POLYUNSATURATED FATTY ACIDS, GENETIC SUSCEPTIBILITY, AND BREAST CANCER INCIDENCE AND SURVIVAL

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ABSTRACT

Nikhil K. Khankari: Polyunsaturated Fatty Acids, Genetic Susceptibility, and Breast Cancer Incidence and Survival
(Under the direction of Marilie D. Gammon)

Laboratory studies have demonstrated that ω-3 polyunsaturated fatty acids (PUFAs) inhibit inflammatory eicosanoids generated by ω-6 PUFAs metabolism. Additionally, ω-3 PUFAs have been shown to induce a cytotoxic environment thereby increasing apoptosis and reducing cell growth in breast cancer cells. Despite this biologic plausibility, epidemiologic investigations of dietary PUFA intake and breast cancer are inconclusive among Western populations. This ancillary study examined the impact of dietary PUFA and fish (a primary source of beneficial long-chain (LC) ω-3 PUFAs) intake, and genetic susceptibility in biologically relevant pathways (i.e., inflammation, oxidative stress, and estrogen metabolism) on: the risk of breast cancer (Aim 1); and survival following a first, primary breast cancer diagnosis (Aim 2). To address these aims, resources from the Long Island Breast Cancer Study Project (LIBCSP), a case-control study of 1463 breast cancer cases and 1500 controls were utilized. Additionally, vital status for the population-based cases was determined through 2011, yielding a median follow-up time of 14.7 years and 485 deaths. Adjusted odds ratios (ORs) and hazard ratios (HRs), and corresponding 95% confidence intervals (CIs), were estimated using unconditional logistic regression and Cox-proportional hazards regression, respectively. We observed a super-additive interaction (Relative Excess Risk Due to Interaction=0.43; 95% CI=0.09, 0.78) between ω-3 and ω-6 intake in association with

breast cancer risk, though the CIs for the joint exposure of low ω-3 and high ω-6 compared to high ω-3 and low ω-6 intake were imprecise (OR=1.21; 95% CI=0.86, 1.70). No interactions were observed with polymorphisms considered, but odds were elevated for low ω-3/ω-6 ratio across genotypes. All-cause mortality was reduced by 25-29% among women with breast cancer reporting the highest quartile of intake (compared to never) for: tuna (HR=0.71, 95% CI=0.55, 0.92); other baked/broiled fish (HR=0.75, 95% CI=0.58, 0.97); and dietary long-chain ω-3 PUFAs docosahexanoic (DHA, HR=0.71, 95% CI=0.55, 0.92) and eicosapentanoic (EPA, HR=0.75, 95% CI=0.58, 0.97) acid. Breast cancer risk reduction may be possible for U.S. women with dietary consumption of both higher ω-3 and lower ω-6. Additionally, LC ω-3 PUFA intake from fish and other dietary sources may provide a potential strategy to improve survival after breast cancer.

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ABBREVIATIONS

AA	arachidonic acid
ALA	α-linolenic acid
BMI	body mass index (kg/m^2)
BRCA	Breast Cancer Gene
CI	confidence interval
COX	cyclooxygenase
CYP	cytochrome p450
DAG	directed acyclic graph
DCIS	ductal carcinoma in-situ
DHA	docosahexanoic acid
DNA	deoxyribonucleic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
ER	estrogen receptor
FFQ	food frequency questionnaire
g	gram
GST	glutathione *S*-transferase
HR	hazard ratio
HCFA	Health Care Finance Administration
HER	human epidermal growth factor receptor
HETE	hydroxyeicosatetraenoic acid
HODE	hydroxyoctadecadienoic acid

IL	interleukin
IRR	incidence rate ratio
kg	kilogram
LA	linoleic acid
LC ω-3	long-chain ω-3
LCIS	lobular carcinoma in-situ
LOX	lipoxygenase
LIBCSP	Long Island Breast Cancer Study Project
LRT	likelihood ratio test
m	meter
miRNA	micro ribonucleic acid
NCI	National Cancer Institute
NDI	National Death Index
NHANES	National Health and Nutrition Examination Survey
NSAID	non-steroidal anti-inflammatory drug
nsSNP	non-synonymous single nucleotide polymorphism
OR	odds ratio
PGE2	prostaglandin E_2
PLA2	phospholipase A_2
PPAR	peroxisome proliferator-activated receptor
PR	progesterone receptor
PTGS	prostaglandin synthase
PUFA	polyunsaturated fatty acid

RERI	relative excess risk due to interaction
RR	risk ratio
RNA	ribonucleic acid
SHBG	sex hormone binding globulin
SNP	single nucleotide polymorphism
TFBS	transcription factor binding site
TNF	tumor necrosis factor
U.S.	United States
USDA	United States Department of Agriculture
VITAL	VITamins and Lifestyle
WHEL	Women's Healthy Eating and Living
WINS	Women's Intervention Nutrition Study

CHAPTER 1:
BACKGROUND AND INTRODUCTION

This dissertation examines the association between polyunsaturated fatty acids (PUFAs) and the risk of breast cancer incidence and mortality, and explores whether these associations vary by PUFA class (ω-3, ω-6, the relative balance of ω-3 and ω-6, and ω-3 and ω-6 subtypes), or by fish (the major source of long-chain ω-3), or by genetic polymorphisms in biologically related pathways (inflammation, oxidative stress, and estrogen metabolism). This first chapter provides the rationale for the dissertation aims, including a review of: the descriptive and analytic epidemiology of breast cancer incidence and mortality; the biologic and epidemiologic characteristics of the primary study exposure, PUFAs, as well as the primary group of effect modifiers of interest, polymorphisms in biologically related pathways; and critically evaluates the previously reported epidemiologic studies that have examined the putative PUFA-breast cancer association to underscore the novel aspects of the dissertation aims.

1.1 Epidemiology of Breast Cancer Incidence and Mortality

Breast cancer is the second most common cancer among women in the United States (U.S.), and accounts for nearly one third of all cancer diagnoses in the U.S. [1, 2]. It is also the second leading cause of cancer mortality among women in the U.S. [1]. Nearly 230,500 women were expected to be diagnosed with invasive breast cancer and roughly 40,000 women will die from breast cancer in 2013 [2]. In the U.S., breast cancer incidence rates are

highest among Caucasian white women, followed by African American, Asian/Pacific Islanders, American Indian/Alaska Native, and lowest among Hispanic women [1].

Majority of breast cancers are invasive, where the tumor spreads past the lobules and ducts into the surrounding tissue. *In situ* breast cancer occurs when the tumor is contained within either the duct (ductal carcinoma *in situ*, DCIS) or the lobule (lobular carcinoma *in situ*, LCIS). It is estimated that about 58,000 new cases of *in situ* breast cancer were diagnosed in 2013, and approximately 83% of these were DCIS [2].

Breast cancer rates are much lower among Asian populations than among Western populations (see **Figure 1.1** below). Among Western populations, breast cancer incidence increases with age, with rate of increase slowing around age fifty, but continuing to increase after age fifty [2]. However, among Asian women, the incidence rate of breast cancer increases with age until fifty, and then stabilizes (**Figure 1.1**). This distinct pattern seen among Western and Asian populations points to the direct role of reproductive factors in breast cancer etiology [3]. Reproductive risk factors for breast cancer include: early age at menarche; late age at menopause; nulliparity; late age at first full-term pregnancy; no lactation; and use of hormone replacement therapy. Also, migration studies of Asian immigrants have shown that the breast cancer incidence patterns begin to reach those of Western countries after only a few generations after migration [4-7], suggesting a role for other environmental risk factors for breast cancer. As discussed in more detail below, other risk factors for breast cancer include: post-menopausal obesity; alcohol use; physical inactivity; and family history of breast cancer [8].

Five-year relative survival rates for breast cancer were lower among women who were diagnosed prior to 40 years of age (84%) compared to women diagnosed at 40 years or

older (90%) [2]. Five-year relative survival rates are highest for women whose tumors are localized (99%) compared to regional (84%) and distant tumors (23%). Mortality rates are highest and 5-year survival rates are lowest among African American women [1]. As discussed below, prognostic factors for breast cancer include: tumor size; tumor stage; breast cancer treatment; breast cancer subtypes; and obesity at diagnosis [9].

1.2 Breast Cancer Risk Factors

1.2.1 Reproductive risk factors

There are several established breast cancer risk factors that are associated with reproduction, including early age at menarche, late age at menopause, late age at first full-term pregnancy, nulliparity, lack of or short duration of breast-feeding, and hormone replacement therapy. Many of these reproductive risk factors are thought to affect breast cancer via regulation of long-term exposure to endogenous hormones, including estrogen and progesterone, which are thought to play an important role in breast cancer etiology [8]. The tumor inducing and promoting potential of estrogens has been demonstrated [10, 11], and removal of the ovaries or administration of an anti-estrogenic drug can prevent this effect [12]. Early age at menarche and late age at menopause maximizes a women's lifetime exposure to estrogen, and this prolonged exposure to estrogen has been shown to increase breast cancer risk among many different populations [13-18].

Additionally, later age at first and last full-term pregnancy is thought to increase breast cancer risk and has been reported in different studies across different populations [14-16, 18]. An earlier first-full term pregnancy is thought to reduce the likelihood that cells could become initiated due to the terminal differentiation of breast tissue during pregnancy,

and these terminally differentiated cells have been shown to have lower proliferation due to longer cell cycles and increased time spent in the resting phase (G1) of the cell cycle [19]. A similar mechanism has been proposed for the risk reductions observed among women who have increased duration of lactation, which has been reported in a number of studies [15, 16, 18, 20, 21]. This risk reduction conferred by breastfeeding may be due to the increase in terminal differentiation of the breast epithelium, as well as the delay in the restoration of the ovulatory cycle post-pregnancy [14].

Many women are prescribed hormone replacement therapy in order to help ameliorate the symptoms associated with menopause. However, the use of hormone replacement therapy has been demonstrated to affect breast cancer risk, and is dependent upon the type of hormones that are prescribed. In 2001, among participants in the Women's Health Initiative trial, there was a reported 26% (95% CI = 1.00, 1.59) increased hazard for breast cancer among postmenopausal women on estrogen and progestin replacement therapy compared to those taking placebo [22]. Increased risks also were observed among the Million Women Study in the United Kingdom, where breast cancer risk was doubled for estrogen-progestin replacement therapy [23].

In sum, examination of the reproductive risk factors for breast cancer has helped to elucidate an important role of endogenous and exogenous estrogen and progesterone in breast cancer carcinogenesis.

1.2.2 Family History

Another established risk factor includes inherited susceptibility for breast cancer. Family history of breast cancer in a first-degree relative has been shown to increase breast

cancer risk. Women, whose mothers or sisters have breast cancer, are 1.5 to 3 times as likely to have breast cancer compared to other first-degree relatives without breast cancer [24]. A meta-analysis reported the risk for breast cancer was nearly double for women with any relative, first-degree relative, mother, or sister with breast cancer [25]. The highest risk was observed for women who had a mother and sister with breast cancer (RR = 3.6; 95% CI = 2.5, 5.0).

In the mid-1990s, breast cancer gene 1 (*BRCA1*) on chromosome 17q21 and breast cancer gene 2 (*BRCA2*) on chromosome 13q12-13 were identified [26]. These two genes are suggested to act as tumor suppressors, and mutations in these genes are highly penetrant and account for 2-5% of breast cancer risk [24]. In the general population, estimated prevalence of *BRCA1* and *BRCA2* mutation carriers range from 0.1- 0.2% [26, 27], and these mutations may be responsible for early-onset breast cancer among high risk families [27].

However, family history cannot solely explain geographic variation in breast cancer rates. Studies conducted among Asian immigrants have shown that the breast cancer incidence patterns for these populations mimic those of Western countries only a few generations after migration [4-7]. Therefore, other environmental risk factors, for which the prevalence varies by geographic residence, may help to explain this observed geographic variation in breast cancer rates.

1.2.3 Obesity and Physical Activity

Obesity is another source for endogenous estrogen production. Adipose tissue and visceral fat has been shown to be metabolically active [28]. Androgens present in adipose tissue can be converted to estradiol via aromatase, resulting in an additional source of

metabolically active estrogen that is not produced by the ovaries [28, 29]. These laboratory findings are supported by epidemiologic results. For example, a pooled analysis based on data collected primarily among women of European descent examined the effect of body mass index (BMI) on breast cancer risk and reported that increasing BMI among postmenopausal women (BMI exceeding 28 kg/m^2) was associated with an increase in breast cancer risk of 26% (95% CI = 1.09, 1.46) [30]. Changes in weight (greater than 15 kg since age 20) compared to maintaining weight, has been reported to increase risk for postmenopausal breast cancer (OR = 1.6; 95% CI = 1.11, 2.26) [31], and this increased risk has been consistently reported among several other studies [32-34]. Asian studies also reported a similar increased risk for postmenopausal breast cancer with increases in body weight [35] or BMI [36, 37].

In contrast, the association between obesity and premenopausal breast cancer appears to be more complex. Estrogen production via metabolism of adipose tissue is not the primary production source for estradiol among premenopausal women, for whom ovarian production remains active; therefore the potential for increased risk for breast cancer due to excess adipose tissue may be reserved for postmenopausal women only. For example, in the pooled analysis discussed above, a risk reduction (RR = 0.54; 95% CI = 0.34, 0.85) was observed for premenopausal women with higher BMI (BMI exceeding 31 kg/m^2 versus BMI less than 21 kg/m^2) [30]. Further, only one Asian study reported a risk reduction for increased BMI and premenopausal breast cancer [36]. Other studies have similarly reported breast cancer risk reductions among premenopausal women for increasing weight [33, 38-40]; however, one of these studies reported that this risk reduction was limited only to those with early-stage, lower grade breast cancer [38]. Some theories have been postulated for the risk reductions

seen among premenopausal women, and include obesity-triggered anovulation [41], which can result in lower levels of exposure to both progesterone and estradiol [42].

Physical activity also is thought to reduce breast cancer risk via a variety of mechanisms, including lower levels of estrogens and increased levels of sex hormone binding globulin (SHBG) resulting from reduced adipose tissue and visceral fat [43, 44], improved immune response [45], and lower inflammatory markers [46, 47]. A systematic review reported risk reductions ranging from 20-80% were observed for postmenopausal breast cancer with increasing levels of physical activity [48]. A smaller risk reduction (15-20%) was observed for premenopausal women [48-50]. Similar risk reductions were observed for recreational physical activity among Chinese women [51]. A recent study examined the joint effects of physical activity, weight gain, and body size on breast cancer risk, and reported risk reductions for breast cancer and recreational physical activity during both reproductive years and postmenopausal [52]. However, it also was reported that excessive weight gain during postmenopausal years may negate any of the beneficial effects of any physical activity.

In summary, physical activity, obesity, and weight maintenance provide a potential opportunity for breast cancer risk reduction. However, increasing physical activity, reducing obesity or maintaining weight may not be an easily implemented option for all women. Therefore, other opportunities for breast cancer risk reduction need to be explored, including nutritional factors.

1.2.4 Non-Steroidal Anti-inflammatory Drugs

Another potential risk reduction strategy for breast cancer is non-steroidal anti-

inflammatory drugs (NSAIDs), such as aspirin and ibuprofen. NSAIDs are drugs that inhibit cyclooxygenase (COX) activity and thereby result in reduced levels of prostaglandins, which have been implicated in breast carcinogenesis [53]. There is evidence from animal studies that NSAIDs may also have an inhibitory effect that is independent of prostaglandin synthesis. Animal and laboratory studies suggest that NSAIDs may inhibit the effects of estrogen in the pituitary gland [54] and may inhibit binding of estradiol to the estrogen receptor [55]. Observational studies have reported a modest risk reduction (10-20%) for NSAID use and breast cancer risk [56], though this inverse association is not consistently reported across all studies [56, 57]. Risk reductions were slightly stronger among case-control studies compared to cohort studies, which may reflect differential recall bias present in case-control studies resulting in exaggerated effect estimates. Recent studies have been inconsistent regarding NSAID use and breast cancer, with one reporting risk reduction [58], another reporting null effects [59], and yet another reporting increased risk [60]. NSAID use was reported to decrease breast cancer risk by 20% among postmenopausal women [58], but had no effect among premenopausal women [61]. A recent study reported stronger risk reductions for NSAIDs that were selective COX-2 inhibitors (e.g., celecoxib, rofecoxib, and valdecoxib) in comparison to non-specific NSAIDs [62]. Inconsistencies in the literature may arise from differences in control selection (e.g., population- versus hospital-based), differences in exposure assessments (e.g., questionnaire versus health care prescription data), or differential recall in case-control studies versus cohort studies.

Considering genetic susceptibility in NSAID metabolism may help to clarify the NSAID breast cancer association. For example, Brasky et al. [63] examined the interaction between NSAID use and genetic polymorphisms in *COX-2*, and reported strongest risk

reductions for aspirin use among those with the variant allele rs4648261 (C-to-T base pair change), a single nucleotide polymorphism (SNP) in *COX-2*. In the Long Island Breast Cancer Study Population (LIBCSP), risk reductions were reported for regular use of aspirin and breast cancer [64], but largely no interactions were seen for *COX-2* polymorphisms and NSAID use [65].

In sum, although many studies have reported risk reductions for NSAID use and breast cancer, the results are not conclusive. NSAIDs inhibit cyclooxygenase activity thereby reducing levels of inflammatory prostaglandins. It has also been noted that long-term use of NSAIDs could have potentially adverse outcomes on health [66, 67]. Similar to NSAIDs, ω-3 PUFAs are also known to competitively inhibit binding of ω-6 PUFAs to cyclooxygenase enzyme (see below), thereby reducing levels of inflammatory prostaglandins [68]. Thus, dietary intake of ω-3 PUFAs may provide a safer alternative for breast cancer risk reduction compared to NSAIDs.

1.2.5 Alcohol

Alcohol is thought to affect breast carcinogenesis via multiple mechanisms, including increasing levels of endogenous estrogen, production of acetaldehyde and reactive oxygen species resulting from alcohol metabolism, and interference with absorption of essential nutrients [69]. An early meta-analysis [70] reported a 24% increased risk (95% CI = 1.15, 1.34) for women consuming two drinks per day compared to non-drinkers. Women consuming three drinks per day had nearly a 40% increase in risk (95% CI = 1.23, 1.55) [70]. A 1998 pooled analysis reported 10% (95% CI = 1.04, 1.13) and 41% (95% CI = 1.18, 1.69) increases in breast cancer risk for 10-gram and 30-60 gram increase in alcohol consumption

per day, respectively [71]. More recent studies examining alcohol intake and breast cancer have reported similar effect estimates. The Million Women Study reported a 12% increase in breast cancer risk for a 10-gram increase [72]. The Nurses' Health Study reported a 15% increase in breast cancer risk for 5-10 gram increase in alcohol consumption [73], and the American Cancer Society Nutrition Cohort reported a 26% increase for 15 gram per day increase in alcohol consumption [74]. However, among the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, no effects were observed for 10-gram per day increase in alcohol intake and breast cancer risk [75].

In sum, alcohol is an established breast cancer risk factor which acts via multiple mechanisms to affect breast cancer risk, including increased estrogen production. Thus, epidemiologic and supporting laboratory evidence has demonstrated that an ingested compound can influence estrogen levels among adult women and increase breast cancer risk.

1.2.6 Dietary Fat

Dietary fat and breast cancer risk has been extensively examined, however, there is limited evidence suggesting an association between total fat intake and breast cancer. Dietary fat is known to increase endogenous estrogen production [76]. Also, dietary fat can increase levels of serum-free fatty acids thereby displacing estradiol from serum albumin, and thus resulting in increased levels of free estradiol [77]. A null association has been reported for total fat intake among all ages or premenopausal breast cancer [78]. Numerous studies have examined the effect of total fat on postmenopausal breast cancer, where prospective cohort studies tend to report inconsistent associations and case-control studies tend to report modest increases in risk. Seven cohort studies reported increased risks for

postmenopausal breast cancer [79-82, 82-85], whereas two other studies reported risk reductions [86, 87]. The majority of case-control studies report an increased risk for postmenopausal breast cancer with total fat intake [88-100], with a relatively fewer number of studies reporting a decrease in risk [89, 101-104]. A summary estimate was derived from seven case-control studies reporting a modest 11% increase in risk for total fat intake [78]. This difference in effect estimates derived from the case-control and prospective studies may reflect differential recall bias of total fat intake, which would exaggerate effects in case-control studies. Therefore, there is limited evidence regarding total fat intake and breast cancer, with no associations reported for premenopausal women, and a suggested increase in risk for postmenopausal breast cancer. However, conclusions regarding total fat and breast cancer risk are still unclear.

The inconsistent reported effects of the association between breast cancer incidence and fat intake may be due to type of dietary fat consumed. A pooling project reported differential effects for different types of fat, where saturated and monounsaturated fats increased risks by 9% and 5%, respectively [105]. Modest risk reductions (2-13%) were reported when substituting either saturated or monounsaturated fats with PUFAs [105]. In order to better understand the effects of fat on breast cancer risk, it may be important to examine the different types of fat in relation to breast cancer, such as PUFAs.

1.2.7 Summary

A number of breast cancer risk factors have been identified. Reproductive risk factors include: reproductive history; menstrual history; and exogenous hormone use. Other risk factors include: family history of breast cancer, such as inherited genetic susceptibility;

and select nutritional factors such as obesity, physical activity, and alcohol. With the exception of these select nutritional factors, few risk factors are easily modifiable. Identification of other potentially modifiable nutritional factors, such as dietary intake of PUFAs, may be warranted in order to provide additional avenues for breast cancer prevention.

1.3 Prognostic Factors for Breast Cancer

1.3.1 Age, Socioeconomic Status, and Race

A few established prognostic factors for breast cancer survival include age at diagnosis, socioeconomic status, and race. Women with younger age at diagnosis have been reported to have reduced survival and increased relapse compared to women who are diagnosed at older ages [106-108]. Younger women at diagnosis have been reported to have more aggressive tumors, consisting of worse histopathology (e.g., higher stage, larger tumor size), thus contributing to their lower survival rates [106]. In addition to age at diagnosis, African American women are more likely to have reduced survival compared to Caucasian women [108]. It has also been reported that African American women tend to have a higher prevalence of basal-like breast cancer, which is reported to have poor prognosis [109]. Although, basal-like breast cancer has poor prognosis, it is not an inherently more aggressive disease among African American women compared to Caucasian women [110]. This racial difference in survival also could be attributed to differences in screening rates resulting in more African American women presenting at older ages with higher tumor grade and stage [108]. It is possible that socioeconomic status is associated with many of the factors relating to differences in survival, since it has been observed that women with low socioeconomic

status have poor prognosis, irrespective of race [111].

1.3.2 Tumor Size and Stage at Diagnosis

The size of the tumor is known to predict survival. Women with larger tumors have worse prognosis than those with smaller tumors [107, 112]. Women with tumors greater than two centimeters are more likely to die from breast cancer five years post diagnosis [107], and are more likely to have distant metastatic disease [113]. Metastases to regional lymph nodes are a strong prognostic indicator, among whom women with greater number of lymph nodes with worse survival than those with negative lymph nodes [112, 114]. Tumor stage is also a strong indicator of disease survival, where women with higher stage have poor prognosis compared to those with lower stage [114, 115]. Thus, tumor size and stage are established breast cancer prognostic factors.

1.3.3 Breast Cancer Subtypes

The estrogen receptor plays a vital role in breast cancer survival, with approximately 60% to 65% of breast cancers being estrogen receptor-positive (ER+) [116]. The ER+ subtype of breast cancer is an important indicator for treatment, as those with this tumor type can be more effectively treated with anti-estrogenic drugs, such as tamoxifen and raloxifene. In addition to the possibility for more effective treatment, ER+ tumors are more likely to be better differentiated and are known to have better prognosis [117], whereas as estrogen receptor-negative (ER-) tumors tend to have worse histologic characteristics (e.g., tumor size, tumor grade, regional spread) resulting in lower survival [118, 119]. Women with ER+ tumors and progesterone receptor-positive (PR+) tumors are reported to have better five-year

relative survival rates than women with ER- or PR- tumors [120].

Gene expression analysis has documented multiple intrinsic subtypes, in addition to the ER/PR subtypes. The ER+/ER- subtypes can be further classified into separate groups, which include: (1) *luminal A*: ER+ and/or PR+, human epidermal growth factor receptor-2 negative (HER2-); (2) *luminal B*: ER+ and/or PR+, HER2+; (3) *HER2+/ER-*: ER-, PR-, (HER2+); and (4) *basal-like*: ER-, PR-, HER2-, cytokeratin 5/6+, and/or HER1+ [109]. Basal-like tumors are more prevalent among African American women (premenopausal), and both basal-like and HER2+/ER- tumors were more likely to be of higher grade, greater mitotic index, higher prevalence of *p53* mutations, and more likely to be poorly differentiated [109]. These factors may account for the poor prognosis observed among women with basal-like and HER2+/ER- tumors [109]. Understanding the prognostic influence of breast cancer subtypes is not only important for identifying successful treatments for women diagnosed with breast cancer, but also could help to identify women who would benefit from additional interventions to help improve survival.

1.3.4 Treatment

Treatment for breast cancer is often dependent upon the subtype of cancer. For example, tamoxifen treatment has been shown to improve survival among women with ER+ tumors, and provide modest improvement for ER- tumors [121-124]. Chemotherapy also reduced the annual breast cancer death rate by nearly 40% for women younger than 50 years of age, and about 20% for women 50-69 years of age at diagnosis [121]. Among patients treated with radical mastectomy, combined radiation and chemotherapy treatment was shown to improve recurrence, relapse-free survival, and breast cancer-specific survival when

compared to chemotherapy alone [125]. Supplementary treatment strategies, that are both cost-effective and easily-implemented, need to be considered to further improve survival for women diagnosed with breast cancer. Nutritional factors that enhance treatment efficacy, such as PUFAs, should be considered as they may provide an additional means for improving survival among women diagnosed with breast cancer.

1.3.5 Obesity and Physical Activity

There is a strong indication that obesity and physical activity play a role in breast cancer prognosis, though they are not considered established breast cancer prognostic factors. A recently published pooling project reported increased hazards for overall mortality and breast cancer mortality for underweight (BMI < 18.5 kg/m^2) and morbidly obese women (BMI ≥ 40 kg/m^2) [126]. More recently, Cleveland et al. also reported increased mortality among women who were obese at diagnosis among both pre- and post-menopausal women [127]. Also, among premenopausal women, those who gained greater than 16 kg between age 20 and one year prior to diagnosis had more than double the hazard for mortality when compared to those who maintained a stable weight (± 3 kg) [127]. Post-diagnosis maintenance of weight is also important for improving survival [128]. Bradshaw et al. reported more than two-fold increase in mortality for those who gained more than 10% after diagnosis, and this effect was more pronounced in the first two years after diagnosis [128]. Thus, maintenance of weight, whether it is prior to diagnosis or after, is suggested to improve breast cancer survival for both premenopausal and postmenopausal breast cancer patients.

Physical activity prior to breast cancer diagnosis is reported to improve survival after breast cancer. Cleveland et al. reported a nearly 50% reduction in all-cause mortality for

women who engaged in nine or more metabolic equivalent task hour (MET-hour) per week of lifetime recreational physical activity from menarche to diagnosis compared to women who did not exercise [129]. Similar results were reported among the California Teachers Study [130]. Additionally, exercise within three years of diagnosis was inversely associated with both overall and relapse/disease-specific mortality [131, 132]. Similarly, Bradshaw et al. reported improved survival among women who were highly active (>9 weekly MET-hours) post-diagnosis [133]. Studies suggest that both pre-diagnostic and timely post-diagnostic engagement in exercise may help to improve survival among women diagnosed with breast cancer.

1.3.6 Dietary Fat and NSAIDs

The relation between dietary fat intake and breast cancer survival has been increasingly reported by a number of investigators. The majority of studies regarding dietary fat and survival showed an increased risk of dying with higher fat intake [134-137], with the exception of one study showing a risk reduction [138]. An early study examined the relation between dietary fat and breast cancer survival among both Caucasian and Japanese women with breast cancer diagnoses in Hawaii [139]. The study reported more than triple the risk of death for high versus low total fat intake for Caucasian women. In comparison, nearly 40% mortality risk reduction was reported among women of Japanese ancestry for high versus low total fat intake. Though both estimates were imprecise, this difference in the direction of the reported associations among Caucasian and Japanese women may highlight the importance of examining different types of fat on breast cancer, such as ω-3 and ω-6 PUFA.

The majority of epidemiologic studies have reported poor prognosis for total dietary

fat intake. Two large randomized trials were conducted in the mid-1990s in order to examine the effect of reductions in dietary fat intake on prognosis among breast cancer survivors in the U.S. The Women's Intervention Nutrition Study (WINS) was focused primarily on the efficacy of reduction in dietary fat intake [140]. The WINS study included approximately 2,400 postmenopausal women who had completed primary treatment and had been diagnosed with stage one breast cancer in the previous year. Study participants were randomized to receive an intervention targeted to reduce fat intake. The study reported slight reductions in the hazard for overall survival (HR = 0.89; 95% CI = 0.65, 1.21) [140]. In contrast, the Women's Healthy Eating and Living (WHEL) trial focused on the efficacy of changes in dietary pattern, which included reduction in dietary fat intake [141]. The WHEL study included approximately 3,000 women, among whom nearly 80% were postmenopausal, and had been diagnosed with stage one breast cancer within the previous four years. The intervention focused on implementing a dietary pattern with high fruit, vegetable, and fiber intake and low total fat intake. The WHEL study reported null associations for the dietary pattern intervention on overall survival (HR = 0.97; 95% CI = 0.78, 1.22) [142]. A modest reduction in the hazard for recurrence was reported in the WINS study (HR = 0.76; 95% CI = 0.60, 0.98), but not for the WHEL study (HR = 0.99; 95% CI = 0.83, 1.17).

It is important to note the differences in assessment methods used in these two trials. The focus of the WHEL study was on dietary pattern changes, which included reduction in dietary fat intake. Whereas, the WINS study's primary focus was on reduction of dietary fat intake. Also, the studies differ with regards to the time between breast cancer diagnosis and enrollment. The WINS study enrolled women within one year of diagnosis, whereas the WHEL study enrolled women within four years of breast cancer diagnosis. Thus, the WINS

study would focus on more short-term prognosis, or women who could have a breast cancer event within five years of diagnosis. In contrast, the WHEL study under sampled women who had a breast cancer event within four years of the diagnosis, and therefore are unable to examine the effect of the intervention on early recurrence and death. This may explain the differences in the reported HRs for breast cancer recurrence in the two studies.

These previous studies on breast cancer progression and mortality focused on reductions in total fat intake without any consideration given to the type of fat. It is possible that ω-3 and ω-6 PUFAs will have differential effects on survival, and that reduction in dietary intake of the unfavorable ω-6 fatty acids and increases in the more favorable ω-3 fatty acid could improve breast cancer survival. Therefore, examination of these different types of PUFAs and their relative balance could further elucidate the relation between dietary fat and breast cancer survival.

Non-steroidal anti-inflammatory drugs could also improve survival by reducing inflammatory metabolites resulting from metabolism of arachidonic acid via cyclooxygenase enzymes. However, only two studies on NSAID use in relation to survival from breast cancer have been reported to date, and results were conflicting [143, 144]. Thus, other avenues for improving breast cancer survival should be considered.

1.3.7 Summary

There are many established clinical indictors of breast cancer prognosis, including late age at diagnosis, low socioeconomic status, African American race, ER- tumor subtype including triple negative and basal-like breast cancer, and inadequate treatment, which have been demonstrated to worsen prognosis among breast cancer patients. Additionally, there is

growing evidence that lifestyle factors such as weight maintenance, increased physical activity, and reduced fat intake before and after diagnosis improve survival. The promising findings for nutritional factors underscore the hypothesis that some factors may be modified in an effort to improve survival, and support the examination of other possible nutritional factors that could influence breast cancer survival. One possibility is dietary PUFA intake, which may help to provide an opportunity for improving survival among women diagnosed with breast cancer.

1.4 PUFAs

1.4.1 Structure

Fatty acids are long-chain lipids containing primarily two groups: a carboxylic acid; long carbon chain; and methyl end. PUFAs are lipids that contain at least two double bonds resulting in the *cis* configuration [145]. There are two primary classes of PUFAs, ω-6 and ω-3. Omega-6 fatty acids refers to the position of the first double bond in the carbon-chain from the methyl group found at the end of the carbon-chain; whereas, ω-3 fatty acids have the first double bond at the third position from the methyl group. The ω-6 PUFA subtypes include: linoleic acid (LA) and arachidonic acid (AA); and the ω-3 PUFA subtypes include: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). The total number of double bonds present in the carbon-chain differentiates between the individual subtypes of ω-3 fatty acids or ω-6 fatty acids. Please refer to **Table 1.1** (see below) for details regarding the carbon chain length and total number of double bonds for the different ω-3 and ω-6 PUFA subtypes.

1.4.2 PUFA Sources and Biosynthesis

Mammalian cells are unable to endogenously produce ω-3 and ω-6 PUFAs and, thus the primary source of PUFAs in humans is diet. As shown in **Table 1.1** (see below), fatty fish (e.g., halibut, mackerel, herring, and salmon) are the primary source of long-chain ω-3 (LC ω-3) PUFAs. Vegetable oils are the major source of ALA [146]. Other sources contribute only minor quantities of ω-3 to the diet and include nuts and seeds, vegetables and some fruit, egg yolk, poultry and meat [146]. Different cooking methods (e.g., deep frying) have been shown to substantially reduce LC ω-3 PUFA content [147]. Vegetable oils are major sources of ω-6 fatty acids. Corn oil, peanut oil, sunflower oil, safflower oil, margarine, lard, bacon, ham, nuts are sources of LA [145, 148].

In addition to these exogenous sources of PUFAs, it is also important to consider *in vivo* metabolism of ω-3 and ω-6 fatty acids, respectively. Humans do not possess the enzymes necessary to desaturate LA to ALA, thus conversion from one PUFA class to another (e.g., ω-6 to ω-3) is impossible [148]. However, through a series of desaturations (removal of hydrogen and introduction of double bond) and elongations (extension of the fatty acid chain by two carbons), formation of different subtypes of ω-3 and ω-6 fatty acids are possible *in vivo*. For example, desaturation (via δ-5-desaturase and δ-6-desaturase) and elongation of LA (ω-6) and ALA (ω-3) would lead to AA and EPA, respectively [148]. Further elongation of EPA leads to DPA, which subsequently can be elongated (via acetyl-CoA), then desaturated (via δ-6-desaturase), and transported to the peroxisome for β-oxidation forming DHA [148]. Deficiency in ALA can lead to reduced levels of DHA and enhanced levels of ω-6 fatty acids in tissue membranes [149]. This conversion process for certain PUFA subtypes is important to consider since it could affect the availability of other

subtypes *in vivo*. For example, low intake of ω-3 PUFAs that are precursors (e.g., ALA) in fatty acid biosynthesis may affect the bioavailability of downstream LC ω-3 PUFAs (e.g., EPA, DPA, DHA) [150]. Therefore, in addition to considering dietary intake of specific subtypes of ω-3 and ω-6 PUFAs separately, it may be equally important to consider total dietary intake of ω-3, ω-6, and the relative balance of ω-3 and ω-6 PUFAs. **Figure 1.2** shown below provides an overview PUFA biosynthesis.

It is suggested that human beings evolved on an ω-3:ω-6 ratio approximately equivalent to one, and since then our diets have evolved to include more sources of ω-6 fatty acids [151]. This increased consumption of ω-6 fatty acids was due in large part to the development of technology during the early 1900s marking the beginning of the vegetable oil industry, and modern agriculture with the emphasis on feeding domestic livestock with grains rich in ω-6 fatty acids [151]. During the 20th century, the consumption of soybean oil increased dramatically [152]. Thus, the availability of LA (as a percentage of total energy) has increased dramatically in Western populations; whereas, the availability of LC ω-3 PUFA has remained stable [152]. The relative intake of ω-3 and ω-6 PUFA varies geographically, with western diets having an intake ratio of ω-3:ω-6 of 1:15-20, compared to the ratio of 1:5-6 in India and 1:4 in Japan [153]. Also, it has been reported that serum levels of LCω3-PUFA are lower among U.S. Whites when compared to other Asian populations [154]. Since ω-3 and ω-6 fatty acids compete for the same enzymes, it is important to consider the relative balance of the two when considering their effects on chronic disease.

In summary, examination of the relative balance of ω-3 and ω-6 fatty acid intake may be vital to understanding the effects of PUFA on breast cancer, and for identifying an unfavorable ratio that may increase breast cancer risk and reduce survival.

1.5 Metabolism of PUFAs

PUFAs are incorporated into the cellular membrane. After incorporation within this lipid bilayer, PUFAs are then available for metabolism which occurs via the cyclooxygenase, lipoxygenase, and cytochrome p450 pathways. Both ω-6 and ω-3 fatty acids are metabolized via these pathways and compete with one another for the same enzymes, and they have demonstrated effects on the hallmarks of cancer [155]. The differential effect of ω-6 and ω-3 fatty acids on breast cancer etiology has been well demonstrated in animal and laboratory studies. **Figure 1.3** shown below provides an overview of the biologic pathways involved in arachidonic acid metabolism.

1.5.1 Cyclooxygenase pathway

Metabolism of AA via the cyclooxygenase pathway results in the formation of inflammatory intermediates, known as eicosanoids. A key metabolic enzyme in this pathway is prostaglandin synthase 2, or COX-2. Aberrant upregulation of COX-2 expression has been reported in breast tumors [156-158]. COX-2 is known to be overexpressed in most human epithelial cancers, and is overexpressed in 40-50% of human invasive breast cancers [159-166]. Additionally, local COX-2 expression in the mammary gland of mice has been suggested to be sufficient for *in situ* tumor initiation and progression [167]. COX-2 overexpression has been shown to enhance lymphatic invasion of breast cells [168], increase metastasis [165, 166, 169], inhibit apoptosis and differentiation [170], and has been suggested to enhance aromatase [171] in breast cancer cells. COX-2 specific inhibitors have been shown to prevent breast cancer metastasis, tumor growth, and angiogenesis [166, 172-

176].

A key prostaglandin resulting from the cyclooxygenase pathway includes prostaglandin E_2 (PGE2), which has been implicated in breast cancer etiology in animal and laboratory studies. Prostaglandin E_2 is known to be a primary eicosanoid of COX-2 derived AA metabolism [177, 178], and is known to have tumorigenic properties [177]. Overall, PGE2 has been shown to influence Hanahan and Weinberg's hallmarks of cancer [179], which include the following: evasion of apoptosis; autonomy in growth signals; promotion of angiogenesis; and increased cell migration eventually leading to tissue invasion and metastasis.

Specifically, in breast tissue and breast cancer cell lines, PGE2 has been shown to increase angiogenesis, metastasis, and invasiveness. Chang et al. [180] demonstrated the harmful effects of COX-2 derived PGE2 on mammary gland tumor progression by inducing mammary gland angiogenesis in mouse models. Similar metastatic effects of PGE2 in breast cancer have been demonstrated in human breast cancer cell lines [181]. A proposed mechanism for PGE2's contribution to enhanced metastatic activity in breast cells is via the suppression of natural killer cell function. Increasing concentrations of PGE2 resulted in inhibition of natural killer cell function in mouse models [182]. In breast cancer cells, PGE2 was also shown to increase *Id-1* gene expression leading to increased invasiveness [183]. Thus, inhibition of COX-2 enzyme, and resulting PGE2 production, may help to inhibit the tumorigenic effects of this metabolic pathway.

1.5.2 Lipoxygenase pathway

Another pathway for ω-6 fatty acid metabolism is via the lipoxygenase pathway. The

enzymes involved in the lipoxygenase pathway include 15-lipoxygenase-1 and -2 (15-LOX-1 and 15-LOX-2), 5-lipoxygenase (5-LOX), and 12-lipoxygenase (12-LOX). 12-LOX has been shown to be overexpressed in breast tumors [163, 184, 185] and both animal and laboratory studies have observed the tumorigenic and metastatic potential of ω-6 fatty acids such as AA. These enzymes metabolize either LA or AA, both ω-6 fatty acids, and result in different sets of inflammatory eicosanoids. 15-LOX-1 metabolizes LA into the mitogenic metabolite, 13S-hydroxyoctadeca-9Z, 11E-dienoic acid (13-S-HODE). The potential mitogenic activity of 13-S-HODE is considered to be epidermal growth factor (EGF) dependent. Increasing formation of 13-S-HODE has been observed to augment the EGF receptor signaling pathway, and thus increase cellular proliferation in breast cells [186]. Therefore, reduction in LA may help to reduce the tumorigenic effects of 13-S-HODE in breast cells. In addition to increased cellular proliferation, 13-S-HODE has been shown to influence metastasis by decreasing E-cadherin expression in breast cancer cells [187].

The lipoxygenase pathway also has enzymes that metabolize AA, including, 15-LOX-2, 5-LOX, and 12-LOX. The eicosanoids resulting from these enzymes include the hydroxyeicosatetraenoic acids (HETEs), which then are converted to leukotrienes (LKs), lipoxins (LOs), and hepoxilins (HOs) [53]. The 12-LOX-2 metabolite of AA metabolism (12-S-HETE) has been suggested to increase tumor invasiveness in breast cancer cell lines via increased secretion of cathepsin B, a collagen responsible for basement membrane digestion [188]. In addition to 12-S-HETE, the 15-LOX-2 metabolite 15-S-HETE also promotes metastasis by stimulating the adhesion of metastatic breast cancer tumor cells to the extracellular matrix, a key component of the metastatic process [189]. Inhibitors of the LOX metabolism enzymes have demonstrated reductions in HETE production and resulting

mammary tumorigenesis [190].

1.5.3 Cytochrome p450 pathway

In addition to the production of prostaglandins and leukotrienes via the cyclooxygenase and lipoxygenase pathways, respectively, AA can also be metabolized via the cytochrome p450 pathway. The eicosanoids produced via the cytochrome p450 pathway include HETEs and epoxyeicosatrienoic acids (EETs) [191]. The principal pro-inflammatory product derived from arachidonic acid via the cytochrome p450 pathway is 20-HETE [192]. This metabolite has been implicated in cardiovascular disease [192-194] and renal cell carcinoma proliferation and growth [195, 196]. However, the effects of 20-HETE has not been elucidated in animal and laboratory studies with respect to breast cancer.

Another process by which cytochrome p450 enzymes could be involved in breast cancer etiology is via estrogen biosynthesis. Cytochrome aromatase enzymes are involved in endogenous production of estrogen, and these enzymes include CYP19 and CYP17. PGE2, the primary cyclooxygenase-derived AA eicosanoid, increases aromatase expression in breast tissue [197-202]. Studies have shown strong correlations between COX2 expression and aromatase expression in human breast cancer cells [171, 203]. Additionally, COX-2 selective-inhibitors suppress aromatase expression in breast cancer cells [204]. Thus, increased levels of PGE2 resulting from AA metabolism via the cyclooxygenase pathway could increase aromatase activity within breast tissue leading to increased estrogen levels thus contributing to breast growth.

1.5.4 Inflammation

AA metabolism is influenced by different inflammatory enzymes, in addition to the enzymes involved in the cyclooxygenase, lipoxygenase, and cytochrome p450 pathways. The primary cytokines and receptors that influence AA metabolism include: tumor necrosis factor alpha (TNF-α) and peroxisome proliferator-activated receptors (PPAR-α, PPAR-γ). Phospholipase A_2 (PLA2) is responsible for releasing the membrane bound-form of AA into the cytosol, and therefore making it available for metabolism via the different pathways [53]. TNF-α has been shown to indirectly influence AA release by inducing PLA2 activity in human tumor cells [205, 206]. In human breast adipose cells, TNF-α was also shown to increase expression of COX-2 and production of PGE2 [207]. FAS and FAS-L, a ligand-receptor system part of the TNF family, is known to increase apoptosis; however, increased production of FAS-L is seen in many cancer types, including breast cancer [208-211]. PGE2, the cyclooxygenase-derived metabolite resulting from AA metabolism, has demonstrated effects on increasing FAS-L production [212], which may lead to the aberrant regulation of apoptosis in cancer cells. FAS/FAS-L expression may provide an advantage for tumor cells (both late and early in the carcinogenic process) by facilitating tumor immune escape [209, 211, 212]. Thus, in addition to its apoptotic properties, TNF-α and family members, could potentially influence carcinogenesis by increasing cytosolic levels of AA, or increasing expression of FAS/FAS-L thus giving tumor cells the ability to evade immune response.

PPARs are transcriptional factors that belong to the nuclear hormone receptor family [213]. There are three different PPARs, including PPAR-α, PPAR-β, PPAR-γ. There is evidence that PPAR-γ is often up-regulated in breast cancer cells [214]. Activation of

PPAR-γ can inhibit breast cancer growth [215], promote apoptosis [216], and invasion of human breast cancer cells [213]. Ligands required for PPAR-γ activation include eicosanoids from AA metabolism, namely 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (PGJ2) derived from the cyclooxygenase pathway, and leukotriene B4 derived from the lipoxygenase pathway [217]. Also, long-chain fatty acids are known ligands for PPAR-activation, which inