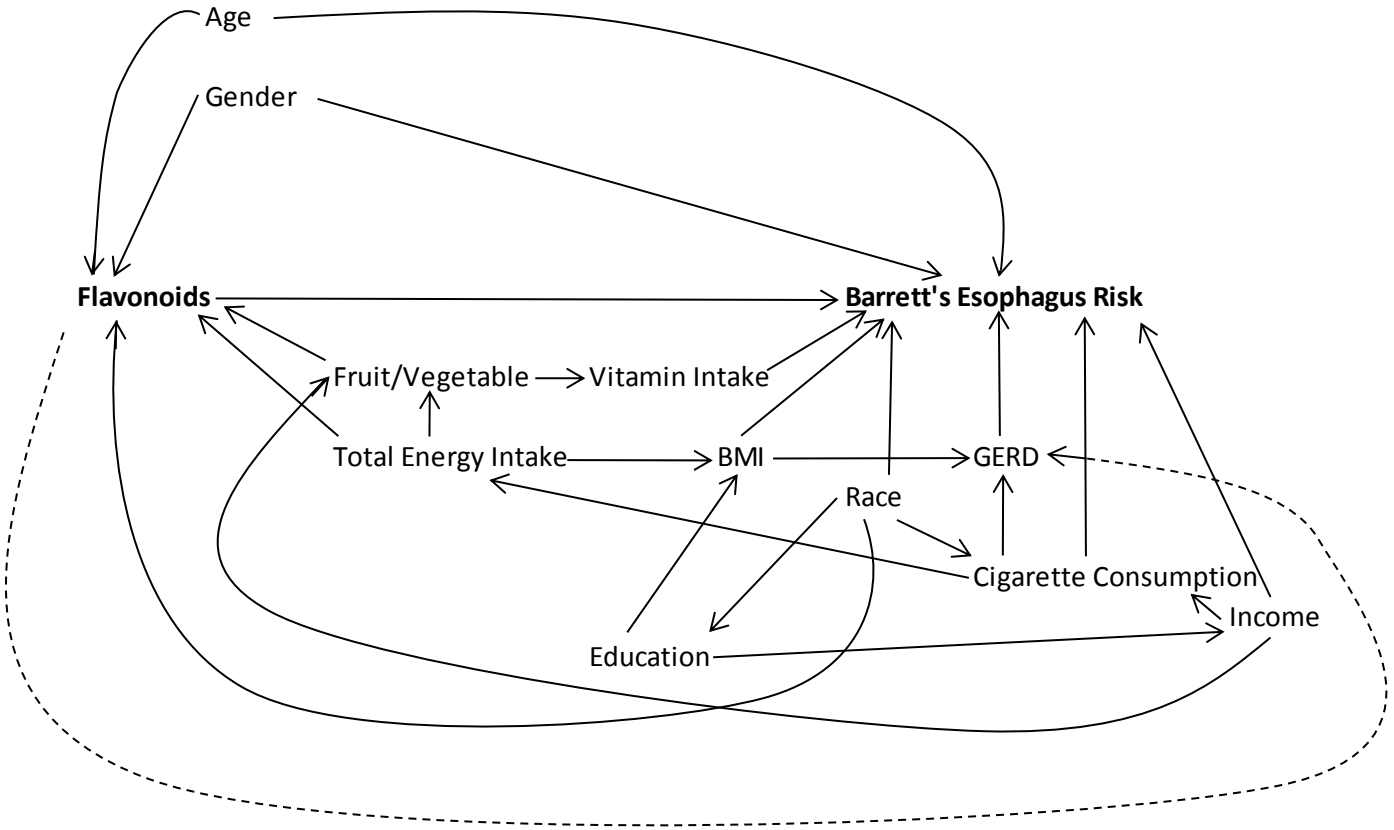


Figure 2.4. Directed Acyclic Graph (DAG) of Potential Confounders of the Association Between Dietary Flavonoid Intake and Survival Among Cases of Esophageal/Gastric Cardia Adenocarcinoma. - - - Denotes a pathway that may not be present for all EA/GCA cases.

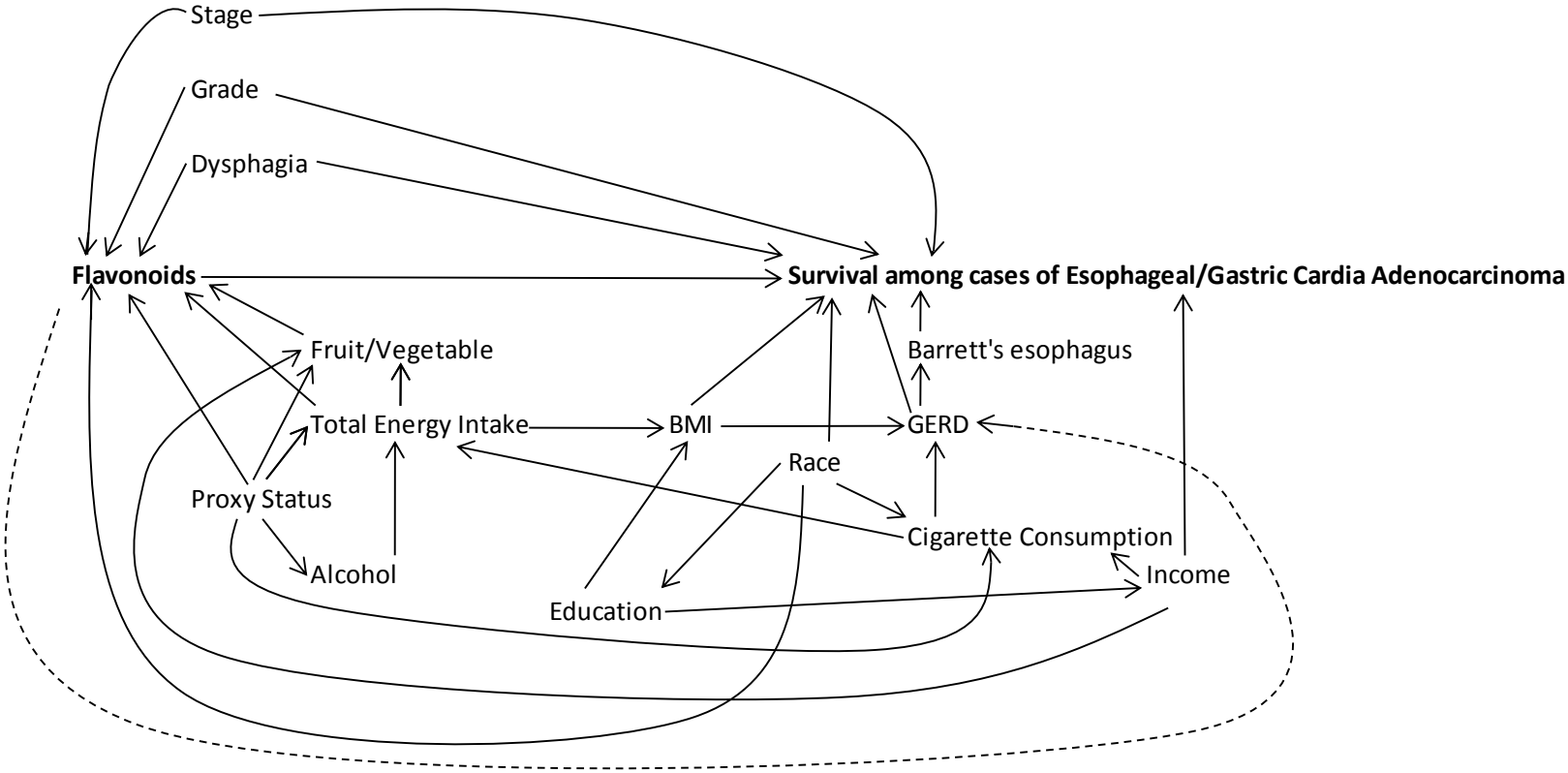


Figure 2.5. Directed Acyclic Graph (DAG) of Potential Confounders of the Association Between Dietary Flavonoid Intake and Survival Among Cases of Esophageal Squamous Cell Carcinoma/Non-cardia Gastric Adenocarcinoma.

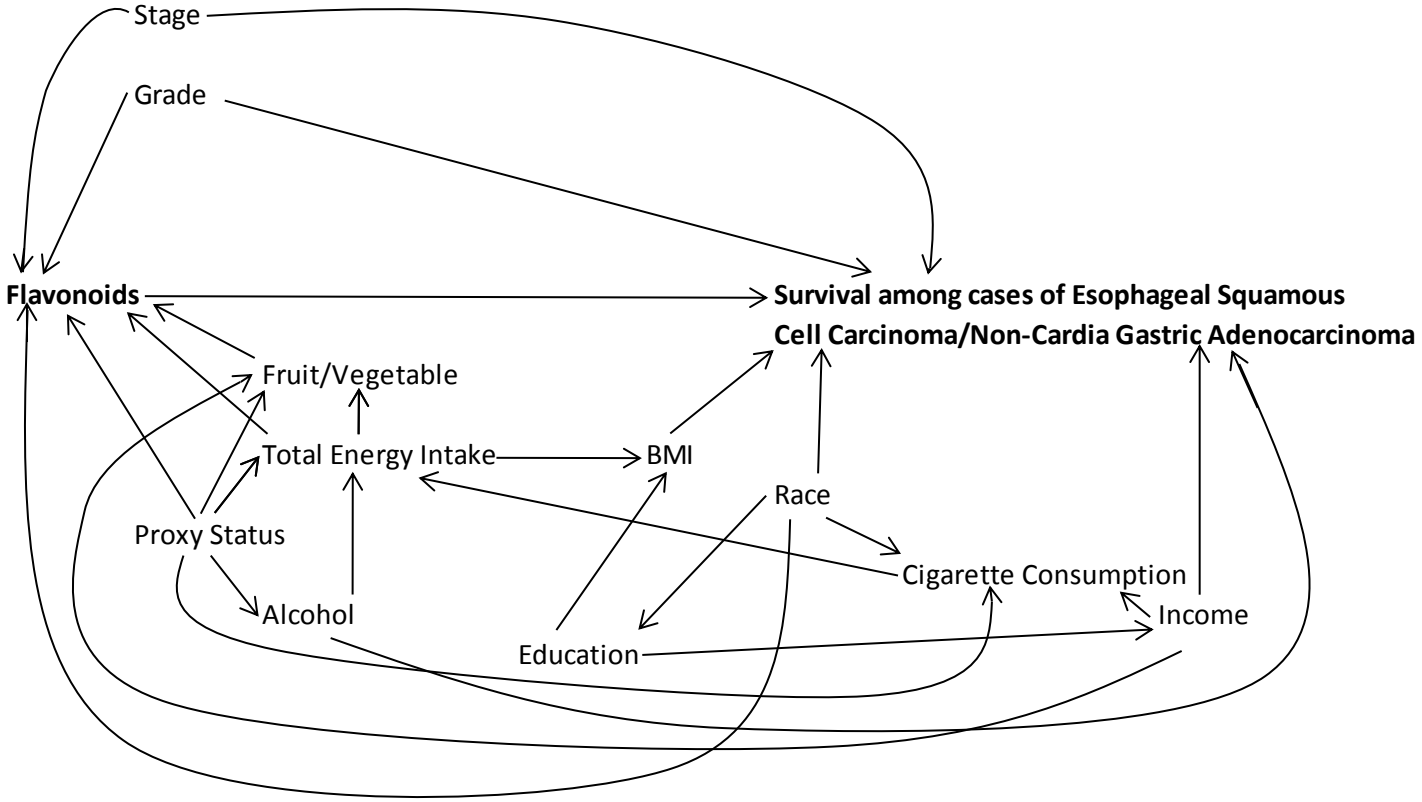


Table 2.3. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Flavonoid and Lignan Intake and Risks of Esophageal and Gastric Cancer, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

			Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma	
Women's intake (mg/day)[2]	Men's intake (mg/day)[2]	Controls (N=662)	Cases (N=274)	OR (95% CI)	Cases (N=248)	OR (95% CI)
Total Flavonoids						
0-63.73	0-63.91	166	84	1.00	59	1.00
63.74-104.32	63.92-96.36	165	52	0.59 (0.39, 0.90)	59	0.99 (0.65, 1.53)
104.33-304.96	96.37-191.03	166	53	0.59 (0.39, 0.90)	59	1.00 (0.65, 1.54)
≥304.97	≥191.04	165	85	0.96 (0.66, 1.42)	71	1.23 (0.81, 1.88)
P for trend[3]				0.61		0.07
Anthocyanidins						
0-7.57	0-7.11	165	99	1.00	67	1.00
7.58-11.25	7.12-11.87	166	69	0.63 (0.43, 0.93)	70	1.00 (0.66, 1.49)
11.26-18.14	11.88-19.08	166	59	0.53 (0.35, 0.79)	60	0.85 (0.56, 1.30)
≥18.15	≥19.09	165	47	0.43 (0.28, 0.65)	51	0.72 (0.46, 1.11)
P for trend[3]				0.06		0.91
Flavan-3-ols						
0-9.70	0-10.55	166	76	1.00	58	1.00
9.71-18.85	10.56-22.16	165	59	0.69 (0.46, 1.04)	51	0.80 (0.52, 1.25)
18.86-215.10	22.17-101.85	165	56	0.65 (0.42, 0.98)	69	1.08 (0.71, 1.66)
≥215.11	≥101.86	166	83	1.02 (0.69, 1.51)	70	1.19 (0.78, 1.81)
P for trend[3]				0.32		0.07
Flavanones						
0-13.22	0-10.74	165	91	1.00	61	1.00
13.23-39.40	10.75-34.47	166	64	0.70 (0.47, 1.03)	57	0.92 (0.60, 1.40)
39.41-51.14	34.48-48.75	166	61	0.65 (0.44, 0.97)	56	0.96 (0.62, 1.47)
≥51.15	≥48.76	165	58	0.61 (0.41, 0.92)	74	1.25 (0.82, 1.90)
P for trend[3]				0.003		0.82
Flavones						
0-1.39	0-1.25	165	84	1.00	59	1.00
1.40-1.90	1.26-1.88	166	55	0.61 (0.40, 0.92)	55	0.87 (0.56, 1.33)
1.91-2.53	1.89-2.62	165	60	0.67 (0.45, 1.01)	65	1.03 (0.67, 1.57)
≥2.54	≥2.63	166	75	0.81 (0.54, 1.21)	69	1.06 (0.69, 1.63)
P for trend[3]				0.81		0.15
Flavonols						
0-8.07	0-8.32	165	84	1.00	51	1.00
8.08-12.55	8.33-11.99	166	51	0.59 (0.39, 0.89)	65	1.28 (0.83, 1.97)
12.56-20.80	12.00-17.58	165	64	0.72 (0.48, 1.07)	56	1.10 (0.71, 1.71)
≥20.81	≥17.59	166	75	0.77 (0.52, 1.14)	76	1.35 (0.88, 2.08)
P for trend[3]				0.71		0.10

(Table 2.3. continued)

Women's intake (mg/day)[2]	Men's intake (mg/day)[2]	Controls (N=662)	Esophageal Adenocarcinoma Cases (N=274)	Esophageal Adenocarcinoma OR (95% CI)	Gastric Cardia Adenocarcinoma Cases (N=248)	Gastric Cardia Adenocarcinoma OR (95% CI)
Isoflavones						
0-0.24	0-0.27	166	49	1.00	38	1.00
0.25-0.37	0.28-0.42	165	50	0.95 (0.60, 1.50)	62	1.48 (0.93, 2.36)
0.38-0.52	0.43-0.59	166	78	1.39 (0.89, 2.18)	69	1.51 (0.93, 2.43)
≥0.53	≥0.60	165	97	1.72 (1.08, 2.74)	79	1.66 (1.00, 2.73)
P for trend[3]				0.07		0.17
Lignans						
0-0.043	0-0.045	166	69	1.00	50	1.00
0.044-0.062	0.046-0.062	165	76	0.96 (0.64, 1.43)	61	1.10 (0.71, 1.71)
0.063-0.081	0.063-0.082	166	56	0.64 (0.42, 0.98)	68	1.15 (0.74, 1.77)
≥0.082	≥0.083	165	73	0.78 (0.52, 1.19)	69	1.07 (0.69, 1.67)
P for trend[3]				0.26		0.63

Women's intake (mg/day)[2]	Men's intake (mg/day)[2]	Esophageal Squamous Cell Carcinoma Cases (N=191)	Esophageal Squamous Cell Carcinoma OR (95% CI)	Other Gastric Adenocarcinoma Cases (N=341)	Other Gastric Adenocarcinoma OR (95% CI)
Total Flavonoids					
0-63.73	0-63.91	48	1.00	80	1.00
63.74-104.32	63.92-96.36	36	0.66 (0.39, 1.09)	86	1.02 (0.69, 1.50)
104.33-304.96	96.37-191.03	62	1.11 (0.70, 1.78)	84	0.95 (0.64, 1.40)
≥304.97	≥191.04	45	0.84 (0.52, 1.37)	91	1.04 (0.71, 1.54)
P for trend[3]			0.42		0.50
Anthocyanidins					
0-7.57	0-7.11	69	1.00	93	1.00
7.58-11.25	7.12-11.87	52	0.66 (0.42, 1.03)	89	0.92 (0.63, 1.33)
11.26-18.14	11.88-19.08	34	0.40 (0.24, 0.65)	94	0.91 (0.63, 1.33)
≥18.15	≥19.09	36	0.43 (0.26, 0.70)	65	0.65 (0.44, 0.98)
P for trend[3]			0.11		0.10
Flavan-3-ols					
0-9.70	0-10.55	45	1.00	70	1.00
9.71-18.85	10.56-22.16	36	0.74 (0.44, 1.24)	98	1.41 (0.96, 2.09)
18.86-215.10	22.17-101.85	65	1.24 (0.78, 1.99)	79	1.06 (0.71, 1.60)
≥215.11	≥101.86	45	0.93 (0.57, 1.53)	94	1.26 (0.85, 1.87)
P for trend[3]			0.68		0.36

(Table 2.3. continued)

Women's intake (mg/day)[2]	Men's intake (mg/day)[2]	Esophageal Squamous Cell Carcinoma Cases (N=191)	OR (95% CI)	Other Gastric Adenocarcinoma Cases (N=341)	OR (95% CI)
Flavanones					
0-13.22	0-10.74	61	1.00	87	1.00
13.23-39.40	10.75-34.47	47	0.73 (0.46, 1.15)	84	0.98 (0.67, 1.43)
39.41-51.14	34.48-48.75	45	0.62 (0.39, 0.99)	81	0.85 (0.58, 1.25)
≥51.15	≥48.76	38	0.50 (0.31, 0.82)	89	0.93 (0.64, 1.37)
P for trend[3]			0.002		0.36
Flavones					
0-1.39	0-1.25	70	1.00	96	1.00
1.40-1.90	1.26-1.88	51	0.71 (0.46, 1.11)	86	0.93 (0.64, 1.36)
1.91-2.53	1.89-2.62	29	0.36 (0.22, 0.60)	70	0.77 (0.52, 1.13)
≥2.54	≥2.63	41	0.55 (0.34, 0.90)	89	1.03 (0.70, 1.53)
P for trend[3]			0.02		0.98
Flavonols					
0-8.07	0-8.32	56	1.00	81	1.00
8.08-12.55	8.33-11.99	34	0.59 (0.36, 0.97)	97	1.16 (0.80, 1.69)
12.56-20.80	12.00-17.58	41	0.74 (0.46, 1.20)	85	1.02 (0.69, 1.50)
≥20.81	≥17.59	60	0.89 (0.56, 1.41)	78	0.90 (0.61, 1.34)
P for trend[3]			0.70		0.92
Isoflavones					
0-0.24	0-0.27	32	1.00	62	1.00
0.25-0.37	0.28-0.42	53	1.34 (0.80, 2.25)	87	1.49 (0.99, 2.24)
0.38-0.52	0.43-0.59	56	1.17 (0.69, 2.01)	96	1.68 (1.10, 2.56)
≥0.53	≥0.60	50	0.84 (0.47, 1.49)	96	1.67 (1.07, 2.62)
P for trend[3]			0.11		0.37
Lignans					
0-0.043	0-0.045	66	1.00	84	1.00
0.044-0.062	0.046-0.062	48	0.66 (0.42, 1.04)	93	1.10 (0.75, 1.60)
0.063-0.081	0.063-0.082	42	0.51 (0.31, 0.82)	100	1.16 (0.79, 1.70)
≥0.082	≥0.083	35	0.39 (0.23, 0.64)	64	0.75 (0.49, 1.14)
P for trend[3]			0.0003		0.13

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]Sex-specific quartiles. [3]P-value for trend for continuous variable.

Table 2.4. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Flavonoid and Lignan Intake and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Women (mg/day)	Men (mg/day)	Controls (N=183)	Cases (N=170)	OR (95% CI)[2]
Total Flavonoids				
0-58.64	0-37.61	46	49	
58.65-100.80	37.62-60.71	45	33	0.82 (0.43, 1.59)
100.81-198.70	60.72-146.89	46	50	1.29 (0.70, 2.39)
≥198.71	≥146.90	46	38	0.97 (0.51, 1.86)
P for trend[3]				
Anthocyanidins				
0-7.04	0-5.70	46	62	
7.05-13.13	5.71-9.37	46	38	0.54 (0.30, 0.99)
13.14-22.21	9.38-17.00	46	34	0.47 (0.25, 0.88)
≥22.22	≥17.01	45	36	0.60 (0.32, 1.14)
P for trend[3]				
Flavan-3-ols				
0-12.50	0-8.31	45	45	
12.51-27.05	8.32-15.60	46	22	0.51 (0.25, 1.02)
27.06-148.91	15.61-68.59	45	60	1.63 (0.88, 3.00)
≥148.92	≥68.60	47	43	1.09 (0.58, 2.07)
P for trend[3]				
Flavanones				
0-5.37	0-3.38	46	60	
5.38-15.79	3.39-11.21	46	38	0.69 (0.37, 1.27)
15.80-30.52	11.22-25.18	45	37	0.68 (0.37, 1.26)
≥30.53	≥25.19	46	35	0.70 (0.37, 1.32)
P for trend[3]				
Flavones				
0-1.09	0-1.24	45	40	
1.10-1.87	1.25-1.93	47	38	0.90 (0.47, 1.73)
1.88-2.41	1.94-3.00	45	50	1.33 (0.69, 2.54)
≥2.42	≥3.01	46	42	0.98 (0.50, 1.92)
P for trend[3]				
Flavonols				
0-7.78	0-6.89	46	54	
7.79-12.00	6.90-9.65	45	39	0.76 (0.41, 1.43)
12.01-15.23	9.66-14.04	46	30	0.56 (0.29, 1.09)
≥15.24	≥14.05	46	47	0.94 (0.50, 1.77)
P for trend[3]				

(Table 2.4. continued)

Women (mg/day)	Men (mg/day)	Controls (N=183)	Cases (N=170)	OR (95% CI)[2]
Isoflavones				
0-0.21	0-0.28	46	41	
0.22-0.49	0.29-0.54	46	55	1.19 (0.64, 2.24)
0.50-0.98	0.55-1.16	46	36	0.78 (0.40, 1.51)
≥0.99	≥1.17	45	38	0.77 (0.39, 1.52)
P for trend[3]				
Lignans				
0-0.036	0-0.032	46	55	
0.037-0.052	0.034-0.051	45	49	0.84 (0.46, 1.56)
0.053-0.076	0.052-0.067	47	29	0.46 (0.23, 0.93)
≥0.077	≥0.068	45	37	0.62 (0.31, 1.23)
P for trend[3]				

[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]Sex-specific quartiles. [3]P-value for trend for continuous variable.

Figure 2.6. Restricted Quadratic Spline Graph of the Unadjusted Odds Ratios (represented by the solid line) and 95% Confidence Intervals (represented by the dotted lines) of the Association Between Total Flavonoid Intake and Esophageal and Gastric Cardia Adenocarcinoma, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Figure 2.6A: Esophageal Adenocarcinoma

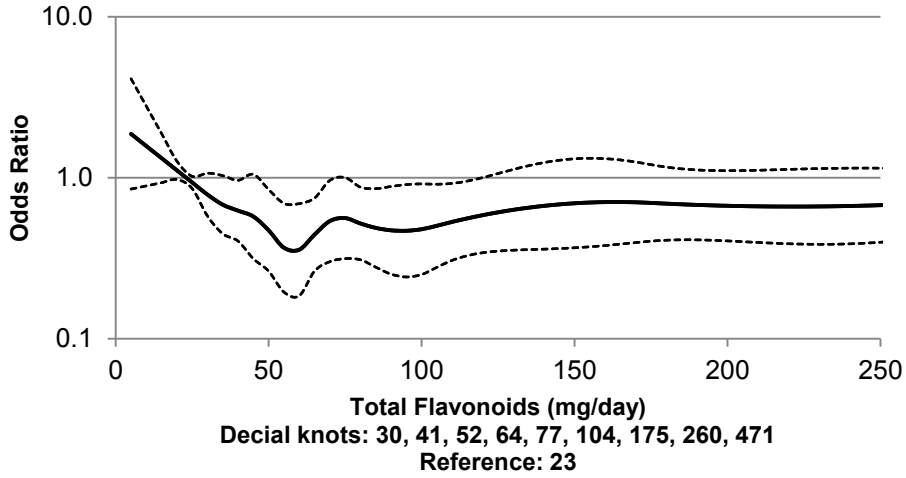


Figure 2.6B: Esophageal Adenocarcinoma

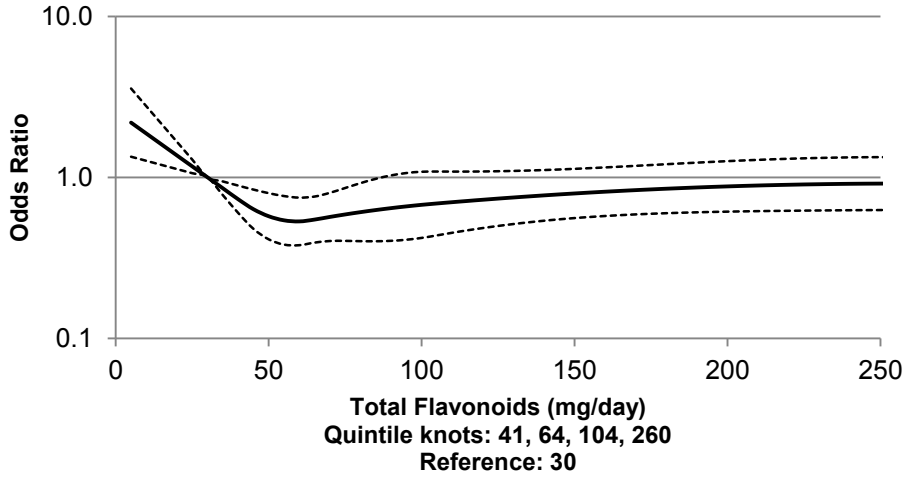


Figure 2.6C: Esophageal Adenocarcinoma

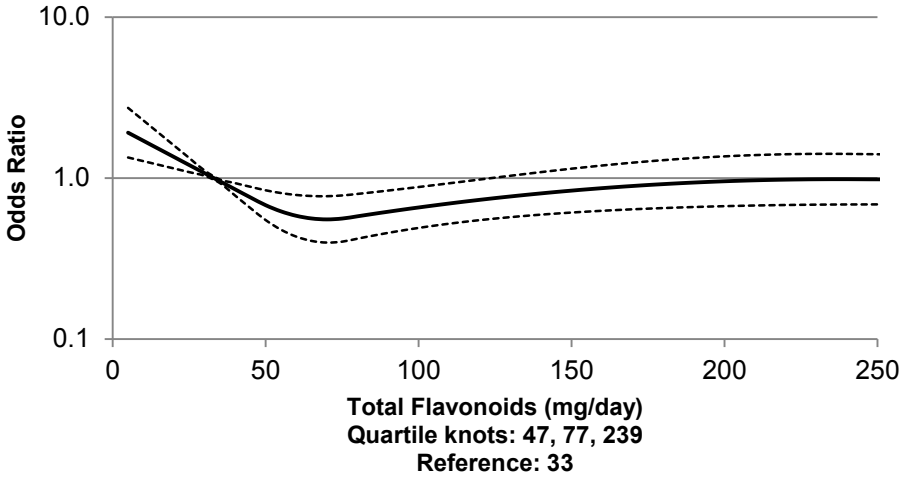


Figure 2.6D: Gastric Cardia Adenocarcinoma

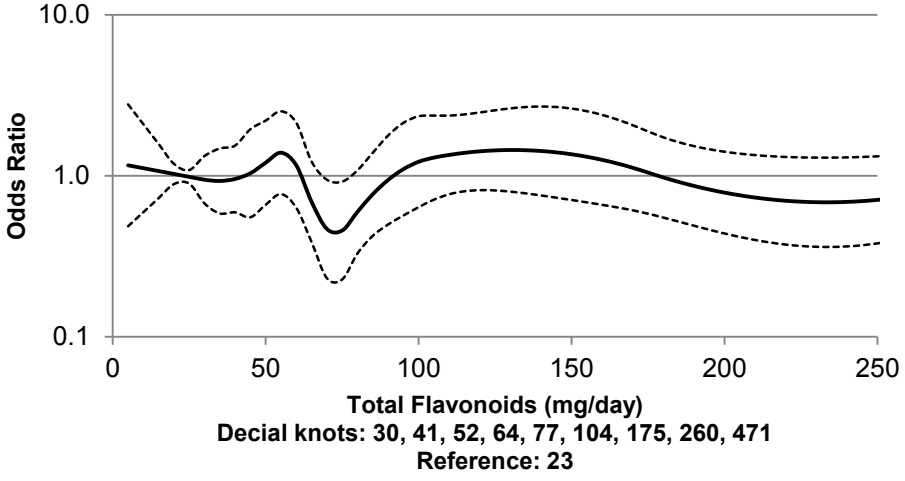


Figure 2.6E: Gastric Cardia Adenocarcinoma

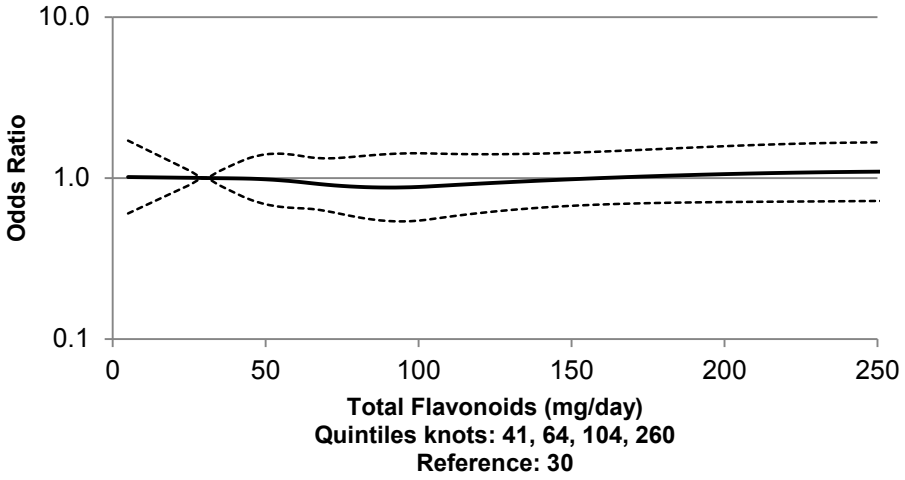


Figure 2.6F: Gastric Cardia Adenocarcinoma

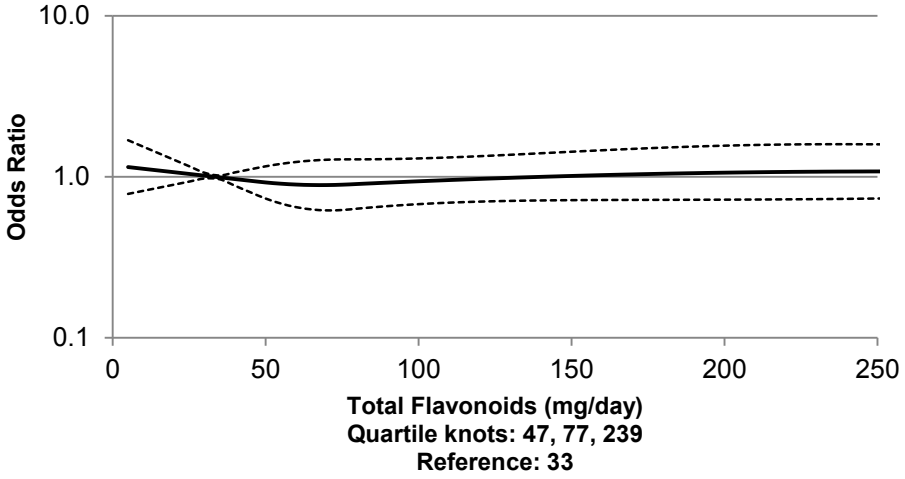


Table 2.5. Adjusted[1] Odds Ratios for Cyclin D1 Positive (+) and Cyclin D1 Negative (-) Esophageal and Gastric Cancer in Relation to Total Flavonoid Intake by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Tumor type and intake (mg/day)	Controls (N=662)	Cyclin D1+ Cases		Cyclin D1- Cases		Ratio of the ORs (95% CI)
		Cases (N=266)	OR (95% CI)[2]	Cases (N=333)	OR (95% CI)[2]	
Esophageal Adenocarcinoma						
0-63.81	166	14	1.00	25	1.00	
≥63.82	496	64	1.39 (0.74, 2.62)	50	0.53 (0.31, 0.91)	2.70 (1.22, 5.99)
Gastric Cardia Adenocarcinoma						
0-63.81	166	13	1.00	22	1.00	
≥63.82	496	39	0.91 (0.46, 1.80)	68	1.00 (0.58, 1.72)	0.79 (0.33, 1.86)
Esophageal Squamous Cell Carcinoma						
0-63.81	166	13	1.00	8	1.00	
≥63.82	496	69	1.47 (0.76, 2.84)	17	0.61 (0.24, 1.53)	2.63 (0.85, 8.11)
Non-Cardia Gastric Adenocarcinoma						
0-63.81	166	11	1.00	32	1.00	
≥63.82	496	43	1.12 (0.54, 2.30)	111	1.00 (0.63, 1.59)	1.01 (0.44, 2.32)

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]Odds ratio (95% confidence interval).

Table 2.6. Adjusted[1] Odds Ratios for P53 Positive (+) and P53 Negative (-) Esophageal and Gastric Cancer in Relation to Total Flavonoid Intake by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Tumor type and intake (mg/day)	Controls (N=662)	P53+ Cases		P53- Cases		Ratio of the ORs (95% CI)
		Cases (N=389)	OR (95% CI)[2]	Cases (N=225)	OR (95% CI)[2]	
Esophageal Adenocarcinoma						
0-63.81	166	33	1.00	14	1.00	
≥63.82	496	84	0.75 (0.47, 1.20)	32	0.60 (0.30, 1.19)	1.09 (0.49, 2.42)
Gastric Cardia Adenocarcinoma						
0-63.81	166	25	1.00	10	1.00	
≥63.82	496	73	0.99 (0.59, 1.65)	32	0.90 (0.42, 1.93)	1.23 (0.49, 3.07)
Esophageal Squamous Cell Carcinoma						
0-63.81	166	10	1.00	15	1.00	
≥63.82	496	57	1.52 (0.73, 3.14)	18	0.36 (0.16, 0.79)	5.60 (1.80, 17.46)
Non-Cardia Gastric Adenocarcinoma						
0-63.81	166	19	1.00	25	1.00	
≥63.82	496	88	1.27 (0.73, 2.21)	79	0.94 (0.56, 1.58)	1.24 (0.61, 2.51)

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]Odds ratio (95% confidence interval).

Table 2.7. Adjusted[1] Odds Ratios for Esophageal and Gastric Cancer Stage[2] in Relation to Total Flavonoid Intake by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Localized		Regional		Distant		Ratio of Localized/Distant (95% CI)
Tumor type and intake (mg/day)	Controls (N=662)	Cases (N=217)	OR (95% CI)[3]	Cases (N=404)	OR (95% CI)[3]	Cases (N=271)	OR (95% CI)[3]	
Esophageal Adenocarcinoma								
0-63.81	166	31	1.00	24	1.00	15	1.00	
≥63.82	496	42	0.33 (0.20, 0.57)	46	0.61 (0.35, 1.06)	60	1.36 (0.74, 2.53)	0.31 (0.14, 0.70)
Gastric Cardia Adenocarcinoma								
0-63.81	166	8	1.00	24	1.00	17	1.00	
≥63.82	496	23	0.88 (0.37, 2.08)	90	1.22 (0.73, 2.02)	51	1.04 (0.57, 1.90)	0.78 (0.25, 2.43)
Esophageal Squamous Cell Carcinoma								
0-63.81	166	10	1.00	19	1.00	7	1.00	
≥63.82	496	35	1.06 (0.49, 2.30)	53	0.81 (0.45, 1.48)	26	0.98 (0.40, 2.40)	1.09 (0.32, 3.76)
Non-Cardia Gastric Adenocarcinoma								
0-63.81	166	17	1.00	42	1.00	17	1.00	
≥63.82	496	51	0.87 (0.48, 1.60)	106	0.77 (0.51, 1.18)	78	1.44 (0.81, 2.54)	0.67 (0.31, 1.46)

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]ORs not estimated for unknown cancer stage. [3]Odds ratio (95% confidence interval).

Table 2.8. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) from Conditional Logistic Regression Models for the Association Between Flavonoid and Lignan Intake and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Variable and intake (mg/day)	Controls (N=155)	Cases (N=144)	OR	95% CI
Total Flavonoids				
0-42.38	38	38	1.00	
42.39-75.36	39	32	1.05	0.48, 2.29
75.37-166.98	38	42	1.34	0.65, 2.75
≥166.99	40	32	1.13	0.52, 2.47
P for trend[2]			0.80	
Anthocyanidins				
0-6.12	39	53	1.00	
6.13-9.82	37	20	0.26	0.11, 0.61
9.83-18.26	39	40	0.62	0.31, 1.23
≥18.27	40	31	0.59	0.26, 1.31
P for trend[2]			0.94	
Flavan-3-ols				
0-9.50	37	39	1.00	
9.51-17.35	38	18	0.40	0.16, 0.97
17.36-107.34	42	60	1.49	0.75, 2.97
≥107.35	38	27	0.91	0.42, 1.98
P for trend[2]			0.56	
Flavanones				
0-3.80	36	45	1.00	
3.81-12.90	40	34	0.69	0.30, 1.58
12.91-29.64	38	36	0.79	0.38, 1.62
≥29.65	41	29	0.72	0.35, 1.49
P for trend[2]			0.26	
Flavones				
0-1.15	39	30	1.00	
1.16-1.88	36	32	1.16	0.51, 2.63
1.89-2.82	39	43	1.62	0.74, 3.54
≥2.83	41	39	1.32	0.63, 2.76
P for trend[2]			0.62	
Flavonols				
0-6.99	39	44	1.00	
7.00-10.86	36	36	1.07	0.54, 2.12
10.87-14.89	42	25	0.74	0.36, 1.53
≥14.90	38	39	1.21	0.57, 2.58
P for trend[2]			0.77	
Isoflavones				
0-0.24	39	32	1.00	
0.25-0.52	38	49	1.44	0.67, 3.12
0.53-1.16	42	32	0.75	0.35, 1.61
≥1.17	36	31	0.68	0.27, 1.72
P for trend[2]			0.25	

(Table 2.8. continued)

Variable and intake (mg/day)	Controls (N=155)	Cases (N=144)	OR	95% CI
Lignans				
0-0.033	38	45	1.00	
0.034-0.051	40	43	0.72	0.36, 1.45
0.052-0.070	41	25	0.40	0.17, 0.93
≥0.071	36	31	0.59	0.26, 1.31
P for trend[2]			0.33	

[1]Adjusted for age (continuous), sex, body mass index (continuous), and dietary energy intake (kilocalories, continuous). [2]P-value for trend for continuous variable.

Table 2.9. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Effect Measure Modification[2] Between Total Flavonoid Intake and Body Mass Index (BMI) and Esophageal and Gastric Cancer, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Total Flavonoids	Controls (N=662)	Cases (N=274)	OR (95% CI)	Cases (N=248)	OR (95% CI)	Cases (N=191)	OR (95% CI)	Cases (N=341)	OR (95% CI)
Overall	662	274	1.02 (0.97, 1.07)	248	0.97 (0.90, 1.04)	191	1.05 (0.99, 1.11)	341	1.01 (0.96, 1.07)
BMI <25	325	96	1.07 (0.99, 1.16)	98	1.02 (0.93, 1.12)	119	1.09 (1.00, 1.19)	173	1.04 (0.94, 1.15)
BMI ≥25	337	178	1.25 (0.91, 1.72)	150	0.73 (0.49, 1.09)	72	1.67 (1.16, 2.39)	168	1.95 (1.37, 2.80)

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), body mass index (<25, ≥25 kg/m^2), and dietary energy intake (kilocalories, continuous). [2]Likelihood ratio test (LRT) for continuous total flavonoid intake (per 100 mg/day) and dichotomized BMI (<25 versus ≥25 kg/m^2): χ^2=4.357, d.f.=4, p_{int}=0.36; LRT for continuous flavonoid intake (per 100 mg/day) and continuous BMI (kg/m^2): χ^2=2.725, d.f.=4, p_{int}=0.60.

Table 2.10. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Effect Measure Modification[2] Between Total Flavonoid Intake and Cigarette Smoking and Esophageal and Gastric Cancer, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Total Flavonoids	Controls (N=662)	Cases (N=274)	OR (95% CI)†	Cases (N=248)	OR (95% CI)†	Cases (N=191)	OR (95% CI)†	Cases (N=341)	OR (95% CI)†
Overall	662	274	1.02 (0.97, 1.07)	248	0.97 (0.90, 1.04)	191	1.05 (1.00, 1.11)	341	1.02 (0.96, 1.08)
Never Smoker	237	61	1.07 (0.96, 1.18)	52	0.85 (0.61, 1.19)	16	1.08 (0.95, 1.22)	96	1.05 (0.92, 1.18)
Ever Smoker	425	213	1.73 (1.18, 2.52)	196	4.64 (2.18, 9.87)	175	2.19 (1.40, 3.42)	244	1.96 (1.28, 3.00

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), and dietary energy intake (kilocalories, continuous). [2]Likelihood ratio test (LRT) for continuous total flavonoid intake (per 100 mg/day) and dichotomized cigarette smoking (ever/never): χ^2=1.907, d.f.=4, p_{int}=0.75; LRT for continuous flavonoid intake (per 100 mg/day) and continuous cigarette smoking duration (years): χ^2=1.900, d.f.=4, p_{int}=0.75.

Table 2.11. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Effect Measure Modification[2] Between Total Flavonoid Intake and Gastroesophageal Reflux Disease and Esophageal and Gastric Cancer, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Total Flavonoids	Controls (N=662)	Cases (N=274)	OR (95% CI)†	Cases (N=248)	OR (95% CI)†	Cases (N=191)	OR (95% CI)†	Cases (N=341)	OR (95% CI)†
Overall	662	274	1.02 (0.97, 1.07)	248	0.97 (0.90, 1.05)	191	1.05 (1.00, 1.11)	341	1.02 (0.96, 1.08)
No GERD	473	123	0.96 (0.89, 1.03)	153	0.97 (0.89, 1.05)	154	1.04 (0.98, 1.12)	211	1.01 (0.93, 1.09)
GERD	189	151	1.23 (0.88, 1.72)	95	0.52 (0.33, 0.82)	37	1.47 (1.03, 2.11)	130	2.88 (2.03, 4.08)

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), and dietary energy intake (kilocalories, continuous). [2]Likelihood ratio test (LRT) for continuous total flavonoid intake (per 100 mg/day) and dichotomized GERD (yes/no): χ^2=7.143, d.f.=4, p_{int}=0.13.

Table 2.12. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Effect Measure Modification[2] Between Total Flavonoid Intake and Body Mass Index (BMI) and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Total Flavonoids per 100 mg/day[b]	Controls (N=183)	Cases (N=170)	OR	95% CI
Overall	183	170	1.03	0.86, 1.23
BMI <25	57	33	1.08	0.80, 1.47
BMI ≥25	126	137	1.81	0.89, 3.65

[1]Adjusted for age (continuous), sex, body mass index (<25, ≥25 kg/m^2), and dietary energy intake (kilocalories, continuous). [2]Likelihood ratio test (LRT) for continuous total flavonoid intake (per 100 mg/day) and dichotomized BMI (<25 versus ≥25 kg/m^2): χ^2=0.155, d.f.=1, p_{int}=0.69; LRT for continuous flavonoid intake (per 100 mg/day) and continuous BMI (kg/m^2): χ^2=0.298, d.f.=1, p_{int}=0.59.

Table 2.13. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Effect Measure Modification[2] Between Total Flavonoid Intake and Cigarette Smoking and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Total Flavonoids per 100 mg/day[b]	Controls (N=183)	Cases (N=170)	OR	95% CI
Overall	183	170	1.04	0.87, 1.25
Never Smoker	94	61	1.06	0.80, 1.41
Ever Smoker	89	109	2.17	1.20, 3.90

[1]Adjusted for age (continuous), sex, body mass index (continuous), cigarette smoking (ever/never), and dietary energy intake (kilocalories, continuous). [2]Likelihood ratio test (LRT) for continuous total flavonoid intake (per 100 mg/day) and dichotomized cigarette smoking (ever/never): χ^2=0.035, d.f.=1, p_{int}=0.85; LRT for continuous flavonoid intake (per 100 mg/day) and continuous cigarette smoking (pack-years): χ^2=2.126, d.f.=1, p_{int}=0.14.

Table 2.14. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Flavonoid and Lignan Intake Among Non-proxy Incident Esophageal and Gastric Cancers, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Variable and intake (mg/day)	Controls (N=643)	Cases (N=190)	OR (95% CI)	Cases (N=182)	OR (95% CI)	Cases (N=128)	OR (95% CI)	Cases (N=241)	OR (95% CI)
Total Flavonoids									
0-63.81	161	69	1.00	36	1.00	31	1.00	56	1.00
63.82-97.90	162	33	0.46 (0.28, 0.74)	45	1.21 (0.73, 1.99)	22	0.65 (0.35, 1.19)	56	0.99 (0.64, 1.54)
97.91-217.35	158	33	0.45 (0.28, 0.73)	41	1.09 (0.65, 1.82)	41	1.19 (0.69, 2.07)	59	1.04 (0.66, 1.62)
≥217.36	162	55	0.81 (0.52, 1.25)	60	1.74 (1.07, 2.82)	34	0.99 (0.56, 1.74)	70	1.15 (0.75, 1.77)
P for trend[2]			0.86		0.03		0.97		0.38
Anthocyanidins									
0-7.21	159	71	1.00	47	1.00	47	1.00	66	1.00
7.22-11.53	162	48	0.62 (0.40, 0.96)	51	1.03 (0.65, 1.63)	37	0.68 (0.41, 1.14)	64	0.89 (0.58, 1.35)
11.54-18.47	162	36	0.43 (0.27, 0.70)	42	0.80 (0.49, 1.31)	22	0.34 (0.19, 0.62)	61	0.78 (0.50, 1.20)
≥18.48	160	35	0.43 (0.27, 0.70)	42	0.81 (0.50, 1.31)	22	0.36 (0.20, 0.65)	50	0.67 (0.43, 1.04)
P for trend[2]			0.32		0.64		0.07		0.13
Flavan-3-ols									
0-10.29	160	53	1.00	38	1.00	32	1.00	50	1.00
10.30-22.00	164	45	0.71 (0.44, 1.13)	35	0.78 (0.47, 1.31)	19	0.54 (0.29, 1.02)	72	1.40 (0.90, 2.17)
22.01-130.69	157	37	0.59 (0.36, 0.97)	54	1.20 (0.73, 1.95)	43	1.23 (0.71, 2.13)	47	0.96 (0.60, 1.54)
≥130.70	162	55	0.99 (0.63, 1.55)	55	1.39 (0.86, 2.25)	34	0.97 (0.55, 1.70)	72	1.35 (0.87, 2.08)
P for trend[2]			0.48		0.03		0.76		0.31
Flavanones									
0-11.57	161	71	1.00	40	1.00	39	1.00	56	1.00
11.58-34.95	163	47	0.65 (0.42, 1.00)	42	1.02 (0.63, 1.68)	32	0.80 (0.47, 1.37)	58	1.04 (0.67, 1.61)
34.96-49.52	160	39	0.56 (0.36, 0.89)	43	1.15 (0.70, 1.88)	34	0.81 (0.47, 1.39)	59	1.02 (0.66, 1.59)
≥49.53	159	33	0.47 (0.29, 0.76)	57	1.48 (0.92, 2.40)	23	0.48 (0.27, 0.87)	68	1.10 (0.71, 1.70)
P for trend[2]			0.0001		0.40		0.02		0.82

(Table 2.14. continued)

Variable and intake (mg/day)	Controls (N=643)	Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
		Cases (N=190)	OR (95% CI)	Cases (N=182)	OR (95% CI)	Cases (N=128)	OR (95% CI)	Cases (N=241)	OR (95% CI)
Flavones									
0-1.29	157	62	1.00	40	1.00	50	1.00	65	1.00
1.30-1.90	164	33	0.47 (0.29, 0.76)	41	0.91 (0.55, 1.49)	35	0.63 (0.38, 1.06)	65	0.95 (0.63, 1.45)
1.91-2.62	160	42	0.62 (0.39, 0.99)	47	1.06 (0.65, 1.73)	18	0.27 (0.15, 0.51)	49	0.70 (0.45, 1.10)
≥2.63	162	53	0.74 (0.47, 1.16)	54	1.16 (0.71, 1.89)	25	0.42 (0.24, 0.76)	62	0.94 (0.61, 1.47)
P for trend[2]			0.83		0.10		0.008		0.82
Flavonols									
0-8.31	163	62	1.00	37	1.00	40	1.00	62	1.00
8.32-12.16	160	31	0.49 (0.30, 0.79)	45	1.18 (0.72, 1.94)	17	0.40 (0.21, 0.74)	60	0.96 (0.63, 1.48)
12.17-17.81	160	42	0.65 (0.41, 1.02)	38	1.00 (0.60, 1.67)	27	0.68 (0.39, 1.19)	58	0.95 (0.62, 1.46)
≥17.82	160	55	0.80 (0.51, 1.25)	62	1.52 (0.94, 2.45)	44	0.94 (0.56, 1.58)	61	0.92 (0.60, 1.43)
P for trend[2]			0.78		0.04		0.65		0.94
Isoflavones									
0-0.27	158	34	1.00	22	1.00	25	1.00	36	1.00
0.28-0.41	160	31	0.78 (0.45, 1.34)	46	1.70 (0.97, 3.00)	33	1.01 (0.55, 1.85)	64	1.83 (1.13, 2.96)
0.42-0.59	165	55	1.27 (0.76, 2.12)	56	1.84 (1.04, 3.26)	41	1.16 (0.63, 2.13)	73	2.21 (1.35, 3.62)
≥0.60	160	70	1.54 (0.90, 2.63)	58	1.77 (0.97, 3.25)	29	0.61 (0.31, 1.22)	68	1.97 (1.16, 3.34)
P for trend[2]			0.06		0.21		0.14		0.30
Lignans									
0-0.045	159	52	1.00	32	1.00	46	1.00	59	1.00
0.046-0.063	160	53	0.87 (0.55, 1.36)	39	1.04 (0.62, 1.77)	33	0.67 (0.40, 1.15)	61	1.07 (0.69, 1.66)
0.064-0.082	163	34	0.51 (0.31, 0.84)	59	1.49 (0.90, 2.45)	26	0.46 (0.26, 0.81)	70	1.14 (0.74, 1.77)
≥0.083	161	51	0.70 (0.44, 1.11)	52	1.19 (0.71, 1.98)	23	0.37 (0.20, 0.67)	51	0.82 (0.52, 1.31)
P for trend[2]			0.41		0.30		0.002		0.42

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]P-value for trend for continuous variable.

Table 2.15. Adjusted[1] Hazard Ratios (HR) and 95% Confidence Internals (CI) for Flavonoid and Lignan Intake and Overall Mortality Among Non-proxy Esophageal and Gastric Cancer Cases by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993–1995 through 2000.

	Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Variable and intake (mg/day)	Cases (N=190)	HR (95% CI)	Cases (N=182)	HR (95% CI)	Cases (N=128)	HR (95% CI)	Cases (N=238)	HR (95% CI)
Total Flavonoids								
0-62.35	69	1.00	35	1.00	30	1.00	54	1.00
62.36-103.39	36	1.18 (0.76, 1.85)	50	1.44 (0.88, 2.34)	27	1.45 (0.81, 2.59)	64	1.15 (0.74, 1.78)
103.40-253.24	41	1.23 (0.80, 1.90)	46	1.01 (0.62, 1.65)	41	1.03 (0.60, 1.77)	58	0.78 (0.49, 1.24)
≥253.25	44	0.78 (0.49, 1.22)	51	1.08 (0.65, 1.79)	30	1.03 (0.58, 1.82)	62	1.08 (0.71, 1.65)
P for trend[2]		0.02		0.98		0.29		0.65
Anthocyanidins								
0-6.23	61	1.00	34	1.00	41	1.00	50	1.00
6.24-10.11	42	0.88 (0.55, 1.40)	52	0.96 (0.60, 1.54)	38	0.78 (0.46, 1.33)	63	1.46 (0.93, 2.28)
10.12-16.23	43	0.78 (0.50, 1.22)	42	0.88 (0.53, 1.46)	24	1.48 (0.83, 2.63)	58	1.41 (0.88, 2.26)
≥16.24	44	0.82 (0.53, 1.29)	54	0.74 (0.45, 1.22)	25	0.88 (0.51, 1.52)	67	1.25 (0.79, 1.98)
P for trend[2]		0.25		0.35		0.77		0.63
Flavan-3-ols								
0-10.90	56	1.00	39	1.00	34	1.00	53	1.00
10.91-26.67	45	1.00 (0.65, 1.56)	39	0.77 (0.46, 1.28)	23	1.24 (0.68, 2.26)	74	1.43 (0.92, 2.20)
26.68-210.51	44	1.19 (0.77, 1.85)	55	0.89 (0.56, 1.40)	41	0.87 (0.51, 1.48)	47	0.98 (0.61, 1.60)
≥210.52	45	0.93 (0.59, 1.45)	49	0.75 (0.46, 1.22)	30	1.03 (0.59, 1.81)	64	1.33 (0.86, 2.05)
P for trend[2]		0.02		0.92		0.25		0.60
Flavanones								
0-8.63	63	1.00	35	1.00	32	1.00	46	1.00
8.64-32.94	52	1.01 (0.67, 1.54)	46	1.36 (0.81, 2.27)	37	1.10 (0.63, 1.92)	62	0.78 (0.49, 1.24)
34.95-49.00	41	1.57 (1.02, 2.41)	42	1.57 (0.95, 2.60)	35	1.36 (0.78, 2.38)	61	0.87 (0.54, 1.39)
≥49.01	34	0.91 (0.55, 1.48)	59	1.01 (0.62, 1.64)	24	2.00 (1.06, 3.79)	69	0.71 (0.45, 1.12)
P for trend[2]		0.40		0.76		0.36		0.65

(Table 2.15. continued)

Variable and intake (mg/day)	Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
	Cases (N=190)	HR (95% CI)	Cases (N=182)	HR (95% CI)	Cases (N=128)	HR (95% CI)	Cases (N=238)	HR (95% CI)
Flavones								
0-1.20	60	1.00	31	1.00	45	1.00	49	1.00
1.21-1.81	30	0.76 (0.46, 1.26)	45	1.26 (0.76, 2.11)	33	1.56 (0.95, 2.56)	73	0.93 (0.61, 1.43)
1.82-2.64	49	0.84 (0.55, 1.28)	52	1.05 (0.63, 1.74)	26	0.92 (0.52, 1.63)	57	0.87 (0.55, 1.40)
≥2.65	51	0.79 (0.51, 1.21)	54	0.94 (0.56, 1.58)	24	0.94 (0.54, 1.66)	59	0.91 (0.57, 1.45)
P for trend[2]		0.08		0.69		0.20		0.44
Flavonols								
0-8.16	59	1.00	35	1.00	39	1.00	59	1.00
8.17-12.30	36	1.21 (0.75, 1.94)	49	1.62 (0.99, 2.67)	19	0.76 (0.40, 1.45)	62	0.83 (0.54, 1.30)
12.31-19.34	53	1.18 (0.78, 1.80)	44	1.16 (0.70, 1.94)	33	0.68 (0.40, 1.16)	60	1.16 (0.76, 1.76)
≥19.35	42	0.80 (0.50, 1.27)	54	1.06 (0.64, 1.75)	37	0.85 (0.51, 1.42)	57	0.92 (0.60, 1.41)
P for trend[2]		0.01		0.42		0.66		0.52
Isoflavones								
0-0.31	46	1.00	40	1.00	35	1.00	53	1.00
0.32-0.46	32	0.73 (0.44, 1.21)	38	1.32 (0.80, 2.19)	40	0.88 (0.52, 1.48)	66	1.61 (1.02, 2.54)
0.47-0.62	52	0.67 (0.42, 1.06)	52	0.97 (0.58, 1.62)	29	1.12 (0.63, 1.99)	60	1.04 (0.64, 1.68)
≥0.63	60	0.77 (0.47, 1.25)	52	1.26 (0.75, 2.13)	24	0.89 (0.48, 1.67)	59	1.20 (0.71, 2.01)
P for trend[2]		0.50		0.21		0.75		0.63
Lignans								
0-0.044	50	1.00	32	1.00	42	1.00	57	1.00
0.045-0.060	52	0.83 (0.54, 1.28)	34	1.46 (0.83, 2.55)	35	0.71 (0.41, 1.22)	56	1.33 (0.84, 2.09)
0.061-0.079	34	0.89 (0.54, 1.47)	55	1.32 (0.80, 2.19)	26	0.55 (0.31, 0.97)	72	1.13 (0.74, 1.72)
≥0.080	54	0.69 (0.44, 1.08)	61	1.17 (0.71, 1.93)	25	0.51 (0.29, 0.90)	53	1.16 (0.73, 1.86)
P for trend[2]		0.28		0.90		0.07		0.88

[1]Adjusted for stage (localized, regional, distant, unknown) and dietary energy intake (kilocalories, continuous). [2]P-value for trend of continuous variable.

Table 2.16. Sensitivity Analysis of Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Total Flavonoid Intake and Esophageal and Gastric Cancer, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Non-Cardia Gastric Adenocarcinoma	
Total Flavonoids	Controls (N=662)	Cases (N=274)	OR (95% CI)	Cases (N=248)	OR (95% CI)	Cases (N=191)	OR (95% CI)	Cases (N=341)	OR (95% CI)
Standard Multivariate Model (flavonoid mg/day)									
0-63.81	165	83	1.00	59	1.00	48	1.00	80	1.00
63.82-97.90	166	54	0.62 (0.41, 0.93)	60	1.00 (0.65, 1.54)	35	0.64 (0.38, 1.07)	84	1.01 (0.69, 1.49)
97.91-217.35	165	57	0.64 (0.42, 0.97)	54	0.90 (0.58, 1.41)	61	1.11 (0.69, 1.77)	78	0.92 (0.62, 1.37)
≥217.36	166	80	0.92 (0.63, 1.37)	75	1.32 (0.87, 2.00)	47	0.87 (0.53, 1.41)	99	1.08 (0.73, 1.58)
P for trend[2]			0.61		0.07		0.42		0.50
Nutrient Density Model (flavonoid mg/1000 kcal)									
0-30.62	165	86	1.00	69	1.00	56	1.00	79	1.00
30.63-48.95	166	59	0.70 (0.47, 1.05)	54	0.84 (0.55, 1.28)	41	0.81 (0.50, 1.30)	83	1.05 (0.71, 1.55)
48.96-102.90	165	51	0.66 (0.43, 1.01)	51	0.88 (0.57, 1.36)	55	1.27 (0.80, 2.01)	74	0.97 (0.65, 1.45)
≥102.91	166	78	1.00 (0.67, 1.48)	74	1.30 (0.86, 1.96)	39	0.84 (0.51, 1.37)	105	1.22 (0.83, 1.80)
P for trend[2]			0.98		0.10		0.21		0.70
Residual Model (energy-adjusted flavonoid intake)									
<-133.68	166	84	1.00	62	1.00	53	1.00	77	1.00
-133.69--103.40	165	58	0.69 (0.46, 1.03)	57	0.96 (0.63, 1.47)	36	0.69 (0.42, 1.14)	86	1.09 (0.74, 1.60)
-103.41-14.64	166	51	0.62 (0.41, 0.94)	55	0.95 (0.62, 1.47)	55	1.14 (0.72, 1.81)	77	0.99 (0.67, 1.47)
≥14.65	165	81	0.98 (0.66, 1.44)	74	1.31 (0.87, 1.99)	47	0.89 (0.56, 1.44)	101	1.17 (0.80, 1.71)
P for trend[2]			0.61		0.07		0.42		0.50

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]P-value for trend for continuous variable.

Table 2.17. Sensitivity Analysis of Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Total Flavonoid Intake and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Total Flavonoids	Controls (N=183)	Cases (N=170)	OR	95% CI
Standard Multivariate Model (flavonoid mg/day)				
0-42.38	46	44	1.00	
42.39-75.36	46	41	1.10	0.59, 2.08
75.37-166.98	45	46	1.37	0.73, 2.58
≥166.99	46	39	1.09	0.56, 2.11
P for trend[2]			0.81	
Nutrient Density Model (flavonoid mg/1000 kcal)				
0-27.47	45	48	1.00	
27.48-49.02	47	36	0.83	0.44, 1.57
49.03-110.97	46	54	1.23	0.67, 2.27
≥110.98	45	32	0.84	0.43, 1.66
P for trend[2]			0.39	
Residual Model (energy-adjusted flavonoid intake)				
<-79.39	46	50	1.00	
-79.40--46.88	46	35	0.76	0.41, 1.38
-46.89-38.00	46	50	1.06	0.59, 1.90
≥38.01	45	35	0.75	0.40, 1.39
P for trend[2]			0.90	

[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]P-value for trend for continuous variable.

Table 2.18. Power Calculations for Risk of Esophageal and Gastric Tumors Development and Survival Among Esophageal and Gastric Cancer Cases for Dichotomous (2 level), Quartiled (4 level) or Tertiled (3 level) Exposures.

	EA, GCA, ESCC, or NCGA Incidence				**Survival among EA, GCA, ESCC, or NCGA Cases**				**BE Development**	
	Overall		Non-proxy		Overall		Non-proxy		Overall	
Exposure Levels:	2	4	2	4	2	4	2	4	2	3
Minimum Detectable Odds/Hazard Ratio										
0.40	1.00	0.99	1.00	0.96	1.00	0.98	1.00	0.93	0.99	0.92
0.45	1.00	0.96	1.00	0.90	1.00	0.95	0.99	0.86	0.95	0.84
0.50	1.00	0.90	0.98	0.81	1.00	0.90	0.97	0.77	0.89	0.74
0.55	0.98	0.81	0.94	0.69	0.98	0.80	0.92	0.65	0.79	0.61
0.60	0.93	0.68	0.85	0.56	0.93	0.68	0.82	0.53	0.66	0.49
0.65	0.83	0.54	0.72	0.43	0.84	0.55	0.69	0.41	0.52	0.37
0.70	0.68	0.40	0.56	0.32	0.69	0.41	0.54	0.30	0.38	0.27

REFERENCES

1. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ, Fraumeni JF, Jr. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1997;89(17):1277-84.

2. Waksberg J. Sampling methods for random digit dialing. *J Am Stat Assoc* 1978;73:40-46.

3. Bunin GR, Spector LG, Olshan AF, Robison LL, Roesler M, Grufferman S, Shu XO, Ross JA. Secular trends in response rates for controls selected by random digit dialing in childhood cancer studies: a report from the Children's Oncology Group. *Am J Epidemiol* 2007;166(1):109-16.

4. Olson SH. Reported participation in case-control studies: changes over time. *Am J Epidemiol* 2001;154(6):574-81.

5. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007;133(2):403-11.

6. *World Health Organization. International Classification of Diseases for Oncology, First Edition*. Geneva, Switzerland: WHO, 1976.

7. Misumi A, Murakami A, Harada K, Baba K, Akagi M. Definition of carcinoma of the gastric cardia. *Langenbecks Arch Chir* 1989;374(4):221-6.

8. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol* 1997;146(10):856-69.

9. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10(10):1055-62.

10. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999;9(3):178-87.

11. Hennessy TP, O'Connell R. Carcinoma of the hypopharynx, esophagus and cardia. *Surg Gynecol Obstet* 1986;162(3):243-7.

12. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence - SEER 17 Regs Research Data + Hurricane Katrina Impacted Louisiana Cases, Nov 2010 Sub (1973-2008 varying), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.

13. Fryzek JP, Lipworth L, Signorello LB, McLaughlin JK. The reliability of dietary data for self- and next-of-kin respondents. *Ann Epidemiol* 2002;12(4):278-83.

14. Herrmann N. Retrospective information from questionnaires. I. Comparability of primary respondents and their next-of-kin. *Am J Epidemiol* 1985;121(6):937-47.

15. Kristal A, Shattuck A, Williams A. Food frequency questionnaires for diet intervention research. *Proceedings of the 17th National Nutrient Databank Conference, June 7-10, 1992, Baltimore, Maryland*. Washington, DC: International Life Sciences Institute, 1994.

16. Wacholder S, Silverman DT, McLaughlin JK, Mandel JS. Selection of controls in case-control studies. II. Types of controls. *Am J Epidemiol* 1992;135(9):1029-41.

17. Willett W. *Nutritional Epidemiology*. Third Edition. ed. New York: Oxford University Press, 2012.

18. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav.

19. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav.

20. Fink BN, Steck SE, Wolff MS, Kabat GC, Gammon MD. Construction of a flavonoid database for assessing intake in a population-based sample of women on Long Island, New York. *Nutr Cancer* 2006;56(1):57-66.

21. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutrition and cancer* 2006;54(2):184-201.

22. Neveu V, Perez-Jimenez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database : the journal of biological databases and curation* 2010;2010:bap024.

23. Perez-Jimenez J, Neveu V, Vos F, Scalbert A. Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the phenol-explorer database. *Journal of agricultural and food chemistry* 2010;58(8):4959-69.

24. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *American journal of epidemiology* 1997;146(10):856-69.

25. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol* 1988;127(1):188-99.

26. Rohan TE, Potter JD. Retrospective assessment of dietary intake. *Am J Epidemiol* 1984;120(6):876-87.

27. van Staveren WA, West CE, Hoffmans MD, Bos P, Kardinaal AF, van Poppel GA, Schipper HJ, Hautvast JG, Hayes RB. Comparison of contemporaneous and retrospective estimates of food consumption made by a dietary history method. *Am J Epidemiol* 1986;123(5):884-93.

28. Byers T, Marshall J, Anthony E, Fiedler R, Zielezny M. The reliability of dietary history from the distant past. *Am J Epidemiol* 1987;125(6):999-1011.

29. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976;24:117-91.

30. Mazza G. Anthocyanins in grapes and grape products. *Crit Rev Food Sci Nutr* 1995;35(4):341-71.

31. Imeh U, Khokhar S. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *J Agric Food Chem* 2002;50(22):6301-6.

32. Re R, Bramley PM, Rice-Evans C. Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radic Res* 2002;36(7):803-10.

33. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem* 2003;51(22):6509-15.

34. Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, Gebhardt S. Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of agricultural and food chemistry* 2006;54(26):9966-77.

35. Gammon MD, Terry MB, Arber N, Chow WH, Risch HA, Vaughan TL, Schoenberg JB, Mayne ST, Stanford JL, Dubrow R, Rotterdam H, West AB, Fraumeni JF, Jr., Weinstein IB, Hibshoosh H. Nonsteroidal anti-inflammatory drug use associated with reduced incidence of adenocarcinomas of the esophagus and gastric cardia that overexpress cyclin D1: a population-based study. *Cancer Epidemiol Biomarkers Prev* 2004;13(1):34-9.

36. Figueroa JD, Terry MB, Gammon MD, Vaughan TL, Risch HA, Zhang FF, Kleiner DE, Bennett WP, Howe CL, Dubrow R, Mayne ST, Fraumeni JF, Jr., Chow WH. Cigarette smoking, body mass index, gastro-esophageal reflux disease, and non-steroidal anti-inflammatory drug use and risk of subtypes of esophageal and gastric cancers by P53 overexpression. *Cancer Causes Control* 2009;20(3):361-8.

37. Arber N, Lightdale C, Rotterdam H, Han KH, Sgambato A, Yap E, Ahsan H, Finegold J, Stevens PD, Green PH, Hibshoosh H, Neugut AI, Holt PR, Weinstein IB. Increased expression of the cyclin D1 gene in Barrett's esophagus. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 1996;5(6):457-9.

38. Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D, Peters G. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. *Cancer research* 1994;54(7):1812-7.

39. Guinee DG, Jr., Travis WD, Trivers GE, De Benedetti VM, Cawley H, Welsh JA, Bennett WP, Jett J, Colby TV, Tazelaar H, et al. Gender comparisons in human lung cancer: analysis of p53 mutations, anti-p53 serum antibodies and C-erbB-2 expression. *Carcinogenesis* 1995;16(5):993-1002.

40. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 1991;39(6):741-8.

41. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 1981;29(4):577-80.

42. Terry MB, Gammon MD, Schoenberg JB, Brinton LA, Arber N, Hibshoosh H. Oral contraceptive use and cyclin D1 overexpression in breast cancer among young women. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2002;11(10 Pt 1):1100-3.

43. Mueller J, Werner M, Stolte M. Barrett's esophagus: histopathologic definitions and diagnostic criteria. *World journal of surgery* 2004;28(2):148-54.

44. Sharma P, Sampliner RE. Short segment Barrett's esophagus and intestinal metaplasia of the cardia--it's not all symantics!!! *The American journal of gastroenterology* 1998;93(11):2303-4.

45. Spechler SJ. Short and ultrashort Barrett's esophagus--what does it mean? *Seminars in gastrointestinal disease* 1997;8(2):59-67.

46. Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. *The American journal of gastroenterology* 2002;97(8):1888-95.

47. Trivers KF, De Roos AJ, Gammon MD, Vaughan TL, Risch HA, Olshan AF, Schoenberg JB, Mayne ST, Dubrow R, Stanford JL, Abrahamson P, Rotterdam H, West AB, Fraumeni JF, Chow WH. Demographic and lifestyle predictors of survival in patients with esophageal or gastric cancers. *Clin Gastroenterol Hepatol* 2005;3(3):225-30.

48. Shaheen N, Ransohoff DF. Gastroesophageal reflux, barrett esophagus, and esophageal cancer: scientific review. *Jama* 2002;287(15):1972-81.

49. Lagergren J, Bergstrom R, Lindgren A, Nyren O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med* 1999;340(11):825-31.

50. Campbell PT, Sloan M, Kreiger N. Utility of proxy versus index respondent information in a population-based case-control study of rapidly fatal cancers. *Ann Epidemiol* 2007;17(4):253-7.

51. McLaughlin JK, Mandel JS, Mehl ES, Blot WJ. Comparison of next-of-kin with self-respondents regarding questions on cigarette, coffee, and alcohol consumption. *Epidemiology* 1990;1(5):408-12.

52. Thompson OM, Beresford SA, Kirk EA, Vaughan TL. Vegetable and fruit intakes and risk of Barrett's esophagus in men and women. *Am J Clin Nutr* 2009;89(3):890-6.

53. Lohman T, Roche A, Martorell M. *Anthropometric standardization reference manual*. Chicago, IL: Human Kinetics Books, 1988.

54. Cameron AJ. Barrett's esophagus: does the incidence of adenocarcinoma matter? *Am J Gastroenterol* 1997;92(2):193-4.

55. Clouston AD. Timely topic: Premalignant lesions associated with adenocarcinoma of the upper gastrointestinal tract. *Pathology* 2001;33(3):271-7.

56. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer* 2008;123(4):852-60.

57. Gammon MD. Personal Communication., 2010.

58. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008.

59. Kleinbaum D. *Logistic regression : a self-learning text* second ed. New York: Springer, 2002.

60. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *The American journal of clinical nutrition* 1997;65(4 Suppl):1220S-1228S; discussion 1229S-1231S.

61. Drossard C, Frohling B, Dietrich H, Kersting M. Anthocyanin analysis in banana fruit--a mistake. *The American journal of clinical nutrition* 2011;93(4):865-6; author reply 866-7.

62. Drossard C, Kersting M. Banana as a relevant source of anthocyani(di)ns in European populations? *The British journal of nutrition* 2012;107(5):765-6; author reply 767.

63. Anderson LA, Johnston BT, Watson RG, Murphy SJ, Ferguson HR, Comber H, McGuigan J, Reynolds JV, Murray LJ. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. *Cancer Res* 2006;66(9):4975-82.

64. Fink BN, Steck SE, Wolff MS, Britton JA, Kabat GC, Schroeder JC, Teitelbaum SL, Neugut AI, Gammon MD. Dietary flavonoid intake and breast cancer risk among women on Long Island. *Am J Epidemiol* 2007;165(5):514-23.

65. Gaudet MM, Britton JA, Kabat GC, Steck-Scott S, Eng SM, Teitelbaum SL, Terry MB, Neugut AI, Gammon MD. Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. *Cancer Epidemiol Biomarkers Prev* 2004;13(9):1485-94.

66. Pierini R, Kroon PA, Guyot S, Ivory K, Johnson IT, Belshaw NJ. Procyanidin effects on oesophageal adenocarcinoma cells strongly depend on flavan-3-ol degree of polymerization. *Mol Nutr Food Res* 2008;52(12):1399-407.

67. Lee LT, Huang YT, Hwang JJ, Lee AY, Ke FC, Huang CJ, Kandaswami C, Lee PP, Lee MT. Transinactivation of the epidermal growth factor receptor tyrosine kinase and focal adhesion kinase phosphorylation by dietary flavonoids: effect on invasive potential of human carcinoma cells. *Biochem Pharmacol* 2004;67(11):2103-14.

68. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF, Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *Jama* 1991;265(10):1287-9.

69. Blot WJ, Devesa SS, Fraumeni JF, Jr. Continuing climb in rates of esophageal adenocarcinoma: an update. *Jama* 1993;270(11):1320.

70. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-53.

71. Brown LM, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002;11(2):235-56.

72. Anderson LA, Watson RG, Murphy SJ, Johnston BT, Comber H, Mc Guigan J, Reynolds JV, Murray LJ. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J Gastroenterol* 2007;13(10):1585-94.

73. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am J Gastroenterol* 2008;103(7):1614-23; quiz 1624.

74. Brown LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, Silverman DT, Pottern LM, Hayes RB, Schwartz AG, et al. Adenocarcinoma of the esophagus: role of obesity and diet. *Journal of the National Cancer Institute* 1995;87(2):104-9.

75. Chen H, Tucker KL, Graubard BI, Heineman EF, Markin RS, Potischman NA, Russell RM, Weisenburger DD, Ward MH. Nutrient intakes and adenocarcinoma of the esophagus and distal stomach. *Nutrition and cancer* 2002;42(1):33-40.

76. Wu AH, Tseng CC, Hankin J, Bernstein L. Fiber intake and risk of adenocarcinomas of the esophagus and stomach. *Cancer causes & control : CCC* 2007;18(7):713-22.

77. Terry P, Lagergren J, Hansen H, Wolk A, Nyren O. Fruit and vegetable consumption in the prevention of oesophageal and cardia cancers. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation* 2001;10(4):365-9.

78. Ibiebele TI, Hughes MC, Whiteman DC, Webb PM. Dietary patterns and risk of oesophageal cancers: a population-based case-control study. *The British journal of nutrition* 2012;107(8):1207-16.

79. Freedman ND, Park Y, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. *International journal of cancer. Journal international du cancer* 2007;121(12):2753-60.

80. Freedman ND, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and gastric cancer risk in a large United States prospective cohort study. *Cancer causes & control : CCC* 2008;19(5):459-67.

81. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. *International journal of cancer. Journal international du cancer* 2011;129(11):2681-93.

82. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr* 2008;99(1):12-22.

83. Erlund I, Meririnne E, Alfthan G, Aro A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. *J Nutr* 2001;131(2):235-41.

84. Manach C, Morand C, Gil-Izquierdo A, Bouteloup-Demange C, Remesy C. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *Eur J Clin Nutr* 2003;57(2):235-42.

85. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *The British journal of nutrition* 2008;99(1):12-22.

86. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, Cameron A, Corley D, Falk G, Goldblum J, Hunter J, Jankowski J, Lundell L, Reid B, Shaheen NJ, Sonnenberg A, Wang K, Weinstein W. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. *Gastroenterology* 2004;127(1):310-30.

CHAPTER 3: DIETARY INTAKE OF FLAVONOIDS AND ESOPHAGEAL AND GASTRIC CANCER: INCIDENCE AND SURVIVAL IN THE UNITED STATES (U.S.)

Introduction

Over the last two decades, esophageal and gastric cardia adenocarcinomas have been among the most rapidly increasing cancer types in the United States (U.S.) and other Western countries.[1,2] Esophageal and gastric cardia adenocarcinoma are often considered as one clinical entity because they are both epithelial cancers originating in or near the gastroesophageal junction, have similar 5-year survival rates of approximately 26%, and have comparable survival decrements according to tumor stage.[3] Still, esophageal squamous cell carcinoma and other (non-cardia) gastric adenocarcinoma are the most common forms of esophageal and gastric cancer worldwide. In addition to geographic variation, these four cancer types have differing risk factors.[4,5] Thus, considering them as distinct entities will help to elucidate the underlying etiology.

Epidemiologic studies have shown that diets high in fruits and vegetables are inversely associated with risk of esophageal and gastric cancer incidence.[6-8] Flavonoids, which are a group of bioactive polyphenolic compounds naturally occurring in fruits, vegetables, and beverages of plant origin, have been hypothesized to account at least partially for such risk reductions.[9] Experimental studies have supported this hypothesis. For example, freeze-dried berries, which are high in anthocyanidins, inhibited 24-56% of esophageal tumor multiplicity (average number of tumors per esophagus) and 8-21% of esophageal tumor incidence in rats caused by N-nitrosomethylbenzylamine (NMBA) compared to controls.[10] Additionally, Flavopiridol, a synthetic flavone, has been studied in Phase I and II clinical trials in patients with metastatic gastric cancer.[11] Lignans are another group of polyphenolic compounds that have

anti-inflammatory and pro-apoptotic effects, antioxidant properties, and promote cell cycle arrest.[12]

Recent epidemiological investigations conducted in the United States,[13] Greece,[14] Italy,[15,16] Europe,[17,18] Sweden,[19] China,[20] and Mexico[21] have analyzed associations between flavonoid or lignan intake and esophageal or gastric cancer incidence. Decreased risk of esophageal or gastric cancer incidence has been seen with specific classes of flavonoids and lignans: anthocyanidins,[13] flavanones,[13-15] flavones,[14] flavonols (overall[14-16] and quercetin[19]), isoflavones,[13] and lignans (secoisolariciresinol).[21] However, no epidemiologic studies to date have examined associations between flavonoid intake and survival among individuals diagnosed with esophageal or gastric cancer. Clarification of whether total flavonoids, or specific flavonoid classes, influence the incidence of these tumor subtypes or survival once diagnosed would provide empirical support for developing potential risk reduction strategies utilizing these compounds.

In this population-based study, we investigated whether intakes of total flavonoids, including specific flavonoid classes, and lignans are associated with: (i) risk of esophageal or gastric cancer incidence; and (ii) survival among individuals diagnosed with esophageal or gastric cancer. For both aims, we consider associations for all four cancer types separately.

Methods

To conduct this ancillary study, we built upon the resources from the U.S. Multi-Center Study, a population-based investigation conducted in the state of Connecticut [CT, Principal Investigator (PI): HA Risch], a 15-county area of New Jersey (NJ, PI: MD Gammon), and a three-county area of western Washington state (WA, PI: TL Vaughan), which was initiated as a case-control study[22] and then continued as a follow-up study to determine vital status among the cases.[23] This study was approved by all Institutional Review Boards of the participating institutions.

Study Population. The three geographic areas (CT, NJ, and WA) each had a population-based cancer registry, which was used to identify the cases through rapid reporting methods. Eligible case participants were English-speaking men and women between the ages of 30 and 79 years, diagnosed with a first primary invasive esophageal or stomach cancer between 1993 and 1995. The parent study goal was to recruit all individuals newly diagnosed with esophageal or gastric cardia adenocarcinoma (which were considered the target case participants), while participants with esophageal squamous cell carcinoma or non-cardia gastric adenocarcinoma were frequency matched to the target case participants by geographic location and 5-year age group (CT, NJ, WA), sex (NJ, WA), and race (white or other, NJ). Final determination of case participant eligibility and classification was made by study pathologists (Drs. Heidi Rotterdam at Columbia University for NJ and A. Brian West at New York University for CT and WA) after review of medical records and pathology specimens.[22]

Population-based control participants were frequency matched to the target case participants by 5-year age group and sex. Control participants 30-64 years of age were identified using a modified Waksberg random digit dialing technique.[24] Control participants 65-79 years of age were identified by random sampling of Health Care Financing Administration rosters (now Centers for Medicare and Medicaid Services).[22]

Study participants included 293 persons with esophageal adenocarcinoma and 261 with gastric cardia adenocarcinoma (80.6% of eligible target case participants), 221 with esophageal squamous cell carcinoma and 368 with other gastric adenocarcinoma (74.1% of eligible comparison case participants), and 695 population control participants (74.1% of eligible control participants).[22] The 93.4% of participants with dietary intake information (see Exposure Assessment below) are the focus of the current study, and the distribution of the demographic characteristics of this subsample did not differ substantially from those of all study participants[25] and are shown in **Table 3.5**. Males comprised 83.9% of target cases, 72.9% of comparison

cases, and 79.8% of controls; 98.1% of target cases, 83.3% of comparison cases, and 93.2% of controls were white; and 78.4% of target cases, 66.0% of comparison cases, and 79.2% of controls had more than a high school education.

