POLYUNSATURATED FATTY ACIDS AND THE BARRETT'S ESOPHAGUS-ESOPHAGEAL ADENOCARCINOMA CONTINUUM

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ABSTRACT

Kathleen M. McClain: Polyunsaturated Fatty Acids and the Barrett's Esophagus-Esophageal Adenocarcinoma Continuum
(Under the direction of Marilie D. Gammon)

Barrett's esophagus (BE) is a precursor lesion for esophageal adenocarcinoma/gastric cardia adenocarcinoma (EA/GCA), which are cancers with increasing incidences in the United States (US) and very poor prognoses. In experimental studies, the impact of polyunsaturated fatty acids (PUFAs) on carcinogenesis varies, with ω-3 PUFAs (primarily found in fish) and ω-6 PUFAs (often found in oils and other foods) displaying anti- and pro-carcinogenic effects, respectively. My hypotheses were that the risk of developing BE/EA/GCA and/or dying from EA/GCA would be inversely associated with non-fried fish intake and other measures of ω-3 PUFAs, but positively associated with ω-6 PUFAs. In my dissertation, I pooled two case-control studies of BE and two case-control studies of EA/GCA with case follow-up for mortality. The total sample size included 471 BE cases with 490 controls, 1027 EA/GCA cases with 2027 controls, and 884 EA/GCA deaths. Using study-specific food frequency questionnaires, I harmonized and pooled dietary information to estimate PUFA measures including intake of fish (with consideration given to cooking methods), ω-3, ω-6, and ω-6:ω-3 ratio. Using logistic, polytomous logistic, and Cox proportional hazards regression models, I estimated odds ratios and hazards ratios, respectively, with 95% confidence intervals. Higher intake of baked/broiled fish was associated with

approximately 30% decreased risk for development of BE (particularly the more severe long-segment BE), EA, and GCA. ω-6 intake was also associated with an increased risk of EA and GCA; however, so was ω-3. Finally higher ω-6:ω-3 was associated with lower EA mortality, but not GCA mortality. There was no evidence of modification by inflammation-related factors for any of the outcomes assessed. My findings of inverse associations for baked/broiled fish intake with BE/EA/GCA development and positive associations for ω-6 with EA/GCA development are consistent with my hypotheses. But the positive association between ω-3 intake and EA/GCA development, and the inverse association between ω-6:ω-3 and EA mortality, are not. If findings are confirmed, increasing intake of baked/broiled fish may be a plausible risk reduction strategy for BE (especially long-segment BE), EA, and GCA, and could reduce the disease burden of these lethal cancers.

To my parents, who have always believed I could succeed at anything I set my mind to and who have inspired me through their own work.

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LIST OF ABBREVIATIONS

ω-3	Omega-3 fatty acid
ω-6	Omega-6 fatty acid
AA	Arachidonic acid
ALA	α-linolenic acid
BE	Barrett's esophagus
BE-EA	Barrett's esophagus-esophageal adenocarcinoma
BEACON	International Barrett's and Esophageal Adenocarcinoma Consortium
BMI	Body mass index
CagA	*Cytotoxin-associated gene A*
CI	Confidence interval
CIM	Cardia intestinal metaplasia
COX	Cyclooxygenase
CT	Connecticut
CYP	Cytochrome P450
DAG	Directed acyclic graph
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentaenoic acid
EA	Esophageal adenocarcinoma
EET	Epoxyeicosatrienoic acids
EMM	Effect measure modification

EPA	Eicosapentaenoic acid
ESCC	Esophageal squamous cell carcinoma
FFQ	Food frequency questionnaire
FHCRC	Fred Hutchinson Cancer Research Center
FINBAR	Factors Influencing the Barrett's/Adenocarcinoma Relationship Study
GCA	Gastric cardia adenocarcinoma
GEJ	Gastroesophageal junction
GERD	Gastroesophageal reflux disease
HCFA	Health Care Financing Administration
HETE	Hydroxyeicosatetraenoic acids
HGD	High-grade dysplasia
HH	Hiatal hernia
Hp	*Helicobacter pylori*
HR	Hazard ratio
ICD-9	International Classification of Disease, Ninth Revision
ICD-O	International Classification of Diseases for Oncology
ICR	Interaction contrast ratio
IRB	Institutional Review Board
IRR	Incidence rate ratio
KPNC	Kaiser Permanente Northern California
LA	Linoleic acid
LAC	Los Angeles County
LES	Lower esophageal sphincter

LGD	Low-grade dysplasia
LOX	Lipoxygenase
LSBE	Long-segment Barrett's esophagus
LT	Leukotriene
NBOR	United Kingdom National Barrett's Oesophagus Register
NCC	University of Minnesota Nutrition Coordinating Center
NDI	National Death Index
NIH-AARP	National Institutes of Health-AARP Diet and Health Study
NJ	New Jersey
NOK	Next-of-kin
NSAID	Nonsteroidal anti-inflammatory drug
OR	Odds ratio
PA	Physical activity
PG	Prostaglandin
PH	Proportional hazards
PUFA	Polyunsaturated fatty acid
RDD	Random digit dialing
RERI	Relative excess risk due to interaction
ROR	Ratio of the odds ratio
RR	Risk ratio/relative risk
SAD	Sagittal abdominal diameter
SAT	Subcutaneous adipose tissue
SEER	Surveillance, Epidemiology, and End Results Cancer Registry

SES	Socioeconomic status
SIM	Specialized intestinal metaplasia/specialized metaplastic epithelium
SIR	Standardized incidence ratio
SSBE	Short-segment Barrett’s esophagus
TXA	Thromboxane
US	United States
USDA	United States Department of Agriculture
VAT	Visceral adipose tissue
VBE	Visible Barrett’s esophagus
WA	Washington state
WC	Waist circumference
WHR	Waist-hip ratio
WHtR	Waist-height ratio

CHAPTER I: BACKGROUND

Introduction

Barrett's esophagus (BE) is hypothesized to be a complication of long-term gastric reflux [1], and a precursor to esophageal adenocarcinoma (EA). EA and gastric cardia adenocarcinoma (GCA) are cancers often considered as one clinical entity, due to anatomical proximity, shared risk factors and treatments, and similar very poor prognoses [2-7]. The Barrett's esophagus-esophageal adenocarcinoma (BE-EA) continuum (**Figure 1.1**) presents a schematic to help determine plausible windows of susceptibility along the cancer continuum that could be targeted for interventions. The BE-EA continuum represents two possible routes of histological changes: normal esophageal tissue → BE → EA/GCA → mortality following EA/GCA, and normal esophageal tissue → EA/GCA → mortality following EA/GCA. **Figure 1.2** shows the histological changes of the esophageal lining throughout the BE-EA continuum.

The goals of this dissertation were to assess the associations between the BE-EA continuum and polyunsaturated fatty acids (PUFAs), which are found in the human diet and may act as anti-carcinogenesis and/or carcinogenesis promoting agents [8]. The study's hypotheses were that PUFAs will be differentially associated with the risk of the three outcomes along the BE-EA continuum (development of BE, risk of developing EA/GCA, and mortality following a diagnosis of EA/GCA), and the direction of the associations will be based on the PUFA measure considered.

The rationale for this study was built upon population research, in particular time trends and geographical trends. The incidences of BE and EA/GCA have been increasing in the western world over the last 50 years [4,7,9-21]. A major dietary shift in the intake of PUFAs in western countries has occurred in the last century as well, mainly due to the increase in production and consumption of vegetable oils [22,23]. The ratio of the intake of ω-6:ω-3 (the detrimental PUFAs:the beneficial PUFAs) has increased substantially over this time period, particularly among westernized countries [24,25]. Additionally, the prevalence of BE [9,13-15,26-28] and the incidence EA/GCA [10-12,29-36] are notably lower in Asian populations than in the western world. These countries with lower rates of BE and EA/GCA, typically have a much different diet when compared to western countries. The diets in Eastern countries, such as Japan, have a much higher intake of ω-3 fatty acids and consequently a lower ratio of ω-6:ω-3 intake [37].

The hypotheses for this dissertation were driven by laboratory research and epidemiological research. In the laboratory, ω-3 PUFAs have been shown to reduce inflammation [38], inhibit cell growth [39-41], and enhance apoptosis [42,43], and in epidemiologic studies are associated with reduced risk of breast [44] and prostate [45] cancer, as well as colorectal adenomas [46]. In contrast, ω-6 PUFAs have been shown to promote cancer cell proliferation in animal models [47,48], and to be adversely associated with risk in the same diseases in human populations [46,49,50].

A more detailed discussion of the scientific background, biologic foundation, and the study's significance and innovation is presented below. I first summarize the descriptive epidemiology of BE along with the established and suspected risk factors for

BE, followed by corresponding sections on EA/GCA incidence and survival. I then provide more detail on previous laboratory and epidemiologic studies focused on PUFA intake in association with the BE-EA/GCA continuum.

Barrett's Esophagus

BE is defined as the transformation of the esophageal mucosa from normal squamous epithelium into metaplastic columnar epithelium [51,52]. BE is the only known precursor lesion to EA. GCA is often considered one clinical entity along with EA, and these two tumors are highly fatal with increasing incidence in the western world [4,10,17-20,53].

Descriptive Epidemiology of BE

The true prevalence of BE is not known because many individuals with BE can be asymptomatic (estimates as high as 65% of BE cases do not exhibit symptoms [54]) and often do not seek medical care for this condition, resulting in only an estimated 5% of patients with BE receiving a diagnosis [55]. Estimates suggest that between 1.5 million and 2 million individuals in the United States (US) are affected by BE [51]. As for high-risk groups, it is suggested that 5-15% of those with gastroesophageal reflux disease (GERD) symptoms have BE [56], and around 15% or more of the US population suffers from GERD [57].

Multiple studies consistently report that the prevalence of BE is increasing worldwide [4,9,58,59], independent of the number of endoscopies performed [58]. Using a database from the Netherlands with over 500,000 patient records, diagnoses of BE

increased from 16.6/1000 upper endoscopies in 1996 to 37.1/1000 upper endoscopies in 2003 [58]. This study also showed that the prevalence of BE had increased more than 60% between 1997 and 2002 [58]. Comparing data from the Rochester Epidemiology Project in the US, the diagnoses of BE had increased 28-fold from 1965-1969 to 1995-1997 [60]. However, the authors concluded that this drastic of an increase in prevalence was due to the increase in endoscopies over that period. The Northern Ireland Barrett's Oesophagus Register (NBOR) found evidence of a 159% increase in BE comparing the 2002-2005 data with the 1993-1997 data [59]. This study also found that two demographic groups had the greatest increase, individuals under the age of 60 and particularly males under the age of 40.

There are geographic differences in the prevalence of BE. Studies in Asia have shown a much lower prevalence of BE. In Eastern China, a study of almost 140,000 participants undergoing gastroscopy found a prevalence of 0.17% [26]. This difference in prevalence extends to high-risk populations in these geographic areas. A study of individuals undergoing endoscopy for upper gastrointestinal symptoms in China observed a BE prevalence of 1.0% [27]. A Korean study of patients receiving upper gastrointestinal endoscopy with clinical symptoms had similar results, with a BE prevalence of 1.0% [28].

BE prevalence decreases with the severity of disease. The prevalence of BE differs by segment length: short-segment BE (SSBE), typically defined as less than 3 cm in length, is estimated to be three times more frequent than long-segment BE (LSBE; ≥3 cm) [61-63]. BE is also classified by the presence and degree of dysplasia, and can be graded as negative, indefinite for dysplasia, low-grade dysplasia (LGD), and

high-grade dysplasia (HGD) [64]. The presence of dysplasia differs by segment length of BE [61,62]: for SSBE about 8% of patients have specialized intestinal metaplasia (SIM), and for LSBE estimates range between 15% and 25%.

Summary of BE Descriptive Epidemiology

Although the true prevalence of BE is unknown [55], researchers acknowledge that the prevalence has been increasing worldwide [4,9,58,59]. This increase is particularly noteworthy among individuals under the age of 60, and males under the age of 40 [59]. BE is distributed differentially in geographic regions: it is highly prevalent in the developed western world [13,14,58,59] and much less frequently diagnosed in Asian countries [26-28]. BE severity, measured by segment length or presence of dysplasia, affects prevalence with less severe disease more prevalent than LSBE or HGD [61-63].

BE Risk Factors

Medical Conditions and BE

GERD: The most well established risk factor for BE is GERD. GERD is a chronic digestive disease that develops when the reflux of gastric contents into the esophagus irritates the lining [65]. A meta-analysis of 26 studies by Taylor et al. examining the association between GERD and BE found an overall odds ratio (OR)=2.90 (95%CI=1.86-4.54) [66]. However there was a significant amount of heterogeneity among these studies. When restricting to studies of “highest quality” and studies that collected information on BE segment length, the association with SSBE was null (OR=1.15, 95%CI=0.76-1.73) but the estimate for LSBE was much more pronounced (OR=4.92, 95%CI=2.01-12.0) [66]. When examining GERD characteristics such as age

of onset, frequency of symptoms, and severity of GERD, Thrift et al. found that the risk of BE increased with frequency of symptoms, severity of symptoms, and earlier age at onset [67].

Esophagitis: Inflammation and ulceration of the esophagus, or esophagitis, is a complication of GERD [68]. Two studies, one from Sweden [69] and one from China [27], have demonstrated that esophagitis may be a risk factor for BE (controlling for GERD) with effect estimates of 5.2 (risk ratio (RR), 95%CI=1.2-22.9) and 4.4 (OR, 95%CI=1.2-1.6), respectively.

Hiatal hernia (HH): HH, a condition where the stomach protrudes into the chest cavity by way of a hole in the diaphragm [70]. HH has been of interest in regards to the development of BE because HH may promote gastric reflux by increasing intra-abdominal pressure, or when acid trapped in the HH sac enters the esophagus when the lower esophageal sphincter (LES) relaxes [71]. HH is more prevalent in those with BE, particularly high-grade dysplasia, than in those with GERD [70] or esophagitis [71], and more prevalent in BE patients than in controls scoped without reflux [71]. Similar to what is seen with GERD, HH appears to be more strongly associated with LSBE (OR=12.67, 95%CI=8.33-19.25) than with SSBE (OR=2.87, 95%CI=1.75-4.70) from a meta-analysis of nine studies [72].

Helicobacter pylori (Hp): Hp infection has been hypothesized to suppress gastric acid secretion and lead to gastric atrophy [73,74]. *Hp* is associated with decreased risk of EA [75,76] and BE [77]. A recent meta-analysis of 49 studies found a pooled OR=0.73 (95%CI=0.60-0.88) for *Hp* infection on BE [77]. The inverse association was more pronounced when: restricting to studies with appropriate measurement of *Hp* and

without selection bias (OR=0.46, 95%CI=0.35-0.60), when restricting to studies performed in the US (OR=0.46, 95%CI=0.40-0.53), or limiting to *cytotoxin-associated gene A* (*CagA*) positive *Hp* strains (OR=0.38, 95%CI=0.19-0.78) [77].

Lifestyle Factors and BE

Obesity: Obesity is a well-established risk factor for BE. A meta-analysis of nine studies showed a 35% increase in risk for BE when comparing those with an obese body mass index (BMI; ≥30 kg/m^2) to those who were not obese (BMI <30 kg/m^2) [78]. Additionally, there was a 49% increase in risk for BE when comparing those with an overweight or obese BMI (≥25 kg/m^2) to those who were not overweight (BMI <25 kg/m^2). Other measures of abdominal obesity have also been shown to be associated with BE, including increases in waist circumference (WC) [79,80], waist-hip ratio (WHR) [79], sagittal abdominal diameter (SAD) [79], waist-height ratio (WHtR) [79], visceral adipose tissue (VAT) [81], and subcutaneous adipose tissue (SAT) [81]. Using these measures may be preferential to BMI because investigators have suggested that centralized obesity may be more important to the development of BE through mechanisms such as intragastric pressure, metabolically active tissue, and inflammation [1,82].

Physical Activity (PA): To date there has only been one study assessing the association between PA and development of BE [83]. The Texas-based case-control study conducted by Hilal et al. examined PA in the week prior to interview and found no associations between BE and the highest level of PA ($OR_{High-Low}$=1.19, 95%CI=0.82-1.73) or total amount of PA ($OR_{High-None}$=1.28, 95%CI=0.93-1.75) [83]. Although the estimate for high amounts of PA (compared to none) suggests there may be a positive

relationship, more studies are necessary to determine associations between PA and BE. The potential mechanism of action between PA and BE is unclear, although it has been suggested it may act through reducing obesity [83] or other pathways [83] such as reducing chronic inflammation [84].

Cigarette Smoking: There is no consensus regarding the relationship between BE and cigarette smoking [85-87]. However there is evidence that cigarette smoking may increase risk of BE by relaxing the LES and increasing the likelihood of gastric acid reflux [88,89]. A meta-analysis of 13 studies of BE cases compared with non-GERD controls found that for those who had ever smoked the OR=1.44 (95%CI=1.20-1.74) [90]. There also appear to be a trend of increasing risk with increase in pack-years smoked. Using ten of the studies from the meta-analysis by Andrici et al., when comparing the lowest number of pack-years smoked to the never smokers risk of BE increased (OR=1.41, 95%CI=1.22-1.63) with a slightly more pronounced estimate for the highest number of pack-years (OR=1.53, 95%CI=1.27-1.84). A pooled analysis using the International Barrett's and Esophageal Adenocarcinoma Consortium (BEACON) data has similar results: when compared to never smokers for <15 pack-years OR=1.59 (95%CI=1.02-2.47), and for ≥45 pack-years OR=1.92 (95%CI=1.05-3.51) [85].

Alcohol Use: Alcohol use has been of great interest as a risk factor for BE, but results have been inconclusive. Two recent meta-analyses, both from 2015, have come to different conclusions [91,92]. The first using 15 studies with 42,925 participants and 3,775 BE cases found a null association comparing highest and lowest intake (RR=0.98, 95%CI=0.62-1.34), additionally there was no evidence suggesting a dose

response relationship [91]. However a reduced risk was found when restricting to women (RR=0.51, 95%CI=1.11-1.56), and increased risk was found when examining Asian studies only (RR=1.34, 95%CI=1.11-1.56). The second meta-analyses included 20 studies, 45,181 participants, and 4,432 BE cases [92]. There was a suggestive positive association when comparing any versus no alcohol consumption (RR=1.10, 95%CI=0.96-1.27), and increased risks found in men (RR=1.35, 95%CI=1.13-1.61) and in Asian populations (RR=1.60, 95%CI=1.03-2.49). When assessing alcohol type, liquor was found to increase risk of BE (RR=1.16, 95%CI=1.02-1.32). Another study pooled data from five BEACON-affiliated studies, and found a borderline inverse association (OR=0.77, 95%CI=0.60-1.00) comparing any intake versus no intake; and when examining alcohol type, the authors also reported a modest inverse association with wine intake (OR=0.71, 95%CI=0.51-0.98) [93]. These conflicting results may be explained by the different biological mechanisms alcohol can affect BE through. Alcohol can increase gastric reflux [94] increasing the risk for BE, but may confer benefits by decreasing insulin resistance and wine contains potentially beneficial antioxidants [95].

Nonsteroidal Anti-inflammatory Drug (NSAID) Use: NSAIDs, in particular aspirin, may reduce the risk of BE through inhibition of cyclooxygenase enzyme (COX)-2 [96,97]. BE patients have been shown to overexpress COX-2 [98-100], and COX-2 inhibitors have been shown to slow cell proliferation in BE cell lines [101]. An Irish case-control study showed a reduction in risk of BE for both aspirin use (OR=0.53, 95%CI=0.31-0.90) and non-aspirin NSAID use (OR=0.40, 95%CI=0.19-0.81) [102]. A California study found a reduction in risk for aspirin only, and when examining duration of use a significant trend of decreasing risk with increasing duration was seen

(p_{trend}=0.003) [103]. A study performed in Australia found that non-aspirin NSAIDs were associated with a decreased risk of BE but only in nondysplastic cases (OR=0.69, 95%CI=0.49-0.97) [104].

Dietary Intake: Recently, a variety of dietary components have been examined for relationships with the development of BE. But with the exception of fruits and vegetables, vitamin C, vitamin E, and β-carotene, there are few studies on other dietary factors to compile the evidence. A case-control study in Washington state found a reduced risk for BE for vegetable intake (OR_{T3-T1}=0.33, 95%CI=0.17-0.63) and combined fruit and vegetable intake (OR_{T3-T1}=0.39, 95%CI=0.21-0.75) [105]. A California study found a similar reduction in risk for BE with combined fruit and vegetable intake (OR_{Q4-Q1}=0.27, 95%CI=0.15-0.50), in addition to vitamin C (OR_{Q4-Q1}=0.48, 95%CI=0.26-0.90), β-carotene (OR_{Q4-Q1}=0.56, 95%CI=0.32-0.99), and vitamin E (OR_{Q4-Q1}=0.25, 95%CI=0.11-0.59) [106]. In a separate study from the same California population, fiber from fruits and vegetables was found to reduce the risk of BE with an OR_{Q3-Q1}=0.47 (95%CI=0.25-0.88) [107]. The Factors Influencing the Barrett's/Adenocarcinoma Relationship (FINBAR) study conducted in Ireland, found a null relationship between antioxidants and BE [108], but did observe an inverse association with dietary fiber intake (OR_{T3-T1}=0.44, 95%CI=0.25-0.80) [109]. Fruits and vegetables may have risk reduction properties because of substances within them that have the potential to decrease inflammation and oxidative stress [110,111].

Demographic Characteristics and BE

Age: BE is most prevalent in the 6th and 7th decades of life but may present at an earlier age (very rarely before the age of 40) [9,58,112,113]. There is also evidence

that, on average, males present with BE much earlier than females [58,112]. Increasing age allows for the accumulation of damage to the esophageal mucosa, and is associated with other risk factors such as obesity [114] and GERD [115].

Sex: Males are the predominant sex affected by BE. Estimates of the male:female sex ratio for BE have been estimated to be between 2:1 and 3:1 [112,116,117]. A meta-analysis of 32 studies by Cook et al. showed a pooled male:female sex ratio of 1.96:1 [118]. Males are more likely to suffer from abdominal obesity than females [119], which can increase intragastric pressure, metabolically active tissue, and inflammation [1,82] affecting BE risk.

Race and Ethnicity: BE is also most common among Caucasians [9]. In a study by Abrams et al. the prevalence among whites was 6.1% compared to 1.7% among Hispanics and 1.6% among African Americans [117]; and in another study by Ford et al. the prevalence was 2.8% among Caucasians and 0.3% among South Asians [116]. It has been hypothesized this could be due to unknown genetic factors [120], or may be due to racial differences in obesity distribution [114] and *Hp* infection [121].

Socioeconomic Status (SES): In regards to socioeconomic status (SES), those with higher education and higher annual household income have a decreased risk of BE with ORs of 0.47 (95%CI=0.27-0.82) for college education and beyond vs. high school or less and 0.68 (95%CI=0.42-1.11) for annual income of ≥$75,000 vs. <$50,000 [122]. Education and income are associated with health-seeking behaviors, which may explain the inverse associations [122].

Summary of BE Risk Factors

A variety of medical, lifestyle, and demographic factors have been identified as factors associated with development of BE. Older age [9,58,112,113], white race [9,116,117], male sex [112,116,117], GERD [66], esophagitis [27,69], HH [70,71], obesity [78-81], and cigarette smoking [85] have been positively associated with the risk of BE. The risk reduction factors that have been identified are: higher education level [122], higher annual income [122], *Hp* infection [77], NSAID use [102,103], and fruit and vegetable intake [105-107]. Each of these factors will be considered as potential confounders by including them in the directed acyclic graphs (DAGs) representing the relationship between PUFAs and the development of BE.

Esophageal Adenocarcinoma and Gastric Cardia Adenocarcinoma

EA is one of the two main histological types of esophageal cancer, the other being esophageal squamous cell carcinoma (ESCC). EA is typically found in the lower third of the esophagus, close to the gastroesophageal junction (GEJ) [10]. GCA is a tumor rising from the cardiac epithelium or metaplastic junctional epithelium, in the portion of the stomach closest to the GEJ [7]. Although these two cancers may be distinct diseases, EA and GCA are often considered one clinical entity due to their anatomically adjacent position, mutual risk factors, similar histological features, shared treatment options, and comparable survival rates [123,124].

Descriptive Epidemiology of EA/GCA Incidence

In 2012, there were over 455,000 incident esophageal cancer cases worldwide, making it the eighth most frequently diagnosed cancer [33]. However this includes both EA and ESCC histological subtypes. In the US in 2016 an estimated 16,910 esophageal cancers will be newly diagnosed, and more than half of these will be EA cases [33,120]. In 2009 incidence of EA was estimated at 2.58 per 100,000, and for GCA was about 2.0 per 100,000 in 2008 [7,20].

EA/GCA incidence in the US has rapidly increased over the last few decades [4,7,16-21], and this increase has been greater than any other cancer over the same time period [125]. Using the Surveillance, Epidemiology, and End Results (SEER) data, the incidence of EA in the US has been estimated to have increased from 3.6 per million in 1973 to 25.6 per million in 2006, resulting in a seven-fold increase in incidence [126]. GCA rates have also been estimated from the SEER registry, and have increased from 1.2 per 100,000 to just under 2.0 per 100,000 from 1970 through 2008 [7]. Global data from other North American and European countries, as well as Australia, show similar incidence trends for EA and GCA as seen in the US [12,127-134]. Comparing the two cancer sites, the increase in EA is sharper than the increase in GCA, and EA rates have continued to rise where GCA rates appear to have begun to level off [7,130].

There are major variations in incidence rates in EA/GCA by geographic location [10-12,29-32]. In the highest-risk areas for esophageal cancer (often referred to as the “esophageal cancer belt”, which extends from the Middle East through China), approximately 90% of all esophageal cancer cases are ESCC [33,34]. This is in stark contrast to westernized countries, where in the US for example only 26% of all cases

are ESCC and this proportion is decreasing (thought to be due to the decline in cigarette smoking) [33,34]. Between 1970 and 1990, it was estimated that the proportion of EA cases among esophageal cancers was between 1% and 4% in east Asian countries [35]. Consequently, it has been shown that the ratio of EA:ESCC patients is well over 1.0 in whites and is much less than 1.0 for Asians [35]. Cumulative rates of EA in Asian countries have been estimated as low as 0.01% and the highest being 0.15% (for males in Hong Kong) [35,36]. The highest rates in Asian countries are in the lower end of the rates in western countries with majority whites, for example 0.19% in Canada and 0.28% in the Netherlands for males [36].

Summary of Descriptive Epidemiology of EA/GCA Incidence

EA and GCA are rare tumors, with incidences between 2 and 2.5 per 100,000 [7,20]. But their incidences have been increasing over the last forty to fifty years [4,7,17-21], outpacing the increase in all other non-skin cancers in the US (notably breast and prostate cancer) [125]. And this increase is expected to continue in the coming decades [2,53,135]. When examining EA and GCA individually, the trends observed for EA are more drastic when compared to GCA [7,130]. Incidence rates vary widely dependent upon geographic location, with much higher rates in western countries when compared to developed Asian countries [10-12,29-32].

EA and GCA Risk Factors

Medical Conditions and EA/GCA Incidence

BE: Barrett's esophagus, as the precursor lesion, is the strongest risk factor for EA/GCA [136-139]. A study using data from the Danish Pathology Registry and the

Danish Cancer Registry found that when compared to the general population those with BE were at a much higher risk of EA (standardized incidence ratio (SIR)=11.3, 95%CI=8.8-14.4) [136]. When dysplasia was considered, the risk of EA was higher for those with diagnosed low-grade dysplasia than for those with BE without dysplasia (SIR=4.8, 95%CI=2.6-8.8). A United Kingdom study found a much higher increase in risk of EA for those diagnosed with BE, with a SIR=29.8 (95%CI=9.6-106) [137]. Analysis on the United Kingdom NBOR data showed that segment length of BE was also related to the risk of EA [138]. Compared to those with <3 cm of BE, those with >9 cm of BE had an IRR=2.051 (95%CI=0.614-6.847), and while this estimate was non-significant, the small sample size limited these analyses. A study out of Northern Ireland that combined the EA, GCA, and HGD into one outcome, showed that presence of SIM (hazard ratio (HR)=3.54, 95%CI=2.09-6.00), long segment length (HR=2.31, 95%CI=0.89-6.01), and low-grade dysplasia (HR=5.67, 95%CI=3.77-8.53) increase risk [139].

Esophagitis: Esophagitis is a well-established risk factor for BE, but the evidence for EA/GCA is scarce. One study using the General Practice Research Database from the United Kingdom, found that the risk of EA in a cohort of individuals with reflux esophagitis, when compared to a reference cohort, was increased with a SIR=4.5 (95%CI=1.04-19.6) [137]. A Danish study showed that patients with esophagitis, when compared to the general Danish population, were at an increased risk of EA (SIR=2.2, 95%CI=1.6-3.0) [140]. Combining EA and GCA into a single outcome, an analysis from the Southern California Kaiser Foundation Health Plan found that esophagitis/esophageal ulcers increased risk (OR=5.0, 95%CI=1.5-16.4) [141].

However, the Los Angeles County (LAC) Multiethnic Study found null associations with both EA and GCA [142]. This could be due to small numbers of those with esophagitis diagnosed by a physician.

GERD: GERD, independent of BE, has been of interest as a risk factor for EA/GCA. GERD may predispose individuals to cancer development through the damaging of the mucosa of the esophagus and gastric cardia by gastric acid reflux [141,143]. A pooled analysis of 12 BEACON studies with 1,197 EA cases examined the association between EA cases and reflux symptoms [143]. For those with recurrent heartburn or regurgitation, the ORs for EA were 4.64 (95%CI=3.28-6.57) and 4.57 (95%CI=3.43-6.08), respectively. When examining frequency of heartburn and regurgitation, the associations grew stronger as symptoms were more frequent; those experience daily symptoms compared to those never experiencing them had an OR=7.96 (95%CI=4.51-14.04). The risk increased as duration of these symptoms increased, for those experiencing heartburn and regurgitation for ≥30 years the OR=6.08 (95%CI=3.26-11.34). When examining reports of esophageal reflux, a study using data from Southern California found that the risk of EA/GCA was increased (OR=2.1, 95%CI=1.2-3.6) and risk was more pronounced when these symptoms were first reported >5 years prior (OR=2.7, 95%CI=1.5-4.9) [141].

HH: HH could be risk factor for EA/GCA through the same mechanisms as for BE, through increasing intra-abdominal pressure and promoting gastric reflux [71]. A positive association was seen between the presence of HH and EA and GCA in the LAC Multiethnic Study [142]. The association with HH was stronger for EA (OR=4.85, 95%CI=3.21-7.33) than for GCA (OR=2.26, 95%CI=1.47-3.45). Data from the Southern

California Kaiser Foundation Health Plan also found an increased risk of EA/GCA with the presence of HH (OR=3.8, 95%CI=1.9-7.6) [141]. Another study by Avidan et al. found that large HH was associated with an increased risk of HGD/EA as a combined outcome (OR=2.48, 95%CI=2.13-2.89) [70]. And a smaller study out of Berlin found a similar but more pronounced association between HH and increased risk of HGD/EA (OR=7.68, 95%CI=3.54-16.65) [144].

Hp: Recently, *Hp* has received a lot of attention as a risk reduction factor for EA/GCA. The proposed biological mechanism is the same as for BE, through suppression of gastric acid [120]. A meta-analysis by Islami and Kamangar estimated the association between *Hp* and EA using 13 studies with 840 cases [76]. A significant decreased risk was seen for *Hp* positivity, with an OR=0.56 (95%CI=0.46-0.68). When restricting to the *CagA* positive *Hp* strains, the inverse association was more pronounced (OR=0.40, 95%CI=0.28-0.56). A smaller, more recent German study found a suggested inverse association between *Hp* and HGD/EA (OR=0.50, 95%CI=0.23-1.09) [144]. When examining *Hp* relationship with GCA, a study using the Alpha-Tocopherol, Beta-Carotene Cancer Prevention data estimated a reduced risk with an OR=0.31 (95%CI=0.11-0.89) [145]. They also assessed this association by *CagA* status, where the authors found a reduced risk only for *CagA* negative *Hp* strains (OR=0.21, 95%CI=0.06-0.81), although this study may be limited by small sample sizes.

Family History: Family history has been hypothesized to be a risk factor for EA/GCA, mainly due to observed familial clustering and the knowledge that those who are male and Caucasian tend to be at highest risk of EA/GCA [146-149]. However, there is no consensus across previous studies that address this issue. A study

performed in the US, showed that patients with EA did not have an association with a positive family history of digestive cancers, subsite specific or as a whole [150]. For GCA, there was no association with any subsite family history but there was evidence of a possible increased risk when examining family history of any digestive cancer. A Swedish study found similar results: null associations for EA, and a suggested increased risk with family history of stomach cancer (including the cardia; OR=1.6, 95%CI=1.0-2.6) [151]. A separate Swedish study using the Swedish Family-Cancer Database, found an increased risk for EA when a parent was diagnosed with ESCC (SIR=4.05, 95%CI=1.05-10.46) or any esophageal cancer (SIR=3.52, 95%CI=1.11-8.28) [152].

Lifestyle Factors and EA/GCA Incidence

Obesity: The increasing obesity epidemic has paralleled the increasing rates of EA and GCA. Obesity may increase risk of EA/GCA through the same mechanisms as for BE [1,82], and by promoting the transition of normal esophageal tissue to BE. A meta-analysis from 2003 with 2,488 EA and 2,509 GCA cases found positive associations with overweight/obesity and EA and GCA [153]. The associations were stronger with EA for overweight (OR=1.9, 95%CI=1.5-2.4) and obesity (OR=2.4, 95%CI=2.0-2.8) than for GCA (OR=1.2, 95%CI=1.0-1.5 and OR=1.5, 95%CI=1.2-1.9, for overweight and obesity, respectively). A pooled analysis from BEACON investigated associations between BMI and EA among 1,997 EA cases, 1,900 GCA cases, and 11,159 controls [154]. Compared to those of normal/underweight BMI (<25 kg/m^2), there was increase in risk for overweight BMI (25.0-29.9 kg/m^2; OR=1.54, 95%CI=1.26-1.88), and the risk increased with each category through those with BMI ≥40 kg/m^2 (OR=4.76,

95%CI=2.96-7.66). A study by Abnet et al. using the National Institutes of Health-AARP Diet and Health (NIH-AARP) Study found that those with a baseline BMI ≥30 had an increased risk of GCA (BMI 30-34.9: OR=1.70, 95%CI=1.22-2.36; BMI ≥35: OR=2.46, 95%CI=1.60-3.80) compared to normal BMI (18.5-24.9 kg/m^2) [155].

PA: PA may modify EA/GCA risk by reducing obesity [83] or through cancer-reducing mechanisms [83] including reducing inflammation and improving insulin sensitivity [84]. A pooled study using five prospective studies with 899 EA cases, and six studies with 790 GCA cases examined leisure-time PA and incident cancer [156]. When comparing those with high leisure-time PA (90^{th} percentile) with low leisure-time PA (10^{th} percentile) and adjusting for BMI, risk was reduced for EA (HR=0.62, 95%CI=0.40-0.97), and a suggested risk reduction for GCA (HR=0.85, 95%CI=0.69-1.04). A 2014 meta-analysis based on seven studies of EA and seven studies of GCA, showed an inverse association between PA and EA and GCA [157]. A high level of PA reduced the risk of EA (RR=0.79, 95%CI=0.66-0.94) and GCA (RR=0.83, 95%CI=0.69-0.99) when compared to low levels of PA. Another meta-analysis undertaken in 2014 by Singh et al., used four studies and saw a 32% (95%CI=0.55-0.85) decrease in risk of EA in individuals engaging in the most PA when compared to those who engaged in the least amount [158].

Tobacco Use: Cigarette smoking is one of the most well-studied risk factors for EA/GCA. Cigarette smoke contains a variety of carcinogenic compounds that damage deoxyribonucleic acid (DNA), and smoking has been hypothesized to increase cellular division and the proliferation of columnar epithelial cells (which would speed up the changes from BE to EA) [159]. Tramacere et al. performed a 33-study meta-analysis

assessing the relationship between tobacco use and EA/GCA [160]. Comparing to never smokers, the pooled RR=1.76 (95%CI=1.54-2.01) for ever smokers. There was a more pronounced association for current smokers (RR=2.32, 95%CI=1.96-2.75), and a slightly attenuated estimate for former smokers (RR=1.62, 95%CI=1.40-1.87). A dose-response relationship was seen when examining both dose (cigarettes/day) and duration (years) of cigarette smoking. Using the BEACON data, Cook et al. showed that the longer the amount of time since smoking cessation the risk of EA/GCA decreases ($OR_{\geq 10\text{-}0}$=0.71, 95%CI=0.56-0.89) [161].

Alcohol Use: The relationship between alcohol use and EA/GCA has not been clearly established. But has been continued to be studied because of its clear relationship with ESCC, and because alcohol can increase gastroesophageal reflux by relaxing the LES [94]. Conversely, alcohol can have positive effects on insulin resistance and some types (wine, specific types of beer) may contain beneficial antioxidants [95]. A meta-analysis of 24 studies including 5,500 cases showed a null association when comparing drinkers to non-drinkers (RR=0.96, 95%CI=0.85-1.09) [94]. However, there was evidence of a decreased risk of EA/GCA when comparing light drinkers (≤1 drink/day) with non-drinkers (RR=0.86, 95%CI=0.75-0.99). But no association was seen with moderate or heavy drinkers and EA/GCA. Similar results were seen in a pooled analysis of BEACON data, where there was a suggested association with moderate alcohol intake (0.5-<1 drinks/day) and EA (OR=0.63, 95%CI=0.41-0.99) and GCA (OR=0.78, 95%CI=0.62-0.99) but no other associations were seen [95]. Beer consumption showed evidence of a decreased risk for both EA ($OR_{1\text{-}<3\text{-}None}$=0.72, 95%CI=0.51-1.04) and GCA ($OR_{1\text{-}<3\text{-}None}$=0.64, 95%CI=0.46-0.90),

similar results were seen for wine (EA: $OR_{1-<3-None}$=0.71, 95%CI=0.49-1.03; GCA: $OR_{1-<3-None}$=0.72, 95%CI=0.52-1.02).

NSAID Use: The mechanism by which NSAIDs and aspirin may reduce the risk of EA/GCA is through a COX-2 pathway [96], similar to the one for BE. A meta-analysis of 11 studies (9 case-control and 2 cohort studies) found a RR=0.64 (95%CI=0.52-0.78) for regular aspirin use and the risk of EA/GCA [162]. A separate meta-analysis observed that use of non-aspirin NSAIDs was associated with reduced risk of EA (OR=0.65, 95%CI=0.50-0.85) and GCA (OR=0.80, 95%CI=0.67-0.95) [163]. A study of BE patients found that the risk of developing EA for current users of NSAIDs at baseline was lower than for never users (HR=0.32, 95%CI=0.14-0.76) [164].

Dietary Intake: Many recent epidemiologic studies have examined the association between diet and EA/GCA, but the most consistent results come from assessing fruits and vegetables and their nutrient components. Fruits and vegetables contain components that have the potential to decrease inflammation, oxidative stress, and cellular proliferation, as well as increase apoptosis [110,111]. A meta-analysis by Li et al. investigated the role of fruits and vegetables in the risk of EA using 12 studies with 1572 EA cases [165]. Fruits ($RR_{High-Low}$=0.73, 95%CI=0.55-0.98) and vegetables ($RR_{High-Low}$=0.76, 95%CI=0.59-0.96) were shown to decrease risk of EA, but the greatest decrease was observed when combining fruits and vegetable intake ($RR_{High-Low}$=0.68, 95%CI=0.49-0.93). The authors also observed significant dose-response relationships for all three of these measures when examining 100 g/day increments in intake. When examining carotenoid intake, another meta-analysis of four studies with 638 cases saw a reduction in risk for β-carotene intake (OR=0.46, 95%CI=0.36-0.58)

[166]. Antioxidants have also been shown to reduce risk; a meta-analysis of seven studies showed inverse associations with EA/GCA for vitamin C ($OR_{Q54\text{-}Q1}$=0.65, 95%CI=0.54-0.78), vitamin E ($OR_{Q54\text{-}Q1}$=0.64, 95%CI=0.41-0.78), and β-carotene/vitamin A ($OR_{Q54\text{-}Q1}$=0.57, 95%CI=0.47-0.68) [167].

Demographic Characteristics and EA/GCA Incidence

Similar to what is seen for BE, specific demographic groups have been found to be at higher risk for EA/GCA.

Age: As is the case for the majority of other cancers, the incidence of EA and GCA increases with age. Increasing age allows for the accumulation of damage, and is associated with other risk factors such as obesity [114], GERD [115], and BE [9,58,112,113]. Rates rise with age until about 80 or 85 and begin to decline in these much older age groups. Using SEER data and data from the Danish Cancer Registry, it was seen that rates for EA and GCA are highest in the age range of 75-84 years [168,169].

Race: Whites are much more affected by EA and GCA than are other race groups [169,170]. A study by El-Serag et al. using SEER data found that age-adjusted incidence rates for EA were 3-4 times higher in whites than in African Americans, and for GCA were 1.5-2 times higher [169]. A later study also using SEER data saw that incidence rates for EA at all age groups and for both sexes were higher among whites than African Americans, with Hispanics incidence rates in the middle [170]. Similar to the proposed mechanisms for BE, these racial differences could be due to genetics [120], or may be due to racial differences in obesity [114], *Hp* infection [121], or BE [9,116,117].

Sex: Male sex is one of the major risk factors for EA and GCA [168-170]. This may be due to the sex disparity observed in BE [171] or due to the fact that males are more predisposed to abdominal obesity than females [119]. There are other hypotheses that estrogen may be acting as an inhibitor of carcinogenesis, which has been seen *in vivo* with EA [171]. A Danish study found that the age-adjusted male:female ratio was 5.91 (95%CI=4.4-7.9) for EA and 4.26 (95%CI=2.94-6.17) for GCA [168]. Studies using SEER data found that the sex disparity was wider for EA, estimated as RR ranging from 7.0 in African Americans to 20.5 in Hispanics with white in the middle at 10.8 [170], but similar for GCA with men being affected 3-5 times more than women [169].

SES: Low SES, using income and education, has been identified as a risk factor for both EA and GCA [172,173]. A Swedish study by Lagergren et al, assessed the relationship between income, education, and incident EA and GCA [172]. The highest level of income was associated with decreased risk of EA (incidence rate ratio $(IRR)_{Q5-Q1}$=0.83, 95%CI=0.71-0.97) and GCA (IRR_{Q5-Q1}=0.75, 95%CI=0.65-0.86) for men, but null associations were seen among women. Increasing level of education was associated with decreased risk of EA and GCA for both men and women. In men having completed a higher tertiary education, the risk of EA and GCA were decreased by 33% (95%CI=0.56-0.79) and 26% (95%CI=0.63-0.87) respectively. The decreased risk was similar in women: for EA the IRR=0.74 (95%CI=0.49-1.11), and for GCA the IRR=0.79 (95%CI=0.58-1.09). The US Multicenter Study found that both higher income and higher education levels were associated with decreased risk of EA and GCA [173]. Compared to those whose annual income <$15,000, those who made >$75,000 per year had an OR=0.5 (95%CI=0.3-1.0) for EA, and an OR=0.8 (95%CI=0.4-1.6) for GCA. When

looking at education, those who had graduate level education have a decreased risk of EA (OR=0.7, 95%CI=0.3-1.3) and GCA (OR=0.8, 95%CI=0.4-1.6) compared to those with <12 years of education. Another Swedish study showed that compared to professional workers, those who are unskilled/semiskilled manual workers have a higher risk of EA (OR=2.0, 95%CI=0.9-4.5) [174]. SES could impact the risk of EA/GCA through its association with health-seeking behaviors or by modifying the risk of BE [122].

Marital Status: Studies have previously suggested that married adults are overall healthier than those who are unmarried [175], and this may be more pronounced for men than for women [176]. Using data from the Swedish Cancer Registry, when compared to those who have been married 15 or more years and never divorced, men who have been divorced five or more years have a higher risk of EA (IRR=1.26, 1.11, 1.43) and perhaps GCA (IRR=1.11, 95%CI=0.99-1.26) [172]. Men who have been widowed five or more years, and men who have been never married had similar results where there was an increased risk of EA and a suggested increased risk of GCA. Women showed a similar pattern of results, divorced women had higher risk of EA and GCA although not statistically significant while never married women had higher risks of EA (IRR=1.51, 95%CI=1.09-2.08) and GCA (IRR=1.42, 95%CI=1.10-1.82). A separate Swedish study by Jansson et al. found that those who lived with a partner for <1 year were at higher risk of EA (OR=2.3, 95%CI=1.2-4.5) when compared to those living with a partner for ≥31 years [174].

Summary of Risk Factors for EA/GCA Incidence

The primary risk factors that have been identified to increase risk for EA/GCA are: BE [136-139], esophagitis [137,140,141], GERD [141,143], HH [70,141,142,144], obesity [153-155], cigarette smoking [160,161], increasing age [168,169], white race [169,170], male sex [168-170], and low SES [172,173]. Several risk reduction factors have also been identified, including *Hp* [76,144], PA [156-158], NSAIDs [162-164], having a long-term spouse-like partner [172,174], and dietary factors [165-167]. These characteristics will be important to consider as potential confounders when designing DAGS and models for the relationships between PUFAs and incident EA/GCA.

Esophageal Adenocarcinoma and Gastric Cardia Adenocarcinoma Survival

EA/GCA is not usually detected until after symptoms present and the cancer is at an advanced stage [2,3]. Despite advances in screening, diagnostics, treatment, and improving survival of EA/GCA [19], the prognosis is still very poor [2-7].

Descriptive Epidemiology of Survival after EA and GCA

Similar to the statistics for the incidence of EA/GCA, there are no significant differences between EA and GCA survival [123,177] and it has been shown that the mortality from these cancers has been increasing over the past few decades [20,178]. The mortality rates increased rapidly from the 1970s to the late 1990s where this increase gradually slowed down [20,178]. Estimates of the increases in mortality over the last 30 years have ranged from 2 per 100,000 to 15 per 100,000 for EA/GCA [178], or from 4 per million to 23 per million for EA alone [20]. Between 1973 and 2008,

survival for EA/GCA has increased for all stages of disease [7,179]; GCA five-year survival has increased from less than 10% to 20% [7], and EA median survival times have increased from 11, 10, and 4 months to 35, 15, and 6 months for local, regional, and distant disease [179]. It has been observed that the gradual leveling of the mortality curve appears to coincide with the improvement in survival for localized disease [20]. Over the same 30 year period, the five-year survival of localized EA went from 2% to over 50% [20]. Unfortunately, the majority of EA/GCA cancers are diagnosed at the regional or distant stage because the early stage tumors are often asymptomatic [7,20]. Although the survival times for the later stage tumors have increased from about 1975-2005 [20], the corresponding five-year survival rates for EA are 20% and 3% for regional and distant stage, respectively [20], and for GCA are 12% and 2%.[7]. The local staged tumors only make up about 25% of all cases, where the distant staged account for around 40% [20,123,179]. Because of this, the 5-year survival rate for patients diagnosed with any stage of disease remains low, reported at less than 20% [2,4-7].

Summary of the Descriptive Epidemiology of Survival after EA/GCA

As the incidence of EA/GCA has been increasing [4,7,16-21], so too has the number of deaths from these cancers [20,178]. And although improvements have been made in diagnosing and treating these cancers [19], the prognosis is still very poor [2-7]. EA/GCA is not usually detected until after symptoms present and the cancer is at an advanced stage [2,3]; most patients with EA/GCA present with regional or distant disease [7,20,180]. While survival for these tumors has increased over time [20], because of these issues the overall survival rates for these tumors remain low [2,4-7].

Prognostic Factors for Survival after EA and GCA

Tumor Characteristics & Treatment and Survival after EA/GCA

Stage: In the US Multicenter Study when compared to distant cancers, localized cancers had a much lower risk of mortality for EA (HR=0.22, 95%CI=0.15-0.31) and GCA (HR=0.18, 95%CI=0.11-0.31) [181]. Distant staged cancers indicate more advanced disease and worse prognoses.

Grade: Tumor grade did not appear to be an important prognostic factor. In the same study using US Multicenter Study data, well- and moderately-differentiated tumors had a suggested decreased risk of mortality for both EA and GCA (HR=0.85, 95%CI=0.65-1.11 and HR=0.83, 95%CI=0.62-1.10, respectively) [181].

Tumor Location: Prognoses for EA tumors are better than for GCA tumors [182]. Surveillance and earlier presentation are thought to be driving these differences. There is no current surveillance for the gastric cardia, and tumors in the esophagus are likely to present earlier with dysphagia [182].

Lymphovascular Involvement: It has been suggested that mortality is lower for those patients with tumors without lymphovascular invasion [183,184]. A small study with 99 EA cases with T1 cancer (the cancer has not yet grown into the muscularis propria) who also underwent esophagectomy saw that lymphovascular involvement had an decreased in five-year overall survival (36%) when compared to those without lymphovascular involvement (85%) [183]. A more recent study out of the Mayo Clinic on 269 patients with T1 EA showed that those with involvement of the vascular or lymphatic system had a HR=1.95 (95%CI=1.18-3.22) [184]. Analogous to what is seen

for stage, lymphovascular involvement indicates more spread disease and would be associated with a higher risk for mortality.

Treatment: Treatment regimen has been identified as a prognostic factor [185,186]. A study using SEER data, showed that three-year survival has been improved in the group of patients receiving an esophagectomy (54%) compared to those cases who did not undergo this surgery (16%) [185]. This improvement was seen for all stages of EA and for both overall survival and cancer-specific survival time. Another study using SEER data saw that in EA patients, regardless of receipt of surgery, chemotherapy decreased mortality (HR=0.6, 95%CI=0.4-1.1) [186]. However, this improvement was not seen in GCA patients (HR=1.1, 95%CI=0.7-1.2). Surgery as a treatment option may indicate earlier stage tumors, leading to an observed survival benefit.

Medical Conditions and Survival after EA/GCA

BE: In a small study of 70 EA patients treated at one center during an eight-year period, it was shown that 46% of these patients were diagnosed with BE before their cancer diagnosis [187]. The EA tumors arising from BE were larger and less likely completely respond to chemoradiation, but had better differentiation than those tumors arising in patients without BE. When examining survival, those tumors arising from BE had better one-year and five-year survival (81% and 64%, respectively) than the tumors that did not arise from BE (70% and 32%, respectively). Although the mechanism is not well understood, this reduction in mortality may be due to more frequent interaction with the medical system and potentially more endoscopic screening leading to earlier diagnosis.

GERD: The presence of GERD symptoms may confer a survival benefit in both EA and GCA [181]. A 20% reduction (95%CI=0.63-1.03) in risk of mortality was seen for EA patients, and a 26% reduction (95%CI=0.56-0.98) in risk for GCA. Similar to the mechanism between BE and improved EA/GCA survival, these higher risk patients may be diagnosed earlier because of existing GERD.

Dysphagia: Dysphagia is a condition where swallowing is difficult or uncomfortable due to disease. Patients who present with dysphagia at diagnosis have a worse prognosis, and this is most likely due to more advanced disease than those without dysphagia [182].

Lifestyle Factors and Survival after EA/GCA

Obesity: A meta-analysis by Fahey et al. based on three studies, found a decreased risk of mortality in overweight/obese (≥25 kg/m^2) individuals using pre-diagnosis BMI with a HR=0.80 (95%CI=0.68-0.95), a suggested inverse association was seen when examining only obesity individuals (≥30 kg/m^2; HR=0.85, 95%CI=0.68-1.06) [188].

Weight Loss: Patients who experience weight loss before start of treatment have worse prognoses than patients who do not [182]. This may be explained by weight loss prior to treatment being an indication of advanced disease. Additionally, this may explain why obesity appears to have a mortality reducing effect since higher body mass would allow the wasting nature of this disease to go on for longer at the same rate.

Other lifestyle factors such as cigarette use, alcohol use, NSAID use, and PA have not been shown to have an impact on EA/GCA survival according to results from the previously mentioned meta-analysis [188].

Demographic Characteristics and Survival after EA/GCA

Age [181,189], sex [181,190], education level [181,191] have not been shown to be associated with survival among EA/GCA patients. Racial/ethnic differences in mortality among EA/GCA patients are of interest, but there are typically so few non-white EA/GCA cases it makes these analyses difficult.

SES: Income has been shown to be associated with EA/GCA survival; the US Multicenter Study showed that an annual household income ≥$15,000 had a reduced risk of death for both EA (HR=0.64, 95%CI=0.48-0.87) and GCA (HR=0.62, 95%CI=0.43-0.88) [181]. Income may be a proxy for SES and represent access to quality health care and access to preferred treatments [181], or represents other health-seeking behaviors [122].

Summary of Prognostic Factors for Survival after EA/GCA

Several prognostic factors for EA/GCA have been identified, and include: stage, tumor location, lymphovascular involvement, treatment, dysphagia, BE, GERD, obesity, weight loss, and income. Advanced stage [181], tumors located in the gastric cardia [182], lymphovascular invasion [183,184], presence of dysphagia [182], weight loss before treatment [182], and lower income [181] are all considered risk factors for mortality following an EA/GCA diagnosis. While surgery and chemotherapy [185,186], BE [187] and GERD [181] previous to cancer diagnosis, and obesity [188] are prognostic factors associated with improved mortality following EA/GCA diagnosis. These EA/GCA mortality modifiers will be considered when creating DAGs and statistical models for the association between PUFAs and EA/GCA survival.

Barrett's Esophagus-Esophageal Adenocarcinoma Continuum

Biology and Natural History

The neoplastic transformation from normal esophageal lining into BE is a stepwise process of cellular changes. **Figure 1.2** displays the histopathologic features of this transformation, which follows from normal esophageal tissue, non-dysplastic BE, LGD, HGD, and finally to invasive EA [1,192]. Although many patients do not detect each of these stages and very few BE patients will develop EA, with risk of about 0.5% per patient-year [193-195].

One hypothesis suggests that the normal squamous lining of the esophagus can be damaged by chronic GERD [192,196], which involves heartburn and regurgitation of gastric contents (stomach acid, bile, duodenal secretions etc.) into the esophagus [65,197]. GERD can be further complicated by esophagitis, which is inflammation and ulceration of the esophagus [68]. The most important step is intestinal metaplasia, or the transformation of the esophageal mucosa to goblet cells, which are typically found in the intestines [198]. The damage created by GERD and esophagitis could pave the way for the reepithelialization of the esophagus by these goblet cells, and indicates the incidence of SIM [192,198]. The SIM that now present in the esophagus can progress to LGD and HGD, where dysplasia is defined as neoplastic epithelium within the basement membrane, but is contained there unlike in invasive cancer [199,200]. Finally, this dysplasia of the esophagus can develop into a neoplasm, indicating incident EA.

A similar process is hypothesized for GCA, where the cardia region of the stomach, adjacent to the GEJ, develops intestinal metaplasia (CIM) [197,201-204], but the scientific community has not come to a consensus [196,197].

Summary of BE/EA/GCA-continuum Biology

BE is diagnosed after the lining of the esophagus under neoplastic changes from normal tissue to intestinal metaplasia [198]. This change is thought to occur after chronic damage, potentially from reflux of gastric acid, duodenal secretions, and bile [65,196,197]. BE can then progress from non-dysplastic to LGD and then finally HGD before invasive EA [1], although very few cases of BE will progress to cancer [193-195]. There is evidence to support a similar histological path from normal gastric cardia, to CIM, and finally GCA [197,201-204].

Epidemiology of Polyunsaturated Fatty Acids

One candidate for a modifiable dietary risk factor is PUFAs. PUFAs may be both anticarcinogenic and carcinogenesis promoting compounds [8]. The ω-3 PUFAs, which are not commonly consumed in the US [24], are considered the beneficial fatty acids [8]. And the ω-6 PUFAs, which are very commonly consumed in the US [24], are considered the deleterious fatty acids [8]. The ω-3 and ω-6 PUFAs are named after the position of the final double bond where the methyl group is located [8]. The primary subtypes of ω-3 PUFAs are: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). And the primary subtypes of ω-6 PUFAs are: linoleic acid (LA) and arachidonic acid (AA). These fatty acids are essential, but the human body cannot make these compounds so they need to be consumed through the diet [8]. **Table 1.1** shows major sources of the two types of PUFAs in a typical western diet; ω-3 are most often consumed in fish/shellfish and nuts/seeds, while ω-6 come from margarine, meat products, and mayonnaise [205].

The availability and intake of PUFAs in the US and the western world has changed over time. It is thought that humans evolved eating a ω-6:ω-3 ratios of 1:1, but over time diets have evolved to include more ω-6 PUFAs [22,23]. This change has largely taken place over the last 100-150 years, due to the production of oils high in ω-6 such as corn oil, safflower oil, and soybean oil [22,23]. The ratio of ω-6:ω-3 fatty acids has steadily increased over the last century, with an estimated total increase from 20-40% [24,25].

PUFA dietary intake varies widely dependent upon geography. The intake of ω-6 fatty acids in a typical western diet greatly outweighs the amount of ω-3 fatty acids, with a ω-6:ω-3 ranging between 15:1 and 20:1 [37]. LA, the most abundant ω-6 subtype, comprises at least 85% of total PUFA intake, while ALA, the most abundant ω-3 subtype comprises only 10% [24]. While in countries such as India and Japan, the ratios are much lower and are estimated to be 6:1 and 4:1, respectively [37].

Increasing ω-3 fatty acid intake and/or decreasing ω-6 fatty acid intake may have the potential to decrease the burden associated with this disease progression from normal tissue to BE to invasive cancer.

Summary of PUFA Epidemiology

PUFAs are essential fats need to be ingested in the human diet, and come in two families: ω-3, the beneficial, and ω-6, the deleterious, fatty acids [8]. The intake of PUFAs has drastically changed over time, mainly a drastic increase in ω-6 consumption [24,25], which is assumed to be due to the increase in production and consumption of vegetable oils [22,23]. Intake varies geographically as well; Asian countries consume a much lower ratio of ω-6:ω-3 compared to countries that consume a typical western diet

[37]. Interventions on PUFA intake may be a potential risk reduction strategy for the BE-EA continuum, and identification of the appropriate windows for implementation along the cancer continuum could further refine these strategies.

PUFAs and the BE-EA Continuum: Biological Mechanisms

The two families of PUFAs, ω-3 and ω-6, have different biochemical roles, are necessary fats, and cannot be converted into one another [23]. However through other processes, such as subsequent desaturation (removal of hydrogen and addition of double bond) and elongation (extension of the chain by two carbon molecules) reactions, the ω-3 and ω-6 subtypes can be synthesized from one another *in vivo* [206,207]. **Figure 1.3** displays the methods by which LA and ALA (the essential fatty acids) are synthesized into the other longer ω-6 and ω-3 fatty acids, respectively [206-209].

PUFAs are incorporated into the cellular membrane and are then available for metabolism [209,210]. Both families of PUFAs use the same metabolic pathways and are competitively inhibited by one another, by vying for binding sites on the same enzymes [23,210,211]. The metabolism occurs through three pathways, COX and lipoxygenase (LOX) [23,209,210,212], and finally cytochrome P450 (CYP) [213-215]. The metabolism of AA and EPA results in the formation of eicosanoids, which are intermediate molecules that are very biologically active and may affect inflammatory and immune response, cellular growth and differentiation, and platelet aggregation [23,212]. The enzymes act as catalysts for the formation of these eicosanoid metabolites [208], along with the mediators in the metabolic process [23,210,213]. The eicosanoids can be

AA-derived or EPA-derived, and this is dependent upon the concentration of the two families of PUFAs in the cell membranes [212]. When comparing ω-3 and ω-6 metabolism, the resulting metabolites of these pathways have competing biological functions [23].

The first pathway for PUFA metabolism is COX. The COX pathway has three major metabolic enzymes: COX-1, COX-2, and COX-3 [213]. This pathway generates prostaglandins (PGs) and thromboxanes (TXAs) [23,208,212]. In addition to the function of the metabolites produced through the pathway, overexpression of COX-2 has been implicated in most human cancers and in some cancer precursors, and has been associated with poor prognosis [23,207,216].

The LOX pathway is another pathway for PUFA metabolism. The enzymes involved in the LOX pathway are 5-LOX, 8-LOX, 12-LOX, and 15-LOX [213]. These enzymes synthesize the eicosanoids generated in the LOX pathway: leukotrienes (LTs), along with hydroxyeicosatetraenoic (HETEs) acids [23,208,212].

The final PUFA metabolic pathway is the CYP pathway, which has two separate families of enzymes involved: the ω-hydroxylase and epoxygenase enzymes [213]. ω-hydroxylase and epoxygenase convert AA into HETEs and epoxyeicosatrienoic acids (EETs), respectively [213,214]. The research on this pathway has focused on inflammation, angiogenesis, and cardiovascular disease [213], the interest its role in cancer development and progression has only recently peaked.

The preponderance of AA-derived eicosanoids has been associated with factors that would increase cancer risk. PGE_2, the primary metabolite in the COX path, has been linked to promotion of cell survival, apoptosis (by both reducing pro-apoptotic

proteins and inducing anti-apoptotic proteins), increased invasiveness of malignant cells [210,212]. It has also been found in higher concentrations in cancer cells when compared to normal cells [212]. LTB4, from LOX metabolic path, boosts the generation of reactive oxygen species, which can affect DNA and lead to cancer initiation [212]. TXA_2 has been found to enhance metastasis [216]. 20-HETE, the principal eicosanoid of ω-hydroxylase CYP pathway, stimulates the production of inflammatory cytokines and has been linked to increased tumor mass [213]. 11,12-EET has been suggested to induce angiogenesis, make tumors more active, and increase proliferation [214]. While the majority of AA-derived molecules have cancer-promoting properties, PGI_2 has been found to decrease cellular proliferation and inflammation, increase apoptosis, and inhibit metastasis [212,216]. Overall AA-derived eicosanoids are pro-inflammatory, pro-angiogenic, decrease apoptosis, promote cell survival and progression, and enhance proliferation [213].

Figure 1.4 shows a schematic of AA metabolism including the pathways, enzymes, mediators, and metabolites.

Summary of PUFA Biology

The carcinogenesis promoting effect of ω-6 fatty acids increases AA-derived eicosanoids, while the anti-carcinogenic effect of ω-3 fatty acids have been shown to suppress AA-derived eicosanoid biosynthesis [8,212]. And because ω-3 and ω-6 are competing for the same binding sites on enzymes [23,210,211], higher intake of ω-3 fatty acids leads to increased presence of EPA and decreased presence of AA in the cell membrane [8,23,209]. Increased presence of ω-3 fatty acids decreases the production of AA-derived eicosanoids, which are highly biologically active, and produces

the EPA-derived molecules which are much less active molecules [207,212]. Additionally increased presence of EPA and DHA can lower the expression of COX-2 [207,210], which has been implicated in a variety of human cancers [23,216]. Furthermore COX-2 inhibitors have been shown to suppress tumor growth, angiogenesis, and metastasis [23,207].

A streamlined version of the biological mechanisms from which the proposed study derives its hypotheses about the associations between PUFAs and the BE-EA continuum is shown in **Figure 1.5**.

Epidemiology of PUFAs and the BE-EA Continuum

Details of and results from the previous epidemiologic studies examining the associations between PUFAs and the BE-EA continuum are summarized in **Figure 1.6** and **Table 1.2**.

PUFAs and BE

As the literature stands today, only three studies have examined some measure of PUFAs with the development of BE [107,217,218] and meta-analysis of two of these studies [219].

Two of these studies used fish intake as a measure, an Irish study by O'Doherty et al. [218] and a Dutch study by Keszei et al. [217]. Using the FINBAR case-control study data, the Irish study with 220 BE cases found no association with fish intake and the effect estimate suggest a possible increased risk of BE ($OR_{Q4\text{-}Q1}$=1.39, 95%CI=0.62-3.11) [218]. The Dutch study was a prospective cohort study with 447 BE cases, and these analyses were stratified by sex. There was a null association between fish intake

and BE in both men and women with $ORs_{T3\text{-}T1}$=1.01 (95%CI=0.70-1.47) and 1.13 (95%CI=0.87-1.96), respectively [217].

O'Doherty et al. explored total PUFA intake in the FINBAR study [218], and Kubo et al. also examined total PUFAs in a California study population with 296 BE cases [107]. The FINBAR study showed no association with total PUFAs ($OR_{Q4\text{-}Q1}$=0.94, 95%CI=0.40-2.17) [218]. Kubo et al. also did not find significant association, but the effect estimate suggested a possible inverse association ($OR_{Q4\text{-}Q1}$=0.47, 95%CI=0.17-1.27) [107]. A meta-analysis by Zhao et al. combined the results from these studies and found no association between BE and PUFAs ($OR_{Highestvs.Lowest}$=0.67, 95%CI=0.35, 1.26) [219].

Kubo et al. was the only study to examine ω-3 fatty acids and found a decreased risk of BE with higher intake of ω-3 ($OR_{Q4\text{-}Q1}$=0.36, 95%CI=0.14-0.90) [107].

In a fourth study, the California study population used an alternative method, dietary patterns, that provided evidence supporting fish intake as a possible risk reduction factor. In a separate study by Kubo et al., a "health conscious" dietary pattern was created where non-fried fish was a large contributor along with fruits and vegetables [220]. A high adherence to this "health conscious" diet was associated with decreased risk of BE ($OR_{Q4\text{-}Q1}$=0.35, 95%CI=0.20-0.64), and this trend of decreasing risk held as adherence increased (p_{trend}=0.001).

PUFAS and EA/GCA Incidence

Ten previous epidemiologic studies [218,221-229] and three meta-analyses [230-232] have assessed the associations between PUFAs and EA or GCA.

The association between fish intake and EA/GCA were examined in seven of these studies. Six of these, with a total of 1,610 case participants from five case-control and one cohort, were included in a meta-analysis for fish intake and EA by Han et al. [230]. Individually, two of these studies showed risk reduction for EA [221,225], two had non-significant suggestive increased risks [218,223], and two were null [222,224]. The meta-analysis had a summary RR of 0.86 (95%CI=0.61-1.22) [230]. Three of these studies were included in another meta-analysis by Jiang et al. which also showed a null association between fish intake and EA (summary RR=0.64, 95%CI=0.26-1.60) [231]. One final study examining fish intake and EA, by Navarro Silvera et al., found a non-significant but positive association between increasing intake of fish by one serving and risk of EA (OR=1.39, 95%CI=0.61-3.19) [227]. Three of these studies also examined the relationship between fish intake and GCA [222,225,227]. The LAC Multiethnic Study saw a slightly increased risk of GCA with higher levels of fish intake (OR_{Q4-Q1}=1.16, 95%CI=0.8-1.8) [222]. In a study by Navarro Silvera et al. using data from the US Multicenter Study, the risk of GCA increased with each one serving increase of fish per day (OR=1.79, 95%CI=0.85-3.80) [227]. In a prospective study using the NIH-AARP Study, there was a null association between fish intake and GCA (HR_{Q5-Q1}=0.98, 95%CI=0.71-1.35) [225].

Five studies and a meta-analysis assessed total PUFAs and the risk of EA/GCA [218,222,226]. In the FINBAR study, increasing intake of PUFAs was associated with an increase in the risk of EA (OR_{Q3-Q1}=2.68, 95%CI=1.23-5.85) [218]. There was no association between EA and total PUFAs (OR_{Q5-Q1}=0.91, 95%CI=0.70-1.19), and no association between GCA and total PUFAs (OR_{Q5-Q1}=0.95, 95%CI=0.69-1.30) with the

NIH-AARP Study data [226]. Similarly, there were no associations between PUFAS and EA (OR_{Q4-Q1}=1.07, 95%CI=0.7-1.7) or GCA (OR_{Q4-Q1}=1.24, 95%CI=0.8-1.9) in the LAC Multiethnic Study [222]. Results from the US Multicenter Study found no associations between PUFAs and EA ($OR_{75th\%vs25th\%}$=0.86, 95%CI=0.59-1-24) or GCA $OR_{75th\%vs25th\%}$=0.86, 95%CI=0.60-1-22) [228]. Finally, a Greek study by Tzonou et al. showed a suggestive increased risk for EA with PUFAs (OR=1.35, 95%CI=0.94-1.94), but only included 56 EA cases [229]. The meta-analysis by Du He et al. included five studies and found a null association between PUFAs and EA (summary RR=1.04, 95% CI=0.86, 1.27) [232].

The NIH-AARP Study also examined ω-3 fatty acids: there was no association for EA (OR_{Q5-Q1}=0.98, 95%CI=0.75-1.29) and no association for GCA (OR_{Q5-Q1}=1.08, 95%CI=0.79-1.43) with ω-3 [226].

Two studies have used dietary patterns to examine possible relationships between fish and EA/GCA. A Swedish study created a “healthy” diet, of which fish was a primary component, and those who had the highest “healthy” diet scores had suggestive decreased risks of both EA ($OR_{High-Low}$=0.8, 95%CI=0.5-1.3) and GCA ($OR_{High-Low}$=0.7, 95%CI=0.5-1.1) [233]. In another study using the US Multicenter Study data, high adherence to a dietary pattern of “fish/vitamin C”, where fish intake was an extremely high contributor to this pattern, was associated with a very slight increase in risk of GCA (OR_{Q4-Q1}=1.11, 95%CI=0.70-1.74) but no association with EA [224].

PUFAs and Survival After EA/GCA

To date, there have been no investigations into the associations between PUFAs and survival following a diagnosis of EA/GCA.

Summary of PUFAs and the BE-EA Continuum

Fish intake and BE/EA/GCA associations have been studied previously, but the research has been limited. Only one of these studies, a meta-analysis, has shown a possible risk reduction effect of fish intake [230], while a few of these have suggested a positive association with BE/EA/GCA [218,222]. These inconsistent results may be because none of these studies have considered type of fish eaten, which can have varying nutritional content [24], or the cooking method, which can also affect nutritional values [234].

The evidence for PUFAs and BE/EA/GCA has been inconsistent. One of the two studies for BE suggested a possible inverse association [107], while two of the studies for EA/GCA showed a positive association with EA [218,229]. The varied results may be due to the fact that overall PUFA measures do not take into account the distinctions between the beneficial and deleterious PUFAs. Using the relative balance represents how ω-3 and ω-6 are competitively inhibited by each other, and may differentially affect the carcinogenic process [8,49,212,216,235-237].

The associations between ω-3 fatty acids and BE/EA/GCA have been studied very rarely. An inverse association was found with the development of BE [107], but no association was seen with either EA or GCA [226]. These studies may be limited because the interplay between ω-3 and ω-6 fatty acids could be more biologically relevant [8,49,212,216,235-237], and better reflect how individuals are exposed to these potential risk modifiers.

No studies have been conducted examining ω-6, the relative balance between ω-3 and ω-6, or the subtypes of ω-3 and ω-6 fatty acids and BE/EA/GCA, and there are

no studies assessing survival among EA/GCA cases in relation to any measure of PUFAs.

Specific Aims

Aim 1: PUFAs and BE

Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with the development of BE.

Aim 2: PUFAs and Risk of Developing EA/GCA

Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with the risk of developing EA/GCA.

Aim 3: PUFAs and Mortality after Diagnosis of EA/GCA

Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with mortality among patients diagnosed with EA/GCA.

Hypotheses

Higher intake of fish and ω-3 fatty acids would be associated with reduced risk of BE-EA continuum outcomes, while the relative balance of ω-6 and ω-3 fatty acids (which will be dominated by ω-6 intake) would be associated with increased risk of these outcomes.

Background Summary

The incidence of EA/GCA is increasing [4,7,16-21], this increase is expected to continue with time [2,53,135], and the prognosis remains poor [2-7]. The incidence of the only known precursor lesion, BE, is also increasing [4,9,58,59]. The major risk factors for BE/EA/GCA development include: older age [9,58,112,113,168,169], white race [9,116,117,169,170], male sex [112,116-118,168-170], GERD [66,67,141,143], obesity [78-81,153-155], and cigarette smoking [85,160,161]. The first three risk factors are non-modifiable. GERD is a chronic disease that requires treatment and maintenance therapy, but even with treatment 30-60% of patients experience relapse [238]. Of the other mentioned risk factors, obesity and cigarette smoking are the only potentially modifiable factors. Unfortunately, weight loss [239] and smoking cessation [240] are difficult to achieve and, especially for obesity [241-243], difficult to maintain. A few risk reduction factors have also been identified, including, *Hp* [76,77,144], NSAIDs [102-104,162-164] (but are difficult to utilize long-term because of well-known gastric side effects [244]), PA [83,156-158], and, perhaps, fruits and vegetables [105,106,165]. Dietary factors appear to be amenable to intervention [245-249], and thus provide opportunities for risk reduction. Research suggests that dietary modifications may be an effective method to reduce chronic disease risk [250,251], particularly among individuals already at increased risk for these diseases [252].

The etiology for outcomes along the BE-EA continuum is complex and multiple risk reduction strategies are likely needed. PUFAs have the potential to reduce disease burden of these outcomes; increasing the beneficial ω-3 fatty acid consumption, while simultaneously decreasing the deleterious ω-6 PUFAs, is a plausible risk reduction

strategy [8,24]. This dissertation examined the associations between PUFAs and the three BE-EA continuum outcomes: development of BE, risk of developing EA/GCA, and mortality following a diagnosis of EA/GCA. If associations are found this could lead to continuum-specific strategies to reduce the risk of developing these esophageal/gastric diseases, or to improve the prognoses of these deadly cancers.

Figure 1.1. Graphical representation of the BE-EA continuum

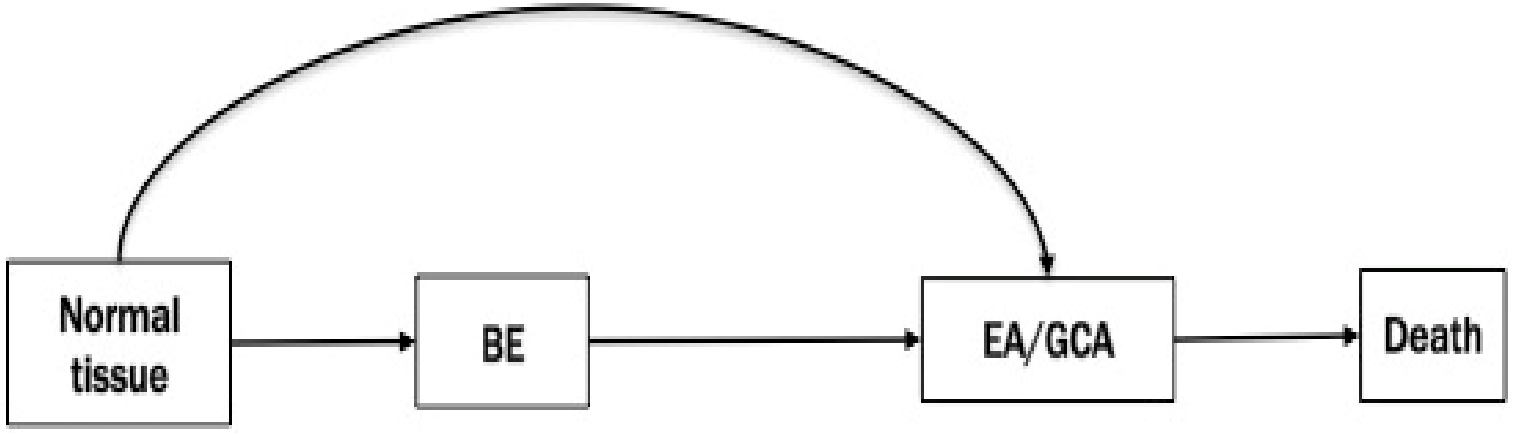
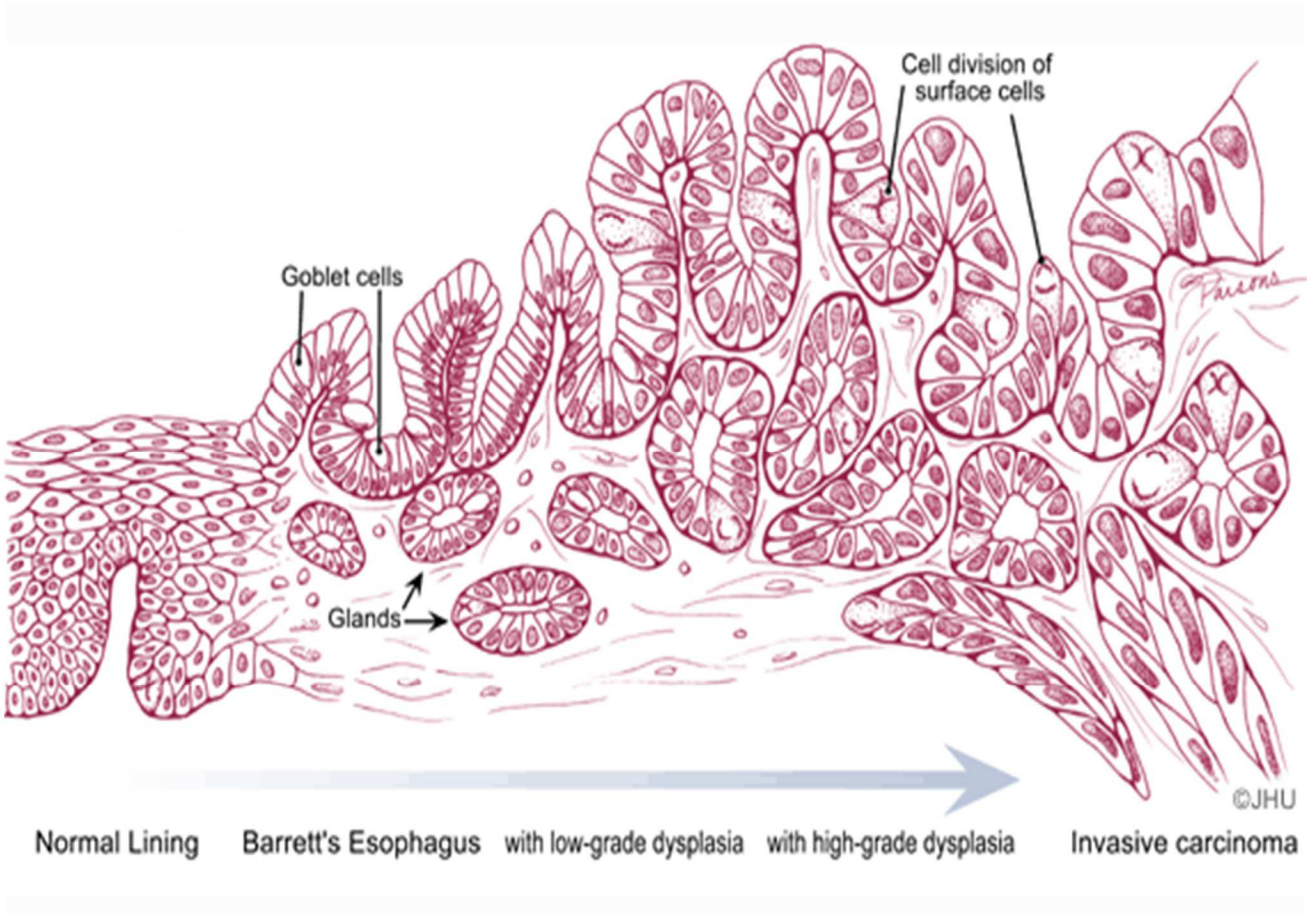


Figure 1.2. Histological changes of esophageal lining from normal tissue though invasive cancer



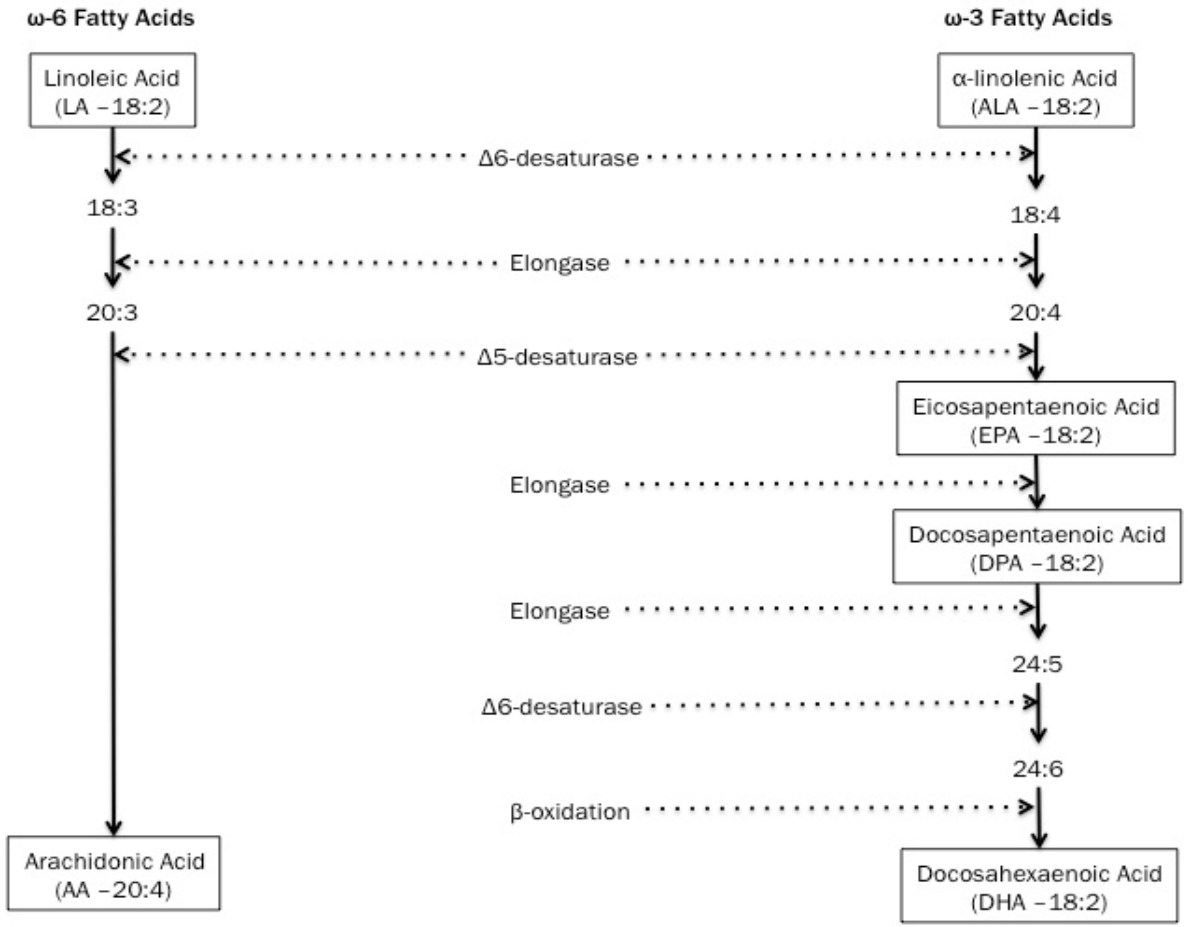
Source: Johns Hopkins Medicine, Pathology Department

Table 1.1. PUFAs, subtypes, and main dietary sources

PUFA Family	Subtype	Examples of foods rich in PUFAs
ω-3	α-linolenic acid (ALA)	Flaxseed, walnuts
	Eicosapentaenoic acid (EPA)	Fish roe, herring, oysters
	Docosahexaenoic acid (DHA)	Swordfish, salmon, mussels
	Docosapentaenoic acid (DPA)	Salmon, mackerel, trout
ω-6	Linoleic acid (LA)	Margarine, canola oil, mayonnaise
	Arachidonic acid (AA)	Pork, lard, egg

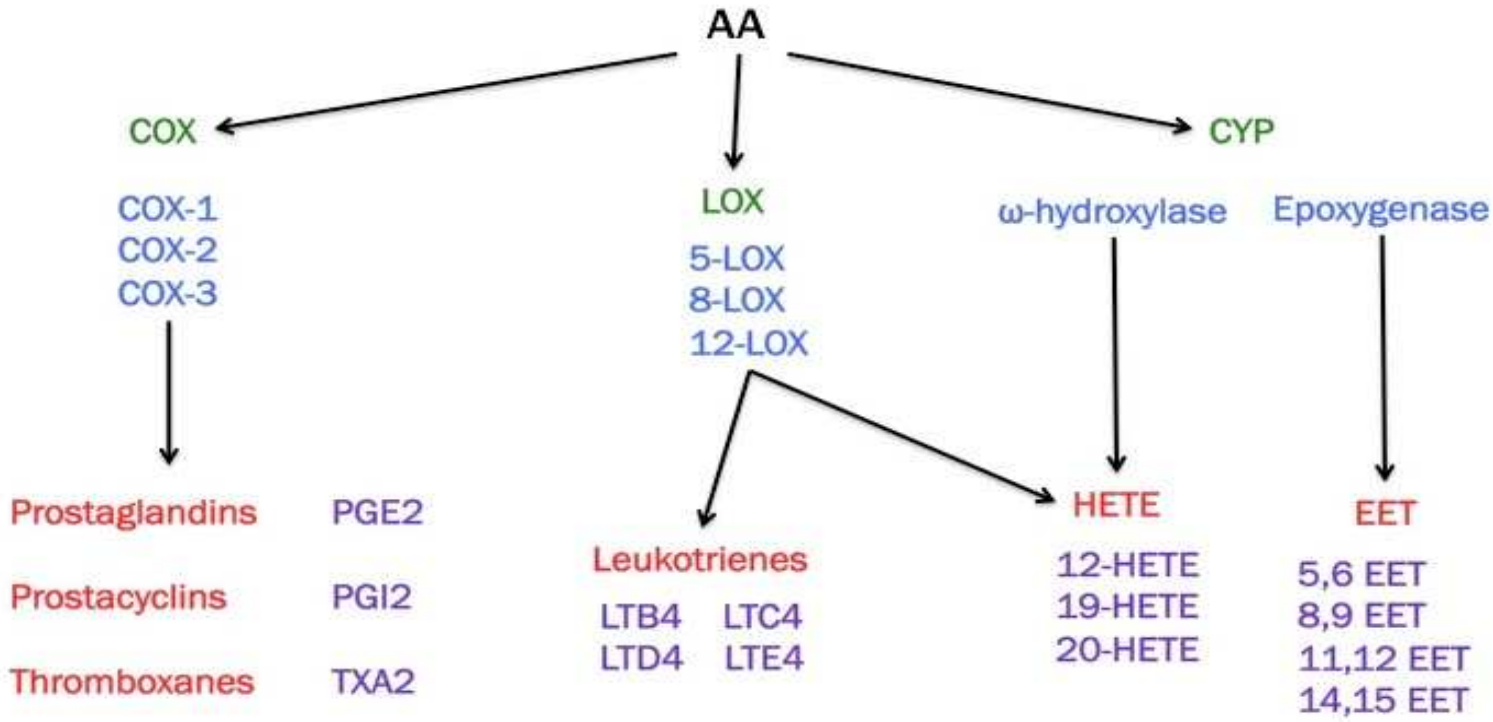
Source: [205]

Figure 1.3. ω-6 and ω-3 PUFA biosynthesis



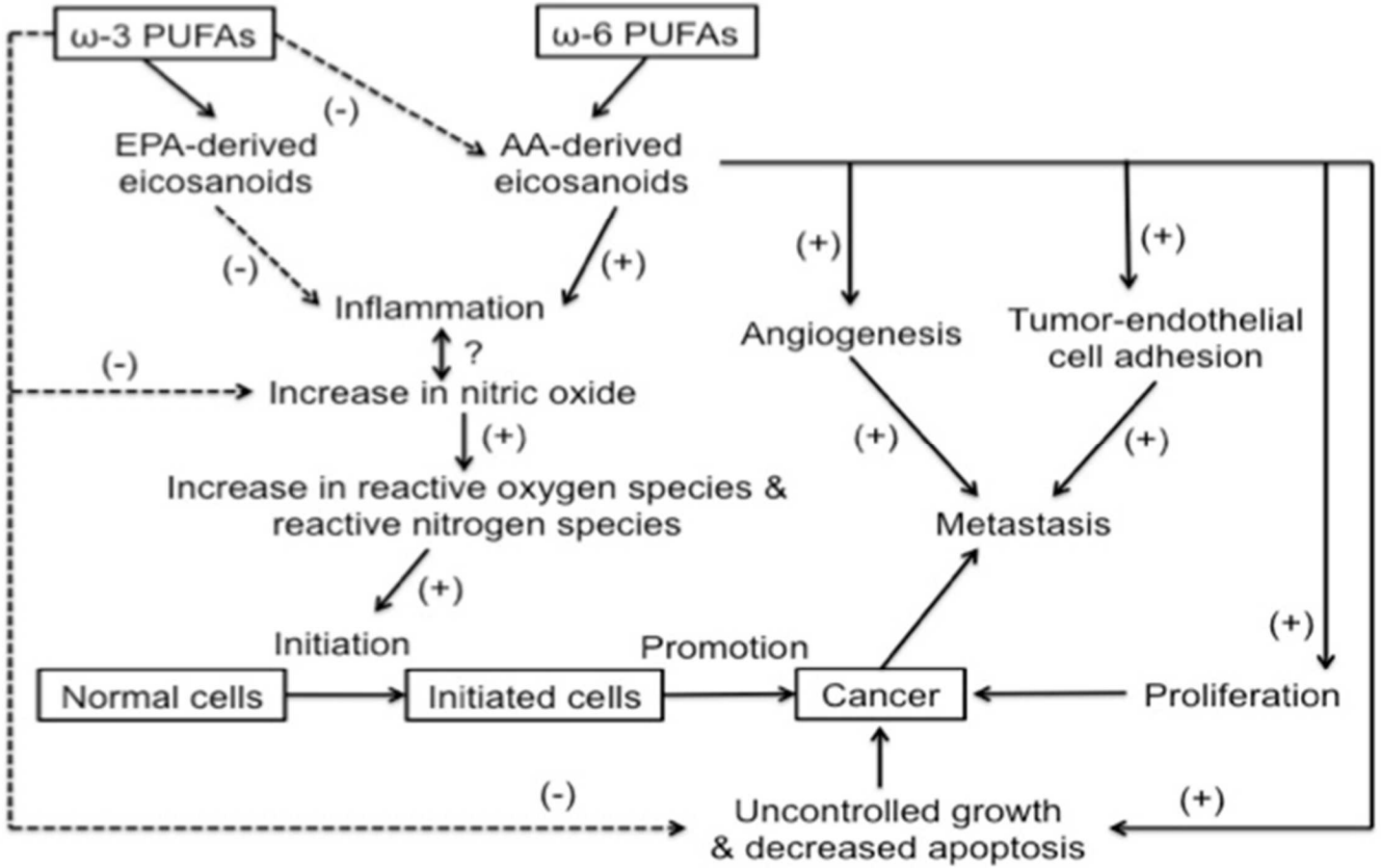
Adapted from [206,207]

Figure 1.4. Metabolism of arachidonic acid including the pathways (green), enzymes (blue), mediators (red), and eicosanoid metabolites (purple)



Adapted from [213]

Figure 1.5. Potential biological mechanisms for the association between PUFAs and outcomes along the BE-EA continuum



Adapted from [212]

Figure 1.6. Evidence from epidemiological studies supporting examination of the associations between PUFAs and the BE-EA continuum

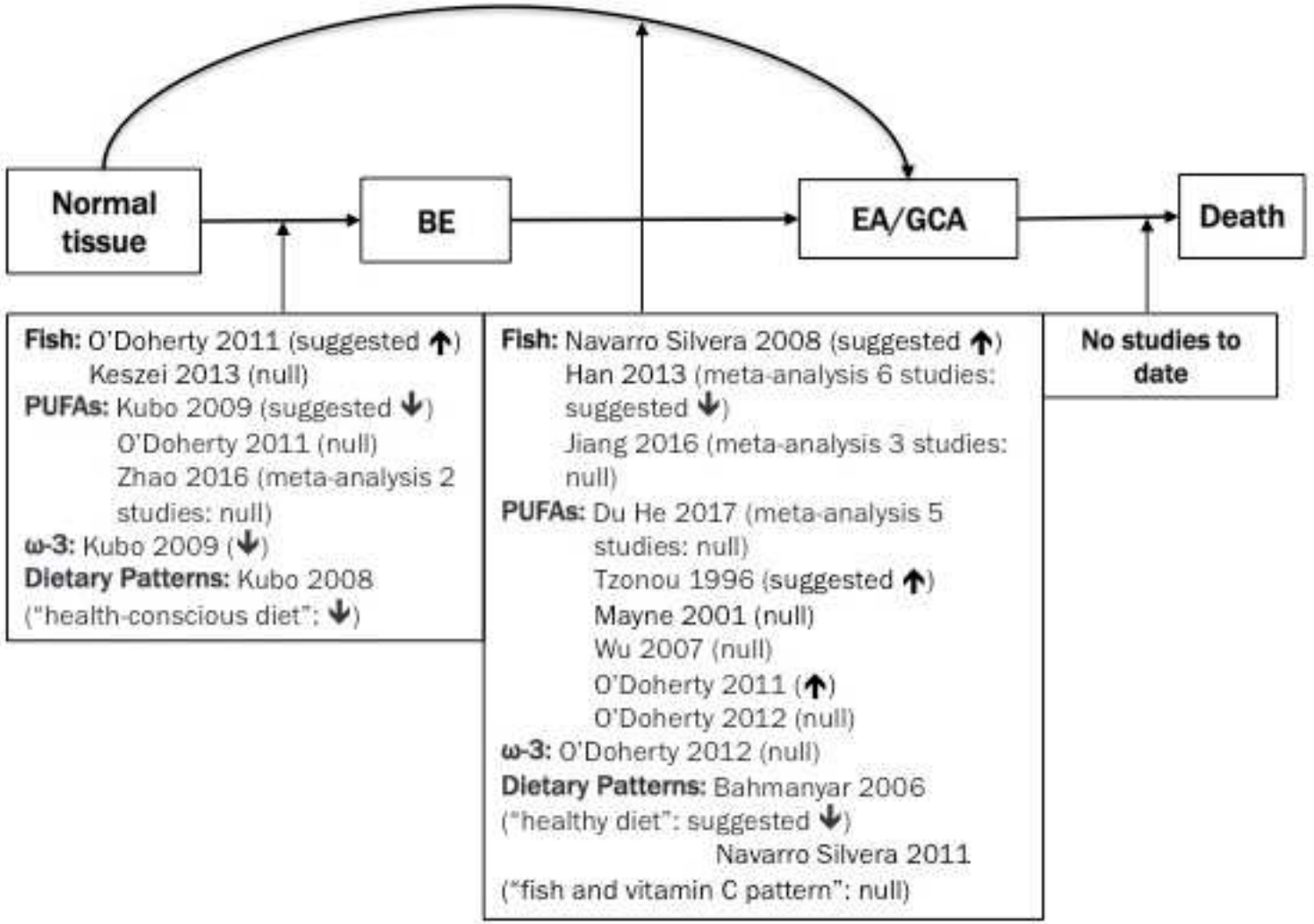


Table 1.2. Epidemiologic studies examining PUFAS and development of BE and risk of developing EA/GCA

Author, Year	Study Population	Study Design	Outcomes Evaluated	Sample Size	Exposure Assessment & Categorization	Results, Adjusted OR/HR (95% CI)
Tzonou et al., 1996 [229]	Greece	Case-control	EA	56 EA cases 200 hospital controls	115-item FFQ Quintiles	PUFA: EA – OR=1.35 (0.94, 1.94)
Mayne et al., 2001 [228]	US Multicenter Study, US	Case-control	EA and GCA	282 EA cases 255 GCA cases 687 population controls	104-item FFQ 75th percentile vs. 25th percentle	PUFA: EA – OR=0.86 (0.59, 1.24) GCA – OR=0.86 (0.60, 1.22)
Chen et al., 2002 [221]	Nebraska, US	Case-control	EA	125 EA cases 449 population controls	54-item Health Habits and History Questionnaire Quartiles	Fish (Q4 vs. Q1): EA – OR=0.14 (0.04, 0.48)
Bahmanyar et al., 2006 [233]	Sweden	Case-control	EA	185 EA cases 815 population controls	63-item FFQ High, Low	"Healthy diet" – high in fruits, vegetables, fish, poultry (high vs. low) EA – OR=0.8 (0.5, 1.3)
Wu et al., 2007 [222]	LAC Multiethnic Study, US	Case-control	EA and GCA	206 EA cases 257 GCA cases 1308 population controls	124-item FFQ Quartiles	Fish/shellfish (Q4 vs. Q1): EA – OR=0.85 (0.5, 1.4) GCA – OR=0.86 (0.6, 1.3) PUFA (Q4 vs. Q1): EA – OR=1.07 (0.7, 1.7) GCA – OR=1.24 (0.8, 1.9)
Kubo et al., 2008 [220]	Epidemiology and Incidence of BE Study, US	Case-control	BE	296 BE cases 309 population controls	110-item FFQ Quartiles	"Health-conscious diet" – high in fruits, vegetable, non-fried fish (Q4 vs. Q1): BE – OR=0.35 (0.20, 0.64)

Table 1.2 (cont'd). Epidemiologic studies examining PUFAS and development of BE and risk of developing EA/GCA

Author, Year	Study Population	Study Design	Outcomes Evaluated	Sample Size	Exposure Assessment & Categorization	Results, Adjusted OR/HR (95% CI)
Navarro Silvera et al., 2008 [227]	US Multicenter Study, US	Case-control	EA and GCA	282 EA cases 255 GCA cases 687 population controls	104-item FFQ Continuous servings/day	Fish (increase intake by 1 serving/day): EA – OR=1.39 (0.61, 3.19) GCA – OR=1.79 (0.85, 3.80)
Kubo et al., 2009 [107]	Epidemiology and Incidence of BE Study, US	Case-control	BE	296 BE cases 309 population controls	110-item FFQ Quartiles	PUFA (Q4 vs. Q1): BE – OR=0.49 (0.22, 1.11) ω-3 (Q4 vs. Q1): BE – OR=0.46 (0.22, 0.97)
Daniel et al., 2011 [225]	NIH-AARP, US	Cohort	EA and GCA	492,186 participants 553 EA cases 510 GCA	124-item FFQ Quintiles	Fish (Q5 vs. Q1): EA – OR=0.78 (0.59, 1.03) GCA – OR=0.98 (0.71, 1.35)
Mulholland et al., 2011 [223]	FINBAR, Northern Ireland & Republic of Ireland	Case-control	EA	218 EA cases 252 population controls	101-item FFQ 0, 0-1, ≥1 portions/week	Oily fish (≥1 portions/week vs. 0 portions/week): EA – OR=1.21, (0.65, 2.27)
Navarro Silvera et al., 2011 [224]	US Multicenter Study, US	Case-control	EA and GCA	282 EA cases 255 GCA cases 687 controls	104-item FFQ Quartiles	"Fish and vitamin C pattern" – high in fish and dietary vitamin C (Q4 vs. Q1): EA – OR=0.94 (0.59, 1.48) GCA – OR=1.11 (0.70, 1.74)

Table 1.2 (cont'd). Epidemiologic studies examining PUFAS and development of BE and risk of developing EA/GCA

Author, Year	Study Population	Study Design	Outcomes Evaluated	Sample Size	Exposure Assessment & Categorization	Results, Adjusted OR/HR (95% CI)
O'Doherty et al., 2011 [218]	FINBAR, Northern Ireland & Republic of Ireland	Case-control	BE and EA	224 BE cases 227 EA cases 260 population controls	101-item FFQ Quartiles	Fish (Q4 vs. Q1): BE – OR=1.39 (0.62, 3.11) EA – OR=1.49 (0.72, 3.10) PUFA (Q4 vs. Q1): BE – OR=0.94 (0.40, 2.17) EA – OR=1.60 (0.73, 3.49)
O'Doherty et al., 2012 [226]	NIH-AARP, US	Cohort	EA and GCA	494,978 participants 630 EA cases 454 GCA cases	124-item FFQ Quintiles	PUFA (Q5 vs. Q1): EA – HR=0.91 (0.70, 1.19) GCA –HR=0.95 (0.69, 1.30) ω-3 (Q5 vs. Q1): EA – HR=0.98 (0.75, 1.29) GCA – HR=1.08 (0.79, 1.47)
Han et al., 2013 [230]	Meta-analysis, US & Europe	5 case-control, 1 cohort	EA	1610 EA cases	FFQs ranging from 54-124 items High, low	Fish (highest intake vs. lowest) EA – RR=0.89 (0.73, 1.09)
Keszei et al., 2013 [217]	Netherlands Cohort Study, Netherlands	Case-cohort	BE	3,919 sub-cohort members 447 BE cases	150-item FFQ Tertiles	Fish (T3 vs. T1): Male BE – HR=0.99 (0.70, 1.41) Female BE – HR=1.13 (0.76, 1.69)
Jiang et al., 2016 [231]	Meta-analysis, US & Europe	2 case-control, 1 cohort	EA	904 EA cases	FFQs ranging 54-124 items	Fish: EA – RR=0.64 (0.26, 1.60)
Du He et al., 2017 [232]	Meta-analysis, US & Europe	4 case-control, 1 cohort	EA	1,398 EA cases	FFQs	Fish: EA – RR=1.04 (0.86, 1.27)

CHAPTER II: METHODS

Overview

The aims of this dissertation were to examine the associations between polyunsaturated fatty acid (PUFA) intake and outcomes along the Barrett's esophagus-esophageal adenocarcinoma (BE-EA) continuum. To examine these associations, I drew upon resources from four existing epidemiologic studies conducted in the United States (US). For my dissertation, I proposed to use both case-control and follow-up study designs to address these aims in three steps.

Step 1: Estimated PUFA dietary intake for the study participants, using the PUFA dietary intake responses assessed as part the four parent studies and which have already been harmonized.

Step 2: Pooled the PUFA dietary intake estimates across the four studies.

Step 3: Estimated odds ratios (ORs)/hazard ratios (HRs) for the associations of PUFAs and three outcomes along the BE-EA continuum: Barrett's esophagus (BE) development, risk of developing esophageal adenocarcinoma (EA)/gastric cardia adenocarcinoma (GCA) incidence, and mortality following an EA/GCA diagnosis.

The case-control study design was used to examine the associations between PUFAs with the development of BE (Aim 1) and risk of developing EA/GCA (Aim 2). Using the cases from the two EA/GCA studies, a follow-up approach examined the associations between PUFAs and mortality following a diagnosis of invasive cancer

(Aim 3). I proposed to pool harmonized data from two BE case-control studies and two EA/GCA case-controls studies to address these aims.

BE and EA/GCA are rare diseases, and thus the most efficient study designs were the case-control and case-only follow-up designs that I employed. Alternative study designs to address these aims were two prospective cohort studies. First, a cohort study of gastroesophageal reflux disease (GERD) patients to examine associations between PUFAs and incident BE. Second, an additional cohort study of BE patients to examine associations between PUFAs and incident EA/GCA. Although BE is hypothesized to be a complication of GERD, it is estimated only 10-15% of GERD patients develop BE [196]. Similarly, even though BE patients are more likely to develop EA/GCA, the likelihood of a BE patient being diagnosed with EA/GCA annually is 0.1-3.5% [136,253-255]. Using the prospective cohort method to examine associations with the risk of developing BE and EA/GCA or with mortality after EA/GCA, would require a very large sample size, due to the rarity of these outcomes [15,19], and a long follow-up, due to lengthy induction periods [256,257], to accrue the necessary number of cases, resulting in an extremely time-intensive and expensive study method. Therefore the proposed study design of pooling existing case-control studies was a practical and time- and cost-effective method, and resulted in a sufficient sample size to examine the associations between PUFAs and the BE-EA continuum. Similarly, the follow-up design, which focused on determining vital status among the case respondents only, was an efficient approach to examining associations with mortality after diagnosis with EA/GCA.

The four studies I proposed to use for my dissertation were conducted by members of the International Barrett's and Esophageal Adenocarcinoma Consortium

(BEACON), an international group of investigators with an open forum for epidemiologic research focusing on these two diseases and the sharing and pooling of data. The studies within BEACON are similar in regards to: study designs, participant selection methods, interview methods, and food frequency questionnaire (FFQ) structure.

Specific Aims

The specific aims of this dissertation were as follows.

Aim 1: Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with the development of BE.

Aim 2: Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with the risk of developing EA/GCA.

Aim 3: Determined if PUFA intake (fish intake, ω-3, ω-6, ω-3*ω-6 interaction, ω-6:ω-3 ratio, and ω-3 and ω-6 subtypes) was associated with mortality among patients diagnosed with EA/GCA.

Hypotheses

I hypothesized that: (1) higher intake of fish and ω-3 fatty acids would be associated with reduced risk of BE/EA continuum outcomes; and (2) higher intake of the ω-6 fatty acids and the relative balance (the interaction or ratio of ω-3 and ω-6, which in the US will be dominated by ω-6 intake [25,258]) would be associated with an increased risk of BE-EA continuum outcomes. The biological mechanisms supporting these hypotheses were discussed in chapter 1 (see **Figure 1.5)**.

Study Populations and Design

To address the study aims, I pooled the harmonized data from two parent case-control studies of BE (Study of Reflux Disease [259] and Epidemiology and Incidence of BE Study [80]) and two parent case-control studies of EA/GCA (US Multicenter Study [173] and Los Angeles County (LAC) Multiethnic Study [260]). Descriptions of each of the four parent study populations are presented below and are summarized in **Table 2.1**.

Study of Reflux Disease

The Study of Reflux Disease [259] was a case-control study of BE conducted in western Washington (WA) state from 1997-2000.

Potential cases were selected from residents 20-80 years of age who underwent an upper endoscopy for GERD symptoms at four community gastroenterology clinics between October 1, 1997 and September 30, 2000, and were newly diagnosed with BE. During the endoscopy procedure the consenting participants had 4-quadrant biopsy specimens taken from the esophagus. Cases were defined as those with the presence of specialized metaplastic epithelium (SIM) on at least one of the four specimens. Physicians also noted the presence of visible columnar epithelium, and its length, during the endoscopy.

Controls were residents of western WA, community-based, and selected using a modified Waksberg random digit dialing (RDD) [261,262]. This RDD technique identifies controls residing in the same geographic area by using the first five digits of each case's

residential telephone number as the primary sampling unit [263,264]. Controls were community-based, and individually matched to cases on age (+/- 3 years) and sex.

Epidemiology and Incidence of BE Study

The Epidemiology and Incidence of BE Study [80] was a case-control study of BE within the Kaiser Permanente of Northern California (KPNC) health care system conducted between 2002 and 2005.

Cases were KPNC members aged 18-79 with an incident diagnosis of BE between October 2002 and September 2005. The newly diagnosed patients were identified using the International Classification of Disease, Ninth Revision (ICD-9) code 530.2.

Controls were KPNC members aged 18-79 without a BE diagnosis before the case selection period, and were selected using risk set sampling [265]. The controls were community-based and frequency matched to the cases on sex, age (5-year groups), and geographic region.

All participants had to be continuously enrolled in KPNC for at least two years before the index date (index date for cases: date of BE diagnosis, and for controls: midpoint of each two- to three-month selection interval for the cases), and had to be able to understand both spoken and written English.

US Multicenter Study

The US Multicenter Study [173] was a case-control study of EA/GCA conducted from 1993-1995 in Connecticut (CT), a 15-county area of New Jersey (NJ), and a three-county area of western WA.

Eligible cases were English-speaking residents aged 30-79 who were diagnosed with a first primary EA/GCA and were identified by state tumor registries through an established rapid-reporting system. Cases had to be diagnosed within specific time periods: from February 1, 1993 through January 31, 1995 in CT; from April 1, 1993 through November 30, 1994 in NJ; and from March 1, 1993 through February 28, 1995 in Washington. Pathology materials were obtained for all potential cases, and final determination of eligibility was based on review by study pathologists using standardized criteria.

Controls were sampled using different methods according to age. Controls who were 30-64 years were identified using Waksberg RDD [261], and controls 65-79 were identified by random sample of Health Care Financing Administration (HCFA) rosters. The controls were population-based and frequency matched on age (5-year groups) and sex, and in NJ matched on race as well.

LAC Multiethnic Study

The LAC Multiethnic Study [260] was a case-control study of EA/GCA conducted in LAC from 1992-1997.

Cases were eligible if they were 30-74 years old when newly diagnosed with EA/GCA and identified by the LAC Cancer Surveillance Program (CSP) between 1992-

1997. All pathology reports were reviewed to consistently confirm cancer subsite; the cancers included were defined by International Classification of Diseases for Oncology (ICD-O) codes for EA (C15.0-15.9) and GCA (C16.0).

Controls were individually matched on gender, race, and age (+/- 5 years), and must not have had a previous diagnosis of esophageal or gastric cancer. These neighborhood controls were identified through a systematic algorithm based on the address of the matched case patient.

Data Collection

Each of the four parent studies received approval from the Institutional Review Board (IRB) of the organizations involved, and informed consent was obtained for all study participants before interview.

Study of Reflux Disease

Of the eligible case and control subjects, 92.8% and 68.7% of these were successfully interviewed. Trained staff conducted structured interviews of study participants in their home or another requested location. The questionnaire took about 45 minutes to complete, and assessed demographics, medical history, medication use, smoking habits, and alcohol consumption. The interviewers also took anthropometric measures (height, weight, waist circumference, hip circumference, and thigh circumference) [266]. Dietary information was collected using the validated, 131 food item Fred Hutchinson Cancer Research Center (FHCRC) FFQ [267]. This diet history assessed the one-year period before the interview date.

Epidemiology and Incidence of BE Study

Interviews were conducted in-person for all study participants by trained interviewers, most frequently at the participant's home. The response rate for the eligible cases and controls were 47% and 37%, respectively. The interview collected information on demographics, GERD symptoms, medical history, medication use, tobacco use, and alcohol consumption in the year prior to the index date. Anthropometric measures (height, weight, abdominal circumference, and mid-thigh circumference) were taken by the interviewers using standardized equipment. Diet history corresponding to the one-year before the index date was assessed using the Block 1998, a validated 110 food item FFQ [268,269].

US Multicenter Study

Interviews were performed in-person for a majority of case (80.6%) and control (73.7%) subjects. Interviews were conducted with the closest next-of-kin (NOK) proxy (typically the spouse) for 29.6% of cases and 3.4% of controls. The mean length of time between cancer diagnosis and interview was 3.7 months for non-proxy interviews, and 8.5 months for proxy interviews. Trained interviewers administered a structured questionnaire, which took about 130 minutes to complete. The questionnaire collected information on demographic characteristics, tobacco use, alcohol consumption, medical history, use of medications, and occupational history. The reference date for this study was defined as the date of diagnosis for the cases and date of identification for the controls. Dietary information was collected using a validated and modified FHCRC FFQ

[270], which included 104 food items. This FFQ assessed the period three to five years prior to diagnosis or interview for cases and controls, respectively.

LAC Multiethnic Study

Interviews were completed with 75% of approached cases, and NOK interviews were used for 30% of the case subjects. The questionnaire was completed during an in-person interview completed by the study subject or NOK. The structure questionnaire used was specifically designed for this study. It assessed demographic information, lifestyle behaviors (tobacco and alcohol use in particular), anthropometric measures, personal medical history and medication use, family history, and occupational history. A reference date was established for the cases and controls: one year before the date of EA/GCA diagnosis for cases, and the same reference date was used for each case's matched controls. Similarly, diet history corresponding to the one year prior to the reference date was assessed using the validated, 124 food item University of Hawaii FFQ [271].

Outcome Assessment

BE

BE cases were defined as those with SIM identified during endoscopy [259] or those who received a new diagnosis with an ICD-9 code of BE in medical records [80]. Board-certified gastroenterologists or pathologists reviewed the endoscopy and pathology records in order to identify eligible BE cases [80,259].

BE Segment Length

BE cases were also described using the segment length observed on endoscopy. The two degrees of BE segment length were defined as short-segment BE (SSBE; <3 cm) and long-segment BE (LSBE; ≥3 cm on endoscopy), which are often used as clinical definitions of the severity of disease [80,272,273].

EA and GCA

EA/GCA cases were defined as those with an incident diagnosis of EA/GCA identified by local tumor or cancer registries during the time periods previously specified [173,260]. ICD-O codes were used to classify tumors: EA cases were identified by C150.0-150.9 [173] and C15.0-15.9 [260] and GCA by C151.0 [173] and C16.0 [260]. Final determination of case status, the anatomical location of the tumor, and other clinical characteristics were determined by a pathologist's review of slides and medical records [173,260].

Vital Status

The follow-up studies were conducted to determine vital status among the EA/GCA cases. The mortality outcome was assessed and date of death were determined by linking participants with the National Death Index (NDI) [181]. An event was defined as death due to any cause during the period of follow-up, and participants alive at the end of follow-up were censored. After 7.5 years the US Multicenter Study reported 476 deaths [181], and the LAC Multiethnic Study reported 426 deaths after 10.7 years of follow-up. Overall survival time was calculated in months from the date of

diagnosis until date of death or censoring. Due to the aggressive nature and short survival time (average of less than a year) of these cancers [2,4-6,181], all-cause mortality was used as an approximation of EA/GCA-specific mortality.

Data Management and Quality Control

The four parent case-control studies collected covariate information during a structured paper-based interview conducted by trained interviewers [80,173,259,260]. Dietary information was either collected by interviewers [106,222,228] or self-administered [105]. Each of the parent studies conducted quality control of the data, and discrepancies were resolved by referencing the original interview documents [105,106,222,228].

Dietary Intake Exposure Assessment

Each of the four parent studies collected dietary information using a validated FFQ, and these dietary measurement tools are summarized in **Table 2.1**. The FFQs that were used were validated using diet records [268,270,274,275] or multiple 24-hour recalls [276,277].

The FFQs used assessed both frequency and portion size [105,106,222] except for the modified FHCRC FFQ (US Multicenter Study), which assumed average portion sizes based on the United States Department of Agriculture (USDA) database [228].

Data Harmonization of Dietary Intake Responses

To harmonize the dietary intake responses, I used each of the four parent studies' FFQs and linked with a nutrient database to estimate with ounces/grams per day of PUFA values.

In each of the four FFQs participants were asked how frequently each of the line items was consumed. In the Study of Reflux Disease, nine choices encompassing never/less than once a month up to two or more time per day were given to the study participants. For the Epidemiology and Incidence of BE Study, participants were given around nine choices ranging from never and a few times per year to twice per week and every day. In the US Multicenter Study FFQ, participants wrote in the number of times the line item was consumed and then circled if this corresponded to times per day, week, month, or year. Options of never eaten, and unsure of how often were also available. Finally for the LAC Multiethnic Study, participants provided the number of times each line item was consumed then chose per day, week, month or year. And if this food was never consumed there was a separate choice.

In three of the FFQs portion size was explicitly assessed. For the Study of Reflux Disease, participants were given a definition of medium serving size for each line item. Then they were given three options to choose from: small serving size (≤1/2 of the medium serving), the medium serving previously defined, and large serving size (≥1 ½ of the medium serving). In the Epidemiology and Incidence of BE Study, four options for serving size specific to the individual line items were provided to choose from. In the LAC Multiethnic Study, participants were asked the usual serving size and the number of servings each time this item was consumed. Participants were given multiple options

for serving sizes for each of the line items. In the US Multicenter Study, medium serving sizes were assumed for the line items.

Nutrient intake was estimated using the University of Minnesota Nutrition Coordinating Center (NCC) database [278], which was primarily based on the USDA nutrient database [205]. However, the NCC database contained more food items, and had a minimal amount of missing nutrient values. If certain values were not available in the USDA database, the NCC included values from other nutrient databases and appropriate information contained in scientific journals. When necessary, values were imputed using one of the following methods: used a value from a similar food; calculated value for another form of the same food; calculated values from components in the same food; or calculated values from recipes or food product formulations [278].

When identifying the specific food items from the NCC database to correspond to the food items on the parent study FFQs, I selected the most similar line items available. For example, a food item from the Kaiser Permanente FFQ is “real 100% orange juice, including fresh, frozen, or bottle”. The corresponding food item chosen from the NCC is “purchased ready-to-go orange juice”. For an item with more open interpretation such as “refried beans”, the item chosen from the NCC is “regular, canned refried beans”.

Because some of the FFQ line items represented multiple food items, the individual foods were weighted according to the estimates of US consumption [279]. The weights used by the original nutrition data processing for the Study of Reflux Disease and the LAC Multiethnic Study were provided. For the Epidemiology and Incidence of BE Study, the original weights were unobtainable and the weighting

scheme used was determined from the FFQs of the other participating studies. In instances where weights were not available for a line item, the weights were evenly split by the number of foods in that particular line item [280]. In the case of the US Multicenter Study, the original weights were unavailable, but a previous verification of the weights was performed using a similar FFQ's weighting scheme [281]. This newer weighting scheme was similar to the original (correlation coefficient=0.97), and was used here. An example of calculating the PUFA intake value for one participant using a weighting scheme is shown in **Table 2.2**.

Data Pooling of Harmonized Dietary Intake Responses

To pool the harmonized dietary intake data I employed two standard approaches. First, using the harmonized dietary data, I generated study-specific quantiles of intake, and then merged the PUFA intake responses within control-based quantiles for each of the two BE and for each of the two EA/GCA studies [282,283]. Second, I pooled the harmonized data using absolute values, with the cut-points determined using responses from the controls only, with the BE studies considered separately from the and EA/GCA studies [282,283]. Absolute value-based quantiles required the inclusion of a covariate for the number of PUFA-containing food items in each of the FFQs. With study-specific quantiles, true differences in population intakes were not considered, potentially resulting in exposure misclassification. When using identical absolute intake cut-points across studies, misclassification was also possible, because intake may increase with the number of relevant items on each FFQ, which varied across study instruments. Thus, study-specific variations in foods that contain PUFAs may have been due to

variations in FFQ design and/or in true intakes. In Smith-Warner's pooled study [283], the diet-cancer risk associations were similar for both approaches.

PUFA Intake Variable Definitions

The proposed study only included participants who completed an FFQ with plausible energy intake values (e.g., from 500-5000 kilocalories per day), and I began with exclusion criterion for those participants greater than ±3 standard deviations on the log-scale [105,106,222,228]. Data were available for: 88% BE cases/86% controls for the BE Study of Reflux Disease [105]; 93% BE cases/97% controls for the Epidemiology and Incidence of BE Study [106]; 96% EA cases/98% GCA cases/99% controls for the US Multicenter Study [228]; and 93% EA cases/93% GCA cases/96% controls for the LAC Multiethnic Study [222]. The final available sample size for my proposed study was: 466 BE cases with 491 controls, and 488 EA/512 GCA cases with 1,995 controls (distribution shown in **Table 2.3**).

Multiple measures of PUFA intake were used to better capture the potential PUFA BE-EA continuum associations. The measures that were used are: fish intake, ω-3, ω-6, ω-3 subtypes (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), docosapentaenoic acid (DPA)), ω-6 subtypes (linoleic acid (LA) and arachidonic acid (AA)), the ω-6:ω-3 ratio, and the interaction of ω-3 and ω-6, (which allows for more flexible modeling then the ratio).

Fish intake was measured in ounces of intake per day, estimated from the FFQ responses. In each of the four FFQs, fish intake was also examined by cooking method: fried (e.g., fried fish, fish sandwich) and other non-frying cooking methods such as

broiled/baked. Cooking method was taken into consideration since a cooking method such as frying would introduce ω-6 PUFAs and may mask potential associations [234]. I would have also liked to differentiate between the intake of white fish and oily/dark fish, due to the higher ω-3 of oily/dark fish [24], however it was not possible due to the specific questions regarding fish intake in each of the four different FFQs.

The PUFA nutrient measures were estimated in grams per day using the responses regarding frequency and portion size from the FFQs and the nutrient content of food items from the NCC database. The ω-3 and ω-6 subtype content were output in the databases, and the total ω-3, and total ω-6 content were calculated from the subtype values. The composite PUFA measures, ω-6:ω-3 ratio and the ω-3 and ω-6 interaction, were unitless and were calculated from the PUFA nutrient measures. An example calculation of the nutrient PUFA measures and the ratio measure for a participant reporting eating 100 grams of wild Atlantic salmon is shown in **Table 2.4**.

Trend analyses were also performed examining associations between the BE-EA continuum outcomes and increasing consumption of PUFAs using continuous variables.

Covariate Assessment

Covariate information was collected by questionnaire of each parent study as previously described [80,173,259,260], except for dietary covariate information, which was collected by each parent study's validated FFQ [105,106,222,228]. Known and suspected risk factors assessed included: demographic information; body size; medical history including GERD symptoms; medication use including use of over-the-counter

drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs); smoking history; and alcohol use.

Harmonization and Pooling of Other Exposure Variables

Non-dietary BEACON data, including most demographic, lifestyle, known, and suspected risk factors, were previously harmonized and pooled by BEACON investigators [95,143,154,161,284].

Covariate Variable Definitions

Covariates of interest in my proposed study that may potentially confound the PUFA BE-EA continuum association included: age (years), sex (male/female), race (white/other), education (highest level attained), income (annual household income in US$), marital status (currently married), body mass index (BMI; kg/m^2), waist circumference (WC; cm), physical activity (PA; frequency and/or duration), smoking status (never/former/current), alcohol intake (drinks/week), total energy intake (kcal per day), fruit intake (servings per day), vegetable intake (servings per day), ω-3/ω-6 intake (grams per day), dietary supplement use (ever/never), NSAID use (ever/never), GERD symptoms (frequency), *Helicobacter pylori* infection (*Hp*; positive/negative), hiatal hernia (HH; yes/no), proxy status (non-proxy/proxy), BE (diagnosis and segment length), tumor stage (I-IV), tumor location (esophagus/gastric cardia), dysphagia (absent/present), lymphovascular involvement (yes/no) cancer treatment (chemotherapy, radiation, surgery, etc.), and study site.

BMI was calculated using either self-reported height and weight for a specified point in time prior to disease for the EA/GCA parent studies, due to the wasting nature of these diseases [173,260], or measurements taken by professional interviewers using standardized equipment on the interview date for the BE parent studies [80,259].

Although there were a variety of methods to adjust for total energy intake, the method that I employed here is the standard method. The standard method included terms in the model for both the dietary exposure of interest and caloric intake [285]. The resulting effect estimate could be interpreted as the effect of an increase in the dietary factor when energy intake is held constant. Another method that may have been of interest was the nutrient density adjustment method. The energy density method included a variable for the dietary exposure as proportion of energy intake and a term for energy intake [285]. The effect estimate interpretation would be the effect of increasing the proportion of intake from the dietary exposure when energy intake is held constant. However, for this particular project the nutrient density method was not the best choice. PUFAs typically do not constitute a large portion of the caloric intake of diets [286], particularly in the US and when examining subtypes of PUFAs. The interpretation of the standard method allowed for easier interpretation, where the messaging was increasing/decreasing absolute amounts of intake of PUFAs. Compared to the interpretation of the nutrient density method, where the message would be to modify the distribution of macronutrients. Which would be particularly difficult for nutrients that do not comprise large portions of the American diet.

GERD may be on the causal pathway of the BE-EA continuum due to the hypothesized relationship between GERD and BE. However, there is evidence in some

cases that the squamous epithelium is replaced by the intestinal-type columnar epithelium without existing damage from GERD [287]. And as previously stated, the majority of BE patients do not exhibit reflux symptoms [54]. Therefore I considered GERD as a potential confounder for the relationship between PUFAs and the BE-EA continuum outcomes.

Statistical Analysis

The first aim of the proposed dissertation was to determine if PUFA intake was associated with development of BE. The second aim of the study was to determine if PUFA intake was associated with incidence of EA/GCA. The third aim was to determine if PUFA intake was associated with mortality following a diagnosis of EA/GCA. Multiple measures of PUFA intake were used to assess these associations, including fish intake, ω-3, and ω-6.

After the data were validated, I linked the dietary data with nutrient databases to estimate nutrient and total energy intake. To facilitate pooling of these data, covariate and dietary data were harmonized across the four studies. As part of my dissertation, I pooled the harmonized dietary intake data in order to estimate PUFA intake and energy intake.

The overall distributions of the exposure, outcomes, and covariates were examined; and the distributions of those variables were also examined by study.

Aim 1: PUFAs and BE

To examine the association between PUFAs and BE, unconditional logistic regression [288] was used to estimate ORs and 95% confidence intervals (CIs).

A secondary analysis was conducted using segment length of BE (SSBE and LSBE) as a nominal polytomous outcome. In order to test if there were differences between SSBE and LSBE, I used the ratio of the ORs (RORs) and the Wald test with a significance level of 0.10.

Aim 2: PUFAs and EA/GCA Incidence

To examine the association between PUFAs and EA/GCA, nominal polytomous logistic regression [288] was used to estimate ORs and 95% CIs, with EA and GCA as distinct outcomes. To determine if there were differences between the associations with EA and GCA, the Wald test was used with a significance level of 0.10.

Since the outcomes for Aims 1 and 2 were dichotomous and the relationship between the outcomes and predictors were non-linear, logistic regression was an appropriate statistical method to use. The outcomes were statistically independent because there were no repeated events, and I could assume that the residuals were binomially distributed because this assumption is rarely violated.

Aim 3: PUFAS and Survival after EA/GCA

To examine the association between PUFAs and survival among EA/GCA cases, Cox proportional hazards models [289] were used to estimate HRs and 95% CIs, with EA and GCA as discrete outcomes. The proportional hazards (PH) assumption was

examined by using Kaplan-Meier curves and an interaction between exposure and follow-up time [289]; if the PH assumption was violated time-specific effects were used and the exposure and follow-up time interaction term was added to the model.

For Aims 2 and 3, EA and GCA were treated as discrete outcomes in order to examine the possibility of heterogeneity of the ORs. If the effect estimates treating EA and GCA as discrete outcomes were consistent (the ratio of the ORs/HRs close to 1.0) the two outcomes were combined in order to increase power.

Model Construction

All analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC), and Stata software, version 15.0 (StataCorp LP, College Station, TX). When modeling continuous or ordinal variables I first checked if they had a linear relationship with the logit form of the outcome, if this assumption for any of the variables was violated I modeled those particular variables using indicator terms [290].

All models included the study matching factors, including age, race, sex, and study indicator.

Confounders for the proposed dissertation were chosen *a priori* by creating directed acyclic graphs (DAGS; **Figures 2.1-2.3**) for each of the three BE-EA/GCA continuum outcomes [291]. These DAGS were created from knowledge of the relationships between the exposure, outcome, and potential covariates. Minimally sufficient adjustment sets were created using the confounders identified in the DAGs.

I explored EMM between total ω-3 and ω-6 intake, and by BMI and WC, as well as NSAID use, smoking status, and GERD symptom frequency. BMI was categorized

into overweight and obese BMI (≥25.0 kg/m^2) and ideal BMI (<25.0 kg/m^2) [292]. WC was assessed in the Study of Reflux Disease [259] and the Epidemiology and Incidence of BE Study [80], therefore I examined EMM by WC among the BE studies only. WC was categorized into two groups based on sex: the increased risk group and the ideal WC group [293]. The increased risk group (based upon metabolic complication and diabetes risk) was those with a WC >94 cm for males and >80 cm for females. The ideal group was those males with a WC ≤94 cm and females with a WC ≤80 cm. NSAID use was categorized into ever and never regular users, as defined in each of the four parent studies. Smoking status was categorized into never smokers and ever smokers. And GERD frequency was dichotomized into those who experience reflux less than weekly, and those who experience it once per week or more. EMM was assessed using multiplicative interaction comparing models with and without interaction terms using the likelihood ratio test with a significance level of 0.10 [290]. Additive interaction was assessed using interaction contrast ratios (ICRs; also known as relative excess risk due to interaction or RERI) [265]. ICRs that were significantly different from 0 using a significance level of 0.10, suggested the presence of additive interaction. Calculating the ICR allowed me to examine the joint effects of the interactions between the exposure and modifier, and thus was easier to interpret.

The first sensitivity analyses performed were including additional confounders to the DAG-identified adjustment sets. The additional confounders under consideration were some of the primary risk factors for the BE-EA continuum outcomes: BMI, reflux frequency, and cigarette smoking status.

Sensitivity analyses were performed to examine if there were differences between the associations by proxy status. Because EA/GCA are diseases with poor prognoses and short survival time [2,4-6], 153 proxies were used in the US Multicenter Study [173] and 140 were used in the LAC Multiethnic study [260]. No proxies were used in either of the BE case-control parent studies. Although it has been seen that proxy and self-report are similar [294], I conducted a sensitivity analysis due to the possibility of misclassification of proxy responses. I performed two separate analyses for the second and third aim, one with non-proxies only and one with proxies only. If the ORs/HRs were consistent (the ratio of the ORs/HRs close to 1.0), the data collected by use of proxies were included in the final analyses. If the estimates were not consistent, I considered how to deal with the data obtained from proxy interviews and perhaps exclude these data.

Another sensitivity analysis was adjusting the exclusion criterion based on daily caloric intake. As stated beforehand, the starting exclusions were for those participants greater than ±3 standard deviations on the log-scale. I examined if the associations between PUFAs and the BE-EA continuum outcomes were stable when relaxing the exclusions to those participants at the highest and lowest 2.5% of daily caloric intake.

As mentioned previously, it was ideal to differentiate fish intake by type of fish consumed. And I would have liked to do this by distinguishing between consumption of white fish (e.g., tilapia or cod) and consumption of dark and oily fish (e.g., salmon or tuna). However, only one of the four FFQs from the parent studies allowed for examination of the associations by type of fish. The Study of Reflux Disease FFQ assessed “white fish (broiled or baked)” and “dark fish (broiled or baked)” individually. I

conducted a sensitivity analysis using on the Study of Reflux Disease data to examine the associations between white fish and dark/oily fish with the development of BE.

Another consideration for a sensitivity analysis was the potential for heterogeneity by center, even though the four parents studies were chosen as members of BEACON due to their high quality and similarities in design. To determine if study heterogeneity was present, an interaction term between the study and the exposure variable was included in the model. Study-specific estimates were reported when necessary. This term was omitted from the model if it was found to be not statistically significant with a significance level of 0.10.

Final sensitivity analyses were conducted using a meta-analytic approach to observe the robustness of the pooled results. Summary ORs/HRs were calculated using random-effects meta-analytic models. The random-effects model was the chosen method for this set of analyses because this model allowed for the true effect size to differ from study to study, whereas in the fixed-effects model it would be assumed that there is one true effect size underlying the included studies to be estimated [265]. The random-effects meta-analysis accomplished this by including a term for the unexplained sources of heterogeneity between studies [265]. First, study-specific ORs/HRs were estimated for the associations between PUFAs and the BE-EA continuum outcomes. Then summary ORs/HRs were generated using the random-effects meta-analytic approach, and these results were compared to the pooled approach ORs/HRs.

Statistical Power

Power calculations were based on the number of study participants with a completed FFQ and plausible energy intake values previously cited, and established using effect estimates observed in previous research. The total sample sizes for the study were: 466 BE cases with 491 controls; and 488 EA cases and 512 GCA cases with 1,995 controls. For Aims 2 and 3, power was calculated for the overall sample size and the non-proxy sample size. The non-proxy sample size was 707 EA/GCA cases with 1995 controls. For Aim 3, power was estimated based on 87% of cases who were no longer alive after maximum follow-up (7.5 years for the Multicenter Study, and 10.7 years for the LAC Multiethnic Study) among the 488 EA patients and the 512 GCA patients.

Multiple measures of PUFAs were used to address the study aims, and each of these measures were categorized using quantiles (quintile, quartile, tertile, and median value cut points) of the control distribution for BE and EA/GCA outcomes and of the case distribution for the mortality outcome. The effect estimates (ORs/HRs) used to calculate power for the effect of PUFAs ranged from 0.60-0.80 (for fish intake and ω-3) [107,226,230] and 1.25-1.67 (for ω-6 and the ω-6:ω-3) [218,227]. Power was estimated using SAS version 9.3 (SAS Institute, Cary, NC), assuming a two-sided test and a 5% significance level.

Aim 1: PUFAs and BE

As is shown in **Table 2.5**, the study power for the association between PUFAs and BE is acceptable (>70%) when the minimal detectable OR was ≤0.70 (or conversely ≥1.43) and the exposure was categorized into tertiles or cut at the median.

Aim 2: PUFAs and EA/GCA Incidence

Power calculations for Aim 2, estimating the association between PUFAs and EA/GCA, are shown in **Table 2.6** and **Table 2.7**. **Table 2.6** displays the power estimates when EA and GCA were treated as discrete outcomes. The estimates presented were based on the number of EA cases; the exact estimates based on GCA cases were similar but slightly higher due to the slightly larger number of GCA cases. The study power when treating EA and GCA as discrete outcomes was good (> 80%) when the minimal detectable OR was ≤0.70 (or conversely ≥1.43) and the exposure was categorized into quartiles, tertiles, or cut at the median. If the ORs for the association between PUFAs and EA and GCA were consistent, the estimates of power for the association with EA/GCA as a combined outcome were great (>90%) when the minimal detectable OR was ≤0.70 (or conversely ≥1.43) as shown in **Table 2.7**.

The power estimates excluding participants who used proxies for EA/GCA as discrete outcomes and as one outcome are shown in **Table 2.8** and **Table 2.9**, respectively. The power using non-proxies only was similar but slightly attenuated than when using all participants.

Aim 3: PUFAs and Survival after EA/GCA

Table 2.10 and **Table 2.11** display the power estimates for the EA/GCA survival aim. The power when treating EA and GCA as discrete outcomes is shown in **Table 2.10**. The estimates shown were based on the number of EA cases; again, the estimates based on GCA cases were similar and slightly higher. The power treating EA and GCA as discrete outcomes was acceptable (>70%) when the minimal detectable HR was ≤0.70 (or conversely ≥1.43), and the exposure was categorized into quartiles, tertiles, or cut at the median. If the HRs for these two cancers were consistent and then combined into one outcome, the power estimates, as shown in **Table 2.11**, were great (>90%) when the minimal detectable HR was ≤0.70 (or conversely ≥1.43).

The power estimates excluding participants who used proxies for EA/GCA as discrete outcomes and as one outcome are shown in **Table 2.12** and **Table 2.13** respectively. Again, the power using non-proxies only was similar but slightly lower than for all participants.

Limitations and Strengths

Issues to consider when interpreting my dissertation results included the following limitations and strengths of the proposed study.

One major limitation was that the study populations were primarily white and male, which limited generalizability. However, BE and EA/GCA primarily affect white males and the rates of these diseases are highest among this demographic group [1,2,123,124,149,256], which allowed for my results to be generalizable to this group of

high-risk individuals. Additionally, this study drew upon data from four studies that were population- or community-based, which enhanced external validity.

Another major consideration was that FFQs were the dietary assessment method in each of the four parent studies. The FFQs used captured dietary intake one to five years prior to diagnosis/interview; and due to the nature of these diseases, patients may change their diet following the onset of clinical symptoms. However, as in most epidemiologic studies of dietary intake, I assumed that participants did not change their diet during the latent period of the diseases. There was also the possibility of recall error due to the time period the dietary assessment was directed towards, but previous research has reported a correlation of assessment of current dietary intake and then assessed again three to ten years later between 0.5 and 0.7 [295-298]. Because of this, I assumed that the dietary intake captured by the parent studies was a reliable estimate of the participants' regular diet before disease. And each of the FFQs were validated using diet records [268,270,274,275] or multiple 24-hour recalls [276,277].

As with all pooled studies, there may have been differences in data collection, variable definitions, and data management between the parent studies. These discrepancies could introduce misclassifications of outcomes, exposures, or covariates. However, the four US population- and community-based BEACON studies were selected because of their high quality and similar data collection procedures. Also, several covariates (BMI, smoking, alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs), and GERD) have already been harmonized and pooled for BEACON [95,143,154,161,284]; this successful pooling showed promise for my proposed

dissertation. Additionally, this concern may have been alleviated by the study-exposure interactions and meta-analytic approach I have proposed as a sensitivity analysis.

In the EA/GCA cancer parent studies proxy interviews were conducted with next of kin (spouse, adult offspring, close friend, in that order) for subjects who were deceased or ill. Proxy interviews were conducted for 188 subjects (164 EA/GCA cases and 24 controls) in the US Multicenter Study [173], and 151 (all EA/GCA cases) in the LAC Multiethnic study [260]. There were no proxy interviews conducted in either of the two BE parent studies. Although it has been seen that proxy and self-report are similar [294], the use of proxies may increase the probability of exposure and covariate misclassification. However, validation studies have found that self-reported and proxy-reported information have good concordance for smoking habits [299,300], anthropometric measures [299], and dietary factors [294,301]. Previous EA/GCA studies from BEACON, excluding proxy responses did not materially change the effect estimates [173,227,228,302,303].

The issue of multiple comparisons was considered. For all analyses, the multiple measures of PUFAs were used individually to determine associations between PUFAs and BE-EA continuum outcomes. Since I used six main measures of PUFAs and three BE-EA continuum outcomes, the total number of comparisons was 18. In addition, secondary aims examined the subtypes of ω-3 and ω-6 as exposures and segment length of BE as an outcome. There was a likelihood of observing statistically significant results due to chance because of the number of comparisons in the proposed study. I did not adjust for the multiple comparisons for each single association examined because it could have undeservedly reduced power. I assessed each of the

associations individually based on biologic plausibility, consistency with current research, and consistency across the cancer continuum [295,304,305].

Other limitations regard data interpretation issues. Because diet is a complex exposure, I considered other elements that could be driving any associations that may have been seen in this study. When interpreting the results for the associations between PUFAs and the BE-EA continuum, I determined what specific food items were the most frequent contributors to the specific PUFA measures. Identifying the particular food items that were the most consumed sources of PUFAs allowed me to consider if there were other dietary components (e.g., saturated fat, glucose) in these food sources that may have been contributing to the associations observed.

A major strength was my proposed pooling of existing case-control studies. First, this study was time- and cost-efficient. My pooled sample size was larger than all previous case-control studies [107,217,218,221-224,226], with increased power to detect associations with PUFA subtypes and provide more precise estimates. I had ample statistical power to evaluate associations between PUFAs and BE-EA/GCA continuum outcomes, including examination of subtypes of ω-3 and ω-6 fatty acids.

Previous studies on PUFAS in relation to BE/EA/GCA focused on one outcome along the continuum [107,217,221-223,226], and none considered mortality. My dissertation was the first study to examine all three outcomes. By examining all three outcomes along the disease continuum, I was able to identify time points best to intervene before disease progression, which is of high priority given the high EA/GCA fatality [2,4-6]. My study was also the first to examine PUFAs in association with mortality following EA/GCA diagnosis. PUFAs have the potential to hasten or delay

mortality through effects on inflammatory pathways. Taking advantage of the richness of the BEACON parent studies, I was able to ascertain any association between PUFAs and risk along the entire spectrum of disease development in EA/GCA. This was especially important in this disease entity, because carcinogenesis may occur over many years, allowing for successful intervention if the appropriate time-points in disease development were identified.

The multiple measures I proposed to use would allow me to better capture the complexity of this dietary exposure, and improve upon the inconsistency in existing research. Most examined fish intake (a major source of long-chain ω-3 fatty acids) as the only PUFA measure [217,221-223,225-227,233], without examining cooking methods, which can mask potential associations (such as with frying, which adds ω-6 fatty acids). A few considered overall PUFAs [107,218,226], which overlook important distinctions between beneficial ω-3 and deleterious ω-6, or examined ω-3 PUFAs only [107,226]. No studies explored ω-6 PUFAs nor, importantly, have they considered the relative ω-3 and ω-6 balance (either the interaction or ratio of ω-6:ω-3). Relative balance is particularly important, because ω-3 and ω-6 are competitively inhibited by each other, are commonly found in the same foods, and may promote or suppress pathogenesis [8,49,212,216]. Studies of other cancer sites have used this approach successfully [235-237], substantiating the importance of relative PUFA balance as a risk modifier.

Additionally, all four parent studies were conducted in US coastal regions with increased opportunity for fish intake (LAC, WA, CT, and NJ); this maximized heterogeneity in intake and enhanced my ability to detect differences, which was an

improvement over other studies since US subjects typically consume a low amount of fish [306].

Methods Summary

The incidence of EA/GCA is among the most rapidly increasing of any cancer type in the US and other western countries [4,17-20], and this increase is suspected to continue [2,53]. The incidence of Barrett's esophagus (BE), the only known potential precursor lesion of these cancers, is also increasing [4,58]. One candidate for a modifiable risk factor for these diseases is PUFAs, which are a group of essential fats. ω-3 PUFAs have been reported to suppress mutations, reduce inflammation, inhibit cell growth, and enhance apoptosis, resulting in decreasing cancer risk [8,44-46]. While ω-6 PUFAs have been shown to promote cancer cell proliferation, and to be associated with increased cancer risk [8,46,49,50]. I proposed to pool harmonized data from two BE case-control studies and two EA/GCA case-control studies to estimate the association between PUFA intake and BE-EA.GCA continuum outcomes using adjusted regression models. Multiple PUFA intake measures were considered, and were based on interviewer-administered, validated FFQ responses. This dissertation was the first to consider the relative balance of ω-3 and ω-6 PUFA intake in relation to BE/EA/GCA, which was important because ω-3 and ω-6 fatty acids are competitively inhibited by each other, are often found in the same food products, and may promote or suppress pathogenesis [8,49,212,216]. My proposed pooling approach was time-efficient and cost-efficient; the sample size and study power was increased, permitting detection of relatively modest associations. My dissertation was the first study to examine all three

outcomes along the BE-EA-GCA continuum and to use multiple measures of PUFAs, which may have made associations more clear. The proposed study had high public health impact because it may identify a modifiable risk factor for a highly fatal cancer [2,4-6], and because it examined the disease continuum the results could identify an appropriate time point to implement this strategy before disease progression.

Table 2.1. Characteristics of the case-control studies included in proposed study

Study	Study of Reflux Disease	Epidemiology and Incidence of BE Study	US Multicenter Study	LAC Multiethnic Study
Period	1997-2000	2002-2005	1993-1995	1992-1997
Location	Western WA	Northern CA	CT, NJ*, western WA*	LAC
Case criteria	Residents aged 20-80 with newly diagnosed BE who underwent an upper endoscopy for GERD symptoms at 4 community gastroenterology clinics	KPNC members 18-79 years newly diagnosed with BE	English-speaking residents 30-79 years diagnosed with first primary EA/GCA identified by state tumor registries	Incident EA/GCA cases identified by the LAC Cancer Surveillance Program
Control criteria	Residents selected by a modified Waksberg RDD. Community-based and individually matched on age (±3 years) and sex.	Sampled KPNC members 18-79 years without BE diagnosis before case selection. Community-based and frequency matched on sex, age (5-year groups), and geographic region.	Sampled by Waksberg RDD for those 30-64 years, and by HCFA rosters for those 65-79 yrs. Population-based and frequency matched on age (5-year groups) and sex.	Neighborhood controls identified by a systematic algorithm based on case's address. Population-based and individually matched on sex, race, and age (5-year groups).
FFQ	FHCRC	Block 1998	Modified FHCRC	University of Hawaii
# of FFQ items	131	110	104	124
Period FFQ assessed	1 year before interview	1 year before diagnosis/midpoint of selection interval	3-5 years before diagnosis/interview	1 year before diagnosis date (same for controls)

*15-county area of NJ and 3-county area of WA; WA-Washington; CA-California; CT-Connecticut; NJ-New Jersey; LA-Los Angeles; KPNC-Kaiser Permanente Northern California; RDD-Random digit dialing; HCFA-Health Care Financing Administration; FHCRC-Fred Hutchinson Cancer Research Center

Table 2.2. Example of linoleic acid (LA) calculation using frequency, portion size, weights, and nutrients for the line item “Bacon, including Canadian bacon”

Food Item	**Bacon**	**Canadian Bacon**
Frequency	1 serving/day	
Portion size	2 slices/strips	
LA content per 100 g	5.327 g	0.690 g
Serving size	8.1 g	13.8 g
Line item weight	0.80	0.20
LA intake per line item	=[(8.1*5.327/100*0.80) + (13.8*0.690/100*0.20)]*1*2 = 0.728 g/day	

Table 2.3. Sample sizes of the four parent studies

	Cases			
Study	**BE**	**EA**	**GCA**	**Controls**
Study of Reflux Disease	170	–	–	182
Epidemiology and Incidence of BE Study	296	–	–	309
US Multicenter Study	–	282	255	687
LAC Multiethnic Study	–	206	257	1308

Table 2.4. Example PUFA nutrient calculation

	Wild Atlantic salmon (100 g)
ω-3*	= 0.378+0.411+1.429+0.368 =2.586
ALA*	0.378
EPA*	0.411
DHA*	1.429
DPA*	0.368
ω-6*	= 0.220 + 0.342 = 0.562
LA*	0.220
AA*	0.342
ω-6: ω-3	= 0.562:2.586 = 0.217:1
*Measured in grams	

Figure 2.1. DAG of potential confounders of the association between PUFAs and risk of developing BE

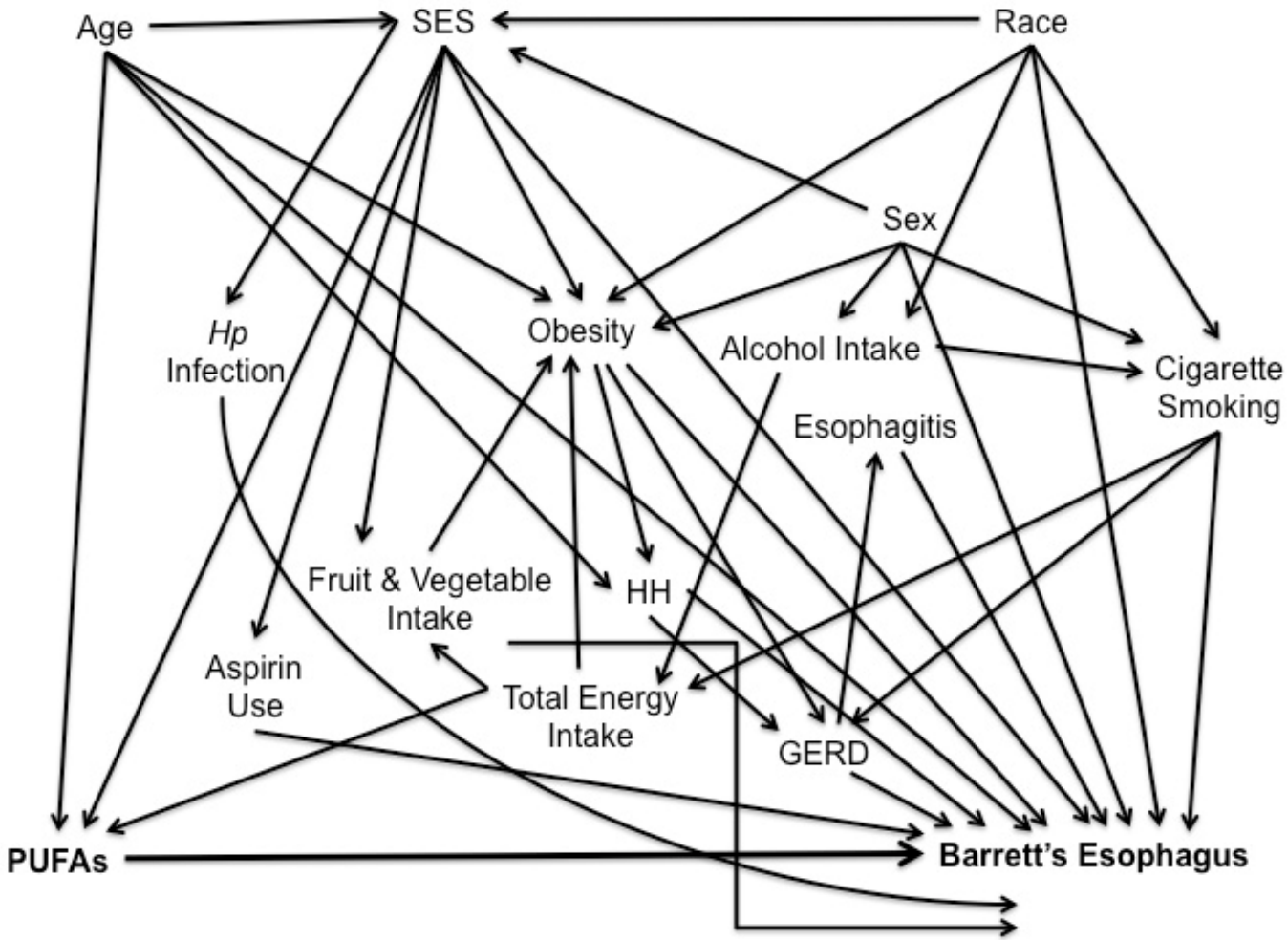


Figure 2.2. DAG of potential confounders of the association between PUFAs and risk of developing EA/GCA

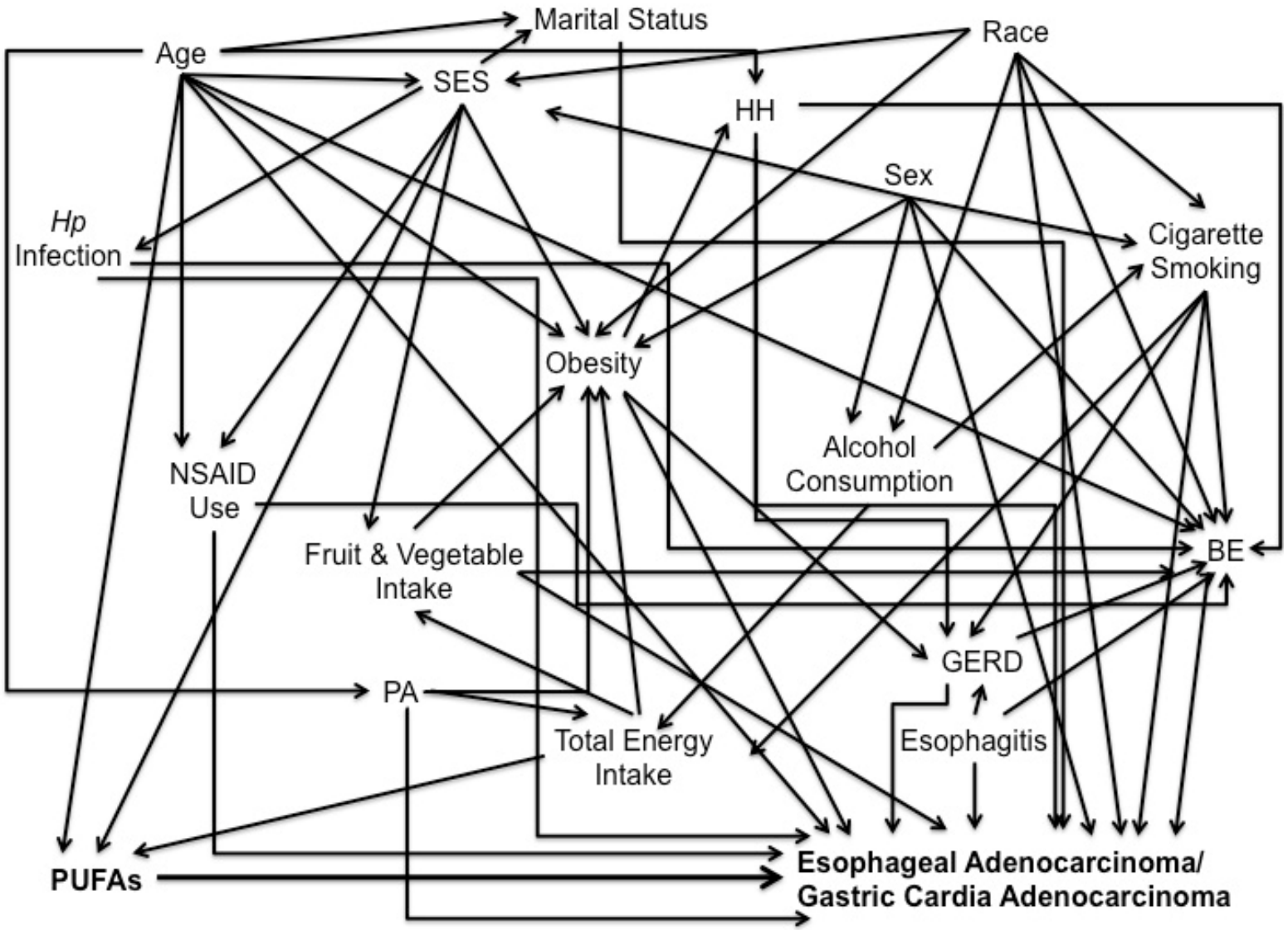


Figure 2.3. DAG of potential confounders of the association between PUFAs and mortality among those diagnosed with EA/GCA

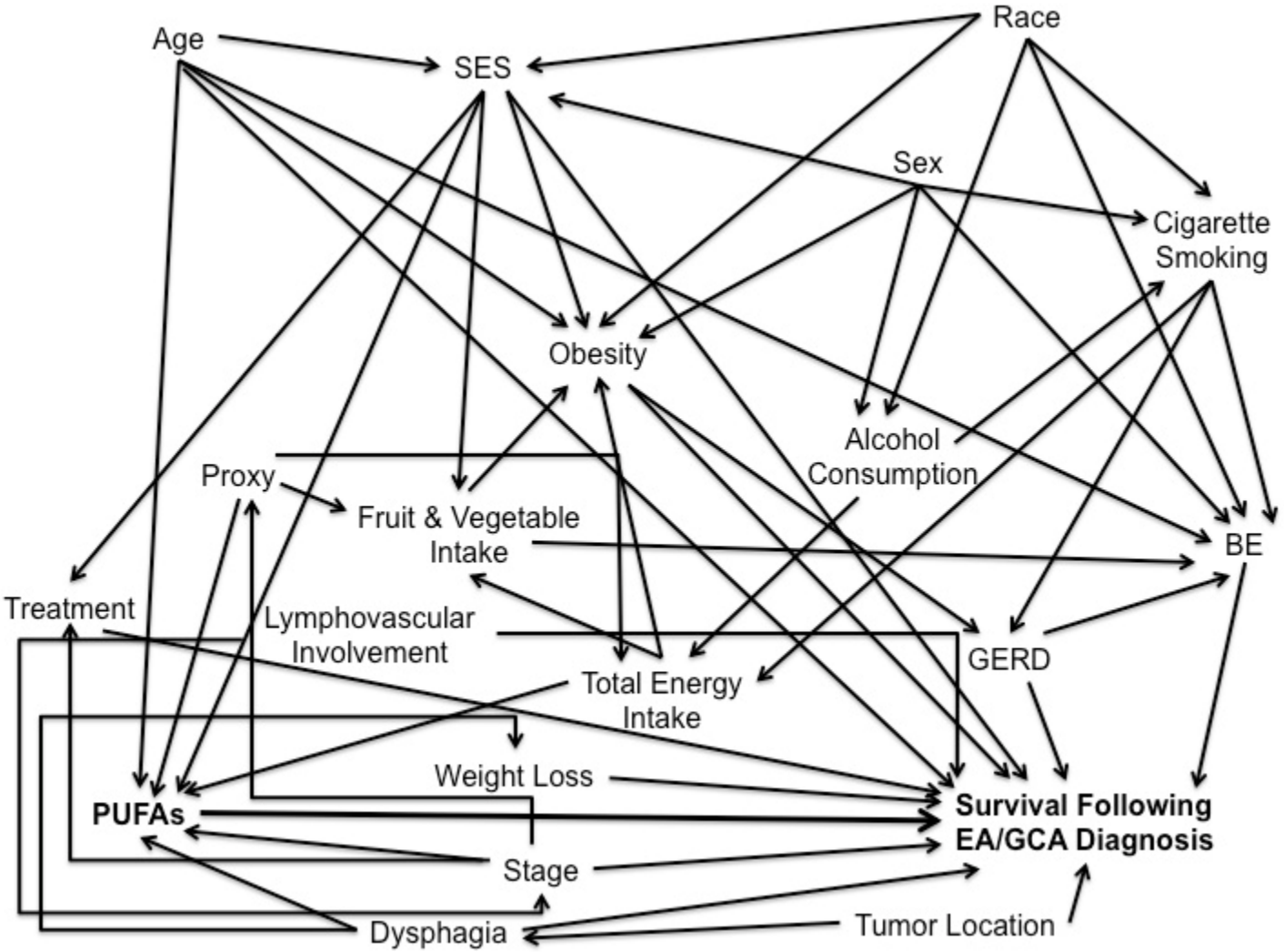


Table 2.5. Power estimates for the effect of PUFAs on development of BE

	Minimum Detectable OR	Power for 4 proportions of exposed controls			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	27%	30%	36%	41%
	0.70	55%	62%	71%	78%
	0.60	83%	89%	94%	98%
ω-6, or ω-6/ω-3 relative balance	1.25	30%	34%	38%	41%
	1.43	64%	70%	76%	79%
	1.67	93%	95%	97%	98%
n=466 BE cases; n=491 controls					

Table 2.6. Power estimates for the association between PUFAs and risk of developing EA or GCA

	Minimum Detectable OR	Power for 4 proportions of exposed controls			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	38%	44%	52%	60%
	0.70	75%	82%	89%	94%
	0.60	96%	98%	>99%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	46%	51%	57%	60%
	1.43	85%	90%	93%	94%
	1.67	>99%	>99%	>99%	>99%
n=488 EA cases; n=512 GCA cases; n=1995 controls					

Table 2.7. Power estimates for the association between PUFAs and risk of developing EA/GCA

	Minimum Detectable OR	Power for 4 proportions of exposed controls			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	60%	67%	75%	82%
	0.70	93%	97%	99%	>99%
	0.60	>99%	>99%	>99%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	67%	73%	79%	82%
	1.43	97%	99%	>99%	>99%
	1.67	>99%	>99%	>99%	>99%
n=1000 EA/GCA cases; n=1995 controls					

Table 2.8. Power estimates for the association between PUFAs and risk of developing EA or GCA among non-proxies only

	Minimum Detectable OR	Power for 4 proportions of exposed controls			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	29%	34%	40%	47%
	0.70	61%	69%	78%	86%
	0.60	89%	94%	97%	99%
ω-6, or ω-6/ω-3 relative balance	1.25	37%	41%	45%	47%
	1.43	74%	80%	84%	86%
	1.67	96%	98%	99%	99%
n=340 EA cases; n=367 GCA cases; n=1995 controls					

Table 2.9. Power estimates for the association between PUFAs and risk of developing EA/GCA among non-proxies only

	Minimum Detectable OR	Power for 4 proportions of exposed controls			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	49%	56%	65%	72%
	0.70	86%	92%	96%	98%
	0.60	99%	>99%	>99%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	57%	63%	69%	72%
	1.43	93%	96%	98%	98%
	1.67	>99%	>99%	>99%	>99%
n=707 EA/GCA cases; n=1995 controls					

Table 2.10. Power estimates for the association between PUFAs and mortality following EA or GCA diagnosis

	Minimum Detectable HR	Power for 4 proportions of exposed EA/GCA cases			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	30%	36%	46%	63%
	0.70	62%	72%	83%	95%
	0.60	89%	95%	98%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	32%	39%	49%	66%
	1.43	67%	76%	87%	97%
	1.67	93%	97%	>99%	>99%
n=488 EA cases; n=512 GCA cases; assuming 13% of cases survived after a maximum follow-up of 7.5 years and 10.7 years					

Table 2.11. Power estimates for the association between PUFAs and mortality following EA/GCA diagnosis

	Minimum Detectable HR	Power for 4 proportions of exposed EA/GCA cases			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	54%	64%	76%	90%
	0.70	90%	95%	99%	>99%
	0.60	>99%	>99%	>99%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	57%	67%	79%	92%
	1.43	93%	97%	>99%	>99%
	1.67	>99%	>99%	>99%	>99%
n=1000 EA/GCA cases; assuming 13% of cases survived after a maximum follow-up of 7.5 years and 10.7 years					

Table 2.12. Power estimates for the association between PUFAs and mortality following EA or GCA diagnosis among non-proxies only

	Minimum Detectable HR	Power for 4 proportions of exposed EA/GCA cases			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	23%	27%	34%	48%
	0.70	47%	56%	68%	85%
	0.60	76%	84%	93%	99%
ω-6, or ω-6/ω-3 relative balance	1.25	24%	29%	36%	50%
	1.43	52%	61%	73%	89%
	1.67	81%	89%	96%	99%
n=340 EA cases; n=367 GCA cases; assuming 13% of cases survived after a maximum follow-up of 7.5 years and 10.7 years					

Table 2.13. Power estimates for the association between PUFAs and mortality following EA/GCA diagnosis among non-proxies only

	Minimum Detectable HR	Power for 4 proportions of exposed EA/GCA cases			
		0.20	**0.25**	**0.33**	**0.50**
Fish intake, or ω-3	0.80	41%	49%	61%	78%
	0.70	78%	86%	94%	99%
	0.60	97%	99%	>99%	>99%
ω-6, or ω-6/ω-3 relative balance	1.25	43%	52%	64%	81%
	1.43	82%	90%	96%	>99%
	1.67	98%	>99%	>99%	>99%
n=707 EA/GCA cases; assuming 13% of cases survived after a maximum follow-up of 7.5 years and 10.7 years					

CHAPTER III: ASSOCIATION OF FISH AND OTHER MEASURES OF POLYUNSATURATED FATTY ACIDS WITH BARRETT'S ESOPHAGUS

Introduction

Barrett's esophagus (BE) is the transformation of esophageal mucosa from normal squamous epithelium into metaplastic columnar epithelium [51]. BE is the only known precursor lesion to esophageal adenocarcinoma (EA), a fatal cancer with increasing incidence in the United States (US) [4,53]. The prevalence of BE may affect approximately 2% of adults in westernized countries [13,307], and is increasing worldwide [59]. But prevalence estimates are uncertain, because many individuals are asymptomatic, and most are never diagnosed [54]. Severity of BE is often classified by segment length; short-segment BE (SSBE) is typically defined as <3 cm, whereas long-segment BE (LSBE) is defined as ≥3 cm on endoscopy [272]. The latter is more strongly associated with the risk of EA, most likely due to greater diseased surface area [272].

Most risk factors for BE are non-modifiable, such as male sex [117] and white race [117], or not easily modifiable, including reflux [143] and obesity [78] where relapse [238] and recidivism [239,242] rates are high. Dietary factors appear to be amenable to intervention [245,246,248] and have therefore been of interest as risk reduction factors for BE [165]. Polyunsaturated fatty acids (PUFAs) may be one such risk reduction factor. In experimental studies, the impact of PUFAs on carcinogenesis varies by PUFA type; ω-3 PUFAs (of which fish, in particular oily fish, are a primary source [22]) and ω-6

PUFAs (often found in vegetable oils [22]) display anti- and pro-carcinogenic effects [8], respectively. Additionally, intake of PUFAs in Westernized diets has changed over time [25] where ω-6 intake has increased over the last century, similar to the increase in BE the last few decades [58,59]. Further, geographic variation of PUFA intake corresponds to the variation of BE/EA prevalence/incidence worldwide [15,26,28,34-36]. However, associations of ω-3 PUFAs and ω-6 PUFAs with BE risk have infrequently been examined in epidemiologic studies [107,217-219]. BE risk reduction may be possible by increasing intake of ω-3 PUFAs [8], while perhaps simultaneously reducing intake of ω-6 PUFAs.

To examine associations epidemiologically between PUFA intake and development of BE, we harmonized and pooled food frequency questionnaires (FFQs) from two US community-based case-control studies [80,259]. We hypothesized that higher intake of ω-3 fatty acids and non-fried fish (the primary dietary source of long-chain ω-3) would be associated with reduced risk of BE, while a higher ω-6 to ω-3 ratio of intake (ω-6:ω-3) would be associated with increased risk. We also hypothesized these associations would be stronger for LSBE as compared to SSBE. We examined whether there is a potential interaction between ω-3 and ω-6, because ω-3 and ω-6 are competitively inhibited by each other and may suppress or promote pathogenesis [8]. Further, because inflammation is the most biologically plausible mechanism linking PUFAs to BE development [23,212], we hypothesized that inflammation-related exposures (such as body mass index (BMI), waist circumference (WC), use of non-steroidal inflammation drugs (NSAIDs), cigarette smoking and reflux symptoms) could modify PUFA-BE associations.

Methods

We used resources from two studies of the International Barrett's and Esophageal Adenocarcinoma Consortium (BEACON): the Study of Reflux Disease and the Epidemiology and Incidence of BE Study. Institutional Review Board approval was obtained from all participating institutions. Details of these case-control studies have been published previously [80,259] and are summarized below.

Study Population

The Study of Reflux Disease was conducted in western Washington from 1997-2000 [259]. Cases were newly diagnosed with BE, 20-80 years old, and underwent endoscopy for gastroesophageal reflux disease (GERD) symptoms. Controls were community-based, residents of western Washington, selected by a modified Waksberg telephone random digit dialing [261], and were matched to cases on age (+/- 3 years) and sex. Study participants included 193 cases and 211 controls.

The Epidemiology and Incidence of BE study was conducted within the Kaiser Permanente Northern California health care system from 2002-2005 [80]. All participants had to be continuously enrolled in the Kaiser Permanente health plan for two years prior to study inclusion. Cases were newly diagnosed with BE (using medical records, endoscopic findings, and biopsy results) and aged 18-79. Controls were community-based Kaiser Permanente members and frequency matched to cases on age (5-year age groups), sex, and geographic region. Study participants included 320 cases and 317 controls.

We excluded respondents without dietary intake assessment and those with reported energy intake greater than ±3 standard deviations on the log scale from the individual study means, resulting in a final population of 471 case and 490 control participants.

Study Interviews

Informed consent was obtained before interview. Trained interviewers conducted in-person interviews that assessed demographics, medical history, tobacco use, and alcohol consumption, and recorded anthropometric measures. Diet in the year prior to diagnosis (cases)/interview (controls) was assessed using validated FFQs. The Study of Reflux Disease used the 131-item Fred Hutchinson Cancer Research Center FFQ [267] and the Epidemiology and Incidence of BE Study used the 110-item Block 1998 FFQ [268,269]. These FFQs were similar in design, and both assessed frequency of intake and portion size.

Harmonization and Pooling

Covariates derived from the study-specific interviews and anthropometry were harmonized previously for BEACON at the National Cancer Institute, as described elsewhere [85,308,309].

To harmonize dietary intake responses of the two BEACON studies examined here, FFQs were linked with the University of Minnesota Nutrition Coordinating Center database [278] to estimate grams per day of PUFA exposures. Thirteen exposure measures were assessed. Of these, nine were PUFA nutrient estimates including: ω-3;

ω-3 subtypes (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA)); ω-6; ω-6 subtypes (linoleic acid (LA), and arachidonic acid (AA)); and the ω-6:ω-3. The remaining four measures were assessments of PUFA-rich foods: tuna (tuna canned or fresh, tuna salad, tuna casserole), fried fish, baked/broiled fish (non-fried fish other than tuna and shellfish), and shellfish.

As an example of the approach we used to estimate the PUFA nutrient measures, EPA intake from a single line item was first calculated (*EPA intake for line item = frequency of intake x portion size of intake x EPA grams per 100 grams of line item*). Intake of EPA per day for a participant was then calculated by summing up EPA intake for all line items. In some instances, the line items represented multiple foods items, in which case the individual foods were weighted according to the estimates of US consumption [310].

After the FFQ responses for PUFA intake were harmonized, we generated study-specific quantiles of intake using control distributions within each study [282]. We then pooled the PUFA exposure variables by merging across quantiles for the two BE studies. For the PUFA nutrient exposure variables we created quartiles of exposure, and for the PUFA-rich food intake measures we used an ordinal categorization of non-consumers and tertiles of exposure among consumers.

Statistical Methods

Multivariable-adjusted logistic regression [265] was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between the PUFA

measures and the development of BE. Polytomous logistic regression [265] was used to estimate effect measures with BE classified by segment length: SSBE (<3 cm) and LSBE (≥3 cm). The Wald test was used to detect differences in associations by segment length [265]. Linear trends were examined by modeling PUFA as continuous variables.

Confounders were identified by selecting potential confounders from the literature and creating a directed acyclic graph (DAG) [265]. Covariates considered in the DAG included age, sex, race, education, obesity, cigarette smoking, alcohol use, total energy intake, fruit and vegetable intake, GERD symptoms, and NSAID use. Covariates included in the final models were the matching factors of the two parent studies (age (±3 years for western Washington, and 5-year age groups for northern California) and sex (male, female)), study indicator (northern California, western Washington), plus the DAG-identified confounders (education (≤high school, technical school or some university, ≥university), and total energy intake (kcal, continuous)).

We examined potential effect measure modification (EMM) between total ω-3 and ω-6 intake, as well as exploring EMM by the inflammation-related factors of BMI (≥25kg/m^2, <25kg/m^2 [292]), WC (≤94cm, >94cm for men, and ≤80cm, >80cm for women [293]), NSAID use (ever regular use, never regular use), cigarette smoking (ever, never), and GERD symptom frequency (≥weekly, <weekly reflux). WC assessments were available only for the northern California Epidemiology and Incidence of BE Study, and thus our WC models were restricted to the northern California participants. EMM was assessed on a multiplicative scale by comparing models with and without interaction terms using the likelihood ratio test with a

significance level of 0.10 [265]. EMM was also assessed on the additive scale by using single referent models to obtain interaction contrast ratios (ICRs) and 95% CIs [265].

For sensitivity analyses, we first considered potential confounding by other factors not identified as confounders using the DAG-identified approach. We added to the DAG-identified models established BE risk factors [66,78,80,81,85] of BMI (<25kg/m^2, 25-29.9kg/m^2, ≥30 kg/m^2 [292]), cigarette smoking (former, current, never), and reflux symptom frequency (<once/week, once/week, once/week-<daily, ≥daily, never). Second, we conducted a sensitivity analysis that used the absolute value-based pooling approach deriving quantiles using all combined controls [282]. Third, we widened the acceptable caloric intake by only excluding participants in the highest and lowest 2.5% of daily caloric intake. The fourth sensitivity analysis was to use a random effects meta-analytic approach [265] to pool the study-specific effect estimates. In the final sensitivity analysis, we considered BE associations with white fish and dark fish, which was only available in the western Washington Study of Reflux Disease. For this particular sensitivity analysis, we categorized intake into consumers and non-consumers due to small variability in intake.

Analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC), and Stata software, version 15.0 (StataCorp LP, College Station, TX).

Results

The distribution of participant characteristics is shown by study and case-control status in **Table 3.1**. Those in the northern California Epidemiology and Incidence of BE Study were older, more likely to be male, non-white, experience reflux more frequently,

had a higher daily caloric intake and higher proportion of LSBE compared to the western Washington Study of Reflux Disease participants.

When examining daily mean intake of PUFAs and fish by study (**Table 3.2**), there was a higher intake of ω-3, ω-6, and fried fish and a lower shellfish intake among the participants of the western Washington Study of Reflux Disease. The intake for cases and controls were similar for a majority of the PUFA measures within each of the two BE studies, with one exception; in the northern California Epidemiology and Incidence of BE study, controls reported eating more baked/broiled fish than cases.

Table 3.3 presents the number of FFQ items and highest contributing (a combination of the most frequently eaten and PUFA-dense) foods for each PUFA measure by study. In both studies, the same food items were the highest contributors to both the ω-3 and ω-6 measures. For example, in the western Washington Study of Reflux disease, the two highest contributing line items for ω-3 and ω-6 were “beef, pork, lamb” and “chicken, turkey”.

When we considered intake of specific PUFA-rich foods (**Table 3.4**), the risk of developing BE was reduced by 34% for those eating the highest amount of baked/broiled fish ($OR_{T3vs.None}$=0.66, 95%CI=0.45-0.98, p_{trend}=0.06). Other measures of fish intake were not associated with BE.

When we considered the PUFA nutrient measures, including the ω-6:ω-3 ratio (**Table 3.5**), there were no significant associations with BE. There was no evidence of EMM by any of the inflammation-related factors considered (BMI, WC, NSAID use, smoking, GERD symptom frequency) on the multiplicative or additive scales (**Table 3.6**).

As shown in **Table 3.7**, associations between PUFA measures and BE differed by segment length. Increased intake of ω-3 was associated with a decreased risk of LSBE ($OR_{>Median vs. \leq Median}$=0.62, 95%CI=0.40-0.96), and this differed significantly from SSBE ($OR_{>Median vs. \leq Median}$=0.98, 95%CI=0.67-1.43; $p_{heterogeneity}$=0.07). Also, higher intake of baked/broiled fish showed a suggestive inverse association with LSBE ($OR_{Consumers vs. Non\text{-}consumers}$=0.68, 95%CI=0.51-1.02). However, in contrast to our hypotheses, higher intake of the ω-6 subtype AA was also associated with a decreased risk of LSBE ($OR_{>Median vs. \leq Median}$=0.67, 95%CI=0.45-0.99), and was different from SSBE ($OR_{>Median vs. \leq Median}$=1.08, 95%CI=0.77-1.54; $p_{heterogeneity}$=0.03). No associations were observed with any other fish or PUFA measures, and none were found between PUFAs and SSBE.

Results from the five sets of sensitivity analyses we conducted did not differ substantially from the results already shown, with a few exceptions. For the sensitivity analysis where we added BMI, cigarette smoking status, and reflux frequency to the DAG-driven adjustment models, the majority of effect estimates were not substantially altered, as shown in **Table 3.8**. Although the OR for tuna intake moved away from the null, interpretation of the effect estimate remained unchanged. Next, for the sensitivity analysis where we considered the absolute value-based pooling approach (deriving quantiles using all combined controls), most results (**Table 3.9**) were not substantially different from the results from the study-specific pooling approach shown in **Tables 3.4** and **3.5**. However, for ω-3 there was a suggestive association with reduced risk of BE ($OR_{Q4 vs. Q1}$=0.61, 95%CI=0.36–1.03), which is slightly different from the null result we obtained when we used the study-specific pooled approach. The results from when we

excluded subjects who reported energy intake in the extreme 2.5% of intake (data not shown) and the results using the random effects meta-analytic approach (data not shown) did not differ substantially from the results shown in **Tables 3.4** and **3.5**. In the final sensitivity analysis examining white fish and dark fish, we found no associations with BE (data not shown).

Discussion

Consistent with our hypotheses, higher intake of baked/broiled fish, one of the most ω-3-dense food items, was associated with a significant 34% decrease in the risk of developing BE, and the association may be limited to patients diagnosed with LSBE. In contrast to our hypotheses, the ω-6 subtype AA was also inversely associated with LSBE. Intake of tuna (which included tuna salad/tuna casserole), fried fish, and other fish and PUFA nutrient measures were not associated with BE.

An inverse association between ω-3-rich baked/broiled fish and BE is biologically plausible. Both families of PUFAs use the same metabolic pathways and are competitively inhibited by one another, by vying for binding sites on the same enzymes [23]. Because of this, higher intake of foods high in ω-3 fatty acids leads to decreased production of ω-6-derived byproducts. Experimental studies demonstrate that ω-6-derived byproducts are highly metabolically active, have cancer-promoting properties, and are pro-inflammatory [212]. Further, increased presence of ω-3 subtypes can lower the expression of COX-2 [212], which has been implicated in a variety of human cancers [23,216]. However, there is currently no consensus for a positive association between ω-6 and chronic disease in humans [311]. Our unexpected finding of an

inverse association between ω-6 subtype AA with LSBE is inconsistent with our hypotheses, and may be due to chance.

Previous studies examining intake of PUFA nutrients or foods with high levels of ω-3 (such as fish) in association with BE are scarce and inconsistent. Two studies, a case-control study conducted in Ireland [218] and a case-cohort study in the Netherlands [217], reported no association between fish intake and BE. The inconsistencies between our results and these studies may be due to the fact that we were able to distinguish between fried fish (which often add ω-6 in the form of oils [22]) and non-fried fish, while the previous studies did not. The Irish study also examined a measure of total PUFA intake (which combined ω-3 and ω-6 intake) with null results [218]. However, in a separate previous analysis, the Northern California Epidemiology and Incidence of BE (the data from which were included in the present pooled study) observed a suggestive inverse association between total PUFAs and BE [107]. A previous meta-analysis by Zhao et al. [219] combined results from the Northern California and Irish studies and observed no association between total PUFAs and BE. These results are similar to our results for ω-3 and ω-6, where null relationships were seen with BE overall. However one previous report, based on the Northern California Epidemiology and Incidence of BE Study, found that ω-3 was associated with a halving of BE risk [107]. This finding from the northern California study is similar to our pooled results for LSBE reported here, and results were consistent in sensitivity analyses when we restricted our models to the western Washington Study of Reflux Disease. Interestingly, more than half of the Northern California Epidemiology and Incidence of BE Study cases were diagnosed with LSBE, as opposed to only one quarter in the

western Washington Study of Reflux Disease. Differentiation between associations with LSBE and SSBE is important, because LSBE is both more strongly associated with reflux than SSBE, and is a stronger risk factor for the development of EA than SSBE.

There are several limitations of our study. For example, we harmonized and pooled data in order to increase sample size, but there may be differences in data collection and management between the studies, which may have resulted in exposure misclassification. To mitigate this potential impact, we selected two US community-based studies in BEACON with similar data collection procedures. Future studies should consider a design that insures *a priori* uniform field procedures for all study participants. Another concern centers on exposure assessment. Patients were asked to recall diet in the year prior to study interview, with the assumption that intake during this time period can be recalled with some accuracy and that it correlates with usual adult diet [296]. Nonetheless, it is possible that recent onset of clinical symptoms and any corresponding changes in diet would influence the accuracy of reporting usual adult diet among cases only. Future studies could consider a prospective cohort design in order to rule out recall bias as a possible reason for our study findings. Yet, such an approach would be costly to implement. Finally, our pooled population is primarily white and male, which limits generalizability. Although the rates of BE are the highest among this demographic group [117], future studies should consider expanding the racial and gender diversity of the target population.

There are several strengths to the study presented here. A major strength is harmonizing and pooling of two existing studies, which is a very time- and cost-efficient design compared to undertaking a new field study effort. This approach is also superior

to conducting a meta-analysis, because creating uniform definitions for our exposure of interest and for the other covariates improves accuracy of the resulting estimates. Our pooled sample size is also larger than previous case-control studies [107,218], with more cases than the only case-cohort study [217], allowing us to provide more precise effect estimates and facilitates our examination of effect measure modification. Another strength of our study was the comprehensive use of multiple exposure measures, which allowed us to consider the complexity of this dietary exposure and helped to clarify previous findings. For example, prior to our study, no BE studies had considered cooking methods of ω-3-rich foods (such as fish), which can aid in unmasking potential associations (by separating, for example, broiled/baked from fried) [217,218]. A few previous studies considered overall PUFA intake [107,218,219], which overlooks important distinctions between ω-3 and ω-6, or examined ω-3 PUFAs only [107]. Ours is also the first BE study to explore ω-6 PUFAs, or to consider the relative ω-3 and ω-6 balance (either the interaction or ratio). Another benefit is that we were able to identify a measure – baked/broiled fish – that was most strongly associated with BE risk, at least among a western US population. For example, contributors to ω-3 intake among respondents were not limited to foods known to be high in beneficial fatty acids such as fish, but were commonly the same foods also contributing to ω-6 (e.g.," chicken, turkey;" and "beef, pork, lamb", as shown in **Table 3.3**). This likely led to our inability to detect associations with either ω-3 or ω-6. Similarly, the parent study FFQs included a composite assessment of tuna, a fish with high levels of ω-3 [24], combining tuna with tuna casserole and tuna salad; the latter two are typically prepared with other foods high in ω-6 using contrasting cooking methods. Instead our strongest effect estimate was for

baked/broiled fish, where food items and the cooking method were clearly delineated. Future studies conducted among US populations should include assessments of PUFA-rich foods such as fish, making sure to consider fish type as well as cooking or other preparation methods. Finally, both parent studies were conducted in western US coastal regions with increased opportunity for fish intake (western Washington, and northern California); this maximized heterogeneity in intake and enhanced our ability to detect differences, since US residents overall typically consume a low amount of fish [306].

In summary, our pooled study of two US-based case-control studies of BE found that high intake of baked/broiled fish was associated with a 34% reduction of BE. Additionally, higher intake of baked/broiled fish, ω-3, and the ω-6 subtype AA, were associated with a 32-38% reduction of risk of LSBE. If our findings are confirmed, increasing non-fried fish intake could be a risk reduction strategy for BE, the precursor to an extremely fatal cancer.

Table 3.1. Demographic characteristics of participants of two US-based case-control studies of BE

Characteristic	Study of Reflux Disease		Epidemiology and Incidence of BE	
	Cases N=176	Controls N=191	Cases N=295	Controls N=301
Age (yrs)	54.8 (12.8)	53.4 (12.1)	62.4 (10.6)	62.4 (10.3)
Sex				
Male	105 (59.7)	119 (62.3)	216 (73.2)	203 (67.4)
Female	71 (40.3)	72 (37.7)	79 (26.8)	98 (32.6)
Race				
White	157 (89.2)	175 (91.6)	255 (86.4)	256 (85.1)
Other	19 (10.8)	16 (8.4)	45 (13.6)	40 (14.9)
Education				
≤High school	46 (26.1)	34 (17.8)	78 (26.4)	58 (19.3)
Technical school or some university	9 (5.1)	6 (3.1)	135 (45.8)	114 (37.9)
≥University	121 (68.8)	151 (79.1)	82 (27.8)	129 (42.9)
BMI (kg/m^2)	29.3 (5.2)	27.6 (5.1)	29.4 (5.5)	29.5 (5.7)
Cigarette smoking status				
Never	62 (35.2)	99 (51.8)	97 (33.0)	131 (43.5)
Former	89 (50.6)	63 (33.0)	157 (53.4)	132 (43.9)
Current	25 (14.2)	29 (15.2)	40 (13.6)	38 (12.6)
NSAID use				
Never	65 (37.1)	100 (52.4)	161 (54.6)	158 (52.8)
Ever	110 (62.9)	91 (47.6)	134 (45.4)	141 (47.2)
Reflux frequency				
<Weekly	102 (58.3)	167 (88.8)	115 (39.3)	245 (81.7)
≥Weekly	73 (41.7)	21 (11.2)	178 (60.8)	55 (18.3)
Total energy intake (kcal/day)	1694.8 (718.2)	1636.1 (720.9)	1804 (856.7)	1843.1 (799.4)
BE segment length				
SSBE	132 (75.0)	--	109 (44.0)	--
LSBE	44 (25.0)	--	139 (56.1)	--

Missing values (N): BMI (7), smoking status (1), NSAID use (3), reflux frequency (7), BE segment length (47)

Table 3.2. Daily mean intake of PUFAs and fish among participants of two US-based case-control studies of BE

Measure	Study of Reflux Disease		Epidemiology and Incidence of BE	
	Cases N=176	Controls N=191	Cases N=295	Controls N=301
Tuna (g/day)	7.59 (9.48)	6.91 (10.04)	7.76 (14.21)	8.43 (13.82)
Fried fish (g/day)	6.02 (8.74)	5.93 (11.01)	3.77 (7.05)	3.96 (6.87)
Baked/broiled fish (g/day)	9.66 (15.62)	8.85 (9.24)	6.06 (12.03)	10.27 (23.74)
Shellfish (g/day)	2.79 (4.49)	2.96 (4.30)	4.74 (8.07)	4.87 (7.91)
ω-3 (g/day)	3.38 (2.48)	3.09 (2.28)	1.67 (0.94)	1.81 (0.92)
ALA (g/day)	2.81 (1.95)	2.60 (1.94)	1.57 (0.88)	1.68 (0.83)
EPA (g/day)	0.11 (0.15)	0.09 (0.09)	0.02 (0.03)	0.03 (0.04)
DHA (g/day)	0.28 (0.40)	0.25 (0.27)	0.07 (0.07)	0.09 (0.13)
DPA (g/day)	0.18 (0.23)	0.15 (0.17)	0.02 (0.02)	0.02 (0.03)
ω-6 (g/day)	30.47 (25.90)	27.90 (22.02)	15.03 (10.61)	16.17 (9.88)
LA (g/day)	29.50 (24.82)	27.04 (21.11)	14.93 (10.57)	16.06 (9.84)
AA (g/day)	0.97 (1.11)	0.86 (0.94)	0.10 (0.07)	0.11 (0.08)
ω-6:ω-3	8.78 (1.79)	8.84 (1.97)	8.94 (3.18)	9.07 (3.46)

Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish

Table 3.3. Number of FFQ items and foods with highest contribution for PUFA nutrient and fish intake measures among control participants (N=492) of two US-based case-control studies of BE

Measure	Study of Reflux Disease (N=131 line items)		Epidemiology and Incidence of BE (N=110 line items)	
	# of FFQ items	Five highest contributing FFQ items	# of FFQ items	Five highest contributing FFQ items
Tuna	1	Canned tuna, tuna salad, tuna casserole	1	Tuna, tuna salad, tuna casserole
Fried fish	1	Fried fish/shellfish, fish sandwich,	1	Fried fish, fish sandwich
Baked/broiled fish	2	White fish; dark oily fish	1	Other fish, not fried
Shellfish	1	Shellfish	2	Oysters; other shellfish
ω-3	116	Beef, pork, lamb; chicken, turkey; mayonnaise, mayonnaise type spreads; salad dressing; French fries, fried potatoes, fried rice	103	Salad dressing; mayonnaise, sandwich spreads; cooking fat (e.g. Pam, olive oil, lard); salty snacks; other fish, not fried
ALA	116	Beef, pork, lamb; chicken, turkey; mayonnaise, mayonnaise type spreads; salad dressing; French fries, fried potatoes, fried rice	103	Salad dressing; mayonnaise, sandwich spreads; cooking fat (e.g. Pam, olive oil, lard); snacks like chips, popcorn; spinach
EPA	36	Chicken, turkey; dark oily fish; beef, pork, lamb; white fish; shellfish	32	Other fish, not fried; shellfish (not oysters); oysters; chicken, turkey; Chinese or other Asian food
DHA	38	Chicken, turkey; dark oily fish; eggs; white fish; fried fish/shellfish, fish sandwich	33	Other fish, not fried; eggs, including egg biscuits; tuna, tuna salad, tuna casserole; fried fish; chicken, turkey

Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish

Table 3.3 (cont'd). Number of FFQ items and foods with highest contribution for PUFA nutrient and fish intake measures among control participants (N=492) of two US-based case-control studies of BE

Measure	Study of Reflux Disease (N=131 line items)		Epidemiology and Incidence of BE (N=110 line items)	
	# of FFQ items	Five highest contributing FFQ items	# of FFQ items	Five highest contributing FFQ items
DPA	30	Chicken, turkey; beef, pork, lamb; dark oily fish; Swiss, cheddar, cream cheeses; white fish	26	Other fish, not fried; chicken, turkey; beef; fried chicken; Chinese or other Asian food
ω-6	118	Chicken, turkey; beef, pork, lamb; mayonnaise, mayonnaise type spreads; peanut butter, peanuts, other nuts/seeds; salad dressing	102	Peanuts, other nuts/seeds; salad dressing; snacks like chips, popcorn; mayonnaise, sandwich spreads; cooking fat (e.g. Pam, olive oil, lard)
LA	118	Chicken, turkey; beef, pork, lamb; mayonnaise and mayonnaise type spreads; peanut butter, peanuts, other nuts/seeds; salad dressing	102	Peanuts, other nuts/seeds; salad dressing; snacks like chips, popcorn; mayonnaise, sandwich spreads; cooking fat (e.g. Pam, olive oil, lard)
AA	60	Chicken, turkey; beef, pork, lamb; eggs; dark oily fish; ground meat (e.g. hamburgers, meatloaf)	52	Eggs, including egg biscuits; chicken, turkey; other fish, not fried; lunchmeat; beef
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish				

Table 3.4. Odds ratios and 95% confidence intervals for associations between PUFAs using fish intake measures and development of BE among participants of two US-based case-control studies of BE

Measure	Cases N=471	Controls N=492	OR* (95%CI)
Tuna			
None	89	85	1.0
T1	174	183	0.89 (0.61, 1.31)
T2	105	117	0.90 (0.60, 1.35)
T3	103	107	0.95 (0.62, 1.44)
			p_{trend}=0.94
Fried fish			
None	169	186	1.0
T1	115	122	1.01 (0.72, 1.42)
T2	113	109	1.13 (0.80, 1.59)
T3	74	75	1.08 (0.72, 1.62)
			p_{trend}=0.76
Baked/broiled fish			
None	189	98	1.0
T1	85	140	0.92 (0.64, 1.32)
T2	98	121	0.68 (0.46, 1.01)
T3	99	133	0.66 (0.45, 0.98)
			p_{trend}=0.06
Shellfish			
None	131	129	1.0
T1	123	113	1.04 (0.72, 1.51)
T2	76	97	0.77 (0.51, 1.16)
T3	141	153	0.90 (0.63, 1.30)
			p_{trend}=0.75

* Model adjusted for age, sex, education, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake

Table 3.5. Odds ratios and 95% confidence intervals for associations between PUFA nutrient measures and development of BE among participants of two US-based case-control studies of BE

Measure	Cases N=471	Controls N=492	OR* (95%CI)
ω-3			
Q1	121	123	1.0
Q2	129	122	1.10 (0.76, 1.60)
Q3	106	124	0.89 (0.59, 1.34)
Q4	115	123	0.94 (0.57, 1.54)
			p_{trend}=0.59
ALA			
Q1	124	123	1.0
Q2	134	124	1.10 (0.76, 1.59)
Q3	100	123	0.79 (0.52, 1.20)
Q4	113	122	0.86 (0.52, 1.42)
			p_{trend}=0.67
EPA			
Q1	136	124	1.0
Q2	126	123	0.93 (0.65, 1.33)
Q3	110	122	0.90 (0.62, 1.31)
Q4	99	123	0.79 (0.53, 1.18)
			p_{trend}=0.56
DHA			
Q1	136	124	1.0
Q2	115	122	0.87 (0.60, 1.25)
Q3	125	123	1.03 (0.71, 1.49)
Q4	95	123	0.78 (0.52, 1.17)
			p_{trend}=0.79
DPA			
Q1	129	123	1.0
Q2	119	122	0.90 (0.62, 1.29)
Q3	116	123	0.94 (0.64, 1.38)
Q4	107	124	0.88 (0.59, 1.32)
			p_{trend}=0.28
ω-6			
Q1	123	123	1.0
Q2	114	123	0.89 (0.61, 1.31)
Q3	131	124	1.04 (0.70, 1.57)
Q4	103	122	0.80 (0.48, 1.33)
			p_{trend}=0.54

* Model adjusted for age, sex, education, caloric intake, and study indicator
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.5 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA nutrient measures and development of BE among participants of two US-based case-control studies of BE

Measure	Cases N=471	Controls N=492	OR* (95%CI)
LA			
Q1	123	122	1.0
Q2	115	124	0.88 (0.60, 1.29)
Q3	129	124	1.03 (0.68, 1.54)
Q4	104	122	0.80 (0.48, 1.33)
			p_{trend}=0.56
AA			
Q1	117	122	1.0
Q2	127	124	1.03 (0.71, 1.49)
Q3	104	123	0.89 (0.60, 1.31)
Q4	123	123	1.08 (0.70, 1.65)
			p_{trend}=0.22
ω-6:ω-3			
Q1	117	124	1.0
Q2	108	122	0.93 (0.64, 1.35)
Q3	130	124	1.12 (0.78, 1.61)
Q4	116	122	1.05 (0.73, 1.52)
			p_{trend}=0.72
* Model adjusted for age, sex, education, caloric intake, and study indicator			

Table 3.6. Odds ratios and 95% confidence intervals for associations between PUFAs and development of BE when examining effect measure modification among participants of two US-based case-control studies of BE

Effect modifier		Cases N=471	Controls N=492	Multiplicative scale		Additive scale	
				Stratified ORs* (95% CIs)	$p_{interaction}$	Single referent ORs* (95% CIs)	ICR (95% CI)
ω-3	**ω-6**						
≤Median	>Median	217	212	1.0		1.0	
	≤Median	33	33	0.90 (0.74, 1.10)		0.90 (0.74, 1.10)	
>Median	>Median	201	213	1.0		0.85 (0.70, 1.04)	
	≤Median	20	34	0.79 (0.59, 1.07)	0.21	0.68 (0.44, 1.05)	-0.08 (-0.22, 0.07)
BMI (kg/m^2)	**Baked/broiled fish**						
25+	≤Median	224	189	1.0		1.0	
	>Median	153	174	0.81 (0.69, 0.94)		0.81 (0.69, 0.94)	
<25	≤Median	59	66	1.0		0.82 (0.70, 0.95)	
	>Median	35	63	0.78 (0.59, 1.02)	0.60	0.63 (0.45, 0.88)	0.01 (-0.08, 0.10)
WC (cm)**	**Baked/broiled fish**						
>Recommendations	≤Median	150	115	1.0		1.0	
	>Median	72	96	0.83 (0.72, 0.96)		0.83 (0.72, 0.96)	
≤Recommendations	≤Median	51	48	1.0		0.77 (0.67, 0.90)	
	>Median	20	42	0.85 (0.66, 1.09)	0.80	0.66 (0.48, 0.89)	0.05 (-0.04, 0.14)

* Adjusted for age, sex, caloric intake, and study indicator
** WC data available in the Epidemiology and Incidence of BE study only
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median is derived from the controls' distribution of intake

Table 3.6 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and development of BE when examining effect measure modification among participants of two US-based case-control studies of BE

Effect modifier		Cases N=471	Controls N=492	Multiplicative scale		Additive scale	
				Stratified ORs* (95% CIs)	$p_{interaction}$	Single referent ORs* (95% CIs)	ICR (95% CI)
NSAID use	**Baked/broiled fish**						
Ever	≤Median	139	113	1.0		1.0	
	>Median	105	119	0.84 (0.74, 0.96)		0.84 (0.74, 0.96)	
Never	≤Median	143	141	1.0		0.91 (0.80, 1.04)	
	>Median	82	117	0.83 (0.69, 1.00)	0.91	0.76 (0.60, 0.96)	0.01 (-0.09, 0.11)
Smoking status	**Baked/broiled fish**						
Ever	≤Median	244	153	1.0		1.0	
	>Median	167	118	0.87 (0.76, 1.00)		0.87 (0.76, 1.00)	
Never	≤Median	37	100	1.0		0.78 (0.68, 0.89)	
	>Median	20	117	0.95 (0.77, 1.16)	0.24	0.74 (0.58, 0.94)	0.08 (-0.01, 0.18)
Reflux frequency	**Baked/broiled fish**						
≥Weekly	≤Median	158	51	1.0		1.0	
	>Median	93	25	0.95 (0.81, 1.12)		0.95 (0.81, 1.12)	
<Weekly	≤Median	123	202	1.0		0.38 (0.32, 0.45)	
	>Median	94	210	0.84 (0.71, 0.99)	0.11	0.32 (0.25, 0.40)	-0.01 (-0.14, 0.12)

* Adjusted for age, sex, caloric intake, and study indicator
** WC data available in the Epidemiology and Incidence of BE study only
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median is derived from the controls' distribution of intake

Table 3.7. ORs and 95% CIs for PUFAs and development of BE characterized by BE segment length* among participants of two US-based case-control studies of BE

Measure	Controls N=492	Short-segment BE(<3cm) Cases N=241	OR** (95%CI)	Long-segment BE (≥3cm) Cases N=183	OR** (95%CI)	Ratio of OR**	$p_{heterogeneity}$
Tuna							
Non-consumers	85	49	1.0	37	1.0	0.73 (0.44, 1.21)	0.22
Consumers	407	192	0.97 (0.65, 1.45)	146	0.70 (0.45, 1.10)		
Fried Fish							
Non-consumers	186	90	1.0	68	1.0	0.84 (0.55, 1.27)	0.40
Consumers	306	151	1.10 (0.79, 1.53)	115	0.92 (0.64, 1.33)		
Baked/broiled fish							
Non-consumers	98	55	1.0	53	1.0	0.76 (0.48, 1.21)	0.25
Consumers	394	186	0.90 (0.61, 1.32)	130	0.68 (0.51, 1.02)		
Shellfish							
Non-consumers	129	80	1.0	42	1.0	1.18 (0.74, 1.90)	0.49
Consumers	363	161	0.90 (0.63, 1.29)	141	1.06 (0.69, 1.63)		
ω-3							
≤Median	245	120	1.0	106	1.0	0.63 (0.39, 1.04)	0.07
>Median	247	121	0.98 (0.67, 1.43)	77	0.62 (0.40, 0.96)		
ALA							
≤Median	247	128	1.0	105	1.0	0.78 (0.47, 1.29)	0.33
>Median	245	113	0.83 (0.56, 1.23)	78	0.65 (0.41, 1.00)		
EPA							
≤Median	247	129	1.0	106	1.0	0.81 (0.53, 1.25)	0.34
>Median	245	112	0.92 (0.66, 1.30)	77	0.75 (0.51, 1.10)		

* Missing values (N): BE segment length (47)
** Adjusted for age, sex, caloric intake, education, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median is derived from the controls' distribution of intake

Table 3.7 (cont'd). ORs and 95% CIs for PUFAs and development of BE characterized by BE segment length* among participants of two US-based case-control studies of BE

Measure	Controls N=492	Short-segment BE(<3cm) Cases N=241	OR** (95%CI)	Long-segment BE (≥3cm) Cases N=183	OR** (95%CI)	Ratio of OR**	$p_{heterogeneity}$
DHA							
≤Median	246	123	1.0	104	1.0	0.77 (0.50, 1.19)	0.25
>Median	246	118	1.03 (0.74 1.44)	79	0.80 (0.54, 1.17)		
DPA							
≤Median	245	120	1.0	106	1.0	0.69 (0.44, 1.08)	0.10
>Median	247	121	1.06 (0.75, 1.50)	77	0.73 (0.50, 1.08)		
ω-6							
≤Median	246	111	1.0	102	1.0	0.56 (0.34, 0.93)	0.02
>Median	246	130	1.27 (0.87 1.86)	81	0.71 (0.46, 1.11)		
LA							
≤Median	246	112	1.0	102	1.0	0.58 (0.35, 0.95)	0.03
>Median	246	129	1.23 (0.84, 1.81)	81	0.71 (0.46, 1.10)		
AA							
≤Median	246	117	1.0	105	1.0	0.62 (0.39, 0.97)	0.03
>Median	246	124	1.08 (0.77, 1.54)	78	0.67 (0.45, 0.99)		
ω-6:ω-3							
≤Median	246	113	1.0	85	1.0	0.92 (0.61, 1.38)	0.68
>Median	246	128	1.18 (0.86, 1.62)	98	1.08 (0.76, 1.54)		

* Missing values (N): BE segment length (47)
** Adjusted for age, sex, caloric intake, education, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median is derived from the controls' distribution of intake

Table 3.8. Odds ratios and 95% confidence intervals for associations between PUFAs using fish intake measures and development of BE among participants of two US-based case-control studies of BE, adjusting for additional covariates

Measure	Cases N=471	Controls N=492	OR* (95%CI)
Tuna			
None	89	85	1.0
T1	174	183	0.80 (0.52, 1.23)
T2	105	117	0.71 (0.45, 1.13)
T3	103	107	0.77 (0.48, 1.23)
			p_{trend}=0.61
Fried fish			
None	169	186	1.0
T1	115	122	1.06 (0.72, 1.54)
T2	113	109	1.03 (0.69, 1.52)
T3	74	75	1.12 (0.71, 1.76)
			p_{trend}=0.76
Baked/broiled fish			
None	189	98	1.0
T1	85	140	0.82 (0.55 1.23)
T2	98	121	0.78 (0.50, 1.21)
T3	99	133	0.68 (0.44, 1.06)
			p_{trend}=0.08
Shellfish			
None	131	129	1.0
T1	123	113	1.12 (0.74, 1.69)
T2	76	97	0.71 (0.45, 1.13)
T3	141	153	0.93 (0.62, 1.40)
			p_{trend}=0.61
ω-3			
Q1	121	123	1.0
Q2	129	122	1.16 (0.76, 1.77)
Q3	106	124	0.92 (0.58, 1.46)
Q4	115	123	0.95 (0.55, 1.65)
			p_{trend}=0.86

* Model adjusted for age, sex, education, BMI, reflux frequency, smoking status, caloric intake and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs using fish intake measures and development of BE among participants of two US-based case-control studies of BE, adjusting for additional covariates

Measure	Cases N=471	Controls N=492	OR* (95%CI)
ALA			
Q1	124	123	1.0
Q2	134	124	1.15 (0.75, 1.75)
Q3	100	123	0.82 (0.51, 1.32)
Q4	113	122	0.84 (0.48, 1.46)
			p_{trend}=0.79
EPA			
Q1	136	124	1.0
Q2	126	123	0.96 (0.64, 1.44)
Q3	110	122	0.87 (0.57, 1.33)
Q4	99	123	0.74 (0.47, 1.15)
			p_{trend}=0.96
DHA			
Q1	136	124	1.0
Q2	115	122	0.91 (0.60, 1.36)
Q3	125	123	1.07 (0.70, 1.61)
Q4	95	123	0.77 (0.49, 1.20)
			p_{trend}=0.86
DPA			
Q1	129	123	1.0
Q2	119	122	0.82 (0.54, 1.23)
Q3	116	123	0.94 (0.61, 1.43)
Q4	107	124	0.87 (0.56, 1.37)
			p_{trend}=0.59
ω-6			
Q1	123	123	1.0
Q2	114	123	0.89 (0.58, 1.37)
Q3	131	124	0.90 (0.57, 1.42)
Q4	103	122	0.65 (0.37, 1.14)
			p_{trend}=0.71

* Model adjusted for age, sex, education, BMI, reflux frequency, smoking status, caloric intake and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs using fish intake measures and development of BE among participants of two US-based case-control studies of BE, adjusting for additional covariates

Measure	Cases N=471	Controls N=492	OR* (95%CI)
LA			
Q1	123	122	1.0
Q2	115	124	0.90 (0.58, 1.38)
Q3	129	124	0.87 (0.55, 1.38)
Q4	104	122	0.66 (0.37, 1.16)
			p_{trend}=0.68
AA			
Q1	117	122	1.0
Q2	127	124	0.95 (0.63, 1.44)
Q3	104	123	0.84 (0.54, 1.30)
Q4	123	123	0.98 (0.61, 1.58)
			p_{trend}=0.57
ω-6:ω-3			
Q1	117	124	1.0
Q2	108	122	0.89 (0.59, 1.36)
Q3	130	124	0.92 (0.61, 1.39)
Q4	116	122	0.92 (0.61, 1.40)
			p_{trend}=0.29

* Model adjusted for age, sex, education, BMI, reflux frequency, smoking status, caloric intake and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.9. Odds ratios and 95% confidence intervals for associations between PUFA nutrient measures and development of BE among participants of two US-based case-control studies of BE, using absolute value-based cutpoints

Measure	Cases N=471	Controls N=492	OR* (95%CI)
Tuna			
None	89	85	1.0
T1	155	163	0.88 (0.59, 1.31)
T2	124	137	0.89 (0.60, 1.33)
T3	103	107	0.93 (0.61, 1.43)
			p_{trend}=0.94
Fried fish			
None	169	186	1.0
T1	136	129	1.14 (0.81, 1.62)
T2	60	69	0.94 (0.62, 1.44)
T3	106	108	1.12 (0.78, 1.61)
			p_{trend}=0.76
Baked/broiled fish			
None	121	98	1.0
T1	152	136	0.94 (0.65, 1.36)
T2	99	130	0.63 (0.42, 0.94)
T3	99	128	0.69 (0.46, 1.02)
			p_{trend}=0.06
Shellfish			
None	131	129	1.0
T1	154	154	0.94 (0.66, 1.34)
T2	86	96	0.86 (0.57, 1.30)
T3	100	113	0.88 (0.60, 1.30)
			p_{trend}=0.75
ω-3			
Q1	142	123	1.0
Q2	106	123	0.72 (0.50, 1.06)
Q3	118	123	0.77 (0.51, 1.16)
Q4	105	123	0.61 (0.36, 1.03)
			p_{trend}=0.59

* Model adjusted for age, sex, education, caloric intake, study indicator, and number of FFQ items for each exposure
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.9 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA nutrient measures and development of BE among participants of two US-based case-control studies of BE, using absolute value-based cutpoints

Measure	Cases N=471	Controls N=492	OR* (95%CI)
ALA			
Q1	128	123	1.0
Q2	122	123	0.98 (0.67, 1.43)
Q3	103	123	0.81 (0.53, 1.24)
Q4	118	123	0.87 (0.52, 1.47)
			p_{trend}=0.67
EPA			
Q1	143	123	1.0
Q2	96	123	0.71 (0.48, 1.03)
Q3	130	123	0.98 (0.66, 1.44)
Q4	102	123	0.80 (0.50, 1.27)
			p_{trend}=0.56
DHA			
Q1	140	123	1.0
Q2	118	123	0.88 (0.61, 1.27)
Q3	113	123	0.86 (0.58, 1.27)
Q4	100	123	0.77 (0.50, 1.19)
			p_{trend}=0.79
DPA			
Q1	148	123	1.0
Q2	99	123	0.70 (0.48, 1.01)
Q3	108	123	0.75 (0.50, 1.12)
Q4	116	123	0.82 (0.48, 1.38)
			p_{trend}=0.28
ω-6			
Q1	127	123	1.0
Q2	120	123	0.95 (0.65, 1.39)
Q3	117	123	0.88 (0.57, 1.36)
Q4	107	123	0.81 (0.48, 1.36)
			p_{trend}=0.54

* Model adjusted for age, sex, education, caloric intake, study indicator, and number of FFQ items for each exposure
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 3.9 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA nutrient measures and development of BE among participants of two US-based case-control studies of BE, using absolute value-based cutpoints

Measure	Cases N=471	Controls N=492	OR* (95%CI)
LA			
Q1	127	123	1.0
Q2	119	123	0.94 (0.64, 1.37)
Q3	119	123	0.90 (0.59, 1.39)
Q4	106	123	0.80 (0.47, 1.35)
			p_{trend}=0.56
AA			
Q1	134	123	1.0
Q2	101	123	0.79 (0.54, 1.15)
Q3	117	123	0.85 (0.57, 1.28)
Q4	119	123	0.99 (0.59, 1.68)
			p_{trend}=0.22
ω-6:ω-3			
Q1	117	123	1.0
Q2	115	123	0.98 (0.68, 1.42)
Q3	123	123	1.08 (0.75, 1.57)
Q4	116	123	1.03 (0.71, 1.49)
			p_{trend}=0.72

* Model adjusted for age, sex, education, caloric intake, study indicator, and number of FFQ items for each exposure
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

CHAPTER IV: FISH AND POLYUNSATURATED FATTY ACID DIETARY INTAKE MEASURES IN RELATION TO ESOPHAGEAL AND GASTRIC CARDIA ADENOCARCINOMA

Background

Esophageal adenocarcinoma (EA)/gastric cardia adenocarcinoma (GCA) incidence in the United States (US) has rapidly increased over the last few decades [18], one of the most pronounced increases in cancer incidence over this time period [19]. These tumors are also highly fatal, with a median survival of ~11 months [2]. EA typically occurs in the lower third of the esophagus near the gastroesophageal junction [10]. GCA is a tumor of the metaplastic junctional epithelium, in the portion of the stomach closest to the gastroesophageal junction [7]. EA and GCA are often considered related entities due to their anatomically adjacent positions, similar histological features, risk factors, and treatments, and poor survival [123].

Established risk factors for EA/GCA include increasing age [169], white race [169], male sex [169], reflux symptoms [143], obesity [154], and cigarette smoking [161]. The first three are non-modifiable, while clinically diagnosed gastroesophageal reflux disease is a chronic condition requiring treatment and maintenance therapy with frequent relapse [238]. Obesity and cigarette smoking are two potentially modifiable factors; however, weight loss [239] and smoking cessation [240] are difficult to achieve and, especially for weight loss [239], difficult to maintain. Several factors associated with

reduced risk of EA/GCA have been identified, including *Helicobacter pylori* [76] (a bacterial infection of the gastric mucosa which predisposes to gastric adenocarcinoma [312]), and nonsteroidal anti-inflammatory drugs (NSAIDs [284]; but are problematic because of well-known gastric side effects [244]). Two additional risk reduction factors that may be promising as potential intervention strategies include physical activity [157], and perhaps fruits and vegetables [165]. Factors associated with EA/GCA prognosis, other than clinical parameters such as stage [181] and treatment [182], are relatively understudied but include an inverse association with obesity [188].

Another factor that may be associated with decreased risk of EA/GCA is fish intake, which is high in ω-3 polyunsaturated fatty acids (PUFAs) [8]. Several, but not all, epidemiologic studies have shown that diets high in fish intake are inversely associated with EA and GCA incidence [221,225]. The ω-3 fatty acids, which are essential dietary fats [8], are hypothesized to partially account for any risk reductions due to their demonstrated anti-carcinogenic properties in experimental studies [8]. In addition to the beneficial ω-3 fatty acids, the PUFA family also includes ω-6 fatty acids (vegetable oils, lard, etc., are examples of ω-6-rich foods [8]), which have demonstrated carcinogenic properties in experimental studies [8]. Trends in PUFA consumption have generally paralleled trends observed for EA/GCA. PUFA intake has drastically changed over time, with marked increase in ω-6 consumption over the last century [25]. Additionally PUFA intake varies by geography. Intake of ω-6 in a typical western diet greatly outweighs ω-3, with ω-6:ω-3 intake ratios ranging between 15:1 and 20:1 [37]. In Asian countries, where incidence rates of EA and GCA are low [35], ratios are estimated to be 4:1-6:1 [37]. The ω-3 and ω-6 PUFAs compete for the same binding sites on enzymes in

metabolic pathways, particularly through inflammation and oxidative stress, and are known to play a role in carcinogenesis and cancer progression [212].

We hypothesized that higher intake of non-fried fish and other measures of ω-3 PUFAs would be associated with reduced risk of EA/GCA incidence and mortality, while ω-6 or the balance of ω-3 and ω-6 PUFAs (which are dominated by ω-6 intake [25]) would be associated with increased risk. If associations with dietary intake of PUFAs are confirmed, increasing ω-3 intake and/or decreasing ω-6 intake may have the potential to decrease the burden associated with EA/GCA. To examine our hypotheses, we harmonized, pooled, and analyzed data from two US-based case-control studies of EA/GCA.

Methods

We used resources from two member studies of the International Barrett's and Esophageal Adenocarcinoma Consortium (BEACON): the US Multicenter Study [173] and the Los Angeles County (LAC) Multiethnic Study [260]. These studies were chosen because of their similarities in study design, participant selection, interview methods, and food frequency questionnaire (FFQ) structure. Participating institutions received study approval from their respective Institutional Review Boards. Details of the included studies have been published previously [173,260], and are summarized below.

Study Populations

The US Multicenter Study was conducted from 1993-1995 in Connecticut, New Jersey, and western Washington state [173]. Case patients were 30-79 years old,

diagnosed with first primary EA/GCA, and identified through rapid reporting systems to state and hospital cancer registries. Controls were population-based, sampled using modified Waksberg random telephone digit dialing [261] for those 30-64 years and through Health Care Financing Administration rosters for those 65 and older, and frequency matched with cases on age, sex, and in New Jersey, race.

The LAC Multiethnic Study was conducted from 1992-1997 [260]. Case patients were aged 30-74 years, newly diagnosed with first primary EA/GCA, and identified by the LAC Cancer Surveillance Program. Neighborhood controls, who were identified through an algorithm based on case address, were matched to cases on age (±5 years), sex, and race.

In the current study, we excluded respondents without dietary intake assessment and those with estimated energy intakes outside of ±3 standard deviations on the log scale from the individual study means. Thus, our final population size consisted of 488 EA case patients, 512 GCA case patients, and 1995 control participants.

Both parent studies included follow-up components to determine vital status of the EA/GCA case patients. Mortality and date of death were determined through linkage with the National Death Index, through 2000 for the US Multicenter Study [181] and 2004 for the LAC Multiethnic Study. An event was defined as death from any cause during follow-up, and participants alive at the end of follow-up were considered censored. Overall survival time was calculated in months from the date of diagnosis until date of death/censoring. Due to the aggressive nature and short survival (average of less than a year) of these cancers [2], all-cause mortality approximates EA/GCA-specific mortality. The US Multicenter Study identified 460 deaths and the LAC

Multiethnic Study identified 424 deaths, for a total of 884 deaths among the 1027 EA/GCA patients in our pooled study.

Study Interviews

Before the interview, informed consent was obtained from all participants or their proxy respondent. Trained interviewers administered structured questionnaires assessing demographic information, lifestyle behaviors (including cigarette smoking and alcohol use), anthropometric measures, and medical history. Diet history was collected using validated FFQs [270,271]. The US Multicenter Study used a modified 104-item Fred Hutchinson Cancer Research Center FFQ [270], where a medium portion size was assumed and dietary intake was assessed three to five years prior to diagnosis date (cases) or interview date (controls). The LAC Multiethnic Study used the 124-item University of Hawaii FFQ [271], where portion size was ascertained and dietary intake was assessed one year prior to date of EA/GCA diagnosis for cases and same reference date for controls.

Exposure Assessment

We harmonized dietary responses by linking study-specific FFQs with the University of Minnesota Nutrition Coordinating Center nutrient database [278] to estimate grams of intake per day of PUFA exposures. The 13 PUFA measures used were: baked/broiled fish (non-fried fish other than tuna and shellfish); fried fish; tuna (tuna/tuna salad/tuna casserole); shellfish; ω-3; ω-3 subtypes (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic

acid (DPA)); ω-6; ω-6 subtypes (linoleic acid (LA) and arachidonic acid (AA)); and ω-6:ω-3 ratio.

As an example, DHA intake from a single line item was calculated as follows: *DHA intake for line item = reported average frequency of intake x reported average portion size x DHA nutrient grams per 100 grams of line item.* Intake of DHA per day for a participant would then be calculated by summing DHA intake for all line items. Many line items represented multiple foods, and in those cases the foods were weighted according to estimates of US consumption [310].

From the harmonized PUFA intakes, we generated study-specific quantiles of intake using the control distributions within each of the studies [282]. The PUFA exposure quantiles were then pooled by merging across the quantiles for the two studies. For the PUFA-rich food measures, we used an ordinal categorization of non-consumers and tertiles of exposure among consumers. For the PUFA nutrient measures, we used quartiles of exposure.

Other covariates used in the pooled study reported here were derived from the parent study-specific questionnaires, and were harmonized as previously described [143,154,161,284].

Statistical Methods

Logistic regression and polytomous logistic regression [265] were used to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) for associations between PUFAs and risk of EA and GCA development. Cox proportional hazards regression models [265] were used to estimate hazard ratios (HRs) and their 95% CIs

for associations between PUFAs and the risk of mortality after EA/GCA. The proportional hazards assumption was assessed by using an interaction between exposure and follow-up time [265]. Linear trends were examined by modeling exposure variables as continuous variables.

Study indicator [282] and total energy intake (kcal/day) [265] were included in all models. The matching factors (age (continuous), sex (women/men), and race (white/black/Hispanic/other)) were included in all logistic regression models. Additional confounders were selected using outcome-specific directed acyclic graphs (DAGs) [265]. Potential confounders for EA/GCA development included covariates that could possibly impact any of the exposure-outcome associations considered, such as education, obesity, reflux symptom frequency, cigarette smoking, alcohol use, fruit and vegetable intake, and proxy status. Potential confounders for mortality after EA/GCA included age, education, obesity, reflux symptom frequency, cigarette smoking, proxy status, and disease stage. The final DAG-identified minimally sufficient adjustment set for EA/GCA development included education (<high school/high school/some college/college) and proxy status (proxy/non-proxy). The final DAG-identified adjustment set for mortality after EA/GCA included age (continuous), education (<high school/high school/some college/college), and proxy status (proxy/non-proxy).

For both outcomes (EA/GCA development and mortality after EA/GCA), modification was assessed for factors thought to act through carcinogenic mechanisms involving inflammation and oxidative stress [154,284,313,314], and included body mass index (BMI; <25.0 kg/m^2/≥25.0 kg/m^2 [292]), NSAID use (never regular users/ever regular users), cigarette smoking status (never/former/current), and reflux symptom

frequency (<once per week/≥once per week). Modification was assessed on the multiplicative scale by comparing models with and without interaction terms using the likelihood ratio statistic with a significance level of 0.05 [265]. Modification was also assessed on the additive scale by using single referent models to obtain interaction contrast ratios (ICRs) and 95% CIs [265]. Interactions between ω-3 and ω-6 were assessed using the same approaches.

Five sensitivity analyses were conducted for both outcomes (EA/GCA development and mortality after EA/GCA). First, we augmented our models by including additional confounders in our models, specifically known risk factors (in addition to the adjustment set identified by DAG). For EA/GCA development we added BMI, reflux frequency, and cigarette smoking status; and for mortality after EA/GCA we added BMI. For the second sensitivity analysis we used the absolute value-based pooling approach, deriving quantiles among all combined controls (EA/GCA development) or all case patients (mortality after EA/GCA), rather than use the study-specific pooling approach described above [282]. In the third sensitivity analysis we examined associations with proxy respondents removed from the analysis. Fourth, we widened the acceptable caloric intake by excluding only participants in the highest and lowest 2.5% of daily caloric intake (rather than outside of ±3 standard deviations on the log scale as the cut-off values for the main analyses). For the fifth sensitivity analysis we used a random effects meta-analytic approach [265] to pool study-specific estimates.

Analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC), and Stata software, version 15.0 (StataCorp LP, College Station, TX).

Results

Characteristics of participants by study and by case-control status are shown in **Table 4.1**. The two studies had similar distributions of age, sex, BMI, cigarette smoking status, and use of proxy interviews. The LAC Multiethnic study had a higher proportion of non-white participants, higher educational attainment, lower NSAID use, more frequent reflux symptoms, and higher daily caloric intake when compared to the US Multicenter Study.

Table 4.2 shows daily mean intake of PUFAs and fish by study and by case-control status. Among controls, the US Multicenter Study had higher intake of ω-6 and lower intake of ω-3. These differences combined to yield a higher average of ω-6:ω-3 intake in the US Multicenter Study. The only other major difference was for baked/broiled fish, where controls in the LAC Multiethnic Study had higher intake.

Table 4.3 presents food items that were the highest contributing (a combination of the most frequently eaten and PUFA-dense) line items to the PUFA exposure variables. Many of the same foods were high contributors to both ω-3 and ω-6 intake. For example, mayonnaise and creamy dressings were primary ω-3 and ω-6 contributors in both studies. Additionally, because of the approach used in the parent study FFQs, the tuna variable included items typically made with mayonnaise (tuna salad) or other creamy dressings (tuna casserole) as well as fresh and canned tuna. In comparison, the baked/broiled fish variable included only non-fried fish.

When we examined associations between the PUFA measures and the risk of developing EA or GCA, results from single and polytomous logistic regression models did not substantially differ, thus only those from the polytomous models are shown here.

Higher intake of baked/broiled fish was associated with an ~30% reduced risk of developing EA ($OR_{T3vs.None}$=0.67, 95%CI=0.44-1.01, p_{trend}=0.3) and GCA ($OR_{T3vs.None}$=0.69, 95%CI=0.48-0.99, p_{trend}=0.4) (**Table 4.4**). In contrast, tuna had a positive association with EA development ($OR_{T3vs.None}$=1.46, 95%CI=1.00-2.13, p_{trend}=0.9), but not GCA ($OR_{T3vs.None}$=0.89, 95%CI=0.63-1.24, p_{trend}=0.9). Fried fish had a positive association with GCA ($OR_{Consumersvs.Non}$=1.33, 95%CI=1.06-1.66), but not EA ($OR_{Consumersvs.Non}$=1.00, 95%CI=0.80-1.26). EA and GCA development was positively associated with ω-6 intake ($OR_{Q4vs.Q1}$=2.62, 95%CI=1.73-3.97, p_{trend}=0.003 and $OR_{Q4vs.Q1}$=1.45, 95%CI=0.99-2.13, p_{trend}=0.04, respectively). In contrast to our hypotheses, ω-3 intake also was associated with an increased risk of developing EA ($OR_{Q4vs.Q1}$=2.58, 95%CI=1.73-3.84, p_{trend}=0.005) and GCA ($OR_{Q4vs.Q1}$=1.72, 95%CI=1.18-2.51, p_{trend}=0.2). There was no evidence of interaction between ω-3 and ω-6, nor modification by any factors examined (**Table 4.5**).

We found no association between the risk of mortality after EA and GCA and baked/broiled fish or most of the other dietary factors examined, with few exceptions (**Table 4.6**). In contrast to our hypotheses, the ω-6:ω-3 ratio was inversely associated with decreased EA mortality ($HR_{Q4vs.Q1}$=0.76, 95%CI=0.58-0.99, p_{trend}=0.07), but not GCA mortality ($HR_{Q4vs.Q1}$=1.21, 95%CI=0.92-1.61, p_{trend}=0.06). Consistent with our hypotheses, EPA ($HR_{Q4vs.Q1}$=0.77, 95%CI=0.58-1.03, p_{trend}=0.3) and DHA ($HR_{Q4vs.Q1}$=0.72, 95%CI=0.54-0.96, p_{trend}=0.08) were associated with decreased EA mortality; but, in contrast to our hypotheses, these ω-3 subtypes were not associated with GCA mortality. There was no evidence of interaction or modification by any factors examined (**Table 4.7**).

In sensitivity analyses, when we added to our models known risk factors for EA/GCA (BMI, reflux frequency, and smoking for EA/GCA development, and BMI for mortality after EA/GCA) that were not DAG-identified as confounders, findings (**Tables 4.8** and **4.9**) were similar to those shown in **Tables 4.4** and **4.6.** Results generated in the other sensitivity analyses outlined in the Methods (data not shown) were also not substantially different from the primary results presented.

Discussion

In this pooled study of US-based participants, higher intake of baked/broiled fish was associated with a 31-33% reduced risk of developing EA and GCA, and ω-6 was positively associated a 2.6-fold increased risk of developing EA and 45% increased risk of developing GCA, all of which were consistent with our hypotheses. However, in contrast to our hypotheses, ω-3 was positively associated with a 2.6-fold increased risk of developing EA and 72% increased risk of developing GCA, whereas ω-6:ω-3 was associated with a 24% reduction in the risk of EA mortality.

Our findings on baked/broiled fish (which are ω-3-dense foods [8]) and ω-6 fatty acids are biologically plausible. The families of PUFAs, ω-3 and ω-6, compete for the same binding sites on enzymes, but they have very different biochemical roles [8,212]. In experimental studies, higher levels of ω-3 fatty acids decrease production of ω-6-derived byproducts and produce ω-3-derived molecules which are considered less biologically active [212]; ω-3 also has been associated with lower expression of COX-2 and COX-2 inhibitors which have been shown to suppress tumor growth, angiogenesis, and metastasis [212]. Excess ω-6 is considered carcinogenesis-promoting in

experimental studies, and the byproducts have been shown to increase inflammation, angiogenesis, cellular proliferation and reduce apoptosis [8,212]. However, in humans, ω-6 intake has been inconsistently linked to chronic diseases [311].

Our biologically plausible results on baked/broiled fish are consistent with some [221,225], but not all previous epidemiologic studies [218,222,223,227]. A case-control study from Nebraska found a substantial decrease in risk of EA comparing those consuming the highest quantities of fish (fresh, frozen, or canned) to those consuming the lowest ($OR_{Q4vs.Q1}$=0.14, 95%CI=0.04-0.48, p_{trend}=0.0001) [221]. Using the National Institutes of Health-AARP Study, Daniel et al. found a decreased risk of EA ($HR_{Q5vs.Q1}$=0.78, 95%CI=0.59-1.03, p_{trend}=0.06) with increased intake of fish (all types of fish/shellfish), but not for GCA [225]. However, the Factors Influencing the Barrett's Adenocarcinoma Relationship (FINBAR) Study, found a suggested increase in risk of EA with higher intake of fish, but CIs were wide [218]. A second analysis using the FINBAR data examined the associations between intake of oily fish and EA and still reported null results [223]. A manuscript using the LAC Multiethnic Study, included in this pooled study, found no associations between fish/shellfish intake and EA or GCA [222]. Previous inconsistencies may be due to the fact that most did not account for cooking methods [218,222,227], which may mask associations with ω-3-rich foods such as fish, given that frying adds ω-6. A few previous studies also observed inconsistent results for overall PUFA intake [218,222,226,228,229]. However, we elected not to use this PUFA measure, given that in western countries it is largely a surrogate measure of ω-6.

In contrast to our hypotheses, we observed a positive association for EA development with ω-3 intake, whereas others have reported no association [226]. Also, we found no association with the relative balance of ω-3 and ω-6 in association with EA/GCA development, which we had hypothesized could help to clarify previous reports of no association with PUFA intake [218,222,226,228,229], given these PUFA subtypes compete for the same enzymes, and their metabolic products may have differential roles in the carcinogenic process [8,212]. We are first to report an inverse association between ω-6:ω-3 and EA mortality, which is also inconsistent with our hypotheses. These unexpected findings could arise from our inability to differentiate ω-3 from ω-6 clearly when we used the nutrient-based measures (rather than the food-based measures that incorporated cooking methods), given that in our parent populations, many of the same foods were high in both PUFA types (e.g., meat, mayonnaise, salad dressing, potatoes, and baked goods, as shown in **Table 4.3**).

Several additional limitations require consideration when interpreting our results. First, the geographically based populations sampled in our two studies comprise relatively homogenous groups, largely whites, which enhances internal validity, but may limit generalizability. However, rates of EA/GCA are highest among this demographic [169], which allows for results to be generalizable to this high-risk group. Second, the time period assessed by the FFQs varied between one to five years prior to diagnosis/interview. Patients may have adjusted diet or altered recall due to unrecognized developing symptoms of these adenocarcinomas. However, as in most epidemiologic studies of dietary intake, we assumed participants did not change their diet during the latent period. Third, recall error is also a possibility due to the time period

assessed, but research has shown moderate correlation between current diet and diet three to ten years later [296]. Fourth, there may be some discrepancies in data collection, variable definitions, and data management across parent studies. We attempted to mitigate this concern by selecting two studies with similar designs and methodologic approaches. Also, we first harmonized individual responses across studies to ensure uniform definitions of all covariates prior to pooling. In addition, our results were similar to those shown here when in a sensitivity analysis we used random-effects meta-analysis, which requires fewer assumptions regarding the true association than does fixed-effect meta-analysis. Fifth, another possible limitation is residual confounding. Although we broadly considered potential confounders for all possible exposure-outcome associations, we were unable to consider the influence of additional interaction terms because of model failure. Finally, in order to maximize our ability to capture the complexity of PUFA intake in association with multiple outcomes in the esophageal/adenocarcinoma cancer continuum, we considered multiple PUFA measures in association with multiple outcomes. However, all of our measures and outcome associations were selected based on biologic plausibility and thus we did not correct for multiple comparisons.

Our pooled study has several additional strengths. Using a pooled approach, our study is larger than most existing studies [218,221-223,227-229]. The larger sample size gave us increased power to detect associations and resulted in more precise estimates of association. Also, a pooling approach allows for uniform definitions of exposures and covariates and improves accuracy of the resulting estimates. Further, the parent studies included in our pooled analysis were conducted in US coastal regions

allowing for increased opportunity for fish consumption. An additional strength of our pooled study is that both parent studies were population-based, which enhances study validity. Another strength of our study is that we used a variety of PUFA measures to better reflect the potential dual (carcinogenic and anti-carcinogenic properties) associated with fatty acid exposure in experimental studies [8]. This facilitated our ability to examine associations with an exposure – namely baked/broiled fish – that appeared to isolate the ω-3-rich fish from the ω-6-rich oils that are introduced using other cooking methods.

In summary, our study pooled two US-based case-control studies of EA/GCA and observed that higher intake of baked/broiled fish was associated with an ~30% decrease in the risk of developing EA and GCA. Also, intake of ω-6 and, surprisingly, ω-3 fatty acids were associated with an increased risk of EA/GCA development. Future studies should include improved PUFA measures, while also accounting for cooking methods, in order to isolate the potentially opposing properties of ω-3 and ω-6 PUFA intake. If confirmed, increasing non-fried fish intake may be a potential promising risk reduction strategy for these lethal cancers with increasing incidence in the US and other western countries.

Table 4.1. Characteristics of participants of two US-based case-control studies of EA/GCA

Characteristic (N (%))	US Multicenter Study			LAC Multiethnic Study		
	EA Cases N=282	GCA Cases N=256	Controls N=687	EA Cases N=217	GCA Cases N=272	Controls N=1340
Age (yrs)*	64.3 (10.7)	63.1 (10.9)	62.8 (10.6)	61.1 (9.4)	60.7 (10.2)	58.9 (11.4)
Sex						
Male	235 (83.3)	218 (85.2)	549 (79.9)	197 (90.8)	226 (83.1)	990 (73.9)
Female	47 (16.7)	38 (14.8)	138 (20.1)	20 (9.2)	46 (16.9)	350 (26.1)
Race						
White	268 (95.0)	243 (94.9)	617 (89.8)	168 (77.4)	206 (75.7)	836 (62.4)
Other	14 (5.0)	13 (5.1)	70 (10.2)	49 (22.6)	66 (24.3)	504 (37.6)
Education						
<High school	62 (22.1)	54 (21.2)	128 (18.6)	47 (21.7)	51 (18.8)	248 (18.5)
High school	90 (32.0)	84 (32.9)	174 (25.3)	50 (23.0)	67 (24.6)	249 (18.6)
Some college	75 (26.7)	59 (23.1)	174 (25.3)	61 (28.1)	85 (31.3)	384 (28.7)
College	54 (19.2)	58 (22.8)	211 (30.7)	59 (27.2)	69 (25.4)	459 (34.3)
BMI (kg/m^2)*	26.3 (4.1)	26.0 (4.3)	24.9 (3.4)	27.1 (5.2)	26.9 (5.3)	25.9 (4.6)
Cigarette smoking status						
Never	56 (20.4)	44 (17.9)	213 (32.4)	48 (22.1)	78 (28.7)	536 (40.0)
Former	141 (51.3)	121 (49.2)	294 (44.8)	103 (47.5)	120 (44.1)	583 (43.5)
Current	78 (28.4)	81 (32.9)	150 (22.8)	66 (30.4)	74 (27.2)	221 (16.5)
NSAID use						
Never	183 (67.8)	151 (60.6)	408 (60.0)	153 (71.2)	174 (64.4)	913 (68.2)
Ever	87 (32.2)	98 (39.4)	272 (40.0)	62 (28.8)	96 (35.6)	425 (31.8)

* Mean (SD)
Missing values (N): education (2), BMI (44) cigarette smoking status (47), NSAID use (32), reflux frequency (22)

Table 4.1 (cont'd). Characteristics of participants of two US-based case-control studies of EA/GCA

Characteristic (N (%))	US Multicenter Study			LAC Multiethnic Study		
	EA Cases N=282	GCA Cases N=256	Controls N=687	EA Cases N=217	GCA Cases N=272	Controls N=1340
Reflux frequency						
<Weekly	181 (69.6)	205 (84.4)	570 (88.2)	121 (56.3)	191 (72.1)	1127 (84.3)
≥Weekly	79 (30.4)	38 (15.6)	76 (11.8)	94 (43.7)	74 (27.9)	210 (15.7)
Total energy intake (kcal/day)*	1993.2 (603.6)	2038.8 (671.2)	1929.2 (673.9)	2835.1 (1237.5)	2757.9 (1341.5)	2644.8 (1197.5)
Proxy interview	87 (30.9)	66 (25.8)	--	65 (30.0)	84 (30.9)	--

* Mean (SD)
Missing values (N): education (2), BMI (44) cigarette smoking status (47), NSAID use (32), reflux frequency (22)

Table 4.2. Daily mean intake of PUFAs and fish among participants of two US-based case-control studies of EA/GCA

Measure (mean (SD))	US Multicenter Study			LAC Multiethnic Study		
	EA Cases N=282	GCA Cases N=256	Controls N=687	EA Cases N=217	GCA Cases N=272	Controls N=1340
Tuna (g/day)	11.17 (13.33)	11.47 (15.03)	10.21 (13.63)	9.16 (12.95)	9.66 (18.77)	10.44 (17.25)
Fried fish (g/day)	3.98 (6.84)	5.61 (6.39)	4.96 (8.70)	4.78 (13.03)	5.83 (13.94)	5.21 (10.36)
Baked/broiled fish (g/day)	7.71 (8.68)	7.86 (8.97)	8.78 (10.10)	9.14 (12.83)	9.18 (18.04)	10.65 (16.97)
Shellfish (g/day)	2.38 (3.20)	2.59 (3.85)	2.71 (4.11)	4.12 (8.38)	5.87 (13.82)	4.10 (8.42)
ω-3 (g/day)	2.64 (1.32)	2.73 (1.60)	2.39 (1.42)	3.46 (4.15)	2.84 (1.76)	2.74 (2.11)
ALA (g/day)	2.35 (1.12)	2.44 (1.36)	2.10 (1.21)	3.30 (4.08)	2.67 (1.72)	2.58 (2.07)
EPA (g/day)	0.06 (0.06)	0.06 (0.07)	0.06 (0.06)	0.04 (0.05)	0.04 (0.05)	0.04 (0.04)
DHA (g/day)	0.16 (0.17)	0.16 (0.21)	0.16 (0.17)	0.10 (0.09)	0.10 (0.10)	0.10 (0.09)
DPA (g/day)	0.10 (0.11)	0.10 (0.13)	0.09 (0.11)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
ω-6 (g/day)	24.71 (13.62)	25.68 (16.75)	22.47 (14.39)	24.47 (39.82)	19.41 (13.19)	18.41 (16.22)
LA (g/day)	24.02 (13.01)	25.0 (16.0)	21.85 (13.75)	24.29 (39.64)	19.26 (13.12)	18.26 (16.14)
AA (g/day)	0.69 (0.75)	0.72 (0.84)	0.62 (0.77)	0.18 (0.20)	0.16 (0.10)	0.14 (0.10)
ω-6:ω-3	9.35 (1.94)	9.38 (1.84)	9.38 (2.07)	6.91 (1.52)	6.85 (1.45)	6.81 (1.39)

Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish

Table 4.3. Number of FFQ items and foods with highest contribution for PUFA nutrient and fish intake measures among control participants (N=2027) of two US-based case-control studies of EA/GCA

Measure	US Multicenter Study (N=104 line items)		LAC Multiethnic Study (N=124 line items)	
	# of items on FFQ	Five highest contributing FFQ items	# of items on FFQ	Five highest contributing FFQ items
Tuna	1	Canned tuna, tuna salad, tuna casserole	1	Canned tuna salmon, sardines, mackerel, plain, salad, or casserole
Fried fish	1	Fried fish/shellfish, fish sandwich	1	Fried/breaded fish sticks, fillets, patties
Baked/broiled fish	1	Other fish, broiled, baked	1	Other fresh or frozen fish, broiled, baked
Shellfish	1	Shellfish, not fried	1	Shellfish
ω-3	104	Beef, veal, lamb, pork (not ham); chicken, turkey; mayonnaise, mayonnaise type spreads; salad dressing; French fries, fried potatoes	112	Margarine on vegetables, rice; mayonnaise or creamy salad dressing; other potatoes, baked, boiled, mashed; cornbread, corn muffin, corn tortilla; doughnuts, cookies, cakes, pastries
ALA	104	Beef, veal, lamb, pork (not ham); chicken, turkey; mayonnaise, mayonnaise type spreads; salad dressing; French fries, fried potatoes	111	Margarine on vegetables, rice; mayonnaise or creamy salad dressing; other potatoes, baked, boiled, mashed; cornbread, corn muffin, corn tortilla; doughnuts, cookies, cakes, pastries
EPA	31	Chicken, turkey; other fish, broiled, baked; beef, veal, lamb, pork (not ham); shellfish, not fried; smoked fish or lox	31	Canned tuna salmon, sardines, mackerel, plain, salad, or casserole; shellfish; other fresh or frozen fish broiled, baked; salted, preserved, pickled fish; chicken, turkey
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish				

Table 4.3 (cont'd). Number of FFQ items and foods with highest contribution for PUFA nutrient and fish intake measures among control participants (N=2027) of two US-based case-control studies of EA/GCA

Measure	US Multicenter Study (N=104 line items)		LAC Multiethnic Study (N=124 line items)	
	# of items on FFQ	Five highest contributing FFQ items	# of items on FFQ	Five highest contributing FFQ items
DHA	33	Chicken, turkey; other fish, broiled, baked; eggs; canned tuna, tuna salad, tuna casserole; fried fish/shellfish, fish sandwich	31	Other fresh or frozen fish broiled, baked; canned tuna salmon, sardines, mackerel, plain, salad, or casserole; fried eggs, scrambled, omelet; shellfish; chicken, turkey
DPA	28	Chicken, turkey; beef, veal, lamb, pork (not ham); other fish, broiled, baked; cheese, cheese spreads; non-smoked poultry lunchmeat	26	Chicken, turkey; beef; other fresh or frozen fish broiled, baked; canned tuna salmon, sardines, mackerel, plain, salad, or casserole; tacos, tostadas, fajitas
ω-6	109	Chicken, turkey; beef, veal, lamb, pork (not ham); mayonnaise, mayonnaise type spreads; salad dressing; peanut butter, peanuts, nuts/seeds	116	Mayonnaise or creamy salad dressing; cornbread, corn muffin, corn tortilla; other potatoes, baked, boiled, mashed; salty snacks, peanuts, chips, pretzels; doughnuts, cookies, cakes, pastries
LA	109	Chicken, turkey; beef, veal, lamb, pork (not ham); mayonnaise, mayonnaise type spreads; salad dressing; peanut butter, peanuts, nuts/seeds	116	Mayonnaise or creamy salad dressing; cornbread, corn muffin, corn tortilla; other potatoes, baked, boiled, mashed; salty snacks, peanuts, chips, pretzels; doughnuts, cookies, cakes, pastries
AA	48	Beef, veal, lamb, pork (not ham); chicken, turkey; eggs; ground beef (e.g. hamburger, meatballs); liver, other organ meats	53	Fried eggs, scrambled, omelet; chicken, turkey; cornbread, corn muffin, corn tortilla; other eggs boiled, poached; beef

Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish

Table 4.4. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
Tuna					
None	504	77	1.0	124	1.0
T1	386	96	1.54 (1.06, 2.24)	90	0.91 (0.64, 1.27)
T2	714	231	1.58 (1.13, 2.21)	216	0.98 (0.73, 1.32)
T3	423	95	1.46 (1.00, 2.13)	98	0.89 (0.63, 1.24)
			p_{trend}=0.92		p_{trend}=0.86
Fried fish					
Non-consumers	1086	268	1.0	251	1.0
Consumers	941	231	1.00 (0.80, 1.26)	277	1.33 (1.06, 1.66)
Baked/broiled fish					
None	662	151	1.0	194	1.0
T1	352	79	1.01 (0.72, 1.43)	79	0.72 (0.52, 1.00)
T2	688	222	1.05 (0.79, 1.41)	191	0.73 (0.56, 0.97)
T3	325	47	0.67 (0.44, 1.01)	64	0.69 (0.48, 0.99)
			p_{trend}=0.32		p_{trend}=0.36
Shellfish					
Non-consumers	966	203	1.0	229	1.0
Consumers	1061	296	1.22 (0.96, 1.55)	299	1.13 (0.90, 1.43)
ω-3					
Q1	507	75	1.0	101	1.0
Q2	507	126	1.98 (1.38, 2.83)	128	1.40 (1.01, 1.95)
Q3	507	131	1.89 (1.30, 2.74)	134	1.34 (0.95, 1.89)
Q4	506	167	2.58 (1.73, 3.84)	165	1.72 (1.18, 2.51)
			p_{trend}=0.004		p_{trend}=0.19

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.4 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
ALA					
Q1	507	68	1.0	99	1.0
Q2	506	125	2.01 (1.39, 2.91)	121	1.26 (0.90, 1.76)
Q3	508	141	2.21 (1.51, 3.23)	143	1.47 (1.04, 2.07)
Q4	506	165	2.82 (1.86, 4.28)	165	1.69 (1.15, 2.49)
			p_{trend}=0.005		p_{trend}=0.28
EPA					
Q1	507	117	1.0	139	1.0
Q2	507	132	1.13 (0.82, 1.56)	140	0.96 (0.71, 1.30)
Q3	507	127	1.14 (0.83, 1.59)	120	0.87 (0.63, 1.18)
Q4	506	123	1.22 (0.87, 1.70)	129	1.00 (0.73, 1.37)
			p_{trend}=0.25		p_{trend}=0.09
DHA					
Q1	507	136	1.0	147	1.0
Q2	507	108	0.83 (0.61, 1.15)	129	0.88 (0.65, 1.18)
Q3	507	142	1.20 (0.88, 1.64)	129	0.97 (0.72, 1.32)
Q4	506	113	0.92 (0.66, 1.28)	123	0.86 (0.63, 1.18)
			p_{trend}=0.49		p_{trend}=0.21
DPA					
Q1	507	99	1.0	122	1.0
Q2	507	121	1.37 (0.98, 1.92)	129	1.17 (0.85, 1.60)
Q3	507	149	1.80 (1.29, 2.50)	140	1.28 (0.94, 1.76)
Q4	506	130	1.41 (0.99, 2.01)	137	1.17 (0.84, 1.64)
			p_{trend}=0.05		p_{trend}=0.04
ω-6					
Q1	507	68	1.0	107	1.0
Q2	507	132	1.97 (1.37, 2.84)	122	1.14 (0.82, 1.58)
Q3	507	137	2.07 (1.41, 3.03)	137	1.17 (0.83, 1.66)
Q4	506	162	2.62 (1.73, 3.97)	162	1.45 (0.99, 2.13)
			p_{trend}=0.003		p_{trend}=0.04

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.4 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
LA					
Q1	506	69	1.0	107	1.0
Q2	508	134	1.94 (1.35, 2.79)	126	1.16 (0.84, 1.61)
Q3	507	134	1.95 (1.33, 2.86)	133	1.11 (0.79, 1.57)
Q4	506	162	2.57 (1.69, 3.89)	162	1.44 (0.98, 2.12)
			p_{trend}=0.003		p_{trend}=0.05
AA					
Q1	506	86	1.0	113	1.0
Q2	507	132	1.71 (1.21, 2.41)	121	1.12 (0.81, 1.55)
Q3	508	127	1.57 (1.10, 2.23)	139	1.27 (0.92, 1.76)
Q4	506	154	2.10 (1.45, 3.03)	155	1.52 (1.08, 2.15)
			p_{trend}=0.01		p_{trend}=0.007
ω-6:ω-3					
Q1	507	129	1.0	116	1.0
Q2	506	120	1.01 (0.74, 1.39)	148	1.41 (1.04, 1.91)
Q3	507	125	0.94 (0.69, 1.29)	144	1.20 (0.88, 1.64)
Q4	507	125	1.01 (0.73, 1.38)	120	1.05 (0.76, 1.44)
			p_{trend}=0.76		p_{trend}=0.98

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.5. Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA and GCA when examining modification among participants of two US-based case-control studies of EA/GCA

Modifier	Exposure	Cases	Controls N=2027	Multiplicative scale Stratified ORs* (95% CIs)	$p_{interaction}$	Additive Scale Single referent ORs* (95% CIs)	Additive ICR (95% CI)
Esophageal adenocarcinoma (N=499 cases)							
ω-3	**ω-6**						
≤Median	>Median	29	111	1.0		1.0	
	≤Median	172	903	0.90 (0.77, 1.06)		0.90 (0.77, 1.06)	
>Median	>Median	270	902	1.0		1.10 (0.93, 1.29)	
	≤Median	28	111	0.89 (0.71, 1.12)	0.84	0.97 (0.69, 1.37)	-0.03 (-0.18, 0.13)
BMI (kg/m^2)	**Baked/broiled fish**						
25+	≤Median	492	149	1.0		1.0	
	>Median	478	147	0.97 (0.87, 1.08)		0.97 (0.87, 1.08)	
<25	≤Median	493	96	1.0		0.77 (0.69, 0.85)	
	>Median	538	99	0.94 (0.80, 1.10)	0.57	0.72 (0.60, 0.87)	-0.02 (-0.06, 0.06)
NSAID use	**Baked/broiled fish**						
Ever	≤Median	342	77	1.0		1.0	
	>Median	355	72	0.96 (0.86, 1.07)		0.96 (0.86, 1.07)	
Never	≤Median	654	166	1.0		1.18 (1.06, 1.32)	
	>Median	667	170	1.00 (0.88, 1.13)	0.50	1.18 (1.00, 1.39)	0.04 (-0.09, 0.16)
Smoking status	**Baked/broiled fish**						
Ever	≤Median	653	199	1.0		1.0	
	>Median	595	189	0.99 (0.88, 1.12)		0.99 (0.88, 1.12)	
Never	≤Median	340	47	1.0		0.73 (0.65, 0.83)	
	>Median	409	57	0.96 (0.78, 1.19)	0.65	0.71 (0.56, 0.89)	-0.02 (-0.09, 0.06)

* Adjusted for age, sex, race, caloric intake, and study indicator
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median derived from the controls' distribution of intake

Table 4.5 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA and GCA when examining modification among participants of two US-based case-control studies of EA/GCA

Modifier	Exposure	Cases	Controls N=2027	Multiplicative scale Stratified ORs* (95% CIs)	$p_{interaction}$	Additive Scale Single referent ORs* (95% CIs)	Additive ICR (95% CI)
Esophageal adenocarcinoma (N=499 cases)							
Reflux frequency	**Baked/broiled fish**						
≥Weekly	≤Median	159	80	1.0		1.0	
	>Median	127	93	1.02 (0.90, 1.15)		1.02 (0.90, 1.15)	
<Weekly	≤Median	821	158	1.0		0.52 (0.47, 0.59)	
	>Median	876	144	0.90 (0.80, 1.02)	0.09	0.48 (0.40, 0.57)	-0.06 (-0.16, 0.04)
Gastric cardia adenocarcinoma (N=528 cases)							
ω-3	**ω-6**						
≤Median	>Median	30	111	1.0		1.0	
	≤Median	199	903	0.96 (0.82, 1.12)		0.96 (0.82, 1.12)	
>Median	>Median	269	902	1.0		1.05 (0.90,1.23)	
	≤Median	30	111	0.97 (0.78, 1.21)	0.86	1.02 (0.74, 1.42)	0.01 (-0.15, 0.17)
BMI (kg/m^2)	**Baked/broiled fish**						
25+	≤Median	492	167	1.0		1.0	
	>Median	478	128	0.86 (0.77, 0.95)		0.86 (0.77, 0.95)	
<25	≤Median	493	120	1.0		0.84 (0.76, 0.93)	
	>Median	538	103	0.86 (0.74, 1.00)	0.95	0.72 (0.60, 0.86)	0.03 (-0.05, 0.10)
NSAID use	**Baked/broiled fish**						
Ever	≤Median	342	97	1.0		1.0	
	>Median	355	97	0.87 (0.79, 0.97)		0.87 (0.79, 0.97)	
Never	≤Median	654	192	1.0		0.99 (0.89, 1.10)	
	>Median	667	133	0.80 (0.71, 0.91)	0.10	0.79 (0.67, 0.94)	-0.07 (-0.15, 0.02)

* Adjusted for age, sex, race, caloric intake, and study indicator
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median derived from the controls' distribution of intake

Table 4.5 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA and GCA when examining modification among participants of two US-based case-control studies of EA/GCA

Modifier	Exposure	Cases	Controls N=2027	Multiplicative scale Stratified ORs* (95% CIs)	$p_{interaction}$	Additive Scale Single referent ORs* (95% CIs)	Additive ICR (95% CI)
Gastric cardia adenocarcinoma (N=528 cases)							
Smoking status	**Baked/broiled fish**						
Ever	≤Median	653	229	1.0		1.0	
	>Median	595	167	0.87 (0.78, 0.98)		0.87 (0.78, 0.98)	
Never	≤Median	340	60	1.0		0.78 (0.70, 0.88)	
	>Median	409	62	0.87 (0.72, 1.06)	1.0	0.68 (0.55, 0.85)	0.03 (-0.04, 0.10)
Reflux frequency	**Baked/broiled fish**						
≥Weekly	≤Median	159	63	1.0		1.0	
	>Median	127	49	0.87 (0.76, 0.99)		0.87 (0.76, 0.99)	
<Weekly	≤Median	821	223	1.0		0.75 (0.66, 0.85)	
	>Median	876	173	0.83 (0.74, 0.93)	0.49	0.63 (0.53, 0.74)	0.004 (-0.10, 0.10)

* Adjusted for age, sex, race, caloric intake, and study indicator
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
Median derived from the controls' distribution of intake

Table 4.6. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR** (95%CI)	Cases N=528	Deaths N=450	HR** (95%CI)
Tuna						
None	77	66	1.0	124	100	1.0
T1	143	126	1.21 (0.88, 1.65)	129	117	1.00 (0.76, 1.33)
T2	179	159	1.21 (0.89, 1.66)	174	144	0.98 (0.74, 1.28)
T3	100	83	1.02 (0.73, 1.43)	101	89	1.16 (0.86, 1.57)
			p_{trend}=0.30			p_{trend}=0.44
Fried fish						
Non-consumers	268	239	1.0	251	224	1.0
Consumers	231	195	0.95 (0.78, 1.16)	277	226	0.88 (0.72, 1.07)
Baked/broiled fish						
None	151	130	1.0	194	168	1.0
T1	79	69	1.10 (0.82, 1.49)	79	67	0.98 (0.73, 1.31)
T2	221	191	1.00 (0.78, 1.27)	186	155	0.84 (0.65, 1.10)
T3	48	44	0.80 (0.55, 1.16)	69	60	0.99 (0.73, 1.35)
			p_{trend}=0.08			p_{trend}=0.76
Shellfish						
Non-consumers	203	176	1.0	229	197	1.0
Consumers	296	258	1.01 (0.83, 1.24)	299	253	0.92 (0.75, 1.13)

* Missing (N): vital status (5)
** Model adjusted for age, education, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.6 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
ω-3						
Q1	116	99	1.0	141	118	1.0
Q2	129	118	1.19 (0.90, 1.57)	127	115	1.05 (0.80, 1.37)
Q3	126	108	0.97 (0.72, 1.31)	131	115	0.73 (0.55, 0.96)
Q4	128	109	1.03 (0.74, 1.45)	129	102	0.76 (0.55, 1.04)
			p_{trend}=0.21			p_{trend}=0.53
ALA						
Q1	117	102	1.0	140	117	1.0
Q2	126	110	0.96 (0.73, 1.27)	131	118	1.12 (0.87, 1.46)
Q3	123	110	0.98 (0.72, 1.31)	132	115	0.71 (0.53, 0.94)
Q4	133	112	0.90 (0.64, 1.25)	125	100	0.80 (0.58, 1.10)
			p_{trend}=0.26			p_{trend}=0.45
EPA						
Q1	119	105	1.0	138	120	1.0
Q2	129	113	0.92 (0.70, 1.20)	128	109	0.99 (0.76, 1.30)
Q3	127	109	0.85 (0.65, 1.13)	130	107	0.86 (0.66, 1.13)
Q4	124	107	0.77 (0.58, 1.03)	132	114	1.15 (0.87, 1.51)
			p_{trend}=0.29			p_{trend}=0.36

* Missing (N): vital status (5)
** Model adjusted for age, education, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.6 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
DHA						
Q1	127	110	1.0	129	114	1.0
Q2	111	97	0.98 (0.74, 1.31)	146	118	0.73 (0.56, 0.95)
Q3	137	121	1.15 (0.88, 1.50)	121	105	1.04 (0.79, 1.37)
Q4	124	106	0.72 (0.54, 0.96)	132	113	1.00 (0.76, 1.32)
			p_{trend}=0.08			p_{trend}=0.53
DPA						
Q1	84	75	1.0	108	90	1.0
Q2	97	84	1.23 (0.89, 1.70)	115	103	0.95 (0.71, 1.28)
Q3	130	110	0.92 (0.67, 1.27)	122	103	0.89 (0.65, 1.20)
Q4	188	165	0.90 (0.66, 1.24)	183	154	0.99 (0.74, 1.34)
			p_{trend}=0.49			p_{trend}=0.57
ω-6						
Q1	111	97	1.0	147	122	1.0
Q2	134	120	1.13 (0.86, 1.49)	122	110	1.04 (0.80, 1.36)
Q3	122	103	0.82 (0.61, 1.11)	135	117	0.87 (0.66, 1.14)
Q4	132	114	0.84 (0.60, 1.19)	124	101	0.83 (0.59, 1.15)
			p_{trend}=0.14			p_{trend}=0.68

* Missing (N): vital status (5)
** Model adjusted for age, education, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.6 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
LA						
Q1	112	99	1.0	145	120	1.0
Q2	131	115	1.04 (0.79, 1.37)	125	112	1.06 (0.81, 1.38)
Q3	124	106	0.82 (0.61, 1.11)	133	116	0.89 (0.67, 1.17)
Q4	132	114	0.81 (0.57, 1.14)	125	102	0.85 (0.61, 1.19)
			p_{trend}=0.14			p_{trend}=0.68
AA						
Q1	117	104	1.0	139	118	1.0
Q2	132	112	1.13 (0.85, 1.49)	125	109	0.89 (0.67, 1.17)
Q3	127	109	0.82 (0.62, 1.10)	130	112	0.93 (0.70, 1.22)
Q4	123	109	1.01 (0.74, 1.37)	134	111	0.88 (0.65, 1.18)
			p_{trend}=0.89			p_{trend}=0.76
ω-6:ω-3						
Q1	135	119	1.0	122	106	1.0
Q2	116	99	0.93 (0.71, 1.22)	141	112	0.94 (0.71, 1.23)
Q3	114	103	0.86 (0.65, 1.12)	142	122	1.24 (0.95, 1.62)
Q4	134	113	0.76 (0.58, 0.99)	123	110	1.21 (0.92, 1.61)
			p_{trend}=0.07			p_{trend}=0.06

* Missing (N): vital status (5)
** Model adjusted for age, education, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.7. Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA and GCA when examining effect measure modification among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Modifier	Exposure	Cases	Events	Multiplicative Scale: Stratified HRs** (95% CIs)	$p_{interaction}$	Additive Scale: Single referent HRs** (95% CIs)	Additive Scale: Additive ICR (95% CI)
Esophageal adenocarcinoma (N=499 cases, N=434 deaths)							
ω-3	**ω-6**						
≤Median	>Median	31	28	1.0		1.0	
	≤Median	214	189	1.03 (0.68, 1.54)		1.03 (0.68, 1.54)	
>Median	>Median	223	189	1.0		0.93 (0.63, 1.39)	
	≤Median	31	28	1.09 (0.72, 1.63)	0.84	1.02 (0.60, 1.72)	0.06 (-0.49, 0.61)
BMI (kg/m^2)	**Ratio**						
25+	≤Median	158	136	1.0		1.0	
	>Median	138	115	0.95 (0.74, 1.21)		0.96 (0.75, 1.23)	
<25	≤Median	86	77	1.0		1.23 (0.93, 1.63)	
	>Median	109	99	1.08 (0.80, 1.46)	0.53	1.34 (1.03, 1.73)	0.15 (-0.30, 0.59)
NSAID use	**Ratio**						
Ever	≤Median	83	70	1.0		1.0	
	>Median	66	55	1.04 (0.73, 1.49)		1.04 (0.73, 1.49)	
Never	≤Median	159	141	1.0		1.15 (0.86, 1.53)	
	>Median	177	156	1.01 (0.81, 1.27)	0.89	1.17 (0.88, 1.55)	-0.03 (-0.48. 0.43)
Smoking status	**Ratio**						
Ever	≤Median	186	162	1.0		1.0	
	>Median	202	176	1.08 (0.87, 1.34)		1.08 (0.87, 1.34)	
Never	≤Median	59	51	1.0		1.14 (0.83, 1.56)	
	>Median	45	39	0.92 (0.60, 1.41)	0.51	1.05 (0.74, 1.49)	-0.17 (-0.69, 0.35)

* Missing (N): vital status (5)
** Adjusted for caloric intake and study indicator
Median derived the cases' distribution of intake

Table 4.7 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA and GCA when examining modification among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Modifier	Exposure	Cases	Events	Multiplicative Scale: Stratified HRs** (95% CIs)	$p_{interaction}$	Additive Scale: Single referent HRs** (95% CIs)	Additive Scale: Additive ICR (95% CI)
Esophageal adenocarcinoma (N=499 cases, N=434 deaths)							
Reflux frequency	**Ratio**						
≥Weekly	≤Median	81	64	1.0		1.0	
	>Median	92	79	1.16 (0.83, 1.61)		1.16 (0.83, 1.61)	
<Weekly	≤Median	152	138	1.0		1.40 (1.04, 1.88)	
	>Median	150	131	0.98 (0.77, 1.24)	0.42	1.36 (1.01, 1.85)	0.19 (-0.70, 0.32)
Gastric cardia adenocarcinoma (N=528 cases, N=450 deaths)							
ω-3	**ω-6**						
≤Median	>Median	27	25	1.0		1.0	
	≤Median	242	208	0.76 (0.50, 1.17)		0.76 (0.50, 1.17)	
>Median	>Median	233	193	1.0		0.65 (0.42, 0.99)	
	≤Median	26	24	1.12 (0.72, 1.72)	0.22	0.72 (0.41, 1.27)	0.31 (-0.11, 0.73)
BMI (kg/m^2)	**Ratio**						
25+	≤Median	141	117	1.0		1.0	
	>Median	154	129	0.97 (0.75, 1.25)		0.97 (0.75, 1.25)	
<25	≤Median	117	109	1.0		1.42 (1.09, 1.85)	
	>Median	106	87	0.67 (0.50, 0.89)	0.05	0.95 (0.71, 1.26)	-0.44 (-0.90, 0.02)

* Missing (N): vital status (5)
** Adjusted for caloric intake and study indicator
Median derived the cases' distribution of intake

Table 4.7 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA and GCA when examining modification among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Modifier	Exposure	Cases	Events	Multiplicative Scale		Additive Scale	
				Stratified HRs** (95% CIs)	$p_{interaction}$	Single referent HRs** (95% CIs)	Additive ICR (95% CI)
NSAID use	**Ratio**						
Ever	≤Median	95	84	1.0		1.0	
	>Median	99	82	0.84 (0.61, 1.14)		0.84 (0.61, 1.14)	
Never	≤Median	165	143	1.0		1.06 (0.81, 1.39)	
	>Median	160	132	0.83 (0.65, 1.06)	1.00	0.88 (0.67, 1.17)	-0.01 (-0.38, 0.35)
Smoking status	**Ratio**						
Ever	≤Median	191	171	1.0		1.0	
	>Median	205	171	0.81 (0.65, 1.00)		0.81 (0.65, 1.00)	
Never	≤Median	67	56	1.0		0.93 (0.69, 1.26)	
	>Median	55	44	0.85 (0.57 1.27)	0.82	0.79 (0.56, 1.11)	0.05 (-0.33, 0.44)
Reflux frequency	**Ratio**						
≥Weekly	≤Median	55	45	1.0		1.0	
	>Median	57	40	0.82 (0.53, 1.26)		0.82 (0.53, 1.26)	
<Weekly	≤Median	201	178	1.0		1.38 (1.00, 1.92)	
	>Median	195	168	0.84 (0.68, 1.04)	0.92	1.16 (0.83, 1.63)	-0.03 (-0.44, 0.37)

* Missing (N): vital status (5)
** Adjusted for caloric intake and study indicator
Median derived the cases' distribution of intake

Table 4.8. Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, adjusting for additional covariates

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
Tuna					
None	504	77	1.0	124	1.0
T1	386	96	1.54 (1.04, 2.27)	90	0.92 (0.65, 1.30)
T2	714	231	1.60 (1.13, 2.27)	216	0.98 (0.72, 1.33)
T3	423	95	1.39 (0.94, 2.07)	98	0.90 (0.63, 1.28)
			p_{trend}=0.87		p_{trend}=0.72
Fried fish					
Non-consumers	1086	268	1.0	251	1.0
Consumers	941	231	0.97 (0.76, 1.23)	277	1.35 (1.07, 1.70)
Baked/broiled fish					
None	662	151	1.0	194	1.0
T1	352	79	1.09 (0.76, 1.56)	79	0.78 (0.55, 1.10)
T2	688	222	1.12 (0.83, 1.53)	191	0.82 (0.61, 1.09)
T3	325	47	0.69 (0.45, 1.07)	64	0.76 (0.52, 1.10)
			p_{trend}=0.38		p_{trend}=0.62
Shellfish					
Non-consumers	966	203	1.0	229	1.0
Consumers	1061	296	1.19 (0.93, 1.53)	299	1.14 (0.90, 1.45)

* Model adjusted for age, education, BMI, GERD symptom frequency, cigarette smoking status, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, adjusting for additional covariates

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
ω-3					
Q1	507	75	1.0	101	1.0
Q2	507	126	2.07 (1.43, 3.00)	128	1.45 (1.03, 2.04)
Q3	507	131	1.90 (1.29, 2.80)	134	1.34 (0.94, 1.91)
Q4	506	167	2.43 (1.60, 3.69)	165	1.65 (1.12, 2.44)
			p_{trend}=0.004		p_{trend}=0.25
ALA					
Q1	507	68	1.0	99	1.0
Q2	506	125	2.19 (1.49, 3.21)	121	1.34 (0.95, 1.89)
Q3	508	141	2.25 (1.51, 3.34)	143	1.48 (1.04, 2.11)
Q4	506	165	2.71 (1.76, 4.20)	165	1.68 (1.13, 2.50)
			p_{trend}=0.004		p_{trend}=0.34
EPA					
Q1	507	117	1.0	139	1.0
Q2	507	132	1.27 (0.91, 1.78)	140	1.11(0.82, 1.52)
Q3	507	127	1.22 (0.87, 1.72)	120	0.92 (0.66, 1.27)
Q4	506	123	1.30 (0.92, 1.84)	129	1.09 (0.79, 1.51)
			p_{trend}=0.45		p_{trend}=0.23

* Model adjusted for age, education, BMI, GERD symptom frequency, cigarette smoking status, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, adjusting for additional covariates

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
DHA					
Q1	507	136	1.0	147	1.0
Q2	507	108	0.94 (0.67, 1.31)	129	1.03 (0.76, 1.41)
Q3	507	142	1.28 (0.92, 1.77)	129	1.11 (0.81, 1.51)
Q4	506	113	1.01 (0.71, 1.43)	123	0.96 (0.69, 1.34)
			p_{trend}=0.47		p_{trend}=0.25
DPA					
Q1	507	99	1.0	122	1.0
Q2	507	121	1.44 (1.01, 2.04)	129	1.23 (0.89, 1.70)
Q3	507	149	1.79 (1.27, 2.53)	140	1.35 (0.97, 1.87)
Q4	506	130	1.41 (0.98, 2.05)	137	1.13 (0.79, 1.59)
			p_{trend}=0.11		p_{trend}=0.08
ω-6					
Q1	507	68	1.0	107	1.0
Q2	507	132	2.13 (1.46, 3.11)	122	1.23 (0.88, 1.72)
Q3	507	137	2.16 (1.46, 3.21)	137	1.24 (0.87, 1.76)
Q4	506	162	2.64 (1.72, 4.07)	162	1.48 (1.00, 2.20)
			p_{trend}=0.001		p_{trend}=0.04

* Model adjusted for age, education, BMI, GERD symptom frequency, cigarette smoking status, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFAs and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, adjusting for additional covariates

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
LA					
Q1	506	69	1.0	107	1.0
Q2	508	134	2.11 (1.45, 3.07)	126	1.25 (0.90, 1.75)
Q3	507	134	2.03 (1.37, 3.01)	133	1.17 (0.82, 1.67)
Q4	506	162	2.59 (1.68, 3.98)	162	1.47 (0.99, 2.18)
			p_{trend}=0.001		p_{trend}=0.04
AA					
Q1	506	86	1.0	113	1.0
Q2	507	132	1.77 (1.24, 2.54)	121	1.16 (0.83, 1.62)
Q3	508	127	1.63 (1.13, 2.35)	139	1.32 (0.94, 1.84)
Q4	506	154	2.04 (1.39, 2.98)	155	1.44 (1.01, 2.06)
			p_{trend}=0.04		p_{trend}=0.02
ω-6:ω-3					
Q1	507	129	1.0	116	1.0
Q2	506	120	0.97 (0.70, 1.35)	148	1.40 (1.02, 1.92)
Q3	507	125	0.98 (0.71, 1.37)	144	1.23 (0.90, 1.70)
Q4	507	125	1.07 (0.77, 1.49)	120	1.09 (0.79, 1.52)
			p_{trend}=0.41		p_{trend}=0.73

* Model adjusted for age, education, BMI, GERD symptom frequency, cigarette smoking status, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from controls' distribution of intake
Q1-Q4 are quartiles derived from controls' distribution of intake

Table 4.9. Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, adjusted for additional covariates

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR** (95%CI)	Cases N=528	Deaths N=450	HR** (95%CI)
Tuna						
None	77	66	1.0	124	100	1.0
T1	143	126	1.21 (0.88, 1.66)	129	117	1.00 (0.75, 1.32)
T2	179	159	1.20 (0.88, 1.64)	174	144	0.99 (0.76, 1.31)
T3	100	83	1.03 (0.74, 1.45)	101	89	1.17 (0.87, 1.58)
			p_{trend}=0.33			p_{trend}=0.37
Fried fish						
Non-consumers	268	239	1.0	251	224	1.0
Consumers	231	195	0.95 (0.78, 1.16)	277	226	0.87 (0.72, 1.06)
Baked/broiled fish						
None	151	130	1.0	194	168	1.0
T1	79	69	1.08 (0.80, 1.47)	79	67	0.97 (0.72, 1.30)
T2	221	191	1.02 (0.79, 1.30)	186	155	0.84 (0.65, 1.10)
T3	48	44	0.78 (0.53, 1.14)	69	60	0.97 (0.71, 1.31)
			p_{trend}=0.07			p_{trend}=0.61

* Missing (N): vital status (5)
** Model adjusted for age, education, BMI, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.9 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, adjusted for additional covariates

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR** (95%CI)	Cases N=528	Deaths N=450	HR** (95%CI)
Shellfish						
Non-consumers	203	176	1.0	229	197	1.0
Consumers	296	258	1.01 (0.82, 1.25)	299	253	0.91 (0.74, 1.11)
ω-3						
Q1	116	99	1.0	141	118	1.0
Q2	129	118	1.24 (0.93, 1.64)	127	115	1.05 (0.80, 1.37)
Q3	126	108	1.01 (0.75, 1.36)	131	115	0.75 (0.56, 0.99)
Q4	128	109	1.07 (0.76, 1.50)	129	102	0.75 (0.54, 1.03)
			p_{trend}=0.17			p_{trend}=0.45
ALA						
Q1	117	102	1.0	140	117	1.0
Q2	126	110	1.01 (0.76, 1.34)	131	118	1.13 (0.87, 1.47)
Q3	123	110	0.97 (0.72, 1.31)	132	115	0.73 (0.55, 0.97)
Q4	133	112	0.93 (0.67, 1.30)	125	100	0.79 (0.57, 1.10)
			p_{trend}=0.19			p_{trend}=0.38

* Missing (N): vital status (5)
** Model adjusted for age, education, BMI, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.9 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, adjusted for additional covariates

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
EPA						
Q1	119	105	1.0	138	120	1.0
Q2	129	113	0.93 (0.70, 1.22)	128	109	1.00 (0.76, 1.31)
Q3	127	109	0.87 (0.66, 1.15)	130	107	0.86 (0.66, 1.14)
Q4	124	107	0.79 (0.59, 1.05)	132	114	1.14 (0.87, 1.50)
			p_{trend}=0.30			p_{trend}=0.42
DHA						
Q1	127	110	1.0	129	114	1.0
Q2	111	97	1.03 (0.77, 1.37)	146	118	0.74 (0.57, 0.96)
Q3	137	121	1.17 (0.90, 1.54)	121	105	1.06 (0.80, 1.39)
Q4	124	106	0.74 (0.56, 1.00)	132	113	1.01 (0.76, 1.33)
			p_{trend}=0.11			p_{trend}=0.60
DPA						
Q1	84	75	1.0	108	90	1.0
Q2	97	84	1.28 (0.92, 1.77)	115	103	0.95 (0.71, 1.28)
Q3	130	110	0.94 (0.68, 1.30)	122	103	0.89 (0.65, 1.20)
Q4	188	165	0.94 (0.69, 1.30)	183	154	1.00 (0.74, 1.36)
			p_{trend}=0.55			p_{trend}=0.62

* Missing (N): vital status (5)
** Model adjusted for age, education, BMI, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.9 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, adjusted for additional covariates

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
ω-6						
Q1	111	97	1.0	147	122	1.0
Q2	134	120	1.14 (0.86, 1.51)	122	110	1.05 (0.80, 1.36)
Q3	122	103	0.86 (0.64, 1.18)	135	117	0.88 (0.67, 1.17)
Q4	132	114	0.84 (0.60, 1.20)	124	101	0.83 (0.59, 1.16)
			p_{trend}=0.12			p_{trend}=0.79
LA						
Q1	112	99	1.0	145	120	1.0
Q2	131	115	1.06 (0.80, 1.40)	125	112	1.06 (0.81, 1.39)
Q3	124	106	0.87 (0.64, 1.18)	133	116	0.90 (0.68, 1.20)
Q4	132	114	0.81 (0.57, 1.15)	125	102	0.85 (0.61, 1.20)
			p_{trend}=0.11			p_{trend}=0.79
AA						
Q1	117	104	1.0	139	118	1.0
Q2	132	112	1.18 (0.89, 1.57)	125	109	0.88 (0.67, 1.15)
Q3	127	109	0.87 (0.65, 1.17)	130	112	0.93 (0.71, 1.23)
Q4	123	109	1.06 (0.77, 1.45)	134	111	0.89 (0.66, 1.20)
			p_{trend}=0.90			p_{trend}=0.78

* Missing (N): vital status (5)
** Model adjusted for age, education, BMI, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

Table 4.9 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFAs and mortality* following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, adjusted for additional covariates

	EA			GCA		
Measure	**Cases N=499**	**Deaths N=434**	**HR** (95%CI)**	**Cases N=528**	**Deaths N=450**	**HR** (95%CI)**
ω-6:ω-3						
Q1	135	119	1.0	122	106	1.0
Q2	116	99	0.93 (0.71, 1.23)	141	112	0.95 (0.72, 1.25)
Q3	114	103	0.90 (0.68, 1.17)	142	122	1.25 (0.96, 1.64)
Q4	134	113	0.77 (0.59, 1.00)	123	110	1.23 (0.93, 1.63)
			p_{trend}=0.10			p_{trend}=0.06

* Missing (N): vital status (5)
** Model adjusted for age, education, BMI, proxy status, caloric intake, and study indicator
Tuna includes tuna fresh and canned, tuna salad, and tuna casserole
Baked/broiled fish describes non-fried fish that is neither tuna nor shellfish
T1-T3 are tertiles derived from cases' distribution of intake
Q1-Q4 are quartiles derived from cases' distribution of intake

CHAPTER V: DISCUSSION

Overview

The objectives of this dissertation were to examine the associations between PUFAs and the three outcomes along the BE-EA continuum: BE, EA/GCA, and mortality following a diagnosis of EA/GCA. Chapter I provides the background on these diseases, on PUFAs, and on the limited existing research assessing PUFAs and BE/EA/GCA, despite the biologic plausibility for these associations. In Chapter II, I describe the pooled study methods used to achieve my dissertation objectives. Chapter III reports my findings for the associations between PUFAs and the risk of developing BE (Aim 1), while Chapter IV focuses on the results for the risk of developing EA/GCA (Aim 2) and mortality following an EA/GCA diagnosis (Aim 3). In Chapter V, I summarize my dissertation results, compare them to the existing literature, address both the limitations and strengths of this study, discuss the public health impact, and propose potential directions for future research. The significance of this dissertation is that the incidence of BE [4,9,58,59] and EA/GCA [4,7,16-21] are increasing among Western populations, while the prognosis of EA/GCA is very poor [2-7]. Identifying potential modifiable risk factors where a risk reduction strategy may be implemented to reduce the disease burden is crucial. My dissertation is innovative and improves upon existing research for several reasons. First, it is innovative because I assessed PUFA intake more comprehensively by using a variety of measures, rather than focusing on a

single measurement. In addition to examining the ω-3 and ω-6 PUFAs intake, I explored the ratio as a representation of the relative balance between the two PUFA types. I also assessed specific PUFA-rich food items, and was able to consider cooking methods. Additionally, this study was the first to examine the associations between PUFAs and mortality following a diagnosis of EA/GCA. Finally, by pooling four existing U.S.-based studies, I was able to increase study power to facilitate examination of associations with these relatively rare outcomes.

Summary of Results and Comparison to Existing Literature

A summary of the results from my dissertation is shown in **Figure 5.1**.

For Aim 1, higher intake of baked/broiled fish was associated with a 34% reduction in risk of developing BE; when stratifying by segment length, this decrease in risk appeared to be limited to those with LSBE. Two studies have examined fish intake and BE [217,218] and both reported no association. The inconsistencies between my findings and these previous studies may be that I was able to differentiate fish (which is rich in beneficial ω-3) by cooking methods (such as frying, which often add the potentially more detrimental ω-6 in the form of oils [22,205]), whereas the previous studies did not. Further assessments by segment length showed that higher intakes of ω-3 ($OR_{>Median vs. \leq Median}$=0.62, 95%CI=0.40-0.96) and of the ω-6 subtype AA ($OR_{>Median vs. \leq Median}$=0.67, 95%CI=0.45-0.99) were associated with a decreased risk of LSBE, and null associations with SSBE. One previous study using the Epidemiology and Incidence of BE Study (which is included in my pooled study) found that ω-3 was associated with a halving of BE risk [107]. This finding is similar to my pooled results for

LSBE reported here, and in sensitivity analyses when we restricted our models to the Study of Reflux Disease the risk was decreased with a comparable estimate. No previous studies have examined BE with ω-6 or any PUFA subtype.

For Aim 2, baked/broiled fish was also associated with an approximately 30% decreased risk of developing EA and GCA. In contrast, tuna appeared to increase risk of EA development ($OR_{T3vs.None}$=1.46, 95%CI=1.00-2.13), but not with GCA. Similarly fried fish was associated with an increased risk with GCA ($OR_{Consumersvs.Non}$=1.33, 95%CI=1.06-1.66), but not with EA. Existing studies examining fish intake and EA/GCA have found no associations [222,225,227,230]. Similar to what was previously mentioned for BE, the inconsistencies between my results and previous reports are most likely due to the inability of others to account for fish cooking methods, which may mask associations. The risk of developing EA and GCA was increased in association with ω-6 intake ($OR_{Q4vs.Q1}$=2.62, 95%CI=1.73-3.97 and $OR_{Q4vs.Q1}$=1.45, 95%CI=0.99-2.13, respectively). However, ω-3 intake was also associated with an increased risk of developing EA and GCA ($OR_{Q4vs.Q1}$=2.58, 95%CI=1.73-3.84 and $OR_{Q4vs.Q1}$=1.72, 95%CI=1.18-2.51, respectively). No studies have examined ω-6 with EA/GCA, and the one study that assessed the association between ω-3 intake and EA/GCA found no associations. This difference may be explained by the parent assessment methods, where specific food items that were high contributors to ω-3 intake (such as tuna) were grouped with foods that also include high amounts of ω-6 (such as tuna casserole and tuna salad). Similarly, at least among the American-based study populations included in my pooled study, intake of foods that were high in ω-3 were the same foods that were

also high in ω-6 (for example, “beef, veal, lamb, pork” and “mayonnaise or creamy salad dressing”).

Finally, for Aim 3, the ω-6:ω-3 was associated with a 24% decreased risk of EA mortality, but was associated with a suggestive 21% increase in risk of GCA mortality (particularly among those with BMI<25 who consume a diet with a high ω-6:ω-3). Because this was the first study to examine PUFAs with mortality following an EA/GCA diagnosis, there are no results for comparison.

Limitations

One major limitation of my pooled study was that the study populations are primarily white and male, which limits generalizability. However BE and EA/GCA primarily affect white males and rates of these diseases are highest among this demographic group [1,2,123,124,149,256], which allowed my results to be generalizable to this group. Additionally, my study pooled data from four studies that were population- or community-based, which enhanced generalizability.

Another major limitation to consider was the dietary assessment method used in each of the four parent studies. Patients were asked to recall diet in the year prior to study interview using FFQs, with the assumption that intake during this time period can be recalled with some accuracy and that it correlates with usual adult diet [295-298]. Nonetheless, it is possible that recent onset of clinical symptoms and any corresponding changes in diet would influence the accuracy of reporting pre-diagnosis diet among cases only. However, dietary recommendations for patients experiencing reflux typically only target avoiding food items such as coffee, chocolate, spicy foods, highly acidic

foods, or fatty foods, in addition to any other food items that may exacerbate symptoms for an individual [315]. But it is important to note that there is no empirical evidence showing decrease of reflux symptoms by limiting those foods listed. Additionally, each of the parent studies' FFQs was validated using diet records [268,270,274,275] or multiple 24-hour recalls [276,277].

As with all pooled studies, there may have been differences in data collection, variable definitions, and data management between the parent studies. These discrepancies could introduce misclassifications of outcomes, exposures, or covariates. However, the four studies were selected because of their high quality and similar data collection procedures. Also, several covariates have already been harmonized and pooled for BEACON [95,143,154,161,284], which was cost-efficient and also showed promise for the harmonization and pooling of the food frequency questionnaire data needed to complete this study. Additionally, this concern was alleviated by the study-exposure interactions and meta-analytic approach I performed as a sensitivity analysis.

In the EA/GCA cancer parent studies, proxy interviews were conducted with next of kin for subjects who were deceased or ill, roughly 30% of all cases used proxy interviews. There were no proxy interviews conducted in either of the BE parent studies. Although it has been shown that proxy- and self-report are similar [294], the use of proxies may have increased the probability of misclassification. However, studies have found that self-reported and proxy-reported information have good concordance for many of the variables used in this study [294,299-301]. Additionally, for the previous EA/GCA studies from BEACON, excluding proxy responses did not substantially change the effect estimates [173,227,228,302,303].

The issue of multiple comparisons was considered. For all analyses, the multiple measures of PUFAs were used individually to determine associations between PUFAs and the BE-EA continuum outcomes. There was a likelihood of observing statistically significant results due to chance because of the large number of comparisons in my study. I did not adjust for the multiple comparisons for each single association examined because it would have reduced statistical power. I assessed each of the associations individually based on biologic plausibility, consistency with current research, and consistency across the cancer continuum [295,304,305].

Finally, because diet is a complex exposure, I had to consider other elements that could be driving results seen in my study. For example, the highest contributing food items to ω-3 intake were not limited to foods known to be high in beneficial fatty acids such as fish, but were commonly the same foods also contributing to ω-6. This no doubt hindered my ability to detect associations with either ω-3 or ω-6:ω-3 ratio. Similarly, the parent study FFQs included a composite assessment of tuna, a fish with high levels of ω-3 [24], combining tuna with tuna casserole and tuna salad; the latter two are typically prepared with other foods high in ω-6 using contrasting cooking methods. Identifying the particular food items that were the most frequently consumed sources of PUFAs allowed me to consider if there are other factors that may be contributing to the associations observed.

Strengths

A major strength of this dissertation is the harmonization and pooling of existing case-control studies of BE and EA/GCA. This approach was time- and cost-efficient

compared to beginning a new field study effort, and was superior to a meta-analytic approach because of our uniform definitions for exposures and covariates. The pooled sample size was larger than previous case-control studies [107,217,218,221-224,226], resulting in increased statistical power to evaluate associations between PUFAs and BE-EA/GCA continuum outcomes and allowed for more precise estimates.

Previous studies examining PUFAS and BE/EA/GCA focused on one outcome along the continuum [107,217,221-223,226], this study was the first to examine multiple outcomes and more specifically the first to examine mortality following an EA/GCA diagnosis. My dissertation was the first to examine all three outcomes, and allowed for identification of windows along the disease continuum to implement potential risk reduction strategies.

Another strength of my study was the comprehensive use of multiple measures, which allowed me to better capture the complexity of this dietary exposure and helped to clarify previous findings. Most existing studies examined fish intake (a major source of long-chain ω-3 fatty acids) as the only PUFA measure [217,221-223,225-227,233], without examining cooking methods, which can mask potential associations (such as with frying, which adds ω-6 fatty acids). I was able to use information on cooking method and identify a measure – baked/broiled fish – that was most strongly associated with BE/EA/GCA risk, at least among the US populations included in my pooled study. A few existing studies considered overall PUFAs [107,218,226], which overlook important distinctions between beneficial ω-3 and potentially deleterious ω-6, or examined ω-3 PUFAs only [107,226]. My study was the first to examine ω-6 PUFAs and the first to consider the relative ω-3 and ω-6 balance, which was particularly important because ω-

3 and ω-6 are competitively inhibited by each other and may promote or suppress pathogenesis [8,49,212,216].

Finally, all four parent studies were conducted in US coastal regions with increased opportunity for fish intake (LAC, WA, CT, and NJ); this maximized heterogeneity in intake and enhanced my ability to detect differences, since US subjects typically consume small amounts of fish [306].

Public Health Impact

The incidence of EA/GCA has been increasing, particularly in westernized countries, [4,7,16-21], and it is expected to continue to increase over time [2,53,135]. The incidence of BE, the only known precursor to these cancers, has also been increasing [4,9,58,59]. Many of the major risk factors for these diseases are non-modifiable, or not easily modifiable. Identification of risk reduction strategies for these diseases could have high public health impact, given the poor prognoses of these cancers [2-7]. If the results of my dissertation are confirmed, there is a potential to reduce the burden of these diseases by encouraging increased intake of baked/broiled fish.

Future Directions

Future studies should consider improvements in the following areas: study design, study populations, and exposure assessment.

The case-control study design that I employed here was time- and cost-efficient approach, and allowed for adequate sample sizes for the three main outcomes.

However, I had to pool four case-control studies in order to achieve my objectives. Future studies could consider a cohort design, which could begin with those initially without BE/EA/GCA and examine the three outcomes I examined, as well as the risk of EA/GCA among those who develop BE. Additionally, using a single cohort study would allow for *a priori* uniform field procedures for all study participants, which cannot be guaranteed in a pooled study. A cohort design would also rule out recall bias as a possible reason for my study findings, since usual adult diet could be captured before disease onset, and allow for multiple measures of diet with time.

Future studies should aim to examine more diverse populations. My study population was primarily white and male, which is the group with highest incidence rates of these disease in the US. However, in order to enhance generalizability of the findings, future studies could expand the racial and gender diversity of the target population. The study population used here is a US population that consumes a typical westernized diet. In the future, researchers should consider populations with different dietary intake distributions, particularly populations that consume greater quantities of ω-3s or fish such as Asian or Mediterranean populations.

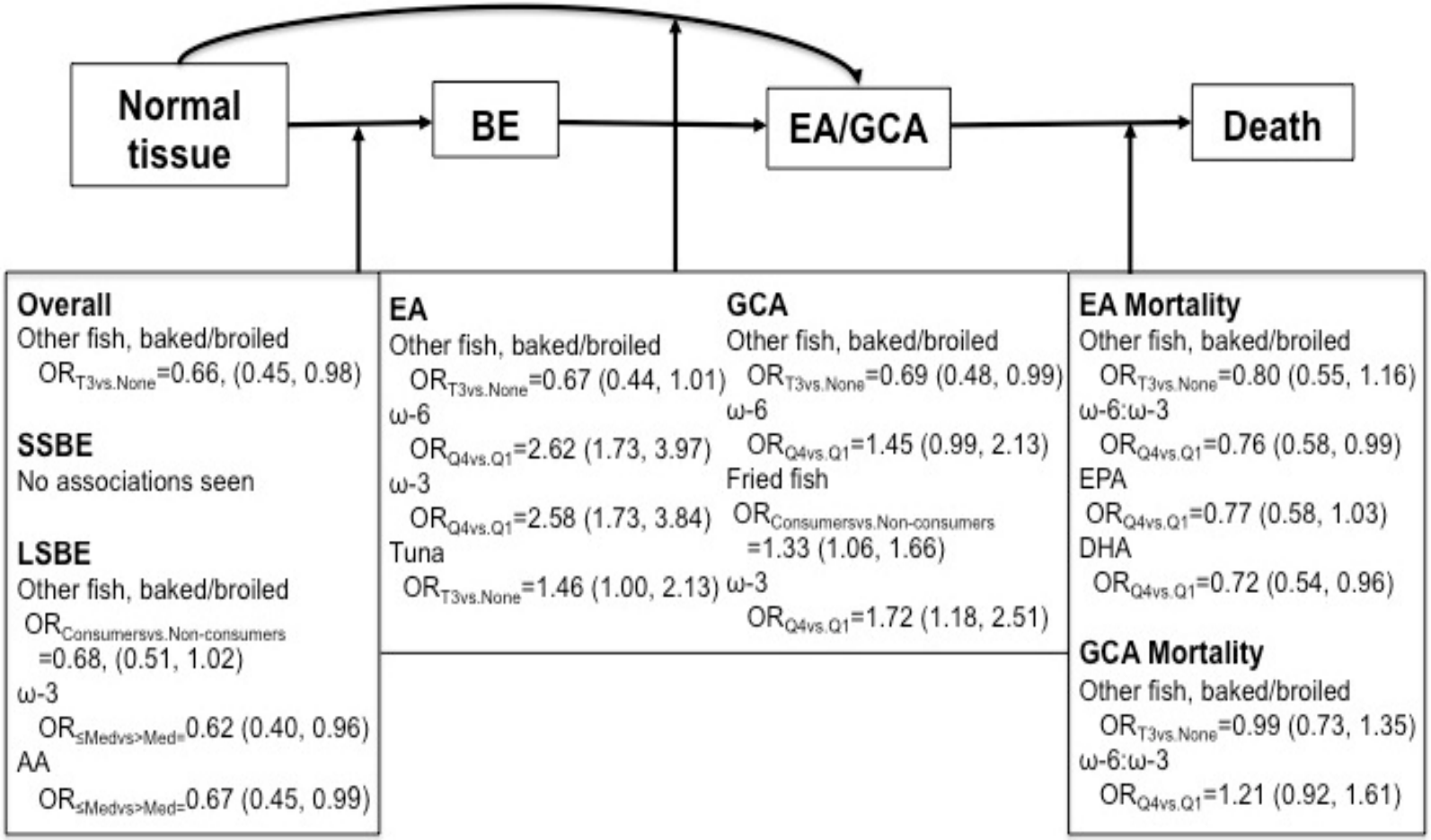
While the exposure measures used in my study are an improvement upon the existing literature, future studies can continue to progress on PUFA exposure assessment. Because my study was the first to examine the relative balance between ω-3 and ω-6, future studies (particularly among those using populations with different PUFA intake) should further investigate the ω-6:ω-3 and the interaction between the two, specifically to confirm the potential relationships I found between the ω-6:ω-3 and EA and GCA mortality. Most importantly, dietary assessment methods used in these

future studies should include more detailed assessments of PUFA-rich foods, making sure to consider fish type (e.g., marine fish, dark and oily fish, white fish) as well as cooking methods or other preparation methods. Finally, if possible future studies should examine the reliability of biomarkers as a potential way to objectively measure PUFA intake.

Conclusions

My dissertation was the first to comprehensively examine the associations between PUFAs and the outcomes along the BE-EA continuum. This was achieved by using pooled resources from four population- or community-based case-control studies of BE and EA/GCA in the US. Each of the parent studies was a member of BEACON and was conducted using similar procedures. In order to better capture the complexity of this dietary exposure and to improve upon the inconsistencies of existing research, I used a variety of PUFA measures, both nutrient-based and PUFA-rich food items, and examined the relative balance between ω-3 and ω-6. I found that higher intake of baked/broiled fish was associated with a ~30% reduction in risk of developing BE/EA/GCA, while ω-3 and ω-6 intakes were associated with an increased risk of EA/GCA. The ω-6:ω-3 was inversely associated with EA mortality, however it was positively associated with GCA mortality. If my findings are confirmed, increasing baked/broiled fish intake could be a promising risk reduction strategy for the BE and EA/GCA and may reduce the burden of these highly lethal cancers.

Figure 5.1. Summary of results examining PUFAs and the BE-EA continuum



APPENDIX: ADDITIONAL TABLES

Appendix Table 1. Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE

Measure	Study of Reflux Disease OR* (95%CI)	Epidemiology and Incidence of BE OR* (95%CI)
Tuna		
None	1.0	1.0
T1	0.99 (0.54, 1.80)	0.68 (0.40, 1.17)
T2	1.29 (0.72, 2.31)	0.60 (0.33, 1.10)
T3	1.16 (0.64, 2.10)	0.71 (0.38, 1.32)
Fried fish		
None	1.0	1.0
T1	1.10 (0.63, 1.92)	0.95 (0.62, 1.46)
T2	1.27 (0.71, 2.26)	1.03 (0.66, 1.60)
T3	1.37 (0.69, 2.70)	0.94 (0.56, 1.57)
Baked/broiled fish		
None	1.0	1.0
T1	0.77 (0.39, 1.51)	0.94 (0.61, 1.45)
T2	0.72 (0.38, 1.33)	0.61 (0.36, 1.04)
T3	0.78 (0.41, 1.50)	0.57 (0.34, 0.93)
Shellfish		
None	1.0	1.0
T1	0.89 (0.51, 1.56)	1.20 (0.72, 2.02)
T2	0.82 (0.40, 1.67)	0.78 (0.46, 1.33)
T3	0.84 (0.48, 1.47)	1.01 (0.61, 1.67)
ω-3		
Q1	1.0	1.0
Q2	1.18 (0.62, 2.24)	1.06 (0.66, 1.70)
Q3	1.28 (0.65, 2.50)	0.68 (0.40, 1.16)
Q4	1.68 (0.81, 3.47)	0.58 (0.29, 1.17)
ALA		
Q1	1.0	1.0
Q2	1.44 (0.77, 2.69)	0.95 (0.60, 1.52)
Q3	1.13 (0.56, 2.28)	0.63 (0.37, 1.07)
Q4	1.72 (0.81, 3.62)	0.50 (0.25, 1.00)
EPA		
Q1	1.0	1.0
Q2	1.21 (0.66, 2.22)	0.81 (0.52, 1.28)
Q3	1.49 (0.80, 2.76)	0.66 (0.41, 1.07)
Q4	1.06 (0.56, 2.02)	0.68 (0.41, 1.14)
* Model adjusted for age, sex, education, and caloric intake		

Appendix Table 1 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE

Measure	Study of Reflux Disease OR* (95%CI)	Epidemiology and Incidence of BE OR* (95%CI)
DHA		
Q1	1.0	1.0
Q2	0.78 (0.42, 1.41)	0.89 (0.56, 1.40)
Q3	1.25 (0.69, 2.28)	0.91 (0.56, 1.46)
Q4	0.97 (0.53, 1.80)	0.67 (0.39, 1.15)
DPA		
Q1	1.0	1.0
Q2	1.25 (0.68, 2.30)	0.76 (0.48, 1.20)
Q3	1.21 (0.65, 2.28)	0.82 (0.51, 1.33)
Q4	1.44 (0.76, 2.71)	0.64 (0.37, 1.11)
ω-6		
Q1	1.0	1.0
Q2	1.34 (0.70, 2.56)	0.71 (0.44, 1.15)
Q3	1.82 (0.93, 3.59)	0.71 (0.42, 1.20)
Q4	1.75 (0.84, 3.67)	0.39 (0.19, 0.82)
LA		
Q1	1.0	1.0
Q2	1.32 (0.69, 2.52)	0.70 (0.43, 1.13)
Q3	1.87 (0.95, 3.69)	0.68 (0.40, 1.15)
Q4	1.76 (0.84, 3.69)	0.39 (0.19, 0.82)
AA		
Q1	1.0	1.0
Q2	1.11 (0.60, 2.06)	0.99 (0.62, 1.57)
Q3	1.31 (0.70, 2.47)	0.67 (0.40, 1.11)
Q4	1.45 (0.75, 2.80)	0.87 (0.49, 1.55)
ω-6:ω-3		
Q1	1.0	1.0
Q2	0.86 (0.47, 1.56)	1.00 (0.62, 1.61)
Q3	0.89 (0.49, 1.62)	1.26 (0.79, 2.01)
Q4	1.04 (0.58, 1.87)	1.08 (0.66, 1.75)
* Model adjusted for age, sex, education, and caloric intake		

Appendix Table 2. Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE, using caloric exclusions of the lower and upper 2.5%

Measure	Cases N=445	Controls N=475	OR* (95%CI)
Tuna			
None	82	80	1.0
T1	167	177	0.91 (0.61, 1.35)
T2	104	114	0.93 (0.61, 1.42)
T3	92	104	0.90 (0.58, 1.39)
			p_{trend}=0.81
Fried fish			
None	158	181	1.0
T1	108	118	1.03 (0.73, 1.46)
T2	111	105	1.22 (0.85, 1.73)
T3	68	71	1.11 (0.74, 1.69)
			p_{trend}=0.91
Baked/broiled fish			
None	112	91	1.0
T1	146	138	0.89 (0.61, 1.28)
T2	93	117	0.67 (0.45, 1.01)
T3	94	129	0.66 (0.44, 0.98)
			p_{trend}=0.16
Shellfish			
None	122	124	1.0
T1	121	109	1.09 (0.75, 1.59)
T2	69	94	0.74 (0.49, 1.13)
T3	133	148	0.93 (0.64, 1.35)
			p_{trend}=0.57
ω-3			
Q1	112	119	1.0
Q2	126	118	1.19 (0.81, 1.75)
Q3	103	119	0.99 (0.64, 1.52)
Q4	104	119	1.01 (0.61, 1.69)
			p_{trend}=0.54
ALA			
Q1	116	118	1.0
Q2	131	120	1.17 (0.80, 1.71)
Q3	92	118	0.81 (0.52, 1.26)
Q4	106	119	0.93 (0.56, 1.56)
			p_{trend}=0.60
* Model adjusted for age, sex, education, caloric intake, and study indicator			

Appendix Table 2 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE, using caloric exclusions of the lower and upper 2.5%

Measure	Cases N=445	Controls N=475	OR* (95%CI)
EPA			
Q1	129	119	1.0
Q2	121	119	0.95 (0.66, 1.37)
Q3	100	118	0.87 (0.59, 1.28)
Q4	95	119	0.84 (0.56, 1.25)
			p_{trend}=0.60
DHA			
Q1	128	119	1.0
Q2	109	119	0.87 (0.60, 1.26)
Q3	121	119	1.09 (0.74, 1.58)
Q4	87	118	0.80 (0.53, 1.20)
			p_{trend}=0.76
DPA			
Q1	121	118	1.0
Q2	114	119	0.92 (0.63, 1.33)
Q3	109	119	0.97 (0.66, 1.44)
Q4	101	119	0.94 (0.62, 1.43)
			p_{trend}=0.25
ω-6			
Q1	116	118	1.0
Q2	109	120	0.92 (0.62, 1.36)
Q3	129	119	1.15 (0.75, 1.75)
Q4	91	118	0.82 (0.49, 1.39)
			p_{trend}=0.69
LA			
Q1	116	119	1.0
Q2	110	118	0.94 (0.64, 1.39)
Q3	127	120	1.14 (0.75, 1.74)
Q4	92	118	0.84 (0.50, 1.41)
			p_{trend}=0.73
* Model adjusted for age, sex, education, caloric intake, and study indicator			

Appendix Table 2 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE, using caloric exclusions of the lower and upper 2.5%

Measure	Cases N=445	Controls N=475	OR* (95%CI)
AA			
Q1	108	119	1.0
Q2	125	118	1.15 (0.79, 1.68)
Q3	99	120	0.93 (0.63, 1.39)
Q4	113	118	1.16 (0.75, 1.79)
			p_{trend}=0.18
ω-6:ω-3			
Q1	110	118	1.0
Q2	102	119	0.92 (0.63, 1.34)
Q3	126	120	1.15 (0.80, 1.67)
Q4	107	118	1.04 (0.71, 1.52)
			p_{trend}=0.62
* Model adjusted for age, sex, education, caloric intake, and study indicator			

Appendix Table 3. Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE, using a random effects meta-analytic approach

Measure	Cases N=471	Controls N=492	OR* (95%CI)	I^2 (%)	$p_{heterogeneity}$
Tuna					
None	89	85	1.0		
T1	174	183	0.80 (0.54, 1.19)		
T2	105	117	0.86 (0.57, 1.30)		
T3	103	107	0.90 (0.59, 1.37)	0	0.50
Fried fish					
None	169	186	1.0		
T1	115	122	0.99 (0.71, 1.39)		
T2	113	109	1.11 (0.78, 1.57)		
T3	74	75	1.07 (0.71, 1.61)	0	0.96
Baked/broiled fish					
None	189	98	1.0		
T1	85	140	0.91 (0.63, 1.30)		
T2	98	121	0.69 (0.46, 1.03)		
T3	99	133	0.66 (0.45, 0.98)	0	0.65
Shellfish					
None	131	129	1.0		
T1	123	113	1.06 (0.73, 1.54)		
T2	76	97	0.82 (0.53, 1.25)		
T3	141	153	0.95 (0.65, 1.37)	0	0.91
ω-3					
Q1	121	123	1.0		
Q2	129	122	1.13 (0.78, 1.65)		
Q3	106	124	0.87 (0.58, 1.32)		
Q4	115	123	0.98 (0.59, 1.62)	37.7	0.16
ALA					
Q1	124	123	1.0		
Q2	134	124	1.11 (0.79, 2.72)		
Q3	100	123	0.94 (0.59, 1.51)		
Q4	113	122	0.89 (0.53, 1.47)	50.1	0.07
EPA					
Q1	136	124	1.0		
Q2	126	123	0.96 (0.67, 1.37)		
Q3	110	122	0.89 (0.61, 1.29)		
Q4	99	123	0.79 (0.53, 1.18)	20.7	0.28
DHA					
Q1	136	124	1.0		
Q2	115	122	0.85 (0.59, 1.23)		
Q3	125	123	1.00 (0.69, 1.45)		
Q4	95	123	0.77 (0.51, 1.15)	0	0.80

* Model adjusted for age, sex, education, caloric intake, and study indicator

Appendix Table 3 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and development of BE among participants of two US-based case-control studies of BE, using a random effects meta-analytic approach

Measure	Cases N=471	Controls N=492	OR* (95%CI)	I^2 (%)	$p_{heterogeneity}$
DPA					
Q1	129	123	1.0		
Q2	119	122	0.94 (0.66, 1.36)		
Q3	116	123	0.96 (0.66, 1.41)		
Q4	107	124	0.90 (0.75, 1.17)	28.3	0.22
ω-6					
Q1	123	123	1.0		
Q2	114	123	0.92 (0.63, 1.36)		
Q3	131	124	1.01 (0.67, 1.52)		
Q4	103	122	0.84 (0.50, 1.41)	70.0	0.005
LA					
Q1	123	122	1.0		
Q2	115	124	0.92 (0.63, 1.35)		
Q3	129	124	0.98 (0.65, 1.48)		
Q4	104	122	0.84 (0.50, 1.41)	71.0	0.004
AA					
Q1	117	122	1.0		
Q2	127	124	1.05 (0.72, 1.52)		
Q3	104	123	0.88 (0.59, 1.30)		
Q4	123	123	1.07 (0.70, 1.65)	9.8	0.35
ω-6:ω-3					
Q1	117	124	1.0		
Q2	108	122	0.93 (0.64, 1.35)		
Q3	130	124	1.06 (0.74, 1.53)		
Q4	116	122	1.03 (0.71, 1.50)	0	0.95
* Model adjusted for age, sex, education, caloric intake, and study indicator					

Appendix Table 4. Comparison of cutpoints for quantiles based on control intake between study-specific and absolute value-based quantiles for assessing associations between PUFAs and development of BE

Measure	Study of Reflux Disease Quantiles	Epidemiology and Incidence of BE Quantiles	Pooled Absolute Value-Based Quantiles
Tuna (g/day)			
None	0	0	0
T1	0.01 – <3.94	0.01 – <3.37	0.01 – <3.37
T2	3.94 – <8.66	3.37 – <8.42	3.37 – <8.42
T3	≥8.66	≥8.42	≥8.42
Fried fish (g/day)			
None	0	0	0
T1	0.01 – <4.10	0.01 – <1.48	0.01 – <2.96
T2	4.10 – <9.56	1.48 – <5.92	2.96 – <6.15
T3	≥9.56	≥5.92	≥6.15
Baked/broiled fish (g/day)			
None	0	0	0
T1	0.01 – <5.59	0.01 – <2.96	0.01 – <3.70
T2	5.59 – <12.12	2.96 – <11.84	3.70 – <12.12
T3	≥12.12	≥11.84	≥12.12
Shellfish (g/day)			
None	0	0	0
T1	0.01 – <2.41	0.01 – <2.23	0.01 – <2.41
T2	2.41 – <5.62	2.23 – <4.47	2.41 – <5.59
T3	≥5.62	≥4.47	≥5.59
ω-3 (g/day)			
Q1	<1.42	<1.13	<1.25
Q2	1.42 – <2.44	1.13 – <1.64	1.25 – <1.82
Q3	2.44 – <4.02	1.64 – <2.27	1.82 – <2.84
Q4	≥4.02	≥2.27	≥2.84
ALA (g/day)			
Q1	<1.21	<1.06	<1.12
Q2	1.21 – <2.12	1.06 – <1.55	1.12 – <1.67
Q3	2.12 – <3.53	1.55 – <2.11	1.67 – <2.45
Q4	≥3.53	≥2.11	≥2.45
EPA (g/day)			
Q1	<0.027	<0.010	<0.013
Q2	0.027 – <0.065	0.010 – <0.018	0.013 – <0.027
Q3	0.065 – <0.132	0.018 – <0.035	0.027 – <0.065
Q4	≥0.132	≥0.035	≥0.065

Appendix Table 4 (cont'd). Comparison of cutpoints for quantiles based on control intake between study-specific and absolute value-based quantiles for assessing associations between PUFAs and development of BE

Measure	Study of Reflux Disease Quantiles	Epidemiology and Incidence of BE Quantiles	Pooled Absolute Value-Based Quantiles
DHA (g/day)			
Q1	<0.064	<0.030	<0.036
Q2	0.064 – <0.149	0.030 – <0.051	0.036 – <0.073
Q3	0.149 – <0.354	0.051 – <0.098	0.073 – <0.165
Q4	≥0.354	≥0.098	≥0.165
DPA (g/day)			
Q1	<0.031	<0.008	<0.010
Q2	0.031 – <0.095	0.008 – <0.012	0.010 – <0.021
Q3	0.095 – <0.220	0.012 – <0.024	0.021 – <0.067
Q4	≥0.220	≥0.024	≥0.067
ω-6 (g/day)			
Q1	<11.17	<9.35	<9.94
Q2	11.17 – <20.36	9.35 – <13.84	9.94 – <15.84
Q3	20.36 – <37.76	13.84 – <20.53	15.84 – <25.88
Q4	≥37.76	≥20.53	≥25.88
LA (g/day)			
Q1	<10.96	<9.24	<9.87
Q2	10.96 – <19.98	9.24 – <13.71	9.87 – <15.59
Q3	19.98 – <36.41	13.71 – <20.38	15.59 – <25.28
Q4	≥36.41	≥20.38	≥25.28
AA (g/day)			
Q1	<0.152	<0.055	<0.067
Q2	0.152 – <0.531	0.055 – <0.088	0.067 – <0.124
Q3	0.531 – <1.206	0.088 – <0.133	0.124 – <0.357
Q4	≥1.206	≥0.133	≥0.357
ω-6:ω-3			
Q1	<7.50	<7.18	<7.28
Q2	7.50 – <8.53	7.18 – <8.27	7.28 – <8.37
Q3	8.53 – <9.98	8.27 – <10.04	8.37 – <10.04
Q4	≥9.98	≥10.04	≥10.04

Appendix Table 5. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)
Tuna				
None	1.0	1.0	1.0	1.0
T1	1.06 (0.55, 2.05)	0.77 (0.42, 1.42)	1.87 (1.20, 2.89)	0.97 (0.65, 1.45)
T2	1.75 (1.03, 2.99)	1.05 (0.65, 1.71)	1.24 (0.79, 1.94)	0.88 (0.59, 1.31)
T3	1.97 (1.04, 3.71)	1.23 (0.68, 2.22)	1.09 (0.68, 1.74)	0.73 (0.48, 1.11)
Fried fish				
Non-consumers	1.0	1.0	1.0	1.0
Consumers	0.99 (0.72, 1.38)	1.35 (0.96, 1.89)	0.98 (0.70, 1.37)	1.32 (0.97, 1.78)
Baked/broiled fish				
None	1.0	1.0	1.0	1.0
T1	1.10 (0.64, 1.89)	0.86 (0.49, 1.50)	0.85 (0.54, 1.33)	0.67 (0.44, 1.00)
T2	0.93 (0.62, 1.41)	0.86 (0.57, 1.29)	1.11 (0.73, 1.71)	0.53 (0.34, 0.82)
T3	0.51 (0.23, 1.13)	0.72 (0.35, 1.47)	0.73 (0.46, 1.16)	0.65 (0.43, 0.98)
Shellfish				
Non-consumers	1.0	1.0	1.0	1.0
Consumers	1.01 (0.72, 1.43)	0.93 (0.65, 1.31)	1.38 (0.99, 1.90)	1.33 (0.99, 1.80)
ω-3				
Q1	1.0	1.0	1.0	1.0
Q2	2.42 (1.47, 3.99)	1.53 (0.94, 2.49)	1.44 (0.87, 2.39)	1.15 (0.74, 1.78)
Q3	1.97 (1.16, 3.34)	1.08 (0.63, 1.83)	1.46 (0.86, 2.47)	1.23 (0.78, 1.95)
Q4	2.97 (1.74, 5.09)	2.40 (1.43, 4.03)	1.95 (1.07, 3.55)	0.88 (0.50, 1.55)

* Model adjusted for age, sex, race, education, proxy status, and caloric intake

Appendix Table 5 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)
ALA				
Q1	1.0	1.0	1.0	1.0
Q2	2.44 (1.45, 4.09)	1.71 (1.04, 2.80)	1.62 (0.97, 2.70)	0.89 (0.57, 1.41)
Q3	2.60 (1.52, 4.43)	1.14 (0.67, 1.95)	1.53 (0.90, 2.61)	1.39 (0.89, 2.18)
Q4	3.48 (1.96, 6.17)	2.65 (1.53, 4.57)	2.06 (1.12, 3.77)	0.85 (0.48, 1.50)
EPA				
Q1	1.0	1.0	1.0	1.0
Q2	1.18 (0.76, 1.85)	0.90 (0.58, 1.41)	1.02 (0.66, 1.58)	0.98 (0.66, 1.46)
Q3	1.47 (0.94, 2.30)	0.95 (0.60, 1.49)	0.78 (0.49, 1.26)	0.72 (0.47, 1.11)
Q4	1.26 (0.80, 2.00)	1.16 (0.74, 1.80)	1.11 (0.69, 1.77)	0.86 (0.55, 1.33)
DHA				
Q1	1.0	1.0	1.0	1.0
Q2	0.90 (0.58, 1.41)	0.84 (0.54, 1.31)	0.67 (0.43, 1.05)	0.85 (0.57, 1.27)
Q3	1.34 (0.87, 2.06)	0.95 (0.61, 1.48)	0.93 (0.60, 1.43)	0.85 (0.57, 1.28)
Q4	1.09 (0.70, 1.72)	1.09 (0.70, 1.70)	0.67 (0.42, 1.08)	0.67 (0.43, 1.05)
DPA				
Q1	1.0	1.0	1.0	1.0
Q2	1.89 (1.19, 3.00)	1.11 (0.70, 1.75)	0.89 (0.54, 1.45)	1.10 (0.72, 1.70)
Q3	1.92 (1.21, 3.04)	1.18 (0.76, 1.86)	1.61 (1.02, 1.55)	1.35 (0.88, 2.07)
Q4	1.71 (1.06, 2.75)	1.27 (0.80, 1.99)	1.12 (0.66, 1.90)	1.08 (0.66, 1.76)
ω-6				
Q1	1.0	1.0	1.0	1.0
Q2	2.27 (1.37, 3.75)	1.32 (0.82, 2.14)	1.65 (0.99, 2.75)	0.90 (0.58, 1.39)
Q3	2.37 (1.39, 4.04)	1.14 (0.68, 1.91)	1.47 (0.85, 2.55)	1.01 (0.64, 1.60)
Q4	2.81 (1.63, 4.82)	1.81 (1.08, 3.03)	2.15 (1.13, 4.11)	0.97 (0.54, 1.74)

* Model adjusted for age, sex, race, education, proxy status, and caloric intake

Appendix Table 5 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)	OR* (95%CI)
LA				
Q1	1.0	1.0	1.0	1.0
Q2	2.19 (1.33, 3.61)	1.35 (0.84, 2.18)	1.66 (0.99, 2.77)	0.92 (0.59, 1.42)
Q3	2.13 (1.25, 3.62)	1.04 (0.61, 1.75)	1.46 (0.85, 2.53)	0.98 (0.62, 1.56)
Q4	2.69 (1.56, 4.62)	1.77 (1.05, 2.97)	2.18 (1.14, 4.15)	0.97 (0.54, 1.74)
AA				
Q1	1.0	1.0	1.0	1.0
Q2	1.90 (1.18, 3.06)	0.77 (0.48, 1.23)	1.35 (0.83, 2.22)	1.48 (0.95, 2.32)
Q3	1.39 (0.85, 2.26)	0.84 (0.53, 1.34)	1.67 (1.02, 2.74)	1.79 (1.14, 2.83)
Q4	2.11 (1.32, 3.40)	1.21 (0.78, 1.89)	2.03 (1.14, 3.64)	2.03 (1.18, 3.50)
ω-6:ω-3				
Q1	1.0	1.0	1.0	1.0
Q2	1.12 (0.73, 1.74)	1.41 (0.87, 2.26)	0.91 (0.58, 1.43)	1.32 (0.88, 1.98)
Q3	1.00 (0.64, 1.56)	1.36 (0.86, 2.13)	0.88 (0.56, 1.38)	1.17 (0.77, 1.78)
Q4	0.94 (0.60, 1.47)	1.08 (0.67, 1.73)	1.03 (0.66, 1.59)	1.07 (0.69, 1.64)
* Model adjusted for age, sex, race, education, proxy status, and caloric intake				

Appendix Table 6. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, using absolute value-based cutpoints

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
Tuna					
None	504	77	1.0	124	1.0
T1	493	141	1.53 (1.07, 2.17)	120	0.86 (0.63, 1.19)
T2	490	143	1.68 (1.18, 2.38)	143	1.05 (0.77, 1.44)
T3	540	138	1.41 (0.99, 2.01)	141	0.89 (0.65, 1.22)
Fried fish					
Non-consumers	1086	268	1.0	251	1.0
Consumers	941	231	1.00 (0.80, 1.26)	277	1.22 (1.06, 1.66)
Baked/broiled fish					
None	662	151	1.0	194	1.0
T1	450	116	0.92 (0.67, 1.27)	122	0.74 (0.55, 1.00)
T2	548	175	1.14 (0.84, 1.53)	141	0.73 (0.54, 0.97)
T3	367	57	0.73 (0.40, 1.08)	71	0.68 (0.48, 0.97)
Shellfish					
Non-consumers	966	203	1.0	229	1.0
Consumers	1061	296	1.22 (0.96, 1.55)	299	1.13 (0.90, 1.43)
ω-3					
Q1	507	84	1.0	110	1.0
Q2	507	124	1.61 (1.13, 2.28)	125	1.17 (0.84, 1.61)
Q3	506	130	1.78 (1.24, 2.55)	139	1.34 (0.96, 1.87)
Q4	507	161	2.29 (1.55, 3.38)	154	1.46 (1.00, 2.12)

* Model adjusted for age, sex, race, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 6 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, using absolute value-based cutpoints

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
ALA					
Q1	506	83	1.0	102	1.0
Q2	508	121	1.64 (1.15, 2.33)	124	1.29 (0.93, 1.79)
Q3	506	139	1.91 (1.33, 2.74)	153	1.55 (1.10, 2.17)
Q4	507	156	2.47 (1.66, 3.69)	149	1.61 (1.09, 2.37)
EPA					
Q1	506	104	1.0	132	1.0
Q2	508	123	1.13 (0.81, 1.58)	130	0.92 (0.68, 1.26)
Q3	507	144	1.38 (0.99, 1.92)	132	0.95 (0.70, 1.30)
Q4	506	128	1.22 (0.86, 1.72)	134	1.00 (0.73, 1.38)
DHA					
Q1	507	122	1.0	141	1.0
Q2	507	109	0.93 (0.67, 1.29)	124	0.87 (0.64, 1.18)
Q3	507	133	1.29 (0.94, 1.77)	131	1.01 (0.74, 1.37)
Q4	506	135	1.07 (0.77, 1.49)	132	0.91 (0.66, 1.24)
DPA					
Q1	506	77	1.0	104	1.0
Q2	507	98	1.14 (0.79, 1.65)	112	0.98 (0.70, 1.37)
Q3	508	137	1.43 (0.99, 2.06)	148	1.17 (0.84, 1.64)
Q4	506	187	1.51 (1.02, 2.25)	164	1.10 (0.75, 1.59)
ω-6					
Q1	507	59	1.0	94	1.0
Q2	507	123	2.14 (1.46, 3.14)	123	1.30 (0.93, 1.82)
Q3	507	139	2.22 (1.49, 3.32)	140	1.26 (0.88, 1.80)
Q4	506	178	2.87 (1.86, 4.43)	171	1.59 (1.07, 2.38)

* Model adjusted for age, sex, race, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 6 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, using absolute value-based cutpoints

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	GCA Cases N=528	GCA OR* (95%CI)
LA					
Q1	507	59	1.0	94	1.0
Q2	506	124	2.14 (1.45, 3.14)	123	1.29 (0.92, 1.81)
Q3	507	139	2.25 (1.51, 3.36)	140	1.28 (0.89, 1.83)
Q4	507	177	2.89 (1.87, 4.47)	171	1.62 (1.08, 2.41)
AA					
Q1	507	68	1.0	96	1.0
Q2	507	107	1.79 (1.23, 2.60)	123	1.35 (0.97, 1.89)
Q3	507	129	1.96 (1.33, 2.89)	129	1.25 (0.88, 1.79)
Q4	506	195	2.23 (1.49, 3.34)	180	1.50 (1.03, 2.19)
ω-6:ω-3					
Q1	505	79	1.0	124	1.0
Q2	492	139	0.84 (0.58, 1.21)	120	1.07 (0.77, 1.50)
Q3	490	143	1.06 (0.74, 1.52)	143	1.17 (0.83, 1.64)
Q4	540	138	1.06 (0.72, 1.56)	141	1.04 (0.71, 1.50)

* Model adjusted for age, sex, race, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 7. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, restricting to non-proxy interviews

Measure	Controls N=2027	EA Cases N=347	EA OR* (95%CI)	GCA Cases N=378	GCA OR* (95%CI)
Tuna					
None	504	54	1.0	86	1.0
T1	386	64	1.41 (0.94, 2.12)	69	0.97 (0.68, 1.38)
T2	714	160	1.53 (1.06, 2.19)	159	1.00 (0.73, 1.37)
T3	423	69	1.51 (1.01, 2.25)	64	0.84 (0.59, 1.21)
			p_{trend}=0.84		p_{trend}=0.91
Fried fish					
Non-consumers	1086	178	1.0	163	1.0
Consumers	941	169	0.94 (0.74, 1.21)	215	1.41 (1.11, 1.78)
Baked/broiled fish					
None	662	105	1.0	130	1.0
T1	352	56	0.97 (0.67, 1.40)	58	0.75 (0.52, 1.06)
T2	688	156	1.03 (0.76, 1.41)	145	0.75 (0.56, 1.00)
T3	325	30	0.65 (0.41, 1.02)	45	0.71 (0.48, 1.03)
			p_{trend}=0.30		p_{trend}=0.44
Shellfish					
Non-consumers	966	137	1.0	159	1.0
Consumers	1061	210	1.23 (0.95, 1.59)	219	1.13 (0.88, 1.44)
ω-3					
Q1	507	51	1.0	72	1.0
Q2	507	97	2.02 (1.37, 2.97)	97	1.37 (0.97, 1.95)
Q3	507	85	1.88 (1.25, 2.83)	90	1.34 (0.93, 1.94)
Q4	506	114	2.58 (1.67, 3.98)	119	1.72 (1.15, 2.56)
			p_{trend}=0.01		p_{trend}=0.21

* Model adjusted for age, sex, race, education, caloric intake, and study indicator

Appendix Table 7 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, restricting to non-proxy interviews

Measure	Controls N=2027	EA Cases N=347	EA OR* (95%CI)	GCA Cases N=378	GCA OR* (95%CI)
ALA					
Q1	507	49	1.0	70	1.0
Q2	506	92	1.96 (1.32, 2.90)	89	1.27 (0.89, 1.82)
Q3	508	91	2.07 (1.38, 3.11)	101	1.54 (1.07, 2.21)
Q4	506	115	2.71 (1.74, 4.23)	118	1.74 (1.16, 2.62)
			p_{trend}=0.01		p_{trend}=0.32
EPA					
Q1	507	80	1.0	100	1.0
Q2	507	87	1.13 (0.80, 1.59)	100	0.97 (0.71, 1.33)
Q3	507	94	1.24 (0.88, 1.76)	81	0.80 (0.57, 1.12)
Q4	506	86	1.24 (0.87, 1.77)	97	0.99 (0.71, 1.37)
			p_{trend}=0.38		p_{trend}=0.07
DHA					
Q1	507	93	1.0	103	1.0
Q2	507	75	0.82 (0.58, 1.16)	93	0.89 (0.65, 1.22)
Q3	507	105	1.24 (0.90, 1.73)	92	0.94 (0.68, 1.30)
Q4	506	74	0.89 (0.62, 1.27)	90	0.89 (0.64, 1.24)
			p_{trend}=0.55		p_{trend}=0.21
DPA					
Q1	507	61	1.0	86	1.0
Q2	507	91	1.56 (1.08, 2.24)	90	1.05 (0.75, 1.47)
Q3	507	111	1.94 (1.35, 2.78)	105	1.21 (0.87, 1.68)
Q4	506	84	1.47 (0.99, 2.17)	97	1.14 (0.80, 1.62)
			p_{trend}=0.07		p_{trend}=0.04

* Model adjusted for age, sex, race, education, caloric intake, and study indicator

Appendix Table 7 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA/GCA among participants of two US-based case-control studies of EA/GCA, restricting to non-proxy interviews

Measure	Controls N=2027	EA Cases N=347	EA OR* (95%CI)	GCA Cases N=378	GCA OR* (95%CI)
ω-6					
Q1	507	47	1.0	79	1.0
Q2	507	103	2.13 (1.44, 3.15)	86	1.06 (0.75, 1.50)
Q3	507	85	1.96 (1.29, 2.99)	100	1.24 (0.86, 1.77)
Q4	506	112	2.71 (1.73, 4.24)	113	1.41 (0.94, 2.12)
			p_{trend}=0.01		p_{trend}=0.04
LA					
Q1	506	48	1.0	79	1.0
Q2	508	103	2.06 (1.40, 3.05)	90	1.10 (0.78, 1.55)
Q3	507	83	1.86 (1.22, 2.82)	96	1.17 (0.81, 1.68)
Q4	506	113	2.67 (1.71, 4.18)	113	1.39 (0.93, 2.09)
			p_{trend}=0.01		p_{trend}=0.05
AA					
Q1	506	55	1.0	81	1.0
Q2	507	103	1.87 (1.29, 2.71)	86	1.03 (0.73, 1.45)
Q3	508	84	1.60 (1.09, 2.36)	98	1.26 (0.89, 1.77)
Q4	506	105	2.12 (1.42, 3.16)	113	1.52 (1.06, 2.19)
			p_{trend}=0.02		p_{trend}=0.01
ω-6:ω-3					
Q1	507	87	1.0	81	1.0
Q2	506	87	0.98 (0.70, 1.37)	116	1.45 (1.05, 2.00)
Q3	507	85	0.95 (0.68, 1.33)	100	1.20 (0.86, 1.67)
Q4	507	88	1.02 (0.73, 1.42)	81	1.04 (0.74, 1.46)
			p_{trend}=0.68		p_{trend}=0.93

* Model adjusted for age, sex, race, education, caloric intake, and study indicator

Appendix Table 8. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using caloric exclusions of the lower and upper 2.5%

Measure	Controls N=1940	Cases N=483	EA OR* (95%CI)	Cases N=507	GCA OR* (95%CI)
Tuna					
None	482	74	1.0	119	1.0
T1	364	91	1.51 (1.03, 2.22)	88	0.93 (0.66, 1.31)
T2	688	226	1.56 (1.11, 2.19)	208	0.98 (0.73, 1.33)
T3	406	92	1.41 (0.96, 2.06)	92	0.88 (0.63, 1.25)
			p_{trend}=0.79		p_{trend}=0.88
Fried fish					
Non-consumers	1041	255	1.0	242	1.0
Consumers	899	228	1.10 (0.80, 1.28)	265	1.31 (1.05, 1.65)
Baked/broiled fish					
None	621	144	1.0	185	1.0
T1	342	77	0.99 (0.70, 1.41)	76	0.69 (0.49, 0.96)
T2	656	215	1.02 (0.76, 1.38)	185	0.72 (0.54, 0.95)
T3	321	47	0.65 (0.43, 0.99)	61	0.65 (0.45, 0.94)
			p_{trend}=0.26		p_{trend}=0.30
Shellfish					
Non-consumers	932	197	1.0	222	1.0
Consumers	1008	286	1.20 (0.94, 1.53)	285	1.13 (0.89, 1.43)

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator

Appendix Table 8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using caloric exclusions of the lower and upper 2.5%

Measure	Controls N=1940	EA Cases N=483	EA OR* (95%CI)	GCA Cases N=507	GCA OR* (95%CI)
ω-3					
Q1	485	70	1.0	101	1.0
Q2	485	122	2.05 (1.42, 2.96)	123	1.44 (1.03, 2.00)
Q3	486	125	1.88 (1.28, 2.77)	131	1.44 (1.01, 2.04)
Q4	484	166	2.52 (1.68, 3.80)	152	1.76 (1.20, 2.58)
			p_{trend}=0.01		p_{trend}=0.39
ALA					
Q1	486	65	1.0	95	1.0
Q2	484	121	1.99 (1.37, 2.90)	120	1.38 (0.98, 1.94)
Q3	486	131	2.02 (1.37, 2.98)	141	1.61 (1.13, 2.29)
Q4	484	166	2.60 (1.71, 3.96)	151	1.78 (1.20, 2.64)
			p_{trend}=0.01		p_{trend}=0.49
EPA					
Q1	486	116	1.0	138	1.0
Q2	484	122	1.01 (0.73, 1.40)	137	0.95 (0.70, 1.29)
Q3	485	126	1.12 (0.81, 1.55)	114	0.86 (0.63, 1.19)
Q4	485	119	1.12 (0.80, 1.57)	118	0.94 (0.68, 1.29)
			p_{trend}=0.36		p_{trend}=0.17
DHA					
Q1	485	132	1.0	145	1.0
Q2	485	103	0.81 (0.58, 1.12)	126	0.88 (0.65, 1.20)
Q3	486	136	1.15 (0.84, 1.58)	127	0.99 (0.73, 1.34)
Q4	484	112	0.91 (0.65, 1.27)	109	0.80 (0.58, 1.11)
			p_{trend}=0.64		p_{trend}=0.37

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator

Appendix Table 8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using caloric exclusions of the lower and upper 2.5%

Measure	Controls N=1940	EA Cases N=483	EA OR* (95%CI)	GCA Cases N=507	GCA OR* (95%CI)
DPA					
Q1	485	93	1.0	122	1.0
Q2	485	121	1.43 (1.01, 2.01)	123	1.12 (0.82, 1.54)
Q3	485	143	1.80 (1.29, 2.53)	137	1.31 (0.95, 1.80)
Q4	485	126	1.39 (0.97, 2.00)	125	1.12 (0.80, 1.58)
			p_{trend}=0.08		p_{trend}=0.15
ω-6					
Q1	484	61	1.0	105	1.0
Q2	487	128	2.12 (1.46, 3.10)	122	1.23 (0.89, 1.71)
Q3	483	136	2.19 (1.47, 3.26)	130	1.23 (0.86, 1.75)
Q4	486	158	2.63 (1.71, 4.05)	150	1.53 (1.03, 2.27)
			p_{trend}=0.01		p_{trend}=0.17
LA					
Q1	486	63	1.0	104	1.0
Q2	483	130	2.12 (1.46, 3.08)	123	1.28 (0.92, 1.78)
Q3	486	129	2.02 (1.36, 3.00)	130	1.25 (0.88, 1.78)
Q4	485	161	2.66 (1.74, 4.07)	150	1.59 (1.07, 2.36)
			p_{trend}=0.01		p_{trend}=0.18
AA					
Q1	485	81	1.0	117	1.0
Q2	484	132	1.76 (1.24, 2.50)	112	1.03 (0.74, 1.43)
Q3	487	123	1.57 (1.10, 2.25)	135	1.27 (0.92, 1.76)
Q4	484	147	1.99 (1.37, 2.90)	143	1.47 (1.04, 2.08)
			p_{trend}=0.02		p_{trend}=0.06

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator

Appendix Table 8 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using caloric exclusions of the lower and upper 2.5%

Measure	Controls N=1940	EA Cases N=483	EA OR* (95%CI)	GCA Cases N=507	GCA OR* (95%CI)
ω-6:ω-3					
Q1	485	121	1.0	112	1.0
Q2	486	120	1.07 (0.77, 1.47)	142	1.40 (1.03, 1.91)
Q3	483	120	0.93 (0.67, 1.29)	138	1.18 (0.86, 1.62)
Q4	486	122	1.05 (0.76, 1.45)	115	1.08 (0.78, 1.49)
			p_{trend}=0.61		p_{trend}=0.94

* Model adjusted for age, sex, race, education, caloric intake, proxy status, and study indicator

Appendix Table 9. Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using a random effects meta-analytic approach

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	EA I^2 (%)	EA $p_{heterogeneity}$	GCA Cases N=528	GCA OR* (95%CI)	GCA I^2 (%)	GCA $p_{heterogeneity}$
Tuna									
None	504	77	1.0			124	1.0		
T1	386	96	1.57 (1.09, 2.26)			90	0.90 (0.64, 1.26)		
T2	714	231	1.43 (1.01, 2.02)			216	0.94 (0.70, 1.28)		
T3	423	95	1.34 (0.92, 1.95)	8.0	0.37	98	0.86 (0.62, 1.22)	0	0.73
Fried fish									
Non-consumers	1086	268	1.0			251	1.0		
Consumers	941	231	0.99 (0.78, 1.25)	0	0.96	277	1.33 (1.06, 1.67)	0	0.92
Baked/broiled fish									
None	662	151	1.0			194	1.0		
T1	352	79	0.95 (0.67, 1.34)			79	0.73 (0.52, 1.01)		
T2	688	222	1.02 (0.76, 1.37)			191	0.68 (0.51, 0.92)		
T3	325	47	0.66 (0.44, 0.99)	0	0.50	64	0.67 (0.46, 0.95)	0	0.66
Shellfish									
Non-consumers	966	203	1.0			229	1.0		
Consumers	1061	296	1.19 (0.94, 1.51)	37.3	0.21	299	1.14 (0.91, 1.43)	58.7	0.12
ω-3									
Q1	507	75	1.0			101	1.0		
Q2	507	126	1.87 (1.31, 2.67)			128	1.31 (0.94, 1.81)		
Q3	507	131	1.69 (1.17, 2.46)			134	1.16 (0.82, 1.65)		
Q4	506	167	2.46 (1.65, 3.68)	11.2	0.34	165	1.53 (1.04, 2.24)	41.2	0.13

* Model adjusted for age, sex, race, education, caloric intake, and proxy status

Appendix Table 9 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using a random effects meta-analytic approach

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	EA I^2 (%)	EA $p_{heterogeneity}$	GCA Cases N=528	GCA OR* (95%CI)	GCA I^2 (%)	GCA $p_{heterogeneity}$
ALA									
Q1	507	68	1.0			99	1.0		
Q2	506	125	1.98 (1.37, 2.85)			121	1.20 (0.86, 1.68)		
Q3	508	141	1.99 (1.37, 2.91)			143	1.28 (0.91, 1.81)		
Q4	506	165	2.71 (1.79, 4.12)	19.1	0.29	165	1.53 (1.03, 2.27)	60.8	0.03
EPA									
Q1	507	117	1.0			139	1.0		
Q2	507	132	1.10 (0.80, 1.50)			140	0.94 (0.70, 1.27)		
Q3	507	127	1.09 (0.79, 1.52)			120	0.82 (0.60, 1.12)		
Q4	506	123	1.18 (0.85, 1.65)	0	0.53	129	0.99 (0.73, 1.36)	0	0.78
DHA									
Q1	507	136	1.0			147	1.0		
Q2	507	108	0.78 (0.57, 1.07)			129	0.85 (0.63, 1.14)		
Q3	507	142	1.11 (0.82, 1.51)			129	0.89 (0.66, 1.21)		
Q4	506	113	0.87 (0.63, 1.20)	29.5	0.21	123	0.86 (0.63, 1.17)	0	0.77
DPA									
Q1	507	99	1.0			122	1.0		
Q2	507	121	1.32 (0.94, 1.85)			129	1.11 (0.81, 1.51)		
Q3	507	149	1.66 (1.37, 2.01)			140	1.27 (0.93, 1.73)		
Q4	506	130	1.41 (0.99, 2.01)	39.0	0.15	137	1.17 (0.84, 1.64)	0	0.98
ω-6									
Q1	507	68	1.0			107	1.0		
Q2	507	132	1.94 (1.35, 2.78)			122	1.07 (0.77, 1.48)		
Q3	507	137	1.88 (1.28, 2.75)			137	1.06 (0.75, 1.50)		
Q4	506	162	2.51 (1.66, 3.81)	0	0.57	162	1.38 (0.94, 2.03)	3.4	0.40

* Model adjusted for age, sex, race, education, caloric intake, and proxy status

Appendix Table 9 (cont'd). Odds ratios and 95% confidence intervals for associations between PUFA measures and risk of developing EA and GCA among participants of two US-based case-control studies of EA/GCA, using a random effects meta-analytic approach

Measure	Controls N=2027	EA Cases N=499	EA OR* (95%CI)	EA I^2 (%)	EA $p_{heterogeneity}$	GCA Cases N=528	GCA OR* (95%CI)	GCA I^2 (%)	GCA $p_{heterogeneity}$
LA									
Q1	506	69	1.0			107	1.0		
Q2	508	134	1.91 (1.34, 2.74)			126	1.09 (0.79, 1.51)		
Q3	507	134	1.77 (1.21, 2.60)			133	1.01 (0.71, 1.42)		
Q4	506	162	2.46 (1.63, 3.73)	0	0.68	162	1.36 (0.92, 2.00)	0	0.42
AA									
Q1	506	86	1.0			113	1.0		
Q2	507	132	1.61 (1.14, 2.27)			121	1.09 (0.54, 2.16)		
Q3	508	127	1.52 (1.07, 2.15)			139	1.23 (0.89, 1.71)		
Q4	506	154	2.08 (1.44, 3.00)	0	0.72	155	1.49 (1.06, 2.10)	61.3	0.02
ω-6:ω-3									
Q1	507	129	1.0			116	1.0		
Q2	506	120	1.01 (0.74, 1.39)			148	1.35 (0.99, 1.85)		
Q3	507	125	0.94 (0.69, 1.29)			144	1.25 (0.92, 1.70)		
Q4	507	125	0.98 (0.72, 1.34)	0	0.98	120	1.07 (0.78, 1.47)	0	0.93

* Model adjusted for age, sex, race, education, caloric intake, and proxy status

Appendix Table 10. Comparison of cutpoints for quantiles between study-specific and absolute value-based quantiles for assessing associations between PUFAs and risk of developing EA and GCA in two US-based case-control studies

Measure	US Multicenter Study Quantiles	LAC Multiethnic Study Quantiles	Pooled Absolute Value-Based Quantiles
Tuna (g/day)			
None	0	0	0
T1	0.01 – <3.45	0.01 – <5.59	0.01 – <5.59
T2	3.45 – <14.93	5.59 – <14.91	5.59 – <14.91
T3	≥14.93	≥14.91	≥14.91
Fried fish* (g/day)			
Non-consumers	0	0	0
Consumers	8.61 (9.99)	12.81 (12.92)	11.04 (11.95)
Baked/broiled fish (g/day)			
None	0	0	0
T1	0.01 – <3.61	0.01 – <7.46	0.01 – <7.21
T2	3.61 – <15.63	7.46 – <18.64	7.21 – <15.63
T3	≥15.63	≥18.64	≥15.63
Shellfish* (g/day)			
Non-consumers	0	0	0
Consumers	3.75 (4.41)	9.74 (10.65)	6.93 (8.85)
ω-3 (g/day)			
Q1	<1.41	<1.59	<1.53
Q2	1.41 – <2.11	1.59 – <2.32	1.53 – <2.24
Q3	2.11 – <2.97	2.32 – <3.25	2.24 – <3.19
Q4	≥2.97	≥3.25	≥3.19
ALA (g/day)			
Q1	<1.26	<1.46	<1.40
Q2	1.26 – <1.91	1.46 – <2.14	1.40 – <2.05
Q3	1.91 – <2.63	2.14 – <3.07	2.05 – <2.94
Q4	≥2.63	≥3.07	≥2.94
EPA (g/day)			
Q1	<0.020	<0.014	<0.016
Q2	0.020 – <0.036	0.014 – <0.028	0.016 – <0.030
Q3	0.036 – <0.071	0.028 – <0.049	0.030 – <0.056
Q4	≥0.071	≥0.049	≥0.056
DHA (g/day)			
Q1	<0.053	<0.045	<0.047
Q2	0.053 – <0.095	0.045 – <0.081	0.047 – <0.085
Q3	0.095 – <0.193	0.081 – <0.137	0.085 – <0.149
Q4	≥0.193	≥0.137	≥0.149

* Variables did not have enough variability to categorize into quantiles, mean (SD) shown

Appendix Table 10 (cont'd). Comparison of cutpoints for quantiles between study-specific and absolute value-based quantiles for assessing associations between PUFAs and risk of developing EA and GCA in two US-based case-control studies

Measure	US Multicenter Study Quantiles	LAC Multiethnic Study Quantiles	Pooled Absolute Value-Based Quantiles
DPA (g/day)			
Q1	<0.019	<0.010	<0.012
Q2	0.019 – <0.046	0.010 – <0.015	0.012 – <0.019
Q3	0.046 – <0.128	0.015 – <0.023	0.019 – <0.035
Q4	≥0.128	≥0.023	≥0.035
ω-6 (g/day)			
Q1	<12.53	<10.83	<11.28
Q2	12.53 – <19.23	10.83 – <15.25	11.28 – <16.47
Q3	19.23 – <28.54	15.25 – <21.93	16.47 – <24.03
Q4	≥28.54	≥21.93	≥24.03
LA (g/day)			
Q1	<12.35	<10.74	<11.17
Q2	12.35 – <18.97	10.74 – <15.12	11.17 – <16.29
Q3	18.97 – <27.75	15.12 – <21.75	16.29 – <23.70
Q4	≥27.75	≥21.75	≥23.70
AA (g/day)			
Q1	<0.120	<0.819	<0.090
Q2	0.120 – <0.312	0.819 – <0.121	0.090 – <0.140
Q3	0.312 – <0.862	0.121 – <0.178	0.140 – <0.250
Q4	≥0.862	≥0.178	≥0.250
ω-6:ω-3			
Q1	<7.92	<5.90	<6.28
Q2	7.92 – <9.15	5.90 – <6.75	6.28 – <7.37
Q3	9.15 – <10.69	6.75 – <7.66	7.37 – <8.79
Q4	≥10.69	≥7.66	≥8.79
* Variables did not have enough variability to categorize into quantiles, mean (SD) shown			

Appendix Table 11. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)
Tuna				
None	1.0	1.0	1.0	1.0
T1	0.97 (0.60, 1.56)	0.72 (0.46, 1.12)	1.49 (0.97, 2.28)	1.28 (0.88, 1.87)
T2	0.87 (0.55, 1.37)	0.74 (0.48, 1.13)	1.90 (1.23, 2.95)	1.26 (0.88, 1.80)
T3	0.89 (0.53, 1.52)	0.95 (0.58, 1.55)	1.20 (0.77, 1.88)	1.16 (0.79, 1.71)
Fried fish				
Non-consumers	1.0	1.0	1.0	1.0
Consumers	1.09 (0.83, 1.43)	0.91 (0.68, 1.21)	0.72 (0.52, 1.00)	0.86 (0.65, 1.12)
Baked/broiled fish				
None	1.0	1.0	1.0	1.0
T1	1.29 (0.84, 1.99)	0.83 (0.51, 1.35)	1.01 (0.66, 1.55)	1.10 (0.76, 1.59)
T2	0.95 (0.68, 1.32)	0.68 (0.48, 0.97)	1.21 (0.82, 1.79)	1.04 (0.67, 1.60)
T3	0.98 (0.47, 2.04)	0.69 (0.37, 1.30)	0.89 (0.55, 1.45)	1.09 (0.77, 1.55)
Shellfish				
Non-consumers	1.0	1.0	1.0	1.0
Consumers	0.91 (0.69, 1.22)	0.65 (0.48, 0.88)	1.16 (0.85, 1.57)	1.19 (0.91, 1.55)
ω-3				
Q1	1.0	1.0	1.0	1.0
Q2	0.98 (0.67, 1.42)	1.05 (0.71, 1.54)	1.86 (1.19, 2.89)	1.09 (0.75, 1.59)
Q3	0.76 (0.52, 1.12)	0.71 (0.45, 1.11)	1.62 (0.98, 2.66)	0.74 (0.50, 1.08)
Q4	0.95 (0.63, 1.44)	0.78 (0.50, 1.21)	1.33 (0.74, 2.37)	0.67 (0.41, 1.10)
ALA				
Q1	1.0	1.0	1.0	1.0
Q2	0.77 (0.53, 1.12)	1.20 (0.81, 1.78)	1.52 (0.98, 2.37)	1.11 (0.76, 1.60)
Q3	0.80 (0.54, 1.18)	0.78 (0.50, 1.22)	1.49 (0.91, 2.44)	0.66 (0.45, 0.95)
Q4	0.81 (0.54, 1.24)	0.90 (0.56, 1.45)	1.16 (0.65, 2.05)	0.66 (0.40, 1.08)

* Model adjusted for age, education, proxy status, and caloric intake

Appendix Table 11 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)
EPA				
Q1	1.0	1.0	1.0	1.0
Q2	0.77 (0.54, 1.11)	0.76 (0.51, 1.13)	1.18 (0.77, 1.80)	1.32 (0.90, 1.95)
Q3	0.77 (0.53, 1.10)	0.73 (0.49, 1.08)	1.21 (0.78, 1.89)	0.99 (0.67, 1.45)
Q4	0.65 (0.45, 0.95)	0.85 (0.57, 1.25)	1.12 (0.78, 1.89)	1.58 (1.05, 2.37)
DHA				
Q1	1.0	1.0	1.0	1.0
Q2	0.96 (0.66, 1.40)	0.50 (0.34, 0.74)	1.00 (0.64, 1.57)	1.04 (0.72, 1.50)
Q3	1.09 (0.76, 1.57)	1.01 (0.69, 1.50)	1.33 (0.89, 2.00)	1.06 (0.71, 1.58)
Q4	0.67 (0.46, 0.98)	0.79 (0.54, 1.15)	0.92 (0.57, 1.48)	1.23 (0.81, 1.87)
DPA				
Q1	1.0	1.0	1.0	1.0
Q2	1.14 (0.70, 1.88)	1.32 (0.82, 2.12)	1.35 (0.87, 2.11)	0.76 (0.52, 1.12)
Q3	0.82 (0.51, 1.31)	0.99 (0.62, 1.59)	1.15 (0.74, 1.80)	0.82 (0.55, 1.23)
Q4	0.75 (0.49, 1.15)	0.95 (0.63, 1.44)	1.54 (0.90, 2.64)	1.18 (0.75, 1.87)
ω-6				
Q1	1.0	1.0	1.0	1.0
Q2	1.06 (0.74, 1.54)	1.41 (0.95, 2.09)	1.42 (0.91, 2.22)	0.79 (0.55, 1.15)
Q3	0.66 (0.43, 0.99)	0.89 (0.58, 1.35)	1.44 (0.87, 2.37)	0.85 (0.58, 1.25)
Q4	0.73 (0.48, 1.11)	0.84 (0.53, 1.35)	1.28 (0.69, 2.37)	0.82 (0.48, 1.42)
LA				
Q1	1.0	1.0	1.0	1.0
Q2	0.99 (0.68, 1.42)	1.45 (0.97, 2.14)	1.33 (0.85, 2.08)	0.79 (0.54, 1.15)
Q3	0.69 (0.46, 1.04)	0.92 (0.60, 1.41)	1.37 (0.83, 2.27)	0.86 (0.59, 1.25)
Q4	0.70 (0.46, 1.06)	0.89 (0.56, 1.42)	1.24 (0.68, 2.27)	0.83 (0.48, 1.42)
* Model adjusted for age, education, proxy status, and caloric intake				

Appendix Table 11 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases

Measure	US Multicenter Study		LAC Multiethnic Study	
	EA	GCA	EA	GCA
	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)
AA				
Q1	1.0	1.0	1.0	1.0
Q2	0.96 (0.66, 1.40)	0.90 (0.59, 1.38)	1.56 (1.00, 2.43)	0.92 (0.62, 1.37)
Q3	0.67 (0.46, 0.99)	0.98 (0.66, 1.45)	1.31 (0.83, 2.07)	0.87 (0.58, 1.31)
Q4	0.83 (0.57, 1.21)	0.79 (0.54, 1.17)	1.81 (1.03, 3.21)	1.04 (0.63, 1.70)
ω-6:ω-3				
Q1	1.0	1.0	1.0	1.0
Q2	0.93 (0.64, 1.34)	0.82 (0.55, 1.23)	1.06 (0.69, 1.61)	1.04 (0.72, 1.52)
Q3	0.70 (0.48, 1.02)	1.00 (0.68, 1.47)	1.12 (0.74, 1.70)	1.51 (1.03, 2.21)
Q4	0.85 (0.60, 1.20)	0.95 (0.64, 1.41)	0.60 (0.40, 0.92)	1.60 (1.08, 2.38)
* Model adjusted for age, education, proxy status, and caloric intake				

Appendix Table 12. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using absolute value-based cutpoints

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR* (95%CI)	Cases N=528	Deaths N=450	HR* (95%CI)
Tuna						
None	77	66	1.0	124	100	1.0
T1	141	125	1.20 (0.87, 1.64)	120	110	1.00 (0.75, 1.33)
T2	143	127	1.18 (0.86, 1.62)	143	119	1.03 (0.78, 1.35)
T3	138	116	1.07 (0.78, 1.48)	141	121	1.08 (0.81, 1.44)
Fried fish						
Non-consumers	268	239	1.0	251	224	1.0
Consumers	231	195	0.95 (0.78, 1.16)	277	226	0.88 (0.72, 1.07)
Baked/broiled fish						
None	151	130	1.0	194	168	1.0
T1	111	97	1.08 (0.81, 1.42)	117	103	0.91 (0.70, 1.20)
T2	180	155	0.95 (0.74, 1.22)	146	117	0.87 (0.67, 1.13)
T3	57	52	0.93 (0.65, 1.32)	71	62	1.01 (0.75, 1.36)
Shellfish						
Non-consumers	203	176	1.0	229	197	1.0
Consumers	296	258	1.01 (0.82, 1.24)	299	253	0.92 (0.75, 1.13)
ω-3						
Q1	116	101	1.0	140	117	1.0
Q2	131	118	1.11 (0.84, 1.46)	126	114	1.03 (0.79, 1.34)
Q3	125	107	0.92 (0.69, 1.23)	133	117	0.73 (0.55, 0.97)
Q4	127	108	0.99 (0.71, 1.38)	129	102	0.76 (0.55, 1.04)

* Model adjusted for age, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 12 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using absolute value-based cutpoints

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR* (95%CI)	Cases N=528	Deaths N=450	HR* (95%CI)
ALA						
Q1	118	101	1.0	138	115	1.0
Q2	128	115	1.03 (0.78, 1.36)	129	117	1.19 (0.91, 1.55)
Q3	123	107	0.99 (0.74, 1.33)	135	116	0.71 (0.54, 0.94)
Q4	130	111	1.05 (0.75, 1.46)	126	102	0.84 (0.61, 1.16)
EPA						
Q1	115	100	1.0	142	125	1.0
Q2	126	113	1.03 (0.77, 1.36)	131	111	0.93 (0.71, 1.21)
Q3	134	113	0.88 (0.67, 1.17)	122	101	0.81 (0.61, 1.06)
Q4	124	108	0.83 (0.61, 1.12)	133	113	1.07 (0.81, 1.41)
DHA						
Q1	121	106	1.0	136	121	1.0
Q2	122	108	1.09 (0.82, 1.44)	135	114	0.93 (0.72, 1.21)
Q3	126	109	1.04 (0.78, 1.38)	131	106	0.81 (0.62, 1.07)
Q4	130	111	0.75 (0.56, 1.00)	126	109	1.05 (0.79, 1.40)
DPA						
Q1	108	95	1.0	149	126	1.0
Q2	124	107	1.07 (0.80, 1.44)	132	115	1.02 (0.79, 1.33)
Q3	133	118	0.98 (0.70, 1.35)	124	111	1.04 (0.77, 1.41)
Q4	134	114	0.79 (0.57, 1.11)	123	98	0.93 (0.67, 1.29)
ω-6						
Q1	110	98	1.0	147	119	1.0
Q2	128	113	0.98 (0.73, 1.31)	128	119	1.21 (0.93, 1.58)
Q3	131	113	0.85 (0.62, 1.16)	127	107	0.92 (0.69, 1.22)
Q4	130	110	0.72 (0.50, 1.04)	126	105	0.94 (0.67, 1.33)

* Model adjusted for age, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 12 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using absolute value-based cutpoints

Measure	EA			GCA		
	Cases N=499	Deaths N=434	HR* (95%CI)	Cases N=528	Deaths N=450	HR* (95%CI)
LA						
Q1	109	97	1.0	147	120	1.0
Q2	130	115	0.98 (0.74, 1.31)	127	116	1.15 (0.88, 1.50)
Q3	130	111	0.84 (0.62, 1.15)	128	109	0.92 (0.69, 1.22)
Q4	130	111	0.74 (0.52, 1.07)	126	105	0.92 (0.66, 1.30)
AA						
Q1	110	97	1.0	146	124	1.0
Q2	124	107	1.09 (0.81, 1.47)	134	117	1.07 (0.82, 1.40)
Q3	132	118	1.18 (0.86, 1.61)	125	109	0.83 (0.60, 1.13)
Q4	133	112	0.75 (0.53, 1.05)	123	100	0.91 (0.66, 1.26)
ω-6:ω-3						
Q1	119	106	1.0	138	113	1.0
Q2	119	107	0.88 (0.65, 1.19)	138	123	1.55 (1.18, 2.04)
Q3	129	106	0.73 (0.53, 1.00)	128	107	1.40 (1.01, 1.93)
Q4	132	115	0.63 (0.45, 0.90)	124	107	1.62 (1.14, 2.30)

* Model adjusted for age, education, proxy status, caloric intake, study indicator, and number of FFQ items for each exposure

Appendix Table 13. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, restricting to non-proxy interviews

Measure	EA			GCA		
	Cases N=347	Deaths N=285	HR* (95%CI)	Cases N=378	Deaths N=303	HR* (95%CI)
Tuna						
None	54	44	1.0	86	63	1.0
T1	97	81	0.94 (0.64, 1.39)	91	81	1.32 (0.94, 1.87)
T2	97	83	1.18 (0.80, 1.74)	105	82	1.24 (0.88, 1.75)
T3	99	77	0.97 (0.65, 1.45)	96	77	1.09 (0.77, 1.55)
			p_{trend}=0.12			p_{trend}=0.93
Fried fish						
Non-consumers	178	151	1.0	163	136	1.0
Consumers	169	134	0.81 (0.63, 1.04)	215	167	0.91 (0.72, 1.16)
Baked/broiled fish						
None	105	86	1.0	130	104	1.0
T1	78	64	1.00 (0.71, 1.42)	88	75	1.17 (0.85, 1.61)
T2	126	102	0.99 (0.73, 1.35)	112	85	0.96 (0.70, 1.32)
T3	38	33	1.43 (0.92, 2.21)	48	39	1.01 (0.70, 1.47)
			p_{trend}=0.84			p_{trend}=0.60
Shellfish						
Non-consumers	137	112	1.0	159	128	1.0
Consumers	210	173	1.08 (0.84, 1.39)	219	175	1.03 (0.81, 1.32)
ω-3						
Q1	82	67	1.0	99	77	1.0
Q2	91	79	1.42 (1.01, 1.99)	91	79	1.24 (0.89, 1.72)
Q3	84	66	0.95 (0.66, 1.37)	96	81	1.13 (0.79, 1.61)
Q4	90	73	1.05 (0.70, 1.57)	92	66	0.92 (0.62, 1.38)
			p_{trend}=0.36			p_{trend}=0.79

* Model adjusted for age, education, caloric intake, and study indicator

Appendix Table 13 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, restricting to non-proxy interviews

	EA			GCA		
Measure	**Cases N=347**	**Deaths N=285**	**HR* (95%CI)**	**Cases N=378**	**Deaths N=303**	**HR* (95%CI)**
ALA						
Q1	84	68	1.0	97	75	1.0
Q2	89	76	1.33 (0.95, 1.87)	93	82	1.41 (1.01, 1.96)
Q3	78	62	0.92 (0.63, 1.32)	102	83	1.12 (0.78, 1.59)
Q4	96	79	1.10 (0.75, 1.63)	86	63	1.01 (0.66, 1.54)
			p_{trend}=0.34			p_{trend}=0.82
EPA						
Q1	80	66	1.0	102	86	1.0
Q2	84	71	1.20 (0.85, 1.70)	97	77	0.83 (0.61, 1.14)
Q3	99	79	1.06 (0.75, 1.49)	81	62	0.76 (0.54, 1.07)
Q4	84	69	1.07 (0.74, 1.56)	98	78	0.91 (0.65, 1.27)
			p_{trend}=0.84			p_{trend}=0.57
DHA						
Q1	84	70	1.0	98	81	1.0
Q2	87	74	1.08 (0.77, 1.52)	94	76	0.92 (0.66, 1.26)
Q3	91	74	1.02 (0.72, 1.43)	90	67	0.75 (0.54, 1.04)
Q4	85	67	0.93 (0.64, 1.35)	96	79	1.00 (0.72, 1.40)
			p_{trend}=0.82			p_{trend}=0.85
DPA						
Q1	74	61	1.0	107	85	1.0
Q2	91	76	1.18 (0.82, 1.69)	90	74	0.97 (0.71, 1.34)
Q3	93	78	1.37 (0.91, 2.06)	89	76	1.05 (0.75, 1.47)
Q4	89	70	1.02 (0.66, 1.57)	92	68	0.95 (0.64, 1.41)
			p_{trend}=0.79			p_{trend}=0.41

* Model adjusted for age, education, caloric intake, and study indicator

Appendix Table 13 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, restricting to non-proxy interviews

	EA			GCA		
Measure	**Cases N=347**	**Deaths N=285**	**HR* (95%CI)**	**Cases N=378**	**Deaths N=303**	**HR* (95%CI)**
ω-6						
Q1	77	65	1.0	104	79	1.0
Q2	90	76	1.02 (0.71, 1.45)	91	80	1.37 (0.98, 1.92)
Q3	88	71	1.01 (0.68, 1.49)	93	74	1.12 (0.78, 1.62)
Q4	92	73	0.84 (0.54, 1.30)	90	70	1.25 (0.81, 1.92)
			p_{trend}=0.33			p_{trend}=0.22
LA						
Q1	77	65	1.0	104	79	1.0
Q2	91	76	1.00 (0.70, 1.43)	91	80	1.37 (0.98, 1.91)
Q3	86	70	1.04 (0.71, 1.54)	94	75	1.13 (0.79, 1.63)
Q4	93	74	0.84 (0.54, 1.30)	89	69	1.22 (0.79, 1.88)
			p_{trend}=0.31			p_{trend}=0.22
AA						
Q1	77	64	1.0	104	84	1.0
Q2	88	73	1.24 (0.86, 1.79)	93	75	0.99 (0.71, 1.38)
Q3	93	78	1.34 (0.89, 2.00)	89	74	0.95 (0.67, 1.37)
Q4	89	70	1.01 (0.66, 1.54)	92	70	1.04 (0.70, 1.54)
			p_{trend}=0.80			p_{trend}=0.28
ω-6:ω-3						
Q1	84	73	1.0	97	72	1.0
Q2	81	69	0.98 (0.68, 1.43)	100	86	1.34 (0.96, 1.87)
Q3	90	67	0.72 (0.48, 1.08)	91	71	1.14 (0.76, 1.69)
Q4	92	76	0.86 (0.56, 1.32)	90	74	1.42 (0.93, 2.15)
			p_{trend}=0.60			p_{trend}=0.13
* Model adjusted for age, education, caloric intake, and study indicator						

Appendix Table 14. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using caloric exclusions of the lower and upper 2.5%

Measure	EA			GCA		
	Cases N=483	Deaths N=419	HR* (95%CI)	Cases N=507	Deaths N=431	HR* (95%CI)
Tuna						
None	74	63	1.0	119	95	1.0
T1	132	116	1.22 (0.89, 1.69)	116	106	0.99 (0.74, 1.32)
T2	180	160	1.25 (0.91, 1.72)	177	146	0.98 (0.74, 1.30)
T3	97	80	1.03 (0.73, 1.45)	95	84	1.20 (0.88, 1.64)
			p_{trend}=0.27			p_{trend}=0.36
Fried fish						
Non-consumers	255	226	1.0	242	216	1.0
Consumers	228	193	0.97 (0.79, 1.19)	215	215	0.86 (0.71, 1.05)
Baked/broiled fish						
None	144	124	1.0	185	159	1.0
T1	77	67	1.10 (0.81, 1.49)	76	64	0.99 (0.74, 1.34)
T2	214	184	0.98 (0.76, 1.26)	180	151	0.86 (0.66, 1.13)
T3	48	44	0.81 (0.55, 1.18)	66	57	0.99 (0.72, 1.35)
			p_{trend}=0.08			p_{trend}=0.76
Shellfish						
Non-consumers	197	170	1.0	222	191	1.0
Consumers	286	249	1.02 (0.83, 1.26)	285	240	0.90 (0.73, 1.11)
ω-3						
Q1	111	93	1.0	138	116	1.0
Q2	125	115	1.19 (0.89, 1.58)	122	110	1.04 (0.79, 1.37)
Q3	118	101	0.99 (0.73, 1.34)	129	113	0.73 (0.54, 0.97)
Q4	129	110	1.00 (0.71, 1.39)	118	92	0.78 (0.57, 1.08)
			p_{trend}=0.21			p_{trend}=0.28

* Model adjusted for age, education, proxy status, caloric intake, and study indicator

Appendix Table 14 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using caloric exclusions of the lower and upper 2.5%

	EA			GCA		
Measure	**Cases N=483**	**Deaths N=419**	**HR* (95%CI)**	**Cases N=507**	**Deaths N=431**	**HR* (95%CI)**
ALA						
Q1	109	94	1.0	138	116	1.0
Q2	122	106	0.98 (0.73, 1.30)	127	114	1.13 (0.86, 1.47)
Q3	119	106	0.99 (0.73, 1.34)	126	111	0.73 (0.54, 0.97)
Q4	133	113	0.89 (0.64, 1.25)	116	90	0.77 (0.55, 1.06)
			p_{trend}=0.24			p_{trend}=0.23
EPA						
Q1	114	100	1.0	133	115	1.0
Q2	122	106	0.90 (0.68, 1.19)	127	109	0.99 (0.75, 1.30)
Q3	123	106	0.88 (0.66, 1.16)	123	101	0.86 (0.65, 1.13)
Q4	124	107	0.76 (0.57, 1.02)	124	106	1.10 (0.83, 1.46)
			p_{trend}=0.35			p_{trend}=0.41
DHA						
Q1	120	103	1.0	127	112	1.0
Q2	107	93	0.98 (0.73, 1.31)	141	114	0.73 (0.55, 0.95)
Q3	130	116	1.18 (0.90, 1.55)	117	100	1.08 (0.81, 1.43)
Q4	126	107	0.73 (0.54, 0.97)	122	105	0.97 (0.73, 1.28)
			p_{trend}=0.11			p_{trend}=0.63
DPA						
Q1	79	70	1.0	105	87	1.0
Q2	90	78	1.26 (0.90, 1.77)	112	101	0.96 (0.72, 1.30)
Q3	129	108	0.93 (0.68, 1.29)	117	98	0.88 (0.64, 1.21)
Q4	185	163	0.91 (0.67, 1.26)	173	145	1.02 (0.75, 1.38)
			p_{trend}=0.58			p_{trend}=0.93

* Model adjusted for age, education, proxy status, caloric intake, and study indicator

Appendix Table 14 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using caloric exclusions of the lower and upper 2.5%

	EA			GCA		
Measure	**Cases N=483**	**Deaths N=419**	**HR* (95%CI)**	**Cases N=507**	**Deaths N=431**	**HR* (95%CI)**
ω-6						
Q1	103	89	1.0	146	121	1.0
Q2	130	116	1.14 (0.86, 1.52)	115	104	1.05 (0.80, 1.38)
Q3	117	100	0.84 (0.62, 1.15)	132	115	0.88 (0.66, 1.18)
Q4	133	114	0.85 (0.60, 1.20)	114	91	0.86 (0.61, 1.20)
			p_{trend}=0.15			p_{trend}=0.91
LA						
Q1	105	91	1.0	143	118	1.0
Q2	127	112	1.09 (0.82, 1.45)	121	109	1.08 (0.82, 1.41)
Q3	119	103	0.86 (0.63, 1.18)	127	111	0.89 (0.67, 1.19)
Q4	132	113	0.83 (0.59, 1.17)	116	93	0.88 (0.63, 1.25)
			p_{trend}=0.15			p_{trend}=0.92
AA						
Q1	112	99	1.0	137	117	1.0
Q2	128	108	1.12 (0.84, 1.50)	118	102	0.89 (0.67, 1.18)
Q3	117	99	0.78 (0.58, 1.05)	129	111	0.94 (0.71, 1.25)
Q4	126	113	1.07 (0.79, 1.45)	123	101	0.90 (0.67, 1.22)
			p_{trend}=1.00			p_{trend}=0.70
ω-6:ω-3						
Q1	130	114	1.0	117	102	1.0
Q2	113	95	0.93 (0.70, 1.22)	136	108	0.93 (0.71, 1.23)
Q3	108	98	0.88 (0.67, 1.16)	138	117	1.19 (0.91, 1.57)
Q4	132	112	0.79 (0.60, 1.03)	116	104	1.22 (0.92, 1.63)
			p_{trend}=0.13			p_{trend}=0.06
* Model adjusted for age, education, proxy status, caloric intake, and study indicator						

Appendix Table 15. Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using a random effects meta-analytic approach

Measure	EA					GCA				
	Cases N=499	Deaths N=434	HR* (95%CI)	I^2 (%)	$p_{heterogeneity}$	Cases N=528	Deaths N=450	HR* (95%CI)	I^2 (%)	$p_{heterogeneity}$
Tuna										
None	77	66	1.0			124	100	1.0		
T1	143	126	1.19 (0.87, 1.63)			129	117	1.04 (0.76, 1.38)		
T2	179	159	1.31 (0.96, 1.78)			174	144	1.03 (0.78, 1.35)		
T3	100	83	1.06 (0.75, 1.49)	51.1	0.07	101	89	1.01 (0.75, 1.36)	41.5	0.13
Fried fish										
Non-consumers	268	239	1.0			251	224	1.0		
Consumers	231	195	0.92 (0.75, 1.13)	72.4	0.06	277	226	0.86 (0.70, 1.04)	0	0.57
Baked/broiled fish										
None	151	130	1.0			194	168	1.0		
T1	79	69	1.14 (0.84, 1.54)			79	67	0.92 (0.69, 1.23)		
T2	221	191	1.05 (0.82, 1.35)			186	155	0.80 (0.60, 1.05)		
T3	48	44	0.88 (0.60, 1.31)	0	0.75	69	60	0.93 (0.69, 1.26)	0	0.56
Shellfish										
Non-consumers	203	176	1.0			229	197	1.0		
Consumers	296	258	1.03 (0.84, 1.26)	34.0	0.22	299	253	0.90 (0.74, 1.09)	87.1	0.01
ω-3										
Q1	116	99	1.0			141	118	1.0		
Q2	129	118	1.31 (0.99, 1.73)			127	115	1.11 (0.85, 1.45)		
Q3	126	108	1.04 (0.77, 1.41)			131	115	0.71 (0.53, 0.96)		
Q4	128	109	1.10 (0.79, 1.54)	55.0	0.05	129	102	0.73 (0.53, 1.02)	20.0	0.28

* Model adjusted for age, education, proxy status, and caloric intake

Appendix Table 15 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using a random effects meta-analytic approach

	EA					GCA				
Measure	**Cases N=499**	**Deaths N=434**	**HR* (95%CI)**	**I^2 (%)**	**$p_{heterogeneity}$**	**Cases N=528**	**Deaths N=450**	**HR* (95%CI)**	**I^2 (%)**	**$p_{heterogeneity}$**
ALA										
Q1	117	102	1.0			140	117	1.0		
Q2	126	110	1.06 (0.80, 1.40)			131	118	1.19 (0.91, 1.56)		
Q3	123	110	1.05 (0.78, 1.43)			132	115	0.70 (0.52, 0.93)		
Q4	133	112	0.96 (0.69, 1.34)	45.2	0.10	125	100	0.79 (0.56, 1.11)	42.9	0.12
EPA										
Q1	119	105	1.0			138	120	1.0		
Q2	129	113	0.91 (0.70, 1.20)			128	109	0.96 (0.73, 1.26)		
Q3	127	109	0.90 (0.69, 1.19)			130	107	0.83 (0.63, 1.09)		
Q4	124	107	0.81 (0.61, 1.09)	42.6	0.12	132	114	1.05 (0.79, 1.38)	29.3	0.22
DHA										
Q1	127	110	1.0			129	114	1.0		
Q2	111	97	0.98 (0.73, 1.30)			146	118	0.73 (0.56, 0.96)		
Q3	137	121	1.16 (0.88, 1.51)			121	105	0.99 (0.75, 1.30)		
Q4	124	106	0.78 (0.58, 1.04)	17.4	0.30	132	113	0.90 (0.68, 1.19)	51.5	0.07
DPA										
Q1	84	75	1.0			108	90	1.0		
Q2	97	84	1.23 (0.88, 1.70)			115	103	1.00 (0.74, 1.34)		
Q3	130	110	0.94 (0.69, 1.30)			122	103	0.92 (0.68, 1.25)		
Q4	188	165	0.99 (0.71, 1.38)	29.5	0.21	183	154	1.03 (0.76, 1.39)	0	0.72
ω-6										
Q1	111	97	1.0			147	122	1.0		
Q2	134	120	1.21 (0.91, 1.60)			122	110	1.08 (0.82, 1.41)		
Q3	122	103	0.92 (0.67, 1.27)			135	117	0.86 (0.65, 1.14)		
Q4	132	114	0.90 (0.64, 1.27)	43.4	0.12	124	101	0.83 (0.58, 1.19)	2.3	0.40

* Model adjusted for age, education, proxy status, and caloric intake

Appendix Table 15 (cont'd). Hazard ratios and 95% confidence intervals for associations between PUFA measures and mortality following a diagnosis of EA/GCA among participants of two US-based case-control studies of EA/GCA with follow-up for cases, using a random effects meta-analytic approach

	EA					GCA				
Measure	**Cases N=499**	**Deaths N=434**	**HR* (95%CI)**	**I^2 (%)**	**$p_{heterogeneity}$**	**Cases N=528**	**Deaths N=450**	**HR* (95%CI)**	**I^2 (%)**	**$p_{heterogeneity}$**
LA										
Q1	112	99	1.0			145	120	1.0		
Q2	131	115	1.12 (0.85, 1.49)			125	112	1.09 (0.83, 1.43)		
Q3	124	106	0.92 (0.68, 1.26)			133	116	0.87 (0.66, 1.16)		
Q4	132	114	0.87 (0.62, 1.22)	31.1	0.20	125	102	0.86 (0.60, 1.23)	8.0	0.37
AA										
Q1	117	104	1.0			139	118	1.0		
Q2	132	112	1.19 (0.90, 1.58)			125	109	0.92 (0.69, 1.23)		
Q3	127	109	0.88 (0.66, 1.18)			130	112	0.93 (0.70, 1.23)		
Q4	123	109	1.07 (0.78, 1.46)	53.6	0.06	134	111	0.86 (0.64, 1.17)	0	0.98
ω-6:ω-3										
Q1	135	119	1.0			122	106	1.0		
Q2	116	99	1.00 (0.76, 1.32)			141	112	0.95 (0.72, 1.24)		
Q3	114	103	0.90 (0.68, 1.18)			142	122	1.16 (0.89, 1.52)		
Q4	134	113	0.75 (0.57, 0.98)	29.3	0.22	123	110	1.24 (0.94, 1.63)	33.3	0.19

* Model adjusted for age, education, proxy status, and caloric intake

Appendix Table 16. Comparison of cutpoints for quantiles between study-specific and absolute value based quantiles for assessing associations between PUFAs and mortality following a diagnosis of EA and GCA in two US-based case-control studies with follow-up for cases

Measure	US Multicenter Study Quantiles	LAC Multiethnic Study Quantiles	Pooled Absolute Value-Based Quantiles
Tuna (g/day)			
None	0	0	0
T1	0.01 – <6.89	0.01 – <4.66	0.01 – <5.59
T2	6.89 – <14.93	4.66 – <12.12	5.59 – <14.91
T3	≥14.93	≥12.12	≥14.91
Fried fish* (g/day)			
Non-consumers	0	0	0
Consumers	8.09 (10.44)	13.66 (18.82)	10.20 (14.44)
Baked/broiled fish (g/day)			
None	0	0	0
T1	0.01 – <3.61	0.01 – <7.46	0.01 – <5.83
T2	3.61 – <15.63	7.46 – <16.78	5.83 – <15.63
T3	≥15.63	≥16.78	≥15.63
Shellfish* (g/day)			
Non-consumers	0	0	0
Consumers	3.50 (3.73)	11.65 (15.47)	6.43 (10.49)
ω-3 (g/day)			
Q1	<1.65	<1.81	<1.70
Q2	1.65 – <2.37	1.81 – <2.58	1.70 – <2.46
Q3	2.37 – <3.38	2.58 – <3.52	2.46 – <3.45
Q4	≥3.38	≥3.52	≥3.45
ALA (g/day)			
Q1	<1.52	<1.67	<1.59
Q2	1.52 – <2.15	1.67 – <2.37	1.59 – <2.27
Q3	2.15 – <3.00	2.37 – <3.36	2.27 – <3.16
Q4	≥3.00	≥3.36	≥3.16
EPA (g/day)			
Q1	<0.021	<0.014	<0.017
Q2	0.021 – <0.037	0.014 – <0.025	0.017 – <0.032
Q3	0.037 – <0.071	0.025 – <0.048	0.032 – <0.057
Q4	≥0.071	≥0.048	≥0.057
* Variables did not have enough variability to categorize into quantiles			

Appendix Table 16 (cont'd). Comparison of cutpoints for quantiles between study-specific and absolute value based quantiles for assessing associations between PUFAs and mortality following a diagnosis of EA and GCA in two US-based case-control studies with follow-up for cases

Measure	US Multicenter Study Quantiles	LAC Multiethnic Study Quantiles	Pooled Absolute Value-Based Quantiles
DHA (g/day)			
Q1	<0.052	<0.041	<0.046
Q2	0.052 – <0.098	0.041 – <0.075	0.046 – <0.088
Q3	0.098 – <0.178	0.075 – <0.130	0.088 – <0.151
Q4	≥0.178	≥0.130	≥0.151
DPA (g/day)			
Q1	<0.014	<0.011	<0.014
Q2	0.014 – <0.023	0.011 – <0.016	0.014 – <0.023
Q3	0.023 – <0.058	0.016 – <0.024	0.023 – <0.058
Q4	≥0.058	≥0.024	≥0.058
ω-6 (g/day)			
Q1	<15.11	<12.22	<13.50
Q2	15.11 – <21.11	12.22 – <17.38	13.50 – <19.27
Q3	21.11 – <31.39	17.38 – <23.79	19.27 – <28.24
Q4	≥31.39	≥23.79	≥28.24
LA (g/day)			
Q1	<14.80	<12.16	<13.38
Q2	14.80 – <20.80	12.16 – <17.24	13.38 – <18.92
Q3	20.80 – <30.11	17.24 – <23.59	18.92 – <27.56
Q4	≥30.11	≥23.59	≥27.56
AA (g/day)			
Q1	<0.14	<0.092	<0.11
Q2	0.14 – <0.37	0.092 – <0.135	0.11 – <0.17
Q3	0.37 – <1.03	0.135 – <0.192	0.17 – <0.46
Q4	≥1.03	≥0.192	≥0.46
ω-6:ω-3			
Q1	<7.99	<5.91	<6.62
Q2	7.99 – <9.16	5.91 – <6.73	6.62 – <7.90
Q3	9.16 – <10.52	6.73 – <7.70	7.90 – <9.51
Q4	≥10.52	≥7.70	≥9.51
* Variables did not have enough variability to categorize into quantiles			

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