Exposure Assessment. Information on demographic factors, tobacco and alcohol use, intake of other beverages (e.g., coffee and tea), medical history, medication use, and occupational history was obtained by a structured questionnaire administered face-to-face by trained interviewers. Despite efforts to recruit and interview participants rapidly, proxy interviews were required for 29.6% of target case participants, 32.2% of comparison case participants, and 3.4% of control participants. We analyzed the data both including and excluding proxy interviews and the results were consistent (data not shown), therefore we present the analysis including the proxy data. The average time between cancer diagnosis and interview for cases was 3.7 months for self-report and 8.5 months for proxy-report. Interviews averaged 130 minutes. Written informed consent was obtained from each participant prior to the interview.[22]

Dietary data were collected by interviewers using a 104-item food frequency questionnaire (FFQ)[25] modified from one developed and validated by investigators at the Fred Hutchinson Cancer Research Center (FHCRC).[26] Participants were asked to report usual dietary intake for the 3-5 years prior to diagnosis (case participants) or interview (control participants). Completed FFQs were not obtained for 33 participants who provided only abbreviated interviews. Estimated total energy intake of <500 or >4,000 kilocalories/day for women and <800 or >5,000 kilocalories/day for men were considered implausible or unrealistic intake values, thereby excluding 61 case and 28 control participants.[27] Final numbers of participants included in this study were 274 with esophageal adenocarcinoma, 248 with gastric cardia adenocarcinoma, 191 with esophageal squamous cell carcinoma, and 341 with other gastric adenocarcinoma cases, and 662 control participants.

Assessment of Dietary Flavonoid Intake. A study-specific flavonoid database was

created based on values from the 2011 U.S. Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods[28] and the 2008 USDA-Iowa State University Database on the Isoflavone Content of Selected Foods[29] and supplemented with lignan content data (i.e., secoisolariciresinol and matairesinol) from foods consumed by a North American population.[30] Using FFQ reported frequencies of dietary intakes, intake of total flavonoids and six classes of flavonoids (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones) and lignans were estimated using the study-specific flavonoid database for 81 food and beverage items that contained measureable amounts of flavonoids.

When FFQ items represented groups of foods or beverages, the individual foods and beverages were weighted based on usual population consumption. For example, the FFQ item of “apples and pears” was assigned weights of 0.75 for “apples” and 0.25 for “pears”. Flavonoid intake was calculated by multiplying the weight assigned to each food in the FFQ item was multiplied by the flavonoid content of that food, summing across all foods in the FFQ item, and then multiplying by the number of times consumed per day. For example, 100 grams of apple contains 1.29 mg and pears contain 12.18 mg of anthocyanidins. A serving size of apples or pears was estimated as 145 grams. Therefore, if an individual reported consuming one serving of apples or pears per day, the individual’s daily intake of anthocyanidins from apples and pears was calculated as 145g apple or pear/day * [(0.75 apple weight * 1.29 mg/100g apples) + (0.25 pear weight * 12.18 mg/100g pear)] = 5.82 mg anthocyanidins/day.

Outcome Assessment. In follow-up of the Multi-Center study, vital status and date of death were determined by linking state tumor registry data with the National Death Index.[23] Overall survival time (in months) was calculated from the date of diagnosis until death or last follow-up, with a maximum follow-up of 90 months, ending in 2000. Median survival time was 9.6 months for the study participants diagnosed with esophageal adenocarcinoma, 12.8

months for those with gastric cardia adenocarcinoma, 10.7 months for esophageal squamous cell carcinoma, and 12.9 months for other gastric adenocarcinoma. At the end of follow-up, participants who were still alive were considered censored. The outcome was death from any cause.[23]

Statistical Analysis. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). All p-values are two-sided.

Case-control Analysis. Polytomous unconditional logistic regression was used to calculate odds ratios (ORs) and their 95% confidence intervals (CIs) for the association between flavonoid/lignan intake and the risk of incidence for the four tumor types (esophageal adenocarcinoma, gastric cardia adenocarcinoma, esophageal squamous cell carcinoma, and other gastric adenocarcinoma) in comparison with control participants. Effect measure modification by cigarette smoking (evaluated as ever/never and continuous years of duration), usual adult body mass index (BMI, based on usual adult weight, evaluated as continuous and as dichotomized, <25 and ≥25 kg/m^2), and gastroesophageal reflux disease (yes/no) was assessed using likelihood ratio statistics to compare regression models with and without a multiplicative term.[31] There was no evidence for effect modification by any of these variables ($p \geq 0.05$) on the association between flavonoid intake and esophageal or gastric cancer.

Potential confounders[31] for the case-control analysis included proxy status (proxy, non-proxy), income (evaluated as <$15,000, $15,000-29,999, $30,000-49,999, $50,000-74,999, or ≥$75,000 and <$15,000 or ≥$15,000), education (evaluated as <high school, high school, technical school/some college, or ≥college and <high school or ≥high school), cigarette smoking (evaluated as ever/never, continuous pack-years, and continuous cigarettes/day), alcohol consumption (evaluated as ever/never and continuous for beer, wine, and liquor), and BMI (evaluated as continuous and as categorized, <25, 25-29.9, or ≥30 kg/m^2). If the log odds ratio changed by ≥10% due to variable elimination, the variable

was considered a confounder and remained in the model;[31] only cigarette smoking met this criterion. Total energy intake was included for adjustment as an *a priori* confounder.[32] Thus, all final logistic regression models include cigarette smoking (ever/never), kilocalories (continuous), and the frequency matching factors of site (CT, NJ, WA), age (continuous), sex (male, female), and race (white, black, other).

Survival Analysis. Kaplan-Meier curves were constructed to examine flavonoids as a univariate predictor of survival, and Cox proportional hazard regression analysis was used to calculate adjusted hazard ratios (HRs) and their 95% confidence intervals for the association between flavonoid/lignan intake and mortality for each tumor type as distinct outcomes. The proportional hazards assumption was tested utilizing an interaction with log(time) in models with confounders, and it was not observed to be violated.

Potential confounders[31] for the survival analysis included the same covariates listed above in the case-control analysis, as well as study site (WA, NJ, CT), age (continuous), sex (male/female), tumor stage (localized, regional, distant, and unknown), tumor grade (well/moderate, poor/undifferentiated, and not determined), and dysphagia (yes/no). Variables remained in the adjusted model if they were significant predictors of survival ($p<0.05$);[31] only stage met this criterion. Total energy intake was included as an *a priori* confounder.[32] Thus, all final proportional hazard models included tumor stage and kilocalories (continuous).

Examination of Linear Trend. Flavonoid intake was categorized as quartiles, based on the distribution of intake among the controls (logistic regression) or all cases (survival analysis).[27] To examine trends, we evaluated flavonoid intake with restricted quadratic splines as well as linear trends in continuous flavonoid values (mg/day).

Sensitivity Analysis. For anthocyanidins, a value of 7.39 mg/100 g of banana is assigned in the USDA Flavonoid Database.[28] However, this value is controversial.[33] Thus, we conducted sensitivity analyses that excluded this value (**Tables 3.6-3.9**).

Results

As shown in **Table 3.1**, control participants consumed on average similar amounts of total flavonoids (median=97.09 mg/day) as case participants (esophageal adenocarcinoma median=96.89; gastric cardia adenocarcinoma median=104.27; esophageal squamous cell carcinoma median=108.97; other gastric adenocarcinoma median=101.32 mg/day). Flavan-3-ols were the largest contributor to total flavonoid intake in this study population, and control participants consumed less flavan-3-ols than esophageal, gastric cardia, and other gastric adenocarcinoma case participants.

Table 3.2 lists the major sources of flavonoids and lignans in the reported food items of the control participants. For total flavonoids, black tea provided 55.8% (105.11 mg/day) of mean intake, orange/grapefruit juice 14.2% (26.75 mg/day), and wine 4.5% (8.46 mg/day). Black tea was a source of flavan-3-ols, flavonols, and lignans. Orange/grapefruit juice provided flavanones, flavonols, isoflavones, and lignans. Wine was a source of anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and lignans.

As shown in **Table 3.3**, intake of dietary anthocyanidins, for which wine, bananas and fruit juice were the major dietary sources, was inversely associated with risk of incidence for all tumor types. Comparing the highest versus lowest quartile of anthocyanidin intake, decreased risks were shown for incident esophageal adenocarcinoma (OR=0.43, 95% CI: 0.29-0.66, p_{trend}=0.06) and squamous cell carcinoma (OR=0.43, 95% CI: 0.26-0.70, p_{trend}=0.11). More modest decreased risks were shown between anthocyanidin intake and incident gastric cardia (OR=0.71, 95% CI: 0.46-1.10) and other gastric adenocarcinoma (OR=0.70, 95% CI: 0.47-1.03).

For all other flavonoid types, the associations with incident esophageal and gastric cancers were less consistent. For example, as also shown in **Table 3.3**, dietary flavanone intake was inversely associated only with risk of incident esophageal adenocarcinoma (OR=0.56, 95% CI: 0.37-0.85, p_{trend}=0.003) and squamous cell carcinoma (OR=0.48, 95% CI: 0.29-0.78,

p_{trend}=0.002). Dietary flavone intake was only inversely associated with the risk of esophageal squamous cell carcinoma incidence (OR=0.55, 95% CI: 0.34-0.89, p_{trend}=0.02). Dietary lignan intake was also associated with a pronounced reduced risk of esophageal squamous cell carcinoma incidence (OR=0.38, 95% CI: 0.23-0.63, p_{trend}=0.0003) and more modestly associated with risk of incident esophageal adenocarcinoma (OR=0.75, 95% CI: 0.49-1.13) and other gastric adenocarcinoma (OR=0.73, 95% CI: 0.48-1.11).

As also shown in **Table 3.3**, we observed a modest inverse association between dietary intake of isoflavones, for which coffee, chili, and white bread were the major dietary sources, and risk of esophageal squamous cell carcinoma incidence (OR=0.72, 95% CI: 0.40-1.29); in contrast, a positive association was observed for isoflavones in relation to all other tumor types. Similarly, we observed a modest positive association for dietary flavonol intake with gastric cardia adenocarcinoma incidence, but not other tumor types. Little or no consistent association was observed between total flavonoid intake or flavan-3-ols, which accounted for 64.6% of total flavonoid intake among controls, and any tumor types.

As presented in **Table 3.4**, anthocyanidin intake was associated with a decreased risk of mortality among gastric cardia adenocarcinoma cases (highest versus lowest quartile HR=0.63, 95% CI: 0.42-0.95, p_{trend}=0.25) and with more modest decreased risk of mortality among esophageal adenocarcinoma case participants (HR=0.87, 95% CI: 0.60-1.26). Dietary lignan intake was associated with decreased risk of mortality for esophageal squamous cell carcinoma case participants (HR=0.58, 95% CI: 0.37-0.92, p_{trend}=0.07) and with a modest decreased risk of mortality for esophageal adenocarcinoma case participants (HR=0.78, 95% CI: 0.54-1.14). Flavanone intake was associated with decreased risk of mortality only for other gastric adenocarcinoma case participants (HR=0.66, 95% CI: 0.46-0.93, p_{trend}=0.18). Anthocyanidin, lignan, and flavanone intake were associated with little or no decreased risk of mortality for the other tumor types.

As also presented in **Table 3.4**, isoflavone (HR=0.75, 95% CI: 0.49-1.13) and flavone

intake (HR=0.83, 95% CI: 0.58-1.19) were associated with modest decreased risk of mortality for esophageal adenocarcinoma cases. Similar associations were also noted for flavan-3-ol intake and gastric cardia adenocarcinoma cases (HR=0.71, 95% CI: 0.48-1.05), while little or no association was seen among the other tumor types. Little or no association was seen between isoflavones, flavones, flavan-3-ols and the other tumors types for risk of mortality. Additionally, total flavonoid or flavonol intake was associated with little or no decreased risk of mortality for any tumor type.

A sensitivity analysis that excluded the anthocyanidin value for bananas did not substantially alter our results for esophageal or gastric cancer incidence or survival (**Tables 3.6-3.9**).

Discussion

This is the first population-based study, to our knowledge, to examine associations between dietary flavonoid intake and survival among esophageal and gastric cancer cases. In our analysis, the highest intake quartile of anthocyanidins was associated with a 37% and 13% decreased risk of mortality for gastric cardia and esophageal adenocarcinoma cases, respectively. The highest intake quartile of lignans was associated with a 42% and 22% decreased risk of mortality for esophageal squamous cell carcinoma and adenocarcinoma cases, respectively. The highest intake quartile of flavanones was associated with a 34% decreased risk of mortality for other gastric adenocarcinoma cases, and the highest quartile of flavan-3-ol intake was associated with a 29% decreased risk of mortality for gastric cardia adenocarcinoma cases. We also found the highest intake quartile of flavones and isoflavones associated with 17 and 25% decreased risk of mortality for esophageal adenocarcinoma cases, respectively.

Additionally, we observed a 25-30% risk reduction for the association between the highest intake quartile of anthocyanidins and the risk of gastric cardia adenocarcinoma

incidence and for the associations between the highest intake quartile of anthocyanidins and lignans and the risk of other gastric adenocarcinoma incidence. We observed approximately 30-60% risk reductions for the associations between the highest intake quartile of anthocyanidins and flavanones and the risk of esophageal adenocarcinoma incidence; similar risk reductions were noted for the associations between the highest intake quartiles of anthocyanidins, flavanones, flavones, isoflavones, and lignans in relation to esophageal squamous cell carcinoma.

Our findings are consistent with previous epidemiologic studies that have reported inverse associations between anthocyanidin intake and risk of esophageal adenocarcinoma incidence,[13] and with flavanone[16, 18] and isoflavone[13] intake in relation to risk of esophageal squamous cell carcinoma incidence. Previous studies, but not our study reported here, also have noted inverse associations between: flavonols and risk of esophageal squamous cell carcinoma incidence;[15] flavonols,[14,16] flavanones,[14] flavones,[14] and lignans (secoisolariciresinol)[21] and risk of gastric adenocarcinoma (i.e., cardia and non-cardia) incidence; and flavonols (quercetin) and risk of gastric cardia adenocarcinoma incidence.[19] Observed differences between these previous studies and our own could partially be due to considering gastric adenocarcinoma as a combined entity and not according to anatomic subsite, examining single flavonoids and not classes of flavonoids, differences in study populations and dietary patterns across studies, or chance.

In the present study, notable risk reductions were observed for the associations between anthocyanidins and risk of esophageal cancer incidence, regardless of histologic subtype. However, in animal studies, results for anthocyanidins as chemopreventive agents are not consistent across histologic subtypes (i.e., squamous cell carcinoma vs. adenocarcinoma). Multiple experimental studies have shown that anthocyanidin-rich black raspberries have chemopreventive properties in N-nitrosomethylbenzylamine-induced tumors in rats (esophageal squamous cell carcinoma rodent model).[10] In a study of esophagoduodenal anastomosis, a

rodent model for reflux-induced esophageal adenocarcinoma, freeze-dried black raspberries were not effective in chemoprevention of esophageal adenocarcinoma.[34] However, interim clinical trial results for Barrett's esophagus, a potential precursor of esophageal adenocarcinoma, found reduced markers of oxidative stress in Barrett's esophagus patients consuming anthocyanidin-rich freeze-dried black raspberries.[35]

A previous study, based on data from the same parent study as our own, found a modest inverse association between fruit and vegetable intake and the risk of incidence for both histologic subtypes of esophageal cancer.[7] As flavonoids are concentrated in fruits and vegetables,[36] the association between flavonoids and esophageal or gastric cancer incidence may reflect diets with greater consumption of such foods or a healthy lifestyle in general. The parent study assessed many lifestyle factors, including cigarette smoking, alcohol intake, and BMI (as a measure of adiposity), individually[22] and through the use of pattern analyses.[7] Cigarette smoking was the only covariate among these that influenced our findings and was adjusted for in the final models. Thus, lifestyle seems unlikely to account for our results.

When estimating flavonoid content in food, particularly fruits and vegetables, potential sources of error include plant varieties, degree of ripeness, storage conditions, distance transported to market, environmental factors affecting plant growth, horticultural practices, industrial processing, and cooking methods, which may vary over time and by geographic region.[28,29,36] Therefore, the foods utilized to create the nutrient database estimates may differ from the actual food sources reportedly consumed by this study population.[28-30] To estimate the potential impact of such influences, the USDA Food Composition and Nutrient Data Laboratories sampled over 60 fruits, vegetables, and nuts from four U.S. regions at two times of the year and estimated the flavonoid content. Values reported in the USDA databases were similar to average flavonoid content determined in this study, although high variability of flavonoid content was seen within and between foods.[37] Additionally, the FFQ line item for wine did not distinguish between red and white, which have different flavonoid concentrations.[28] In

our study reported here, weights of 50% for red and 50% for white were assigned. However, individuals often preferentially drink red or white wine, and thus, individual estimates of flavonoid types for which wine is a source may be misclassified.

Bioavailability of flavonoid compounds is another potential source of error when estimating the amount of flavonoid intake necessary to reduce risk of esophageal or gastric cancer. However, little is known about flavonoid absorption in the gastrointestinal tract, metabolism of flavonoids varies by individual, and the degree to which flavonoids might have direct effects on epithelial surfaces as they traverse the esophagus and stomach is unclear.[38] Additionally, absorption profiles of flavonoids vary, with maximum concentrations reached between 0.5-9 hours after dietary intake.[39] Thus, serum flavonoid biomarkers may not be highly correlated with usual adult dietary intake, which can vary seasonally and for other reasons, and which for cancer studies is the target exposure. While such variation in estimating representative dietary flavonoid intakes and bioavailability may be a study limitation, this issue would apply to greater or lesser degrees to all studies reliant on nutritional databases to estimate dietary intake.[27]

Patients with gastroesophageal reflux disease or Barrett's esophagus are recommended to omit foods that are mechanically or chemically irritating, including some flavonoid-rich foods (e.g., coffee, tea, alcohol, citrus, tomatoes, chocolate, peppers, and onions).[40] While the FFQ assessed dietary habits 3-5 years prior to diagnosis, esophageal adenocarcinoma patients may already have symptoms prior to diagnosis, causing their usual diet to change, or perhaps, reporting of past diet could be influenced by current dietary habits. However, the foods that are irritating vary by individual;[41] thus, we are unable to estimate how such potential changes in diet would have affected our flavonoid intake values. Additionally, a dietary study showed that intakes of fruits, vegetables, and alcohol did not differ by symptomatic gastroesophageal reflux disease status.[42] While it is unknown if gastroesophageal reflux disease or Barrett's esophagus are necessary precursors of esophageal adenocarcinoma,[43] it is still possible that associations

observed in case-control studies between flavonoid intake and risk of esophageal adenocarcinoma are due to reverse causation. However, examining the comparison case group of esophageal squamous cell carcinoma we see similar associations, with the exception of isoflavones. Thus, it seems unlikely that our observed associations are completely due to reverse causation.

FFQ responses for the 3-5 years prior to diagnosis are assumed to reflect usual adult diet, both pre- and post-diagnosis. Whether such time period, even assessed accurately, reflects intakes during the time relevant to esophageal and gastric cancer development is unknown. However, because all existing studies conducted among esophageal and gastric cancer patients have relied on a FFQ,[6,44-46] one would need to conduct a cohort study and employ multiple alternative dietary assessment methods over time to overcome limitations of existing studies. Such an alternative study design would be very inefficient, because the lifetime risk of esophageal or gastric cancer in the general U.S. population is less than one percent.[47] Similarly for the survival analyses, potential changes in dietary intake after diagnosis may be a relevant exposure but we were not able to assess this from the data available.

Our study FFQ did not assess dietary supplement use of flavonoids; however, it is unlikely that use of flavonoid-rich supplements was widespread during this study time period. In the early 1990s, clinical studies of flavonoid supplements began;[48] however, *Ginkgo biloba* extract, EGb 761, was not patented in the U.S. until 1995,[49] which is after completion of participant interviews in our parent study.[22]

The issues of multiple comparisons needs to be considered when discussing the study results, as there were 64 comparisons within the main analyses, given we considered 8 exposures and 8 outcomes. Thus, there is a possibility that some statistically significant results arose due to chance. Adjusting for multiple comparisons would reduce the likelihood of detecting a false positive association, but would reduce power for detecting a true association if one exists. Instead, we chose to focus on associations based on biologic plausibility and

consistency with published results;[27,50] we also gave more credence to results that were consistent across the continuum of cancer development. For example, in the current study, we noted inverse associations for both incidence and survival for esophageal adenocarcinoma in relation to anthocyanidin intake.

In summary, our population-based findings suggest that dietary intake of some types of flavonoids, particularly anthocyanidins, may lower the risk of esophageal and gastric cancer incidence and may potentially enhance survival. In contrast, total dietary flavonoid intake does not appear to be inversely associated with these tumors in our study population. This is the first epidemiologic study to examine the association of flavonoids and lignans with survival among esophageal and gastric cancer cases, and one of few studies to examine these compounds in associations with the risk of incident esophageal and gastric cancer by tumor type. Thus, further research is needed before definite conclusions can be made about the chemopreventive role of dietary flavonoids and lignans on esophageal and gastric cancer incidence and survival.

Table 3.1. Mean Intakes (mg/day) of Flavonoids and Lignans Among Case and Control Participants, U.S. Multi-Center Study, Connecticut, New Jersey, and Western Washington State: 1993-1995.

	Controls (N=662)			Esophageal Adenocarcinoma (N=274)			Gastric Cardia Adenocarcinoma (N=248)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Total Flavonoids	188.31	240.76	0.77-2182.23	198.19	245.12	8.45-2199.36	219.90	270.10	5.85-1753.52
Anthocyanidins	14.78	11.84	0-83.18	13.34	16.60	0.09-166.59	15.50	16.31	0.41-127.54
Flavan-3-ols	121.61	229.64	0.29-2064.10	138.10	234.35	1.07-2063.16	150.53	256.87	0.68-1650.30
Flavanones	34.93	27.00	0-231.14	29.42	24.46	0.004-125.24	35.04	24.99	0.02-113.73
Flavones	2.07	1.16	0-7.56	2.09	1.39	0-12.50	2.26	1.39	0.17-8.40
Flavonols	14.46	9.41	0.36-78.60	14.70	9.68	2.22-72.82	16.04	10.63	3.18-67.17
Isoflavones	0.47	0.29	0.02-2.81	0.54	0.33	0.03-2.82	0.53	0.31	0.07-1.99
Lignans	0.068	0.032	0.011-0.286	0.069	0.039	0.014-0.304	0.073	0.036	0.016-0.247

	Esophageal Squamous Cell Carcinoma (N=191)			Other Gastric Adenocarcinoma (N=341)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Total Flavonoids	182.98	206.29	7.13-1098.55	219.20	322.57	7.05-2796.17
Anthocyanidins	13.51	14.88	0.12-97.54	13.32	9.78	0.42-67.98
Flavan-3-ols	121.54	198.64	0.68-1037.81	154.01	307.90	0.93-2467.85
Flavanones	29.85	26.08	0-227.42	34.47	26.77	0.06-226.64
Flavones	1.86	1.26	0.02-6.75	2.02	1.16	0.02-8.01
Flavonols	15.74	10.41	1.85-55.62	14.90	11.36	3.34-98.33
Isoflavones	0.49	0.27	0.05-1.67	0.49	0.28	0.03-2.24
Lignans	0.061	0.032	0.011-0.210	0.065	0.030	0.010-0.266

Table 3.2. Major Sources of Flavonoids and Lignans Among a Population-based Sample of Control Participants Without Esophageal or Gastric Cancer With Information on Dietary Intake, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Flavonoid/Phytoestrogen Class	Representative Flavonoids	Main FFQ Line Item Sources (%)
Total Flavonoids		Black tea (55.8), orange/grapefruit juice (14.2), wine (4.5)
Anthocyanidins	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin	Wine (29.1), banana (13.7), fruit juice (10.6), other fruit (fruit cocktail, grapes, pineapple, blueberries, applesauce) (9.0), apples (7.5), pies (6.0), bean soup (5.9)
Flavan-3-ols	(+)-Catechin, (+)-Catechin-3-gallate, (-)-Epicatechin, (-)-Epicatechin-3-gallate, (-)-Epigallocatechin, (-)-Epigallocatechin-3-gallate, (+)-Gallocatechin, (+)-Gallocatechin-3-gallate, Theaflavin, Theaflavin-3-gallate, Theaflavin-3'-gallate, Theaflavin-3,3'-digallate, Thearubigins	Black tea (83.7), beer (3.2), green tea (3.1), wine (2.0) , apples (1.9), bananas (1.4)
Flavanones	Eriodictyol, Hesperetin, Naringenin	Orange/grapefruit juice (75.4), oranges (21.5), wine (1.8)
Flavones	Apigenin, Luteolin	Pizza (27.5), Wine (13.0), Celery (8.3), vegetable soup (7.8), mixed salad (7.8), cream soups (7.0), chicken noodle soup (5.4)
Flavonols	Isorhamnetin, Kaempferol, Myricetin, Quercetin	Black tea (22.9), onions (10.5), beer (9.2), apples (8.1), wine (5.9), mixed salad (5.9)
Isoflavones	Daidzein, Genistein, Glycitein	Coffee (36.8), chili (17.4), white bread (10.8), cake (8.4), fried chicken (5.1), sausage (4.5)
Lignans	Matairesinol, Secoisolariciresinol	Coffee (34.8), orange juice (13.4), wine (10.3), black tea (5.0), onions (3.5)

Table 3.3. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for Associations Between Flavonoid and Lignan Intake and Esophageal and Gastric Cancer Incidence by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Other Gastric Adenocarcinoma	
Variable and intake (mg/day)	Controls (N=662)	Cases (N=274)	OR (95% CI)[2]	Cases (N=248)	OR (95% CI)[2]	Cases (N=191)	OR (95% CI)[2]	Cases (N=341)	OR (95% CI)[2]
Total Flavonoids									
0-63.81	165	83	1.00	59	1.00	48	1.00	80	1.00
63.82-97.90	166	54	0.62 (0.41, 0.93)	60	1.00 (0.65, 1.54)	35	0.64 (0.38, 1.07)	84	1.01 (0.69, 1.49)
97.91-217.35	165	57	0.64 (0.42, 0.97)	54	0.90 (0.58, 1.41)	61	1.11 (0.69, 1.77)	78	0.92 (0.62, 1.37)
≥217.36	166	80	0.92 (0.63, 1.37)	75	1.32 (0.87, 2.00)	47	0.87 (0.53, 1.41)	99	1.08 (0.73, 1.58)
P for trend[3]			0.61		0.07		0.42		0.50
Anthocyanidins									
0-7.21	166	98	1.00	67	1.00	67	1.00	92	1.00
7.22-11.53	165	69	0.65 (0.45, 0.96)	67	0.98 (0.65, 1.47)	51	0.68 (0.43, 1.06)	89	0.91 (0.63, 1.32)
11.54-18.47	165	59	0.54 (0.36, 0.81)	63	0.91 (0.60, 1.38)	37	0.44 (0.27, 0.72)	91	0.89 (0.61, 1.29)
≥18.48	166	48	0.43 (0.29, 0.66)	51	0.71 (0.46, 1.10)	36	0.43 (0.26, 0.70)	69	0.70 (0.47, 1.03)
P for trend[3]			0.06		0.91		0.11		0.10
Flavan-3-ols									
0-10.29	165	75	1.00	59	1.00	45	1.00	71	1.00
10.30-22.00	166	61	0.72 (0.48, 1.09)	50	0.77 (0.50, 1.20)	37	0.78 (0.47, 1.30)	101	1.45 (0.99, 2.14)
22.01-130.69	165	56	0.65 (0.43, 1.00)	69	1.05 (0.69, 1.61)	62	1.24 (0.77, 2.00)	68	0.98 (0.65, 1.48)
≥130.70	166	82	1.02 (0.69, 1.51)	70	1.17 (0.77, 1.78)	47	0.98 (0.60, 1.59)	101	1.30 (0.88, 1.92)
P for trend[3]			0.32		0.07		0.68		0.36
Flavanones									
0-11.57	166	91	1.00	62	1.00	61	1.00	89	1.00
11.58-34.95	165	61	0.67 (0.45, 1.00)	54	0.86 (0.56, 1.33)	45	0.70 (0.44, 1.11)	80	0.92 (0.63, 1.35)
34.96-49.52	165	69	0.75 (0.50, 1.10)	59	1.01 (0.66, 1.55)	49	0.69 (0.43, 1.10)	83	0.86 (0.59, 1.27)
≥49.53	166	53	0.56 (0.37, 0.85)	73	1.23 (0.81, 1.87)	36	0.48 (0.29, 0.78)	89	0.88 (0.60, 1.28)
P for trend[3]			0.003		0.82		0.002		0.36

(Table 3.3. continued)

Variable and intake (mg/day)	Controls (N=662)	Esophageal Adenocarcinoma Cases (N=274)	Esophageal Adenocarcinoma OR (95% CI)[2]	Gastric Cardia Adenocarcinoma Cases (N=248)	Gastric Cardia Adenocarcinoma OR (95% CI)[2]	Esophageal Squamous Cell Carcinoma Cases (N=191)	Esophageal Squamous Cell Carcinoma OR (95% CI)[2]	Other Gastric Adenocarcinoma Cases (N=341)	Other Gastric Adenocarcinoma OR (95% CI)[2]
Flavones									
0-1.29	165	82	1.00	60	1.00	71	1.00	95	1.00
1.30-1.90	166	58	0.67 (0.45, 1.00)	55	0.87 (0.57, 1.34)	51	0.70 (0.45, 1.09)	87	0.92 (0.64, 1.34)
1.91-2.62	166	59	0.68 (0.45, 1.02)	62	0.98 (0.64, 1.50)	28	0.34 (0.20, 0.57)	72	0.77 (0.52, 1.13)
≥2.63	165	75	0.84 (0.56, 1.25)	71	1.09 (0.71, 1.67)	41	0.55 (0.34, 0.89)	87	1.01 (0.69, 1.50)
P for trend[3]			0.81		0.15		0.02		0.98
Flavonols									
0-8.31	166	86	1.00	52	1.00	56	1.00	82	1.00
8.32-12.16	165	49	0.56 (0.37, 0.85)	64	1.24 (0.81, 1.91)	33	0.59 (0.36, 0.98)	94	1.16 (0.80, 1.69)
12.17-17.81	166	61	0.67 (0.45, 1.00)	53	1.01 (0.65, 1.57)	39	0.70 (0.43, 1.14)	79	0.96 (0.65, 1.41)
≥17.82	165	78	0.80 (0.54, 1.18)	79	1.42 (0.93, 2.17)	63	0.97 (0.62, 1.53)	86	0.98 (0.67, 1.46)
P for trend[3]			0.71		0.10		0.70		0.92
Isoflavones									
0-0.27	165	47	1.00	36	1.00	34	1.00	65	1.00
0.28-0.41	166	51	0.98 (0.61, 1.55)	64	1.55 (0.97, 2.49)	50	1.12 (0.67, 1.89)	90	1.45 (0.97, 2.17)
0.42-0.59	166	83	1.51 (0.97, 2.37)	73	1.62 (1.00, 2.63)	59	1.18 (0.69, 2.00)	97	1.68 (1.10, 2.56)
≥0.60	165	93	1.65 (1.02, 2.65)	75	1.56 (0.93, 2.60)	48	0.72 (0.40, 1.29)	89	1.50 (0.96, 2.37)
P for trend[3]			0.07		0.17		0.11		0.37
Lignans									
0-0.045	165	70	1.00	50	1.00	67	1.00	87	1.00
0.046-0.063	166	75	0.91 (0.61, 1.36)	61	1.06 (0.69, 1.65)	47	0.64 (0.40, 1.01)	92	1.09 (0.75, 1.59)
0.064-0.082	165	57	0.65 (0.42, 0.99)	70	1.17 (0.76, 1.81)	42	0.50 (0.31, 0.82)	98	1.11 (0.76, 1.63)
≥0.083	166	72	0.75 (0.49, 1.13)	67	1.01 (0.65, 1.58)	35	0.38 (0.23, 0.63)	64	0.73 (0.48, 1.11)
P for trend[3]			0.26		0.63		0.0003		0.13

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]Odds ratio (95% confidence interval). [3]P-value for trend for continuous variable.

Table 3.4. Adjusted[1] Hazard Ratios (HR) and 95% Confidence Intervals (CI) for Flavonoid and Lignan Intake and Overall Mortality in Esophageal and Gastric Cancer Cases by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995 through 2000.

	Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Other Gastric Adenocarcinoma	
Variable and intake (mg/day)	Cases (N=274)	HR (95% CI)[2]	Cases (N=248)	HR (95% CI)[2]	Cases (N=191)	HR (95% CI)[2]	Cases (N=338)	HR (95% CI)[2]
Total Flavonoids								
0-62.35	83	1.00	57	1.00	47	1.00	76	1.00
62.36-103.39	60	1.37 (0.95, 1.98)	66	1.05 (0.71, 1.54)	42	1.26 (0.81, 1.97)	95	1.01 (0.71, 1.42)
103.40-253.24	65	1.39 (0.97, 1.98)	60	0.79 (0.54, 1.17)	61	0.92 (0.60, 1.40)	77	0.72 (0.50, 1.05)
≥253.25	66	0.98 (0.68, 1.41)	65	0.88 (0.59, 1.32)	41	0.91 (0.58, 1.44)	90	0.99 (0.70, 1.39)
P for trend[3]		0.11		0.80		0.11		0.90
Anthocyanidins								
0-6.23	83	1.00	52	1.00	57	1.00	70	1.00
6.24-10.11	61	0.93 (0.65, 1.35)	64	0.76 (0.51, 1.12)	52	0.77 (0.50, 1.18)	86	1.27 (0.89, 1.82)
10.12-16.23	67	0.88 (0.62, 1.26)	66	0.80 (0.54, 1.18)	41	1.46 (0.94, 2.26)	89	1.22 (0.84, 1.76)
≥16.24	63	0.87 (0.60, 1.26)	66	0.63 (0.42, 0.95)	41	1.01 (0.66, 1.56)	93	1.14 (0.80, 1.63)
P for trend[3]		0.14		0.25		0.94		0.55
Flavan-3-ols								
0-10.90	80	1.00	61	1.00	47	1.00	75	1.00
10.91-26.67	62	0.92 (0.64, 1.32)	53	0.69 (0.46, 1.04)	46	1.68 (1.08, 2.62)	102	1.33 (0.94, 1.87)
26.68-210.51	66	1.20 (0.84, 1.72)	71	0.78 (0.54, 1.13)	56	0.97 (0.63, 1.50)	70	0.96 (0.66, 1.39)
≥210.52	66	0.93 (0.65, 1.33)	63	0.71 (0.48, 1.05)	42	1.09 (0.69, 1.74)	91	1.22 (0.87, 1.73)
P for trend[3]		0.11		0.94		0.10		0.80
Flavanones								
0-8.63	81	1.00	55	1.00	50	1.00	77	1.00
8.64-32.94	66	0.96 (0.67, 1.38)	60	1.12 (0.75, 1.69)	54	0.95 (0.61, 1.47)	83	0.70 (0.49, 1.00)
34.95-49.00	71	1.40 (0.99, 1.98)	57	1.24 (0.83, 1.86)	48	1.05 (0.67, 1.63)	87	0.74 (0.52, 1.05)
≥49.01	56	1.15 (0.79, 1.68)	76	0.90 (0.61, 1.33)	39	1.24 (0.76, 2.03)	91	0.66 (0.46, 0.93)
P for trend[3]		0.05		0.50		0.80		0.18

(Table 3.4. continued)

Variable and intake (mg/day)	Esophageal Adenocarcinoma Cases (N=274)	Esophageal Adenocarcinoma HR (95% CI)[2]	Gastric Cardia Adenocarcinoma Cases (N=248)	Gastric Cardia Adenocarcinoma HR (95% CI)[2]	Esophageal Squamous Cell Carcinoma Cases (N=191)	Esophageal Squamous Cell Carcinoma HR (95% CI)[2]	Other Gastric Adenocarcinoma Cases (N=338)	Other Gastric Adenocarcinoma HR (95% CI)[2]
Flavones								
0-1.20	79	1.00	46	1.00	64	1.00	74	1.00
1.21-1.81	52	0.97 (0.66, 1.41)	63	1.03 (0.68, 1.56)	48	1.23 (0.82, 1.84)	100	0.88 (0.63, 1.24)
1.82-2.64	73	0.89 (0.63, 1.26)	70	0.95 (0.63, 1.44)	39	0.88 (0.56, 1.38)	80	0.88 (0.61, 1.26)
≥2.65	70	0.83 (0.58, 1.19)	69	0.86 (0.56, 1.31)	40	1.00 (0.64, 1.54)	84	0.97 (0.67, 1.39)
P for trend[3]		0.04		0.54		0.17		0.62
Flavonols								
0-8.16	79	1.00	50	1.00	55	1.00	79	1.00
8.17-12.30	60	1.30 (0.89, 1.89)	68	1.25 (0.83, 1.87)	36	1.14 (0.70, 1.83)	98	1.12 (0.80, 1.57)
12.31-19.34	72	1.01 (0.71, 1.43)	63	1.11 (0.74, 1.68)	45	0.78 (0.50, 1.20)	84	1.09 (0.78, 1.54)
≥19.35	63	0.94 (0.65, 1.37)	67	0.93 (0.61, 1.40)	55	0.93 (0.61, 1.40)	77	0.97 (0.68, 1.38)
P for trend[3]		0.05		0.35		0.69		0.71
Isoflavones								
0-0.31	65	1.00	59	1.00	48	1.00	89	1.00
0.32-0.46	56	1.00 (0.68, 1.47)	57	1.26 (0.84, 1.87)	60	1.03 (0.68, 1.58)	90	1.29 (0.92, 1.82)
0.47-0.62	73	0.70 (0.48, 1.03)	68	0.94 (0.62, 1.44)	42	1.14 (0.71, 1.81)	81	0.86 (0.60, 1.25)
≥0.63	80	0.75 (0.49, 1.13)	64	1.01 (0.65, 1.57)	41	0.97 (0.60, 1.58)	78	0.92 (0.62, 1.37)
P for trend[3]		0.65		0.77		0.60		0.88
Lignans								
0-0.044	68	1.00	50	1.00	62	1.00	83	1.00
0.045-0.060	72	0.85 (0.60, 1.23)	55	1.45 (0.95, 2.22)	50	0.73 (0.47, 1.13)	85	1.22 (0.86, 1.73)
0.061-0.079	57	0.98 (0.67, 1.45)	66	1.05 (0.70, 1.59)	41	0.61 (0.39, 0.96)	99	1.08 (0.77, 1.51)
≥0.080	77	0.78 (0.54, 1.14)	77	0.97 (0.65, 1.46)	38	0.58 (0.37, 0.92)	71	1.05 (0.72, 1.53)
P for trend[3]		0.28		0.43		0.07		0.55

[1]Adjusted for stage (localized, regional, distant, unknown), and dietary energy intake (kilocalories, continuous). [2]Hazard ratio (95% confidence interval). [3]P-value for trend for continuous variable.

Table 3.5. Distribution (N [%]) of Characteristics Among Control Participants and Esophageal and Gastric Cancer Patients by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Characteristic	Controls N=662	Esophageal Adenocarcinoma N=274	Gastric Cardia Adenocarcinoma N=248	Esophageal Squamous Cell Carcinoma N=191	Other Gastric Adenocarcinoma N=341
Age					
<57	170 (25.7)	69 (25.2)	59 (23.8)	28 (14.7)	57 (16.7)
57-64	170 (25.7)	46 (16.8)	51 (20.6)	50 (26.2)	57 (16.7)
65-71	145 (21.9)	64 (23.4)	60 (24.2)	52 (27.2)	71 (20.8)
>71	177 (26.7)	95 (34.7)	78 (31.5)	61 (31.9)	156 (45.8)
Sex					
Male	528 (79.8)	228 (83.2)	210 (84.7)	151 (79.1)	237 (69.5)
Female	134 (20.2)	46 (16.8)	38 (15.3)	40 (20.9)	104 (30.5)
Race					
White	617 (93.2)	271 (98.9)	241 (97.2)	151 (79.1)	292 (85.6)
Other	45 (6.8)	3 (1.1)	7 (2.8)	40 (20.9)	49 (14.4)
Education					
<High School	120 (18.1)	62 (22.7)	51 (20.6)	74 (38.7)	107 (31.4)
High School	165 (24.9)	85 (31.1)	82 (33.2)	61 (31.9)	99 (29.0)
Some College	170 (25.7)	72 (26.4)	57 (23.1)	33 (17.3)	77 (22.6)
College or more	207 (31.3)	54 (19.8)	57 (23.1)	23 (12.0)	58 (17.0)
Missing	0	1	1	0	0
Income level, US $					
<15,000	83 (12.5)	52 (19.0)	36 (14.5)	56 (29.3)	78 (22.9)
15,000-29,999	165 (24.9)	84 (30.7)	78 (31.5)	66 (34.6)	105 (30.8)
30,000-49,999	172 (26.0)	63 (23.0)	65 (26.2)	50 (26.2)	95 (27.9)
50,000-74,999	121 (18.3)	40 (14.6)	34 (13.7)	9 (4.7)	43 (12.6)
≥75,000	121 (18.3)	35 (12.8)	35 (14.1)	10 (5.2)	20 (5.9)
Cigarette Use					
Ever	425 (64.2)	213 (77.7)	196 (79.0)	175 (91.6)	244 (71.8)
Never	237 (35.8)	61 (22.3)	52 (21.0)	16 (8.4)	96 (28.2)
Geographic Center					
Connecticut	193 (29.2)	74 (27.0)	77 (31.1)	75 (39.3)	108 (31.7)
New Jersey	322 (48.6)	134 (48.9)	110 (44.4)	84 (44.0)	164 (48.1)
Washington	147 (22.2)	66 (24.1)	61 (24.6)	32 (16.8)	69 (20.2)
Tumor Stage					
Localized		73 (26.6)	31 (12.5)	45 (23.6)	68 (20.1)
Regional		70 (25.6)	114 (46.0)	72 (37.7)	148 (43.8)
Distant		75 (27.4)	68 (27.4)	33 (17.3)	95 (28.1)
Unknown		56 (20.4)	35 (14.1)	41 (21.5)	27 (8.0)

Table 3.6. Sensitivity Analysis of Mean Intakes (mg/day) of Anthocyanidins (Bananas Included versus Excluded), U.S. Multi-Center Study, Connecticut, New Jersey, and Western Washington State: 1993-1995.

	Controls (N=662)			Esophageal Adenocarcinoma (N=274)			Gastric Cardia Adenocarcinoma (N=248)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Anthocyanidins, Bananas Included	14.78	11.84	0-83.18	13.34	16.80	0.09-166.59	15.50	16.31	0.41-127.54
Anthocyanidins, Bananas Excluded	12.76	11.54	0-82.85	11.73	16.60	0.09-166.07	13.65	15.81	0.41-127.54

	Esophageal Squamous Cell Carcinoma (N=191)			Other Gastric Adenocarcinoma (N=341)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Anthocyanidins, Bananas Included	13.51	14.88	0.12-97.54	13.32	9.78	0.42-67.98
Anthocyanidins, Bananas Excluded	11.86	14.69	0.12-97.31	11.06	9.20	0.42-60.43

Table 3.7. Sensitivity Analysis of the Major Sources of Anthocyanidins (Bananas Included versus Excluded) Among a Population-based Sample of Control Participants Without Esophageal or Gastric Cancer With Information on Dietary Intake, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

Flavonoid/Phytoestrogen Class	Main FFQ Line Item Sources (%)
Anthocyanidins, Bananas Included	Wine (29.1), banana (13.7), fruit juice (10.6), other fruit (fruit cocktail, grapes, pineapple, blueberries, applesauce) (9.0), apples (7.5), pies (6.0), bean soup (5.9)
Anthocyanidins, Bananas Excluded	Wine (33.7), fruit juice (12.3), other fruit (fruit cocktail, grapes, pineapple, blueberries, applesauce) (10.4), apples (8.7), pies (7.0), bean soup (6.9)

Table 3.8. Sensitivity Analysis of Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Anthocyanidin Intake (Bananas Included versus Excluded) and Esophageal and Gastric Cancer Incidence by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995.

		Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Other Gastric Adenocarcinoma	
Variable and intake (mg/day)	Controls (N=660)	Cases (N=274)	OR (95% CI)[2]	Cases (N=248)	OR (95% CI)[2]	Cases (N=191)	OR (95% CI)[2]	Cases (N=341)	OR (95% CI)[2]
Anthocyanidins, Bananas Included									
0-7.21	166	98	1.00	67	1.00	67	1.00	92	1.00
7.22-11.53	165	69	0.65 (0.45, 0.96)	67	0.98 (0.65, 1.47)	51	0.68 (0.43, 1.06)	89	0.91 (0.63, 1.32)
11.54-18.47	165	59	0.54 (0.36, 0.81)	63	0.91 (0.60, 1.38)	37	0.44 (0.27, 0.72)	91	0.89 (0.61, 1.29)
≥18.48	166	48	0.43 (0.29, 0.66)	51	0.71 (0.46, 1.10)	36	0.43 (0.26, 0.70)	69	0.70 (0.47, 1.03)
P for trend[3]			0.06		0.91		0.11		0.10
Anthocyanidins, Bananas Excluded									
0-5.53	165	100	1.00	57	1.00	71	1.00	95	1.00
5.54-9.57	166	64	0.59 (0.40, 0.87)	76	1.27 (0.84, 1.92)	50	0.68 (0.43, 1.06)	97	1.04 (0.72, 1.50)
9.58-15.92	165	60	0.56 (0.37, 0.83)	63	1.07 (0.70, 1.65)	36	0.40 (0.25, 0.66)	85	0.85 (0.58, 1.24)
≥15.93	166	50	0.45 (0.30, 0.68)	52	0.84 (0.54, 1.31)	34	0.41 (0.25, 0.67)	64	0.68 (0.45, 1.01)
P for trend[3]			0.14		0.79		0.24		0.07

[1]Adjusted for age (continuous), sex, race (white, other), geographic center (Connecticut, New Jersey, Washington), cigarette smoking (ever/never), dietary energy intake (kilocalories, continuous). [2]Odds ratio (95% confidence interval). [3]P-value for trend for continuous variable.

Table 3.9. Sensitivity Analysis of Adjusted[1] Hazard Ratios (HR) and 95% Confidence Intervals (CI) for Anthocyanidin Intakes (Bananas Included versus Excluded) and Overall Mortality in Esophageal and Gastric Cancer Cases by Tumor Type, U.S. Multi-Center Study: Connecticut, New Jersey, and Western Washington State, 1993-1995 through 2000.

	Esophageal Adenocarcinoma		Gastric Cardia Adenocarcinoma		Esophageal Squamous Cell Carcinoma		Other Gastric Adenocarcinoma	
Variable and intake (mg/day)	Cases (N=274)	HR (95% CI)[2]	Cases (N=248)	HR (95% CI)[2]	Cases (N=191)	HR (95% CI)[2]	Cases (N=338)	HR (95% CI)[2]
Anthocyanidins, Bananas Included								
0-6.23	83	1.00	52	1.00	57	1.00	70	1.00
6.24-10.11	61	0.93 (0.65, 1.35)	64	0.76 (0.51, 1.12)	52	0.77 (0.50, 1.18)	86	1.27 (0.89, 1.82)
10.12-16.23	67	0.88 (0.62, 1.26)	66	0.80 (0.54, 1.18)	41	1.46 (0.94, 2.26)	89	1.22 (0.84, 1.76)
≥16.24	63	0.87 (0.60, 1.26)	66	0.63 (0.42, 0.95)	41	1.01 (0.66, 1.56)	93	1.14 (0.80, 1.63)
P for trend[3]		0.14		0.25		0.94		0.55
Anthocyanidins, Bananas Excluded								
0-4.77	85	1.00	45	1.00	58	1.00	74	1.00
4.78-8.34	58	1.15 (0.79, 1.68)	74	0.84 (0.56, 1.25)	49	0.73 (0.48, 1.12)	82	1.06 (0.74, 1.51)
8.35-13.75	62	0.96 (0.67, 1.37)	62	0.68 (0.45, 1.04)	43	1.11 (0.72, 1.70)	97	1.09 (0.77, 1.54)
≥13.76	69	0.83 (0.58, 1.19)	67	0.66 (0.43, 1.02)	41	0.87 (0.56, 1.36)	85	0.97 (0.67, 1.40)
P for trend[3]		0.12		0.26		0.66		0.35

[1]Adjusted for stage (localized, regional, distant, unknown), and dietary energy intake (kilocalories, continuous). [2]Hazard ratio (95% confidence interval). [3]P-value for trend for continuous variable.

REFERENCES

1. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-53.

2. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. *CA: a cancer journal for clinicians* 2012.

3. Wijnhoven BP, Siersema PD, Hop WC, van Dekken H, Tilanus HW. Adenocarcinomas of the distal oesophagus and gastric cardia are one clinical entity. Rotterdam Oesophageal Tumour Study Group. *Br J Surg* 1999;86(4):529-35.

4. Hongo M, Nagasaki Y, Shoji T. Epidemiology of esophageal cancer: Orient to Occident. Effects of chronology, geography and ethnicity. *Journal of gastroenterology and hepatology* 2009;24(5):729-35.

5. de Martel C, Forman D, Plummer M. Gastric cancer: epidemiology and risk factors. *Gastroenterology clinics of North America* 2013;42(2):219-40.

6. Kubo A, Corley DA, Jensen CD, Kaur R. Dietary factors and the risks of oesophageal adenocarcinoma and Barrett's oesophagus. *Nutrition research reviews* 2010;23(2):230-46.

7. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer* 2008;123(4):852-60.

8. Lunet N, Valbuena C, Vieira AL, Lopes C, David L, Carneiro F, Barros H. Fruit and vegetable consumption and gastric cancer by location and histological type: case-control and meta-analysis. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation* 2007;16(4):312-27.

9. Pierini R, Kroon PA, Guyot S, Ivory K, Johnson IT, Belshaw NJ. Procyanidin effects on oesophageal adenocarcinoma cells strongly depend on flavan-3-ol degree of polymerization. *Mol Nutr Food Res* 2008;52(12):1399-407.

10. Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. *Nutr Cancer* 2006;54(1):33-46.

11. Schwartz GK, Ilson D, Saltz L, O'Reilly E, Tong W, Maslak P, Werner J, Perkins P, Stoltz M, Kelsen D. Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2001;19(7):1985-92.

12. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer* 2010;62(1):1-20.

13. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int J Cancer* 2009;125(5):1147-54.

14. Lagiou P, Samoli E, Lagiou A, Peterson J, Tzonou A, Dwyer J, Trichopoulos D. Flavonoids, vitamin C and adenocarcinoma of the stomach. *Cancer Causes Control* 2004;15(1):67-72.

15. Rossi M, Garavello W, Talamini R, La Vecchia C, Franceschi S, Lagiou P, Zambon P, Dal Maso L, Bosetti C, Negri E. Flavonoids and risk of squamous cell esophageal cancer. *International journal of cancer. Journal international du cancer* 2007;120(7):1560-4.

16. Rossi M, Rosato V, Bosetti C, Lagiou P, Parpinel M, Bertuccio P, Negri E, La Vecchia C. Flavonoids, proanthocyanidins, and the risk of stomach cancer. *Cancer causes & control : CCC* 2010;21(10):1597-604.

17. Vermeulen E, Zamora-Ros R, Duell EJ, Lujan-Barroso L, Boeing H, Aleksandrova K, Bueno-de-Mesquita HB, Scalbert A, Romieu I, Fedirko V, Touillaud M, Fagherazzi G, Perquier F, Molina-Montes E, Chirlaque MD, Vicente Arguelles M, Amiano P, Barricarte A, Pala V, Mattiello A, Saieva C, Tumino R, Ricceri F, Trichopoulou A, Vasilopoulou E, Ziara G, Crowe FL, Khaw KT, Wareham NJ, Lukanova A, Grote VA, Tjonneland A, Halkjaer J, Bredsdorff L, Overvad K, Siersema PD, Peeters PH, May AM, Weiderpass E, Skeie G, Hjartaker A, Landberg R, Johansson I, Sonestedt E, Ericson U, Riboli E, Gonzalez CA. Dietary flavonoid intake and esophageal cancer risk in the European prospective investigation into cancer and nutrition cohort. *American journal of epidemiology* 2013;178(4):570-81.

18. Zamora-Ros R, Agudo A, Lujan-Barroso L, Romieu I, Ferrari P, Knaze V, Bueno-de-Mesquita HB, Leenders M, Travis RC, Navarro C, Sanchez-Cantalejo E, Slimani N, Scalbert A, Fedirko V, Hjartaker A, Engeset D, Skeie G, Boeing H, Forster J, Li K, Teucher B, Agnoli C, Tumino R, Mattiello A, Saieva C, Johansson I, Stenling R, Redondo ML, Wallstrom P, Ericson U, Khaw KT, Mulligan AA, Trichopoulou A, Dilis V, Katsoulis M, Peeters PH, Igali L, Tjonneland A, Halkjaer J, Touillaud M, Perquier F, Fagherazzi G, Amiano P, Ardanaz E, Bredsdorff L, Overvad K, Ricceri F, Riboli E, Gonzalez CA. Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The American journal of clinical nutrition* 2012;96(6):1398-408.

19. Ekstrom AM, Serafini M, Nyren O, Wolk A, Bosetti C, Bellocco R. Dietary quercetin intake and risk of gastric cancer: results from a population-based study in Sweden. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2011;22(2):438-43.

20. Sun CL, Yuan JM, Lee MJ, Yang CS, Gao YT, Ross RK, Yu MC. Urinary tea polyphenols in relation to gastric and esophageal cancers: a prospective study of men in Shanghai, China. *Carcinogenesis* 2002;23(9):1497-503.

21. Hernandez-Ramirez RU, Galvan-Portillo MV, Ward MH, Agudo A, Gonzalez CA, Onate-Ocana LF, Herrera-Goepfert R, Palma-Coca O, Lopez-Carrillo L. Dietary intake of

polyphenols, nitrate and nitrite and gastric cancer risk in Mexico City. *International journal of cancer. Journal international du cancer* 2009;125(6):1424-30.

22. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ, Fraumeni JF, Jr. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1997;89(17):1277-84.

23. Trivers KF, De Roos AJ, Gammon MD, Vaughan TL, Risch HA, Olshan AF, Schoenberg JB, Mayne ST, Dubrow R, Stanford JL, Abrahamson P, Rotterdam H, West AB, Fraumeni JF, Chow WH. Demographic and lifestyle predictors of survival in patients with esophageal or gastric cancers. *Clin Gastroenterol Hepatol* 2005;3(3):225-30.

24. Waksberg J. Sampling methods for random digit dialing. *J Am Stat Assoc* 1978;73:40-46.

25. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10(10):1055-62.

26. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *American journal of epidemiology* 1997;146(10):856-69.

27. Willett W. *Nutritional Epidemiology*. Vol. third. New York: Oxford University Press, 2012.

28. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav.

29. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav.

30. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutrition and cancer* 2006;54(2):184-201.

31. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008.

32. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *The American journal of clinical nutrition* 1997;65(4 Suppl):1220S-1228S; discussion 1229S-1231S.

33. Drossard C, Frohling B, Dietrich H, Kersting M. Anthocyanin analysis in banana fruit--a mistake. *The American journal of clinical nutrition* 2011;93(4):865-6; author reply 866-7.

34. Aiyer HS, Li Y, Losso JN, Gao C, Schiffman SC, Slone SP, Martin RC. Effect of freeze-dried berries on the development of reflux-induced esophageal adenocarcinoma. *Nutrition and cancer* 2011;63(8):1256-62.

35. Kresty LA, Frankel WL, Hammond CD, Baird ME, Mele JM, Stoner GD, Fromkes JJ. Transitioning from preclinical to clinical chemopreventive assessments of lyophilized black raspberries: interim results show berries modulate markers of oxidative stress in Barrett's esophagus patients. *Nutrition and cancer* 2006;54(1):148-56.

36. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. *Ann Ist Super Sanita* 2007;43(4):348-61.

37. Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, Gebhardt S. Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of agricultural and food chemistry* 2006;54(26):9966-77.

38. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews* 1998;56(11):317-33.

39. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *The British journal of nutrition* 2008;99(1):12-22.

40. Ko AH, Dollinger M, Rosenbaum EH. *Everyone's guide to cancer therapy : how cancer is diagnosed, treated, and managed day to day*. Rev. 5th ed. Kansas City: Andrews McMeel Pub., 2008.

41. Brown JE. *Nutrition through the life cycle*. 3rd ed ed. Belmont, CA: Thomson/Wadsworth, 2008.

42. El-Serag HB, Satia JA, Rabeneck L. Dietary intake and the risk of gastro-oesophageal reflux disease: a cross sectional study in volunteers. *Gut* 2005;54(1):11-7.

43. Shaheen N, Ransohoff DF. Gastroesophageal reflux, barrett esophagus, and esophageal cancer: scientific review. *Jama* 2002;287(15):1972-81.

44. Ibiebele TI, Hughes MC, Whiteman DC, Webb PM. Dietary patterns and risk of oesophageal cancers: a population-based case-control study. *The British journal of nutrition* 2012;107(8):1207-16.

45. Freedman ND, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and gastric cancer risk in a large United States prospective cohort study. *Cancer causes & control : CCC* 2008;19(5):459-67.

46. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. *International journal of cancer. Journal international du cancer* 2011;129(11):2681-93.

47. Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KAe. SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, April 2013. Accessed December 16, 2013.

48. EGb 761: ginkgo biloba extract, Ginkor. *Drugs in R&D* 2003;4(3):188-93.

49. U.S. Patent Office. Extract from Ginkgo biloba leaves, its method of preparation and pharmaceuticals containing the extract. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5,399,348.PN.&OS=PN/5,399,348&RS=PN/5,399,348 Accessed July 9, 2013.

50. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1(1):43-6.

CHAPTER 4: DIETARY FLAVONOID INTAKE AND BARRETT'S ESOPHAGUS IN WESTERN WASHINGTON STATE

Introduction

Over the last two decades, the incidence rate for esophageal adenocarcinoma has been among the most rapidly increasing of any cancer type in the United States (U.S.).[1,2] Esophageal adenocarcinoma is thought to arise in Barrett's esophagus, specialized intestinal metaplasia of the lower esophageal epithelium.[3] Studying precursor lesions may provide insight into the etiology of cancer by elucidating risk factors that act early in disease initiation. Epidemiologic studies have shown that diets high in fruit and vegetable intake are inversely associated with risk of Barrett's esophagus.[4] Flavonoids, a group of bioactive polyphenolic compounds naturally occurring in fruits, vegetables, and beverages of plant origin, may partially account for the inverse dietary association of fruits and vegetables with Barrett's esophagus.[5]

Experimental studies support the hypothesis of an inverse association between flavonoid exposure and Barrett's esophagus. For example, flavan-3-ol inhibited Barrett's esophagus cell growth through down-regulation of cyclin D1 protein expression.[6] Lignans are other polyphenolic compounds that have antioxidant properties, anti-inflammatory and pro-apoptosis effects, and promote cell cycle arrest.[7] One epidemiologic investigation to date has examined the association between dietary flavonoid intake and risk of Barrett's esophagus.[8] This case-control study of 151 Barrett's esophagus cases from the Veterans Affairs Medical Center in Houston, Texas, considered one class of flavonoids, isoflavones, and found an inverse association.[8] However, intake of isoflavone-containing foods in the U.S. is limited; whereas the other five flavonoid classes are found in foods more commonly consumed by Americans,[9] yet their associations with Barrett's esophagus have not been considered.

To determine whether intakes of total flavonoids or specific flavonoid classes are associated with risk of Barrett's esophagus, we compared flavonoid intake between patients newly diagnosed with Barrett's esophagus and general population controls who participated in a community-based case-control study.[10]

Methods

To conduct this ancillary study, we built upon data collected for the Study of Reflux Disease, a case-control investigation conducted in western Washington state.[10,11] This study was approved by the Institutional Review Boards of the participating institutions.

Study Population. Eligible case participants were men and women, aged 20-80 years without previously diagnosed Barrett's esophagus who underwent upper endoscopy for gastroesophageal reflux disease (GERD) symptoms between 1997 and 2000 at community gastroenterology clinics.[10,11] Consenting participants had four-quadrant biopsy specimens collected. Specimens were evaluated by one of three university-based pathologists, who were blinded to the endoscopy findings. Barrett's esophagus was considered present if at least one biopsy specimen had specialized intestinal metaplasia (SIM). Case participants were classified into one, two, or three diagnostic categories indicating disease progression, based on the presence (and length) or absence of visible columnar epithelium [visible Barrett's esophagus (VBE)] during endoscopy: 1) SIM (i.e., all cases), 2) SIM and VBE (VBE cases), and 3) SIM and VBE greater than two centimeters [long-segment Barrett's esophagus (LSBE) cases].[10] The first and most inclusive category (SIM cases) adheres to the concept of "ultra-short segment Barrett's esophagus."[12] The latter two categories were selected because they are consistent with the American College of Gastroenterology definition of Barrett's esophagus,[13] enhancing the clinical relevance of our study results.

Community-based control participants were identified using a modified Waksberg random digit dialing technique,[14] which identifies individuals living in the same geographic area

as case participants by utilizing the first five digits of each case's residential telephone number as the sampling unit.[15] Controls were individually matched to cases on age (±3 years) and sex.[10]

Study participants included 193 cases (92.8% of eligible) and 211 community controls (68.7% of eligible).[10] Of those, 87.4% (170 cases, 183 controls) provided adequate dietary intake information (see Exposure Assessment below) and are the focus of the current report. Their demographic characteristics are shown in **Table 4.5**.

Exposure Assessment. Information on potential risk factors, including demographic characteristics, tobacco and alcohol use, consumption of other beverages (e.g., coffee and tea), history of medical conditions, medication use, and occupational history, was obtained by a 45-minute structured questionnaire administered face-to-face by trained interviewers. The time between endoscopy and interview for case participants was 1-2 months.[10] Written informed consent was obtained from each participant prior to interview.

Dietary intake for the one year prior to interview was assessed by a validated self-administered, 131-item food frequency questionnaire (FFQ).[16] In total, 177 cases (91.7%) and 192 controls (91.0%) completed FFQs. Individuals with estimated total energy intake of <500 or >4,000 kilocalories/day for women or <800 or >5,000 kilocalories/day for men were excluded based on implausible energy intake (7 cases, 9 controls).[11,17] With this exclusion, a total of 170 case and 183 control participants were available for the current study.

Assessment of Dietary Flavonoid Intake. Intakes of total flavonoids, six classes of flavonoids (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones), and lignans was estimated from 91 food and beverage FFQ items that contained measureable amounts of flavonoids.[18-20] A study-specific flavonoid database was developed by linking the FFQ data with the 2011 U.S. Department of Agriculture (USDA) Database for the Flavonoid Content of Selected Foods[18] and the 2008 USDA-Iowa State University Database on the Isoflavone Content of Selected Foods.[19] To assess lignan content, specifically secoisolariciresinol and matairesinol, we supplemented the USDA databases with data from

foods consumed by a North American population.[20]

Some FFQ items represented groups of foods or beverages. For flavonoid intake calculations, the individual foods and beverages represented in a single item were weighted, based on the relative frequency of consumption in the general American population.[16] For example, the FFQ item of "apples and pears" was assigned a weight of 0.75 for "apples" and 0.25 for "pears." To calculate the flavonoid intake, the weight assigned to each food in the FFQ item was multiplied by the flavonoid content of that food, summed across all foods in the FFQ item, and then multiplied by the number of times consumed per day and by the serving size.[9]

Statistical Analysis. Unconditional logistic regression was used to calculate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for the association between flavonoid intakes and risk of Barrett's esophagus. Conditional logistic regression was also performed on matched pairs of cases and controls.[21] Results were similar; therefore, only unconditional logistic regression results are reported. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). All p-values are two-sided.

Flavonoid intakes were categorized in quartiles, based on the distributions of intakes among the control participants.[17] To examine linear trend, we also utilized restricted quadratic spline coding (**Figure 4.1**). Tests for linear trends were based on continuous flavonoid values in mg/day.

Effect measure modification by cigarette smoking (evaluated as continuous pack-years and as dichotomous, ever/never) and body mass index (BMI, kg/m^2) at interview (evaluated as continuous and as dichotomous, <25 or ≥25 kg/m^2) was assessed using likelihood ratio tests to compare regression models with and without a multiplicative term;[21] there was no evidence of effect measure modification by either covariate ($p \geq 0.05$) on the association between total flavonoid intake and Barrett's esophagus in any of the models.

Potential confounders included BMI (evaluated as continuous and as dichotomous, <25 or ≥25 kg/m^2), race (white, other), income (<$45,000, ≥$45,000-74,999, ≥$75,000),

education (≤high school, technical school, ≥college), and cigarette smoking (ever/never). If variable elimination changed the log odds ratio by ≥10%, the variable was considered a confounder and included in the model;[21] only BMI met this criterion. Total energy intake was included for adjustment on an *a priori* basis.[22] Thus, the final models included BMI (continuous), total energy intake (kilocalories, continuous), and the matching factors age (continuous) and sex.

To determine whether associations with flavonoids varied by diagnostic category, Barrett's esophagus patients were categorized into progressively more exclusive groups by segment length[12] and then each case subgroup was compared to all of the controls. To explore the threshold associations seen in restricted quadratic splines (**Figure 4.1**), we dichotomized exposures and compared the bottom quartile versus the upper three quartiles.

Sensitivity Analysis. The USDA Flavonoid Database assigns a value of 7.39 mg/100 g of banana for anthocyanidins,[18] which is controversial.[23] We therefore conducted a sensitivity analysis excluding the anthocyanidin value for bananas, which did not substantially alter our results (**Tables 4.6-4.9**).

Results

As shown in **Table 4.1** for this western Washington study population, control participants consumed similar amounts of total flavonoids (median=75.37 mg/day) as Barrett's esophagus case participants (median=75.55 mg/day). However, control participants consumed a smaller dietary intake of flavan-3-ols (median=17.35 mg/day), which were the largest contributor to total flavonoid intake, than case participants (median=25.56 mg/day).

Table 4.2 lists the major sources of flavonoids among the control participants. For total flavonoids, 47.2% of mean intake was from black tea (58.96 mg/day), 12.2% from orange/grapefruit juice (15.31 mg/day), and 6.8% from wine (8.48 mg/day). Black tea contains flavan-3-ols, flavonols, and lignans; orange/grapefruit juice contains flavanones, flavonols,

isoflavones, and lignans; and wine contains anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and lignans.

Barrett's esophagus risk was modestly reduced in relation to intake of anthocyanidins (highest versus lowest quartile of intake, OR=0.59, 95% CI: 0.31-1.12), flavanones (OR=0.71, 95% CI: 0.37-1.35), flavonols (OR=0.89, 95% CI: 0.47-1.69), isoflavones (OR=0.68, 95% CI: 0.34-1.36), and lignans (OR=0.64, 95% CI: 0.32-1.26), but the confidence intervals were wide (**Table 4.3**). In contrast, there was little or no association between total flavonoids (OR=1.09, 95% CI: 0.56-2.11) or flavan-3-ols (OR=0.88, 95% CI: 0.45-1.71) and risk of Barrett's esophagus. A modest increased risk of Barrett's esophagus was observed for flavones (OR=1.26, 95% CI: 0.63-2.52).

As presented in **Table 4.4**, the strength of inverse associations between flavonoid intake and Barrett's esophagus appeared to increase with increasing disease specificity. For example, comparing the upper three quartiles to the bottom one, the odds ratio for the association with anthocyanidin intake (for which wine, bananas and fruit juice were the major dietary sources) was reduced by 51% for SIM (OR=0.49, 95% CI: 0.30-0.80), by 44% for VBE (OR=0.56, 95% CI: 0.31-1.02), and by 56% for LSBE (OR=0.44, 95% CI: 0.21-0.92). The corresponding risk reductions were similarly pronounced for LSBE and flavanones (OR=0.49, 95% CI: 0.24-1.00) and flavonols (OR=0.53, 95% CI: 0.24-1.17).

Discussion

This is the first epidemiologic study, to our knowledge, to examine the association between total and all classes of flavonoid and lignan intakes and risk of Barrett's esophagus. In our analysis, we found modest, imprecise decreases in the odds ratios with increasing intakes of anthocyanidins, flavanones, flavonols, isoflavones, and lignans when all Barrett's esophagus stages were considered together. While we did not observe a significant trend, the inverse associations for anthocyanidins, flavanones, and flavonols were slightly more pronounced when

we considered segment length. For example, risk reductions ranged from 47 to 56% for LSBE in relation to these flavonoid classes.

Our findings are consistent with the one previous epidemiologic study that found a decreased risk of Barrett's esophagus associated with dietary isoflavone intake, the only flavonoid class considered in that investigation.[8] Foods containing high levels of isoflavones are infrequently consumed in the U.S.,[9] which is consistent with reports from our study population (**Table 4.1**). In our study we observed a suggested risk reduction for Barrett's esophagus in relation to anthocyanidin intake. Our findings are consistent with interim clinical trial results that found reduced markers of oxidative stress in Barrett's esophagus patients consuming anthocyanidin-rich freeze-dried black raspberries.[24]

Barrett's esophagus is a potential precursor lesion of esophageal adenocarcinoma, thus risk factors for this lesion could be involved in tumor initiation or promotion, whereas factors associated with tumor invasion should be more closely involved in cancer progression.[25] Our finding of a possible inverse association between anthocyanidin intake and Barrett's esophagus is consistent with a previous study of esophageal adenocarcinoma conducted in the United States [26] that found a significant decrease in invasive cancer risk associated with increased anthocyanidin intake. These observations suggest that anthocyanidin may play a role in both initiation and progression of esophageal adenocarcinoma.

In our study reported here, we observed possible risk reductions for the associations between anthocyanidins, flavanones, and flavonols in relation to all diagnostic Barrett's esophagus categories. Importantly, Barrett's esophagus segment length is related to the risk of developing esophageal adenocarcinoma.[27] However, our study population included a limited number of patients with VBE or LSBE. Because of data sparseness when we examined associations with the case participants categorized by segment length, we grouped flavonoid intake into two categories, rather than four.[21] Categorization of flavonoid exposures into two categories for the segment length analysis was conducted after examining the spline analysis

by collapsing quartiles 2-4 versus quartile 1. Therefore, results from these subgroup analyses should be interpreted with caution.

Our findings do not support an inverse association between total flavonoid intake or flavan-3-ol intake and risk of Barrett's esophagus. Additionally, a modest increased risk of Barrett's esophagus was observed for flavones. These results are at odds with experimental studies that have shown flavan-3-ols and flavones to have important chemopreventive effects against Barrett's esophagus.[6,28] In an *in vitro* study, Polyphenon E, a flavan-3-ol derived from green tea, inhibited growth and down-regulated the expression of cyclin D1 protein, which was associated with G(1) cell cycle arrest, in Barrett's esophagus cells.[6] One animal study has shown that a synthetic flavone derived from *Dysoxylum binectariferum*, Flavopiridol, reduces Barrett's esophagus development.[28] However, it is important to note that these experimental studies administered pure flavonoids derived from plants – green tea and *D. binectariferum*; whereas, our study utilized dietary intake of flavonoids from various foods and beverages. We found that the main sources of flavan-3-ols and flavones in our study population were black tea and pizza, respectively. Thus, our observation of an increased risk of Barrett's esophagus associated with flavone intake may be confounded by other dietary habits and lifestyle choices linked to high pizza intake.

A recent report, based on data from the same parent study as the present report, found an inverse association between fruit and vegetable intake and Barrett's esophagus.[11] Flavonoids are concentrated in fruits and vegetables;[29] therefore, flavonoid intake may be a marker for some other factor associated with a healthy diet and lifestyle, rather than act as a causative factor itself.[11] The parent study assessed a number of relevant lifestyle factors, including cigarette smoking, alcohol intake, and BMI;[10] however, in our ancillary study, BMI was the only covariate that influenced our results and was included in the final adjusted models.

A potential source of error in estimating flavonoid content in food, especially in fruits and vegetables, is the variability of environmental conditions, horticultural practices, degree of

ripeness, plant variety, storage conditions, industrial processing, and cooking methods, all of which may vary regionally and over time.[18,19,29] Organically and sustainably grown foods, compared to those produced by conventional methods, also have higher polyphenol concentrations.[30] Additionally, the FFQ line item for wine did not distinguish between red and white wine, which have different concentrations of flavonoids. Thus, food items reportedly consumed by this study population may differ from the foods utilized to create the estimates included in the databases.[18-20] To estimate the impact such influences, the USDA Food Composition and Nutrient Data Laboratories determined the flavonoid content for more than 60 fruits, vegetables, and nuts by sampling foods from four U.S. regions during two seasons of the year. While flavonoid content variability was high within and between foods, the average flavonoid content was similar to values reported in the USDA databases.[31]

Another potential source of error in estimating an association between flavonoid intake and risk of Barrett's esophagus is the bioavailability of flavonoid compounds. Little is known about the absorption of flavonoids in the body, and metabolism of flavonoids varies by individual.[32] Additionally, currently measured flavonoid biomarkers are of limited usefulness in epidemiologic studies because of the variation in absorption profiles, with maximum concentrations reached between 0.5-9 hours after dietary intake.[32] Thus, these biomarkers may not be highly correlated with usual adult dietary intake, which is the target exposure for cancer etiology studies, including studies of precursor lesions. While variation in dietary flavonoid content and flavonoid bioavailability may be a study limitation, it is a common limitation for all studies that rely on nutritional databases to estimate dietary intakes.[17]

Patients with GERD symptoms are recommended to omit foods that are chemically or mechanically irritating;[33] therefore, Barrett's esophagus patients may have already made changes to their usual diets by the time of FFQ administration. Foods that are irritating vary by individual,[34] so we are unable to determine how such potential changes in diet could have affected our flavonoid intake estimates. While some flavonoid-containing foods may be

recommended for GERD patients to avoid (e.g., coffee, tea, alcohol, citrus, tomatoes, chocolate, peppers, and onions), one dietary study showed that intakes of fruits, vegetables, and alcohol did not differ by symptomatic GERD status.[35] As all cases in this study had GERD, it is still possible that the association between BE and flavonoid intake is due to reverse causation.

For a FFQ, assessing diet for the year prior to diagnosis is a standard time interval, as it does not require extensive recall.[17] Responses are assumed to reflect usual adult diet. Whether the time period assessed accurately reflects intakes during the time relevant to Barrett's esophagus development is unknown. However, because all existing studies conducted among Barrett's esophagus patients have relied on a FFQ,[11,36,37] a cohort study would be required, with employment of multiple alternative dietary assessment methods repeatedly over time, to overcome the limitations of existing studies. Such an alternative study design would be inefficient, because only 10-15% of symptomatic GERD patients develop BE in their lifetime.[38]

Our study FFQ did not assess dietary supplement use. Clinical studies of flavonoid supplements began in the early 1990s,[39] and a U.S. patent was granted for *Ginkgo biloba* extract, EGb 761, in 1995.[40] While it is possible that some participants were taking flavonoid-containing supplements, it is unlikely that use was widespread during this study time period.

The difference that we observed in mean intake of total flavonoids between case and control participants was minimal, roughly equivalent to half of a medium apple per week. However, absolute differences in dietary flavonoid intakes need to be interpreted with caution, as a FFQ was utilized to collect relative, not absolute, dietary information. While FFQs have acknowledged measurement errors, they are useful for ranking individuals' dietary intake relative to one another, which was our primary objective.[17]

In summary, our finding of modest inverse associations between anthocyanidins, flavanones and flavonols in relation to Barrett's esophagus suggests that dietary intake of these compounds may lower risk of this precursor lesion. In contrast, total dietary flavonoid intake does not appear to be associated with decreased risk of developing Barrett's esophagus in our

study sample. This is the first epidemiologic study to determine the association between the six flavonoid classes, total flavonoids and lignans and Barrett's esophagus; therefore, further research is needed before definite conclusions can be made about the role of dietary flavonoids and lignans in relation to Barrett's esophagus risk.

Table 4.1. Mean Intakes (mg/day) of Flavonoids and Lignans Among Cases and Controls With Information on Dietary Intake, Study of Reflux Disease, Western Washington State, 1997-2000.

	Controls (N=183)			Cases (N=170)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Total Flavonoids	125.03	123.10	5.83-819.34	123.55	134.39	10.52-707.45
Anthocyanidins	13.84	10.67	0.29-55.42	13.47	13.15	0.36-85.19
Flavan-3-ols	73.01	114.23	1.71-739.93	78.06	125.34	1.85-659.00
Flavanones	21.97	26.08	0.01-143.37	17.20	21.62	0.02-146.23
Flavones	2.19	1.56	0.19-11.69	2.15	1.19	0.13-6.68
Flavonols	11.89	6.63	1.74-42.60	11.47	6.87	2.04-39.00
Isoflavones	2.14	5.90	0.02-55.18	1.20	2.55	0.04-19.95
Lignans	0.056	0.029	0.011-0.160	0.051	0.030	0.009-0.176

Table 4.2. Major Dietary Sources of Flavonoids and Lignans Among a Community-based Sample of Control Participants Without Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Flavonoid/Phytoestrogen Class	Representative Flavonoids	Main FFQ[1] Line Item Sources (%)
Total Flavonoids		Black tea (47.2), orange/grapefruit juice (12.2), wine (6.8), oranges/grapefruit (4.7), apples/pears (3.5), bananas (3.0)
Anthocyanidins	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin	Wine (31.2), bananas (14.8), fruit juice (11.3), fruit cocktail/applesauce (8.5), strawberries/kiwi (8.0), apples/pears (7.6), bean soups (5.5)
Flavan-3-ols	(+)-Catechin, (+)-Catechin-3-gallate, (-)-Epicatechin, (-)-Epicatechin-3-gallate, (-)-Epigallocatechin, (-)-Epigallocatechin-3-gallate, (+)-Gallocatechin, (+)-Gallocatechin-3-gallate, Theaflavin, Theaflavin-3-gallate, Theaflavin-3'-gallate, Theaflavin-3,3'-digallate, Thearubigins	Black tea (78.2), green tea (3.5), wine (3.3), apples/pears (3.0), beer (2.7), bananas (2.3)
Flavanones	Eriodictyol, Hesperetin, Naringenin	Orange/grapefruit juice (68.6), oranges/grapefruit (26.3), wine (2.8)
Flavones	Apigenin, Luteolin	Pizza (38.4), wine (12.4), vegetable soup (8.0), cream soup (6.8), mixed salad (6.1)
Flavonols	Isorhamnetin, Kaempferol, Myricetin, Quercetin	Black tea (15.6), onions (11.0), apples/pears (9.4), wine (7.2), mixed salad (5.9), beer (5.7)
Isoflavones	Daidzein, Genistein, Glycitein	Tofu (76.5), coffee (5.9), chili with beans (5.7), milk (5.2)
Lignans	Matairesinol, Secoisolariciresinol	Coffee (31.0), wine (12.6), orange/grapefruit juice (9.4), onions (3.8), peanuts (3.7), black tea (3.5)

[1]FFQ: Food frequency questionnaire.

Table 4.3. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Flavonoid and Lignan Intake and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Variable and intake (mg/day)	Controls (N=183)	Cases (N=170)	OR	95% CI
Total Flavonoids				
0-42.38	46	44	1.00	
42.39-75.36	46	41	1.10	0.59, 2.08
75.37-166.98	45	46	1.37	0.73, 2.58
≥166.99	46	39	1.09	0.56, 2.11
P for trend[2]			0.81	
Anthocyanidins				
0-6.12	46	64	1.00	
6.13-9.82	45	24	0.33	0.17, 0.63
9.83-18.26	47	46	0.58	0.32, 1.06
≥18.27	45	36	0.59	0.31, 1.12
P for trend[2]			0.91	
Flavan-3-ols				
0-9.50	45	44	1.00	
9.51-17.35	46	22	0.51	0.25, 1.03
17.36-107.34	46	70	1.78	0.98, 3.23
≥107.35	46	34	0.88	0.45, 1.71
P for trend[2]			0.54	
Flavanones				
0-3.80	45	55	1.00	
3.81-12.90	47	41	0.71	0.39, 1.32
12.91-29.64	46	41	0.81	0.44, 1.48
≥29.65	45	33	0.71	0.37, 1.35
P for trend[2]			0.27	
Flavones				
0-1.15	46	37	1.00	
1.16-1.88	46	39	1.10	0.57, 2.13
1.89-2.82	45	49	1.46	0.75, 2.85
≥2.83	46	45	1.26	0.63, 2.52
P for trend[2]			0.61	
Flavonols				
0-6.99	46	52	1.00	
7.00-10.86	46	44	0.83	0.45, 1.53
10.87-14.89	46	28	0.60	0.31, 1.16
≥14.90	45	46	0.89	0.47, 1.69
P for trend[2]			0.50	
Isoflavones				
0-0.24	46	41	1.00	
0.25-0.52	45	56	1.22	0.65, 2.29
0.53-1.16	47	39	0.82	0.42, 1.60
≥1.17	45	34	0.68	0.34, 1.36
P for trend[2]			0.09	
Lignans				
0-0.033	45	53	1.00	
0.034-0.051	46	52	0.91	0.50, 1.67
0.052-0.070	46	28	0.46	0.22, 0.94
≥0.071	46	37	0.64	0.32, 1.26
P for trend[2]			0.15	

[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]P-value for trend for continuous variable.

Table 4.4. Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Flavonoid and Lignan Intake and Barrett's Esophagus Segment Length,[2] Study of Reflux Disease, Western Washington State, 1997-2000.

		Clinical SIM[2]				Clinical VBE[2]				Clinical LSBE[2]			
Variable and intake (mg/day)	Controls (N=183)	Cases (N=170)	OR	95% CI	P for trend[3]	Cases (N=86)	OR	95% CI	P for trend[3]	Cases (N=48)	OR	95% CI	P for trend[3]
Total Flavonoids													
0-42.38	46	44	1.00			25	1.00			16	1.00		
≥42.39	137	126	1.19	0.70, 2.01	0.81	61	0.93	0.50, 1.73	0.97	32	0.71	0.33, 1.52	0.70
Anthocyanidins													
0-6.12	46	64	1.00			31	1.00			20	1.00		
≥6.13	137	106	0.49	0.30, 0.80	0.91	55	0.56	0.31, 1.02	0.45	28	0.44	0.21, 0.92	0.67
Flavan-3-ols													
0-9.50	45	44	1.00			20	1.00			15	1.00		
≥9.51	138	126	1.06	0.63, 1.78	0.54	66	1.21	0.63, 2.32	0.81	33	0.75	0.35, 1.60	0.95
Flavanones													
0-3.80	45	55	1.00			31	1.00			20	1.00		
≥3.81	138	115	0.74	0.45, 1.22	0.27	55	0.55	0.31, 0.99	0.38	28	0.49	0.24, 1.00	0.12
Flavones													
0-1.15	46	37	1.00			20	1.00			10	1.00		
≥1.16	137	133	1.25	0.71, 2.20	0.61	66	1.11	0.56, 2.19	0.42	38	1.66	0.67, 4.15	0.96
Flavonols													
0-6.99	46	52	1.00			29	1.00			17	1.00		
≥7.00	137	118	0.77	0.46, 1.30	0.50	57	0.59	0.31, 1.10	0.48	31	0.53	0.24, 1.17	0.63
Isoflavones													
0-0.24	46	41	1.00			20	1.00			9	1.00		
≥0.25	137	129	0.92	0.53, 1.60	0.09	66	0.82	0.41, 1.61	0.35	39	0.98	0.40, 2.42	0.30
Lignans													
0-0.033	45	53	1.00			24	1.00			10	1.00		
≥0.034	138	117	0.71	0.41, 1.21	0.15	62	0.80	0.42, 1.54	0.13	38	1.35	0.56, 3.26	0.52

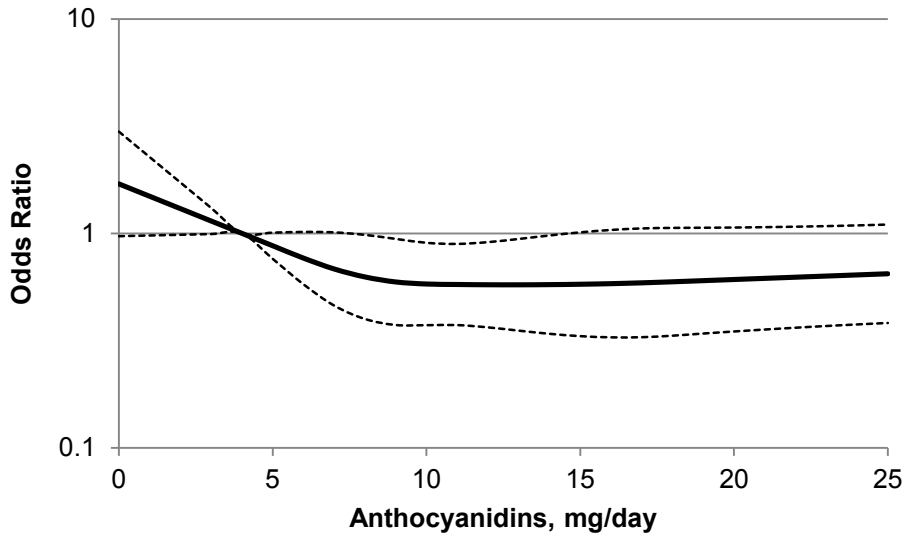
[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]Barrett's esophagus segment length categories are progressively exclusive groups. SIM: Specialized intestinal metaplasia, VBE: visible Barrett's esophagus, LSBE: long-segment Barrett's esophagus. [3]P-value for trend for continuous variable.

Table 4.5. Distribution (n [%]) of Characteristics Among Barrett's Esophagus Cases and Controls with Information on Dietary Intake, Study of Reflux Disease, Western Washington State, 1997-2000.

Characteristic	Control participants N=183	Barrett's Esophagus participants N=170
Age		
20-39	25 (13.7)	22 (12.9)
40-49	46 (25.1)	42 (24.7)
50-59	55 (30.1)	53 (31.2)
60-80	57 (31.1)	53 (48.2)
Sex		
Male	113 (61.7)	100 (58.8)
Female	70 (38.3)	70 (41.2)
Race		
White	169 (92.9)	152 (91.6)
Other	13 (7.1)	14 (8.4)
Education		
≤ High School	32 (17.5)	43 (25.3)
Technical School	6 (3.3)	9 (5.3)
College or more	145 (79.2)	118 (69.4)
Income level, US $		
<45,000	55 (31.4)	50 (32.5)
≥45,000-74,999	51 (29.1)	49 (31.8)
≥75,000	69 (39.4)	55 (35.7)
Cigarette Use		
Ever	89 (48.6)	109 (64.1)
Never	94 (51.4)	61 (35.9)
Body Mass Index		
<25 kg/m^2	57 (31.1)	33 (19.4)
≥25 kg/m^2	126 (68.9)	137 (80.6)
Segment Length[1]		
SIM		170 (100.0)
VBE		86 (50.6)
LSBE		48 (28.2)

[1]Barrett's esophagus segment length categories are progressively exclusive groups. SIM: Specialized intestinal metaplasia, VBE: visible Barrett's esophagus, LSBE: long-segment Barrett's esophagus.

Figure 4.1. Restricted Quadratic Spline Graph of the Adjusted[1] Odds Ratios (represented by the solid line) and 95% Confidence Intervals (represented by the dotted lines) of the Association Between Anthocyanidin Intake and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.



[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous).

Table 4.6. Sensitivity Analysis of Mean Intakes (mg/day) of Anthocyanidins (Bananas Included versus Excluded) Among Cases and Controls With Information on Dietary Intake, Study of Reflux Disease, Western Washington State, 1997-2000.

	Controls (N=183)			Cases (N=170)		
	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
Anthocyanidins, Bananas Included	13.84	11.31	0.29-55.42	13.47	14.21	0.36-85.19
Anthocyanidins, Bananas Excluded	11.80	10.67	0.18-52.97	11.78	13.15	0.36-79.93

Table 4.7. Sensitivity Analysis of the Major Sources of Anthocyanidins (Bananas Included versus Excluded) Among a Community-based Sample of Control Participants Without Barrett's Esophagus With Information on Dietary Intake, Study of Reflux Disease, Western Washington State, 1997-2000.

Flavonoid/Phytoestrogen Class	Main FFQ[1] Line Item Sources (%)
Anthocyanidins, Bananas Included	Wine (31.2), bananas (14.8), fruit juice (11.3), fruit cocktail/applesauce (8.5), strawberries/kiwi (8.0), apples/pears (7.6), bean soups (5.5)
Anthocyanidins, Bananas Excluded	Wine (36.6), fruit juice (13.3), fruit cocktail/applesauce (10.0), strawberries/kiwi (9.3), apples/pears (9.0), bean soups (6.4)

[1]FFQ: Food frequency questionnaire.

Table 4.8. Sensitivity Analysis of Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Anthocyanidin Intake (Bananas Included versus Excluded) and Barrett's Esophagus, Study of Reflux Disease, Western Washington State, 1997-2000.

Variable and intake (mg/day)	Controls (N=183)	Cases (N=170)	OR	95% CI
Anthocyanidins, Bananas Included				
0-6.12	46	64	1.00	
6.13-9.82	45	24	0.33	0.17, 0.63
9.83-18.26	47	46	0.58	0.32, 1.06
≥18.27	45	36	0.59	0.31, 1.12
P for trend[2]			0.91	
Anthocyanidins, Bananas Excluded				
0-4.68	45	54	1.00	
4.69-8.29	46	40	0.65	0.35, 1.19
8.30-15.26	47	42	0.62	0.33, 1.16
≥15.27	45	34	0.64	0.33, 1.22
P for trend[2]			0.90	

[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]P-value for trend for continuous variable.

Table 4.9. Sensitivity Analysis of Adjusted[1] Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Association Between Anthocyanidin Intake (Bananas Included versus Excluded) and Barrett's Esophagus Segment Length,[2] Study of Reflux Disease, Western Washington State, 1997-2000.

		Clinical SIM[2]				Clinical VBE[2]				Clinical LSBE[2]			
Variable and intake (mg/day)	Controls (N=183)	Cases (N=170)	OR	95% CI	P for trend[3]	Cases (N=86)	OR	95% CI	P for trend[3]	Cases (N=48)	OR	95% CI	P for trend[3]
Anthocyanidins, Bananas Included													
0-6.12	46	64	1.00			31	1.00			20	1.00		
≥6.13	137	106	0.49	0.30, 0.80	0.91	55	0.56	0.31, 1.02	0.45	28	0.44	0.21, 0.92	0.67
Anthocyanidins, Bananas Excluded													
0-4.68	45	54	1.00			24	1.00			15	1.00		
≥4.69	138	116	0.64	0.39, 1.05	0.90	62	0.81	0.43, 1.52	0.33	33	0.69	0.32, 1.50	0.43

[1]Adjusted for age (continuous), sex, body mass index (continuous), and kilocalories (continuous). [2]Barrett's esophagus segment length categories are progressively exclusive groups. SIM: Specialized intestinal metaplasia, VBE: visible Barrett's esophagus, LSBE: long-segment Barrett's esophagus. [3]P-value for trend for continuous variable.

REFERENCES

1. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-53.

2. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. *CA: a cancer journal for clinicians* 2012.

3. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, Cameron A, Corley D, Falk G, Goldblum J, Hunter J, Jankowski J, Lundell L, Reid B, Shaheen NJ, Sonnenberg A, Wang K, Weinstein W. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. *Gastroenterology* 2004;127(1):310-30.

4. Kubo A, Corley DA, Jensen CD, Kaur R. Dietary factors and the risks of oesophageal adenocarcinoma and Barrett's oesophagus. *Nutr Res Rev* 2010;23(2):230-46.

5. Pierini R, Kroon PA, Guyot S, Ivory K, Johnson IT, Belshaw NJ. Procyanidin effects on oesophageal adenocarcinoma cells strongly depend on flavan-3-ol degree of polymerization. *Mol Nutr Food Res* 2008;52(12):1399-407.

6. Song S, Krishnan K, Liu K, Bresalier RS. Polyphenon E inhibits the growth of human Barrett's and aerodigestive adenocarcinoma cells by suppressing cyclin D1 expression. *Clin Cancer Res* 2009;15(2):622-31.

7. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutrition and cancer* 2010;62(1):1-20.

8. Jiao L, Kramer JR, Rugge M, Parente P, Verstovsek G, Alsarraj A, El-Serag HB. Dietary intake of vegetables, folate, and antioxidants and the risk of Barrett's esophagus. *Cancer causes & control : CCC* 2013;24(5):1005-14.

9. Fink BN, Steck SE, Wolff MS, Kabat GC, Gammon MD. Construction of a flavonoid database for assessing intake in a population-based sample of women on Long Island, New York. *Nutr Cancer* 2006;56(1):57-66.

10. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007;133(2):403-11.

11. Thompson OM, Beresford SA, Kirk EA, Vaughan TL. Vegetable and fruit intakes and risk of Barrett's esophagus in men and women. *Am J Clin Nutr* 2009;89(3):890-6.

12. Mueller J, Werner M, Stolte M. Barrett's esophagus: histopathologic definitions and diagnostic criteria. *World journal of surgery* 2004;28(2):148-54.

13. Sampliner RE. Updated guidelines for the diagnosis, surveillance, and therapy of Barrett's esophagus. *The American journal of gastroenterology* 2002;97(8):1888-95.

14. Waksberg J. Sampling methods for random digit dialing. *J Am Stat Assoc* 1978;73:40-46.

15. Voigt LF, Davis S, Koepsell TD. Do cases and controls matched on the first eight digits of their telephone number share geographic proximity and socioeconomic characteristics? *Annals of epidemiology* 2006;16(4):299-304.

16. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *American journal of epidemiology* 1997;146(10):856-69.

17. Willett W. *Nutritional epidemiology*. Monographs in epidemiology and biostatistics. Third edition. ed. Oxford ; New York: Oxford University Press, 2012.

18. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav.

19. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav.

20. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutrition and cancer* 2006;54(2):184-201.

21. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology*. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2008.

22. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *The American journal of clinical nutrition* 1997;65(4 Suppl):1220S-1228S; discussion 1229S-1231S.

23. Drossard C, Frohling B, Dietrich H, Kersting M. Anthocyanin analysis in banana fruit--a mistake. *The American journal of clinical nutrition* 2011;93(4):865-6; author reply 866-7.

24. Kresty LA, Frankel WL, Hammond CD, Baird ME, Mele JM, Stoner GD, Fromkes JJ. Transitioning from preclinical to clinical chemopreventive assessments of lyophilized black raspberries: interim results show berries modulate markers of oxidative stress in Barrett's esophagus patients. *Nutrition and cancer* 2006;54(1):148-56.

25. Farber E. Cancer development and its natural history. A cancer prevention perspective. *Cancer* 1988;62(8 Suppl):1676-9.

26. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. *Int J Cancer* 2009;125(5):1147-54.

27. Schottenfeld D, Fraumeni JF. *Cancer epidemiology and prevention*. 3rd ed. Oxford ; New York: Oxford University Press, 2006.

28. Lechpammer M, Xu X, Ellis FH, Bhattacharaya N, Shapiro GI, Loda M. Flavopiridol reduces malignant transformation of the esophageal mucosa in p27 knockout mice. *Oncogene* 2005;24(10):1683-8.

29. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. *Ann Ist Super Sanita* 2007;43(4):348-61.

30. Asami DK, Hong YJ, Barrett DM, Mitchell AE. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of agricultural and food chemistry* 2003;51(5):1237-41.

31. Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, Gebhardt S. Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of agricultural and food chemistry* 2006;54(26):9966-77.

32. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr* 2008;99(1):12-22.

33. Ko AH, Dollinger M, Rosenbaum EH. *Everyone's guide to cancer therapy : how cancer is diagnosed, treated, and managed day to day*. Rev. 5th ed. Kansas City: Andrews McMeel Pub., 2008.

34. Brown JE. *Nutrition through the life cycle*. 3rd ed ed. Belmont, CA: Thomson/Wadsworth, 2008.

35. El-Serag HB, Satia JA, Rabeneck L. Dietary intake and the risk of gastro-oesophageal reflux disease: a cross sectional study in volunteers. *Gut* 2005;54(1):11-7.

36. Anderson LA, Watson RG, Murphy SJ, Johnston BT, Comber H, Mc Guigan J, Reynolds JV, Murray LJ. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J Gastroenterol* 2007;13(10):1585-94.

37. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am J Gastroenterol* 2008;103(7):1614-23; quiz 1624.

38. Clouston AD. Timely topic: Premalignant lesions associated with adenocarcinoma of the upper gastrointestinal tract. *Pathology* 2001;33(3):271-7.

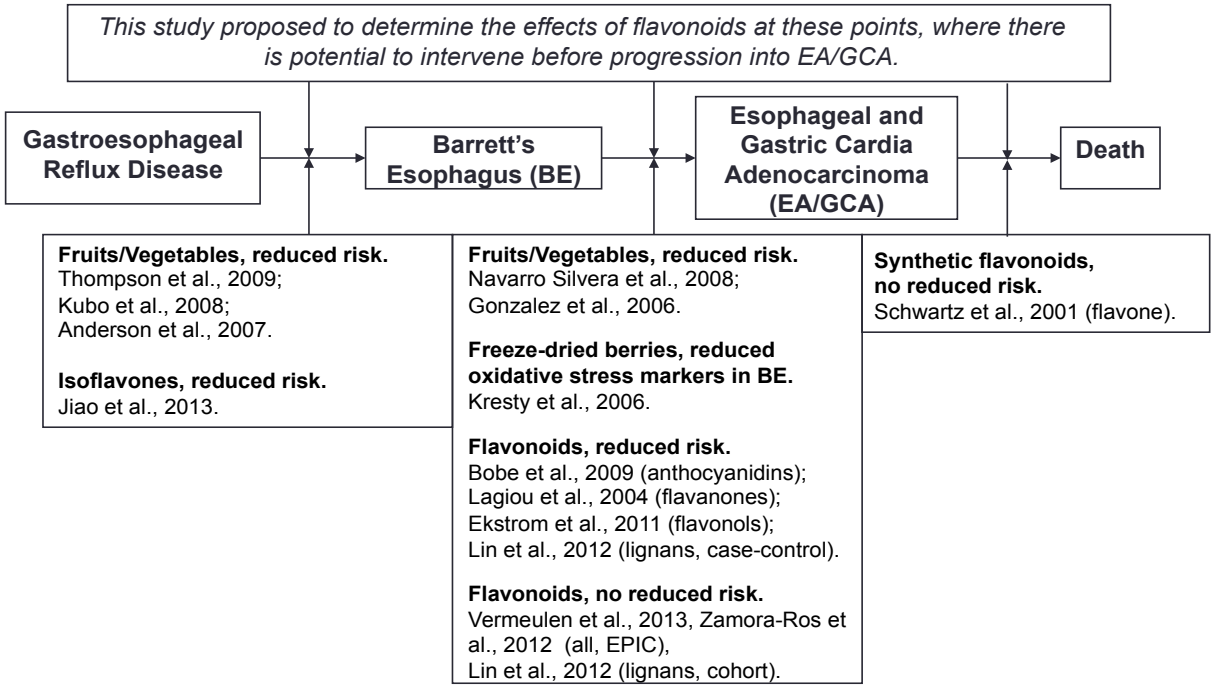
39. EGb 761: ginkgo biloba extract, Ginkor. *Drugs in R&D* 2003;4(3):188-93.

40. U.S. Patent Office. Extract from Ginkgo biloba leaves, its method of preparation and pharmaceuticals containing the extract. http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5,399,348.PN.&OS=PN/5,399,348&RS=PN/5,399,348 Accessed July 9, 2013.

CHAPTER 5: DISCUSSION

The purpose of this dissertation was to examine the association between flavonoid intake and esophageal and gastric cancer along the cancer continuum (normal tissue → precancerous conditions → invasive cancer → mortality). Chapter 4 examines the association between flavonoids and the precancerous lesion, Barrett's esophagus. Chapter 3 examines the association between flavonoids and invasive esophageal and gastric cancer incidence and survival. This novel approach of examining the precancerous lesion and invasive cancer aimed to identify key windows of susceptibility that could signify when along the cancer continuum a potential intervention strategy of flavonoid use could be implemented in an effort to reduce the significantly high disease burden associated with these cancers.

Figure 5.1. Literature of Epidemiologic and Clinical Studies Supporting Examination of the Association Between Flavonoid Intake and the Esophageal and Gastric Cancer Continuum.



Summary of Results

In this study, modest decreasing risks were found with increasing intakes of anthocyanidins, across all stages of the cancer continuum. Anthocyanidin intake was associated with a decreased risk of BE development (quartile 4 versus quartile 1, OR=0.59, 95% CI: 0.31-1.12). This association was also found across all categories of BE segment length (quartiles 2-4 versus quartile 1, SIM OR=0.49, 95% CI: 0.30-0.80; VBE OR=0.56, 95% CI: 0.31-1.02; LSBE OR=0.44, 95% CI: 0.21-0.92). Reduced risk of invasive EA and GCA incidence was shown for increasing levels of anthocyanidin intake (quartile 4 versus quartile 1, EA OR=0.43, 95% CI: 0.29-0.66; GCA OR=0.71, 95% CI: 0.46-1.10). Reduced risk of mortality among EA and GCA cases was also shown for higher levels of anthocyanidin intake (quartile 4 versus quartile 1, EA HR=0.87, 95% CI: 0.60-1.26; GCA HR=0.63, 95% CI: 0.42-0.95). Additionally, anthocyanidin intake was associated with decreased risk of ESCC and NCGA incidence (quartile 4 versus quartile 1, ESCC OR=0.43, 95% CI: 0.26-0.70; NCGA OR=0.70, 95% CI: 0.47-1.03).

Further, flavanones, flavonols, isoflavones, and lignans were associated with a decreased risk of BE development. Flavanones were associated with a decreased risk of EA incidence; flavanones, flavones, isoflavones, and lignans were associated with a decreased risk of ESCC incidence; and lignans were associated with a decreased risk of NCGA incidence. Flavones, isoflavones, and lignans were associated with risk reduction in all-cause mortality for EA cases; flavan-3-ols were associated with a risk reduction in all-cause mortality for GCA cases; lignans were associated with risk reduction in all-cause mortality for ESCC cases; and flavanones were associated with risk reduction in all-cause mortality for NCGA cases.

Study Strengths

Cancer Continuum. Esophageal adenocarcinoma (EA) and gastric cardia adenocarcinoma (GCA) have been among most rapidly increasing cancer types in the U.S. and other Western countries.[1,2] EA/GCA appears to develop through a sequence of pathologic events.[3] Chronic or

severe gastroesophageal reflux disease (GERD) can cause ulceration of the normal esophageal squamous mucosa followed, in some people, by the development of Barrett's esophagus (BE).[4] BE is the only known potential precursor of EA/GCA.[5] This dissertation examined the association between flavonoid intake and esophageal and gastric cancer along the BE-adenocarcinoma cancer continuum (normal tissue → gastroesophageal reflux disease → Barrett's esophagus → invasive esophageal/gastric cardia adenocarcinoma → mortality). This approach is novel because it allows examination of the precancerous lesion and invasive cancer, which allows identification of key windows of susceptibility that could signify when along the cancer continuum a potential intervention strategy of flavonoid use could be implemented. This also lends plausibility to studying a particular class of flavonoids further as a potential chemopreventive agent if it is observed that it affects all stages of carcinogenesis, as was the case with anthocyanidins. Our findings, if confirmed, suggest that adequate dietary anthocyanidin intake may lower risk of these cancers and could potentially be used to reduce the significantly high disease burden associated with these tumors. However, this does not preclude flavonoid classes that do not affect all stages of carcinogenesis as potential risk reduction strategies, as other classes could differentially affect the cancer stages of initiation, promotion, and progression.[6] For example, flavanones reduced the risk of Barrett's esophagus development and esophageal adenocarcinoma incidence but had no association with mortality among esophageal adenocarcinoma cases.

Experimental Studies. Most experimental studies support the hypothesis of an inverse association between flavonoids and BE and esophageal and gastric cancer. For example, flavan-3-ol inhibited growth through down-regulation of cyclin D1 protein expression in BE cells,[7] and anthocyanidin-containing freeze-dried berries inhibited the formation of esophageal tumors in rats caused by N-nitrosomethylbenzylamine (NMBA), which has long been long been used to model ESCC in rats.[8,9]

Geographic Variation. This study is significant because EA/GCA have rapidly increasing incidence rates in the U.S. and other Western countries,[1,2] but incidence rates in Asian countries remain comparatively low.[10] The rates of EA/GCA vary 50- to 60-fold between high and low incidence countries.[10] These geographic differences in incidence do not appear to be only due to genetic variation between racial/ethnic groups, as incidence rates of EA/GCA in Asian migrants to the U.S. tend to approach those of European-American incidence rates within a few generations.[11-13]

The geographic variation in incidence rates of EA/GCA and results of migrant studies have led to the hypothesis that the variability is in part due to differences in dietary composition, including energy intake and micronutrient intake.[1,14] This study was inspired by geographic differences in the incidence of these tumors. As Asian countries have lower incidence rates of EA/GCA and consume more flavonoid-rich foods than Western countries,[15,16] it is possible that flavonoids or a specific flavonoid class may decrease BE and EA/GCA risk. We considered all classes of flavonoids (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, isoflavones), as many of these flavonoid classes occur more frequently in foods consumed by Western populations (e.g., tomatoes, green peppers, berries, and citrus fruits).[17]

Flavonoid and Lignan Database. The most recent U.S. Department of Agriculture (USDA) databases were utilized to create the exposure assessment: 2011 USDA Database for the Flavonoid Content of Selected Foods[18] and the 2008 USDA-Iowa State University Database on the Isoflavone Content of Selected Foods.[19] Additionally, these were supplemented with lignan content (i.e., secoisolariciresinol and matairesinol) from a North American population.[20] Fred Hutchison Cancer Research Center FFQs are generally processed by the University of Minnesota Nutrition Data System for Research (NDSR), which was not linked with the most current USDA databases at time of exposure creation, thus necessitating the creation of study-specific flavonoid composition databases from other sources.

Study Design and Sample Size. BE and esophageal and gastric cancer are rare diseases; therefore, a case-control study is the most efficient study design. Although a cohort study could overcome the limitation of recall bias, this study design is inefficient. The current study is a practical and cost-efficient use of resources because dietary data was previously collected using a valid, reliable FFQ from the Fred Hutchison Cancer Research Center.[21,22] This allowed estimation and ranking of dietary intake of flavonoids. Additionally, the BE and EGA study data collection and management have been performed in similar ways, as Dr. Thomas Vaughan was a principal investigator in both studies.

Our study benefited from a larger population than similar studies published previously. The EGA study is a population-based study of 274 EA, 248 GCA, 191 ESCC, and 341 NCGA cases and 662 frequency-matched controls with plausible dietary intake information. The number of EA cases is larger than in the Black-White Study, a previous study that had examined flavonoids and EA incidence, which had 161 cases.[23] The community-based BE study also has a larger sample size (n=170 BE cases with plausible dietary intake) than the previous study to examine isoflavones, which had 151 BE cases.[24] Additionally, the population-based design of the EGA study allows for generalizability of the results to a broader population than in previous studies.

Study Limitations

Exposure Misclassification: Dietary Recall. FFQs were assessed for the year prior to interview (BE cases) or 3-5 years prior to diagnosis (esophageal and gastric cancer cases). Responses were assumed to reflect usual adult diet, but whether the time period assessed accurately reflects intakes during the time relevant to BE or esophageal/gastric cancer development is unknown. However, because all existing studies conducted among BE[25-27] and esophageal/gastric cancer patients[25,28-36] have relied on FFQs, one would need to conduct a new cohort study and employ multiple alternative dietary assessment methods over time to

overcome this issue of nondifferential misclassification. Such an alternative study design would be inefficient, because only 10-15% of symptomatic GERD patients develop BE in their lifetime[37] and the lifetime risk of esophageal or gastric cancer is less than one percent.[38]

There is also potential for differential exposure misclassification in the EGA study, as many of the case participants were experiencing symptomatic GERD (e.g., 55% of EA cases versus 29% of controls). Thus, a larger proportion of case participants were experiencing symptomatic reflux and considering dietary choices that could impact GERD symptoms more carefully. Therefore, it is possible that a greater number of case participants recalled their dietary intake more accurately than the control participants. This type of recall bias would increase the likelihood of detecting spurious results.

Lack of Certain Flavonoid-rich Foods and Supplements in FFQ. The FFQs did not assess certain foods that contain high-levels of flavonoids. In the BE and EGA FFQ, raspberries, which contain high levels of anthocyanidins,[18] were not included. Additionally, the EGA FFQ did not ask about tofu intake, which is a primary source of isoflavones.[19] Thus, the results could be more pronounced (i.e., if cases consumed higher quantities of raspberries or tofu) or attenuated (i.e., if controls consumed higher quantities of raspberries or tofu) with the assessment of these additional dietary sources of flavonoids.

The study FFQs did not assess dietary supplement use. Clinical studies of flavonoid supplements began in the early 1990s,[39,40] and a U.S. patent was granted for *Ginkgo biloba* extract, EGb 761, in 1995.[34] While it is possible that some study participants were taking flavonoid-containing supplements, it is unlikely that use was widespread during the time period the parent studies were recruiting participants (EGA 1993-1995 and BE 1997-2000).

Bioavailabilty of Flavonoids. A potential source of error in estimating the amount of flavonoid intake necessary to reduce the risk of BE or esophageal/gastric cancer is the bioavailability of flavonoid compounds. However, little is known about the absorption of flavonoids in the body, and metabolism of flavonoids varies by individual.[41] Additionally, the use

of current flavonoid biomarkers is of limited usefulness in epidemiologic studies, because of the variation in absorption profiles, with maximum concentrations reached between 0.5-9 hours after dietary intake.[42] Thus, these biomarkers may not be highly correlated with usual adult diet, which is the target exposure for cancer studies, including precursor lesions. While the variation in dietary flavonoid content and flavonoid bioavailability may be a study limitation, this is a limitation for all studies that rely on nutritional databases to estimate dietary intake.[43]

Flavonoid Classification. In this study, flavonoids were categorized by classes (anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and isoflavones) and also combined into total flavonoids. However, this may mask effects of individual flavonoids. For instance, the flavonoid class anthocyanidins is composed of six individual flavonoids: cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin. Thus, by combining all of these individual flavonoids into the category of anthocyanidins, there is potential that risk reductions are being diluted if the association between exposure and outcome is strong for one flavonoid in the class but not others.

Healthy Lifestyle. Recent reports from the parent studies found modest inverse associations between fruit and vegetable intake and BE[27] and both histologic types of esophageal cancer.[30] Flavonoids are concentrated in fruits and vegetables;[44] therefore, it is possible that flavonoid intake is a marker for a healthy diet and lifestyle, rather than a causative agent.[27] Lifestyle factors, including cigarette smoking, alcohol intake, and BMI, were assessed in the parent studies.[45] BMI was the only covariate that influenced the BE results, and cigarette smoking was the only covariate that influenced the esophageal/gastric cancer results. These were adjusted for in the final models.

Error Estimating Flavonoid Content. A potential source of error in estimating flavonoid content in food, especially fruits and vegetables, may be associated with the variability of environmental conditions, horticultural practices, degree of ripeness, plant variety, storage conditions, industrial processing, and cooking methods, which may vary regionally and over

time.[17-19,41,46-51] Organically and sustainably grown foods, compared to those produced by conventional methods, also have higher polyphenol concentrations.[51,52] To estimate the impact of these potential influences, the USDA Food Composition and Nutrient Data Laboratories determined the flavonoid content for over 60 fruits, vegetables, and nuts by sampling foods from four U.S. regions at two times of the year. While flavonoid content variability was high within and between foods, the average flavonoid content was similar to values reported by the USDA.[53] Additionally, the FFQ line item for wine did not discern between red and white wine, which have different concentrations of flavonoids. Thus, the food sources consumed by this study population may be different than the foods utilized to create the databases estimates.[18-20]

Sample Size Limitations. While this study has a larger sample size than previous studies to examine the association between BE[25-27] and esophageal/gastric cardia adenocarcinoma,[25,28-36] the sample size is still small to examine stratifications of the data – specifically segment length for BE and cyclin D1 status, p53 status, and stage for esophageal and gastric cancer.

Multiple Comparisons. The issues of multiple comparisons needs to be considered when discussing the results of this dissertation, as there were 72 comparisons just within the main analyses. Examination of the exposures of total flavonoids, six classes of flavonoids, and lignans and the outcomes of BE development, EA/GCA/ESCC/NCGA incidence, and survival among EA/GCA/ESCC/NCGA cases was conducted. Additionally, outcomes were stratified by BE tumor segment length, esophageal/gastric cancer stage, and cyclin D1/p53 status. Thus, there is a high likelihood of some statistically significant results arising due to chance, due to the number of comparisons examined. While this is a highly debated topic in epidemiology, it is generally accepted that adjusting for multiple comparisons undeservedly reduces power. Therefore, associations should be evaluated individually based on biologic plausibility and consistency with published results.[54-56]

In this dissertation, more credence was given to results that were consistent across the continuum of cancer development (i.e., anthocyanidins). While it is possible that classes of

flavonoids affect the stages of cancer development differentially, the consistency of risk reduction across the cancer development continuum for anthocyanidins was striking.

Generalizability and Selection Bias. While the population-based and community-based designs of the EGA and BE studies increase the generalizability of the results, generalizability is limited due to potential selection bias. The majority of participants were Caucasian males with low socioeconomic status, and a large proportion of esophageal and gastric cancer participants' report was based on proxy response. However, the overwhelming majority of individuals that develop esophageal and gastric cancer are white males.[57-63] Thus, the results are still largely generalizable to those at highest risk of developing these deadly cancers.

Future Direction

Disentangling Effects of Antioxidants. Flavonoids may be associated with risk reduction of BE or esophageal/gastric cancer due to chemopreventive properties, as demonstrated in experimental studies, such as regulation of cell cycle, proliferation, and apoptosis.[7-9] However, these chemopreventive properties are common to many other constituents of fruits and vegetables, including fiber, vitamins, minerals, and other phytochemicals.[64] Vitamin C, vitamin E, fiber, and beta-carotene are also inversely associated with BE and esophageal/gastric cancer in population studies.[65] However, considering these other nutrients as covariates for the association between flavonoids and risk of BE or esophageal/gastric cancer is problematic, due to the inherently high collinearity between flavonoids and other chemopreventive agents in fruits and vegetables. Thus, more sophisticated statistical techniques, which can be utilized in future analyses, will be required to disentangle the effects of flavonoids from other antioxidants or to examine if the combination of antioxidants, including flavonoids and lignans, in dietary intake of fruits and vegetables is affecting the development of BE or esophageal/gastric cancer incidence and survival.[66]

Utilizing Flavonoid Gram Weights. In this analysis, flavonoid gram weights were utilized to create the exposure variables. The alternative is to utilize the molecular weight of flavonoids. That is, it might not be the grams of flavonoids that are important for risk reduction of esophageal/gastric cancers but the number of molecules of flavonoids consumed. As some flavonoids in the USDA database are presented as glycones (i.e., containing a sugar molecule) and some are presented as aglycones (i.e., not containing a sugar molecule), the issue of determining molecular weights for flavonoids is further complicated.[18] However, this is an issue that should be dealt with in future research, as there is a wide range of molecular weights in the flavonoids contributing to certain classes, particularly flavan-3-ols.[67]

Possible Future Research Designs. In an ideal study to examine the association between flavonoid intake and the EGA cancer continuum, a cohort study would be undertaken whereby individuals were followed for outcomes along the continuum of GERD → BE → EA/GCA → death, and flavonoid intake was assessed utilizing multiple techniques, including biomarkers, 24-hour recall and FFQs, administered repeatedly over time. However, a study of this nature would be extraordinarily inefficient because only 10-15% of symptomatic GERD patients develop BE in their lifetime[37] and the lifetime risk of esophageal or gastric cancer is less than one percent.[38] Thus this "ideal" cohort study would demand extensive effort from each participant annually (because of the need for repeated multiple measures per year over 20+ years of time), which could result in high participant drop-out – affecting internal validity of the study – and still yield only a small number of outcomes.

An alternative study design to consider is a new case-control study whereby BE cases and population-based controls are enrolled. Similarly to the parent BE study examined here, BE cases would be enrolled from individuals experiencing symptomatic GERD. Participants would be asked to provide 24-hour dietary recalls, urine collections, and a FFQ for each of the four seasons. Additionally, all participants would be asked to complete a FFQ for the time prior to symptomatic GERD. As flavonoids are concentrated in fruits and vegetables, which can have

seasonal availability, this study would be able to examine changing intakes of flavonoids by season. This proposed study would also allow a retrospective assessment of flavonoid intake for the time prior to symptomatic GERD. However, this recalled dietary intake could still be influenced by current dietary intake. In the proposed study, though, there would be multiple 24-hour dietary recalls, urine collections, and FFQs. Thus, the correlation between these measures can be examined, and if an individual repeatedly under- or over-reports dietary intake, the FFQ that assessed dietary intake prior to symptomatic GERD could be corrected for this misreporting. One could consider also including a GERD control group, however, over-matching of cases and controls (both of whom would have GERD) could result in detecting no differences, when in fact there should be.

Prior to implementing this "ideal" case-control study, a pilot study would need to be undertaken to determine feasibility. First, determination of whether first morning urine or 24-hour urine collection is needed to adequately capture flavonoid variability. Second, determination of participant compliance for collecting and returning urinary specimen(s) and competing the 24-hour recalls and FFQs is needed. Finally, determination of the associations between urinary biomarkers of flavonoid intake and self-reported intakes from 24-hour recalls and FFQs is needed to assess necessary exposure assessments to include in the "ideal" case-control study.

Due to the highly lethal nature of EA/GCA, a similar study utilizing 24-hour dietary recalls and urinary biomarkers would be challenging. A case-control study could be undertaken utilizing only one time point (i.e., 24-hour recall, urinary collection, and FFQ at interview) with the knowledge that study participants would primarily be individuals with less progressed cancers. In the parent EGA study, rapid case ascertainment was utilized, but 29.6% of EA/GCA cases required a proxy interview.

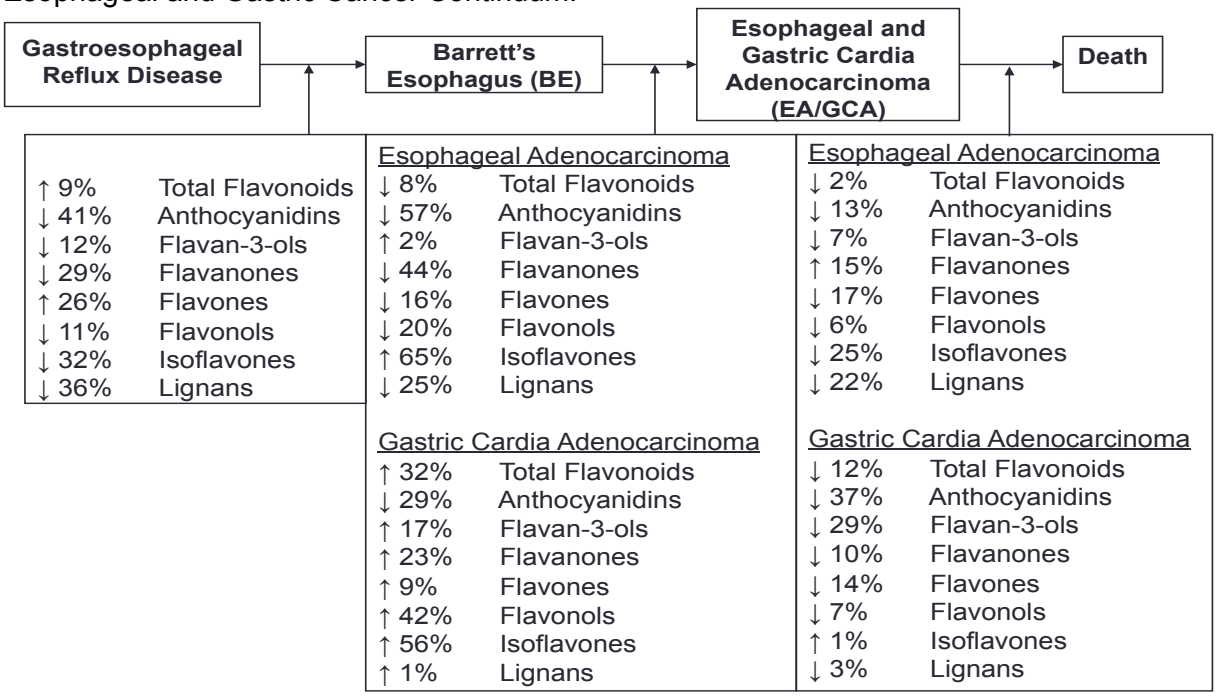
In summary, the "ideal" cohort approach with multiple, repeated measures over time would be costly, and likely to induce low retention rates with low outcome numbers, which would yield results that were unstable and not very informative. In contrast, the "ideal" case-control

approach would be challenging to interpret because of the potential impact of GERD influencing dietary recall, and it would apply to only those individuals with less aggressive disease. Given the multiple challenges associated with identifying an ideal study approach with which to address the proposed research questions, the study design used here, although less than ideal, was very cost-efficient.

Public Health Implications

Esophageal and gastric cancer have very poor survival prognoses (normally less than a year),[68] and the only known potential precursor of esophageal and gastric cardia adenocarcinoma is Barrett's esophagus.[5] These findings, if confirmed, suggest that adequate dietary anthocyanidin intake may lower risk of these cancers. This would allow some esophageal and gastric cancer to be prevented before individuals develop these deadly cancers or offer support for use of flavonoids as novel chemotherapy drugs.

Figure 5.2. Study Results for Dietary Flavonoid Intake (quartile 4 versus 1) and Risk of The Esophageal and Gastric Cancer Continuum.



Conclusion

This study of the esophageal and gastric cardia adenocarcinoma cancer continuum indicates that dietary intake of certain classes of flavonoids, particularly anthocyanidins, may reduce the risk of Barrett's esophagus and esophageal/gastric cancer. Future studies should examine individual flavonoids, disentangle effects of antioxidants, and utilize molecular weights for flavonoids. If it is confirmed in future epidemiologic studies that certain classes of flavonoids reduce the risk of esophageal/gastric cancer, clinical trials utilizing these flavonoid classes as chemopreventive therapies should be undertaken.

REFERENCES

1. Devesa SS, Blot WJ, Fraumeni JF, Jr. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;83(10):2049-53.

2. Simard EP, Ward EM, Siegel R, Jemal A. Cancers with increasing incidence trends in the United States: 1999 through 2008. *CA: a cancer journal for clinicians* 2012.

3. Shaheen N, Ransohoff DF. Gastroesophageal reflux, barrett esophagus, and esophageal cancer: scientific review. *Jama* 2002;287(15):1972-81.

4. Riddell RH. The genesis of Barrett esophagus: has a histologic transition from gastroesophageal reflux disease-damaged epithelium to columnar metaplasia ever been seen in humans? *Arch Pathol Lab Med* 2005;129(2):164-9.

5. Sharma P, McQuaid K, Dent J, Fennerty MB, Sampliner R, Spechler S, Cameron A, Corley D, Falk G, Goldblum J, Hunter J, Jankowski J, Lundell L, Reid B, Shaheen NJ, Sonnenberg A, Wang K, Weinstein W. A critical review of the diagnosis and management of Barrett's esophagus: the AGA Chicago Workshop. *Gastroenterology* 2004;127(1):310-30.

6. Farber E. Cancer development and its natural history. A cancer prevention perspective. *Cancer* 1988;62(8 Suppl):1676-9.

7. Song S, Krishnan K, Liu K, Bresalier RS. Polyphenon E inhibits the growth of human Barrett's and aerodigestive adenocarcinoma cells by suppressing cyclin D1 expression. *Clin Cancer Res* 2009;15(2):622-31.

8. Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. *Nutr Cancer* 2006;54(1):33-46.

9. Stoner GD, Wang LS, Seguin C, Rocha C, Stoner K, Chiu S, Kinghorn AD. Multiple berry types prevent N-nitrosomethylbenzylamine-induced esophageal cancer in rats. *Pharm Res* 2010;27(6):1138-45.

10. Corley DA, Buffler PA. Oesophageal and gastric cardia adenocarcinomas: analysis of regional variation using the Cancer Incidence in Five Continents database. *International journal of epidemiology* 2001;30(6):1415-25.

11. Schottenfeld D, Fraumeni JF. *Cancer epidemiology and prevention*. 3rd ed. Oxford ; New York: Oxford University Press, 2006.

12. Kamineni A, Williams MA, Schwartz SM, Cook LS, Weiss NS. The incidence of gastric carcinoma in Asian migrants to the United States and their descendants. *Cancer causes & control : CCC* 1999;10(1):77-83.

13. Lee J, Demissie K, Lu SE, Rhoads GG. Cancer incidence among Korean-American immigrants in the United States and native Koreans in South Korea. *Cancer control : journal of the Moffitt Cancer Center* 2007;14(1):78-85.

14. Tzonou A, Lipworth L, Garidou A, Signorello LB, Lagiou P, Hsieh C, Trichopoulos D. Diet and risk of esophageal cancer by histologic type in a low-risk population. *International journal of cancer. Journal international du cancer* 1996;68(3):300-4.

15. Sun CL, Yuan JM, Lee MJ, Yang CS, Gao YT, Ross RK, Yu MC. Urinary tea polyphenols in relation to gastric and esophageal cancers: a prospective study of men in Shanghai, China. *Carcinogenesis* 2002;23(9):1497-503.

16. Dai Q, Franke AA, Jin F, Shu XO, Hebert JR, Custer LJ, Cheng J, Gao YT, Zheng W. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2002;11(9):815-21.

17. Fink BN, Steck SE, Wolff MS, Kabat GC, Gammon MD. Construction of a flavonoid database for assessing intake in a population-based sample of women on Long Island, New York. *Nutr Cancer* 2006;56(1):57-66.

18. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Flavonoid Content of Selected Foods, Release 3.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/flav.

19. U.S. Department of Agriculture, Agricultural Research Service. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. Nutrient Data Laboratory Home Page: http://www.ars.usda.gov/nutrientdata/isoflav.

20. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutrition and cancer* 2006;54(2):184-201.

21. Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *American journal of epidemiology* 1997;146(10):856-69.

22. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol* 1999;9(3):178-87.

23. Bobe G, Peterson JJ, Gridley G, Hyer M, Dwyer JT, Brown LM. Flavonoid consumption and esophageal cancer among black and white men in the United States. *International journal of cancer. Journal international du cancer* 2009;125(5):1147-54.

24. Jiao L, Kramer JR, Rugge M, Parente P, Verstovsek G, Alsarraj A, El-Serag HB. Dietary intake of vegetables, folate, and antioxidants and the risk of Barrett's esophagus. *Cancer causes & control : CCC* 2013;24(5):1005-14.

25. Anderson LA, Watson RG, Murphy SJ, Johnston BT, Comber H, Mc Guigan J, Reynolds JV, Murray LJ. Risk factors for Barrett's oesophagus and oesophageal adenocarcinoma: results from the FINBAR study. *World J Gastroenterol* 2007;13(10):1585-94.

26. Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Jr., Buffler P, Corley DA. Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. *Am J Gastroenterol* 2008;103(7):1614-23; quiz 1624.

27. Thompson OM, Beresford SA, Kirk EA, Vaughan TL. Vegetable and fruit intakes and risk of Barrett's esophagus in men and women. *Am J Clin Nutr* 2009;89(3):890-6.

28. Brown LM, Swanson CA, Gridley G, Swanson GM, Schoenberg JB, Greenberg RS, Silverman DT, Pottern LM, Hayes RB, Schwartz AG, et al. Adenocarcinoma of the esophagus: role of obesity and diet. *Journal of the National Cancer Institute* 1995;87(2):104-9.

29. Chen H, Tucker KL, Graubard BI, Heineman EF, Markin RS, Potischman NA, Russell RM, Weisenburger DD, Ward MH. Nutrient intakes and adenocarcinoma of the esophagus and distal stomach. *Nutrition and cancer* 2002;42(1):33-40.

30. Navarro Silvera SA, Mayne ST, Risch H, Gammon MD, Vaughan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF, Jr. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer* 2008;123(4):852-60.

31. Wu AH, Tseng CC, Hankin J, Bernstein L. Fiber intake and risk of adenocarcinomas of the esophagus and stomach. *Cancer causes & control : CCC* 2007;18(7):713-22.

32. Terry P, Lagergren J, Hansen H, Wolk A, Nyren O. Fruit and vegetable consumption in the prevention of oesophageal and cardia cancers. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation* 2001;10(4):365-9.

33. Ibiebele TI, Hughes MC, Whiteman DC, Webb PM. Dietary patterns and risk of oesophageal cancers: a population-based case-control study. *The British journal of nutrition* 2012;107(8):1207-16.

34. Freedman ND, Park Y, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. *International journal of cancer. Journal international du cancer* 2007;121(12):2753-60.

35. Freedman ND, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and gastric cancer risk in a large United States prospective cohort study. *Cancer causes & control : CCC* 2008;19(5):459-67.

36. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Vegetables and fruits consumption and risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. *International journal of cancer. Journal international du cancer* 2011;129(11):2681-93.

37. Clouston AD. Timely topic: Premalignant lesions associated with adenocarcinoma of the upper gastrointestinal tract. *Pathology* 2001;33(3):271-7.

38. Howlader N, Noone AM, Krapcho M, Garshell J, Neyman N, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KAe. SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, April 2013. Accessed December 16, 2013.

39. Agnusdei D, Adami S, Cervetti R, Crepaldi G, Di Munno O, Fantasia L, Isaia GC, Letizia G, Ortolani S, Passeri M, et al. Effects of ipriflavone on bone mass and calcium metabolism in postmenopausal osteoporosis. *Bone and mineral* 1992;19 Suppl 1:S43-8.

40. EGb 761: ginkgo biloba extract, Ginkor. *Drugs in R&D* 2003;4(3):188-93.

41. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews* 1998;56(11):317-33.

42. Spencer JP, Abd El Mohsen MM, Minihane AM, Mathers JC. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *The British journal of nutrition* 2008;99(1):12-22.

43. Willett W. *Nutritional epidemiology*. Monographs in epidemiology and biostatistics. Third edition. ed. Oxford ; New York: Oxford University Press, 2013.

44. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: promising anticancer agents. *Medicinal research reviews* 2003;23(4):519-34.

45. Edelstein ZR, Farrow DC, Bronner MP, Rosen SN, Vaughan TL. Central adiposity and risk of Barrett's esophagus. *Gastroenterology* 2007;133(2):403-11.

46. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 1976;24:117-91.

47. Mazza G. Anthocyanins in grapes and grape products. *Crit Rev Food Sci Nutr* 1995;35(4):341-71.

48. Imeh U, Khokhar S. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *J Agric Food Chem* 2002;50(22):6301-6.

49. Re R, Bramley PM, Rice-Evans C. Effects of food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radic Res* 2002;36(7):803-10.

50. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem* 2003;51(22):6509-15.

51. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and bioavailability. *The American journal of clinical nutrition* 2004;79(5):727-47.

52. Asami DK, Hong YJ, Barrett DM, Mitchell AE. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of agricultural and food chemistry* 2003;51(5):1237-41.

53. Harnly JM, Doherty RF, Beecher GR, Holden JM, Haytowitz DB, Bhagwat S, Gebhardt S. Flavonoid content of U.S. fruits, vegetables, and nuts. *Journal of agricultural and food chemistry* 2006;54(26):9966-77.

54. Willett W. *Nutritional Epidemiology*. Vol. third. New York: Oxford University Press, 2012.

55. Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. *American journal of epidemiology* 1995;142(9):904-8.

56. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1(1):43-6.

57. Falk GW. Risk factors for esophageal cancer development. *Surg Oncol Clin N Am* 2009;18(3):469-85.

58. Pondugula K, Wani S, Sharma P. Barrett's esophagus and esophageal adenocarcinoma in adults: long-term GERD or something else? *Curr Gastroenterol Rep* 2007;9(6):468-74.

59. El-Serag HB, Mason AC, Petersen N, Key CR. Epidemiological differences between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia in the USA. *Gut* 2002;50(3):368-72.

60. van Blankenstein M, Looman CW, Hop WC, Bytzer P. The incidence of adenocarcinoma and squamous cell carcinoma of the esophagus: Barrett's esophagus makes a difference. *Am J Gastroenterol* 2005;100(4):766-74.

61. Rogers EL, Goldkind SF, Iseri OA, Bustin M, Goldkind L, Hamilton SR, Smith RL. Adenocarcinoma of the lower esophagus. A disease primarily of white men with Barrett's esophagus. *J Clin Gastroenterol* 1986;8(6):613-8.

62. Chalasani N, Wo JM, Waring JP. Racial differences in the histology, location, and risk factors of esophageal cancer. *J Clin Gastroenterol* 1998;26(1):11-3.

63. Younes M, Henson DE, Ertan A, Miller CC. Incidence and survival trends of esophageal carcinoma in the United States: racial and gender differences by histological type. *Scand J Gastroenterol* 2002;37(12):1359-65.

64. Slavin JL, Lloyd B. Health benefits of fruits and vegetables. *Advances in nutrition* 2012;3(4):506-16.

65. Kubo A, Corley DA, Jensen CD, Kaur R. Dietary factors and the risks of oesophageal adenocarcinoma and Barrett's oesophagus. *Nutrition research reviews* 2010;23(2):230-46.

66. Elmstahl S, Gullberg B. Bias in diet assessment methods--consequences of collinearity and measurement errors on power and observed relative risks. *International journal of epidemiology* 1997;26(5):1071-9.

67. Bolton E, Wang Y, Thiessen PA, Bryant SH. PubChem: Integrated Platform of Small Molecules and Biological Activities. Chapter 12 IN Annual Reports in Computational Chemistry, Volume 4, American Chemical Society, Washington, DC, 2008 Apr.

68. Trivers KF, De Roos AJ, Gammon MD, Vaughan TL, Risch HA, Olshan AF, Schoenberg JB, Mayne ST, Dubrow R, Stanford JL, Abrahamson P, Rotterdam H, West AB, Fraumeni JF, Chow WH. Demographic and lifestyle predictors of survival in patients with esophageal or gastric cancers. *Clin Gastroenterol Hepatol* 2005;3(3):225-30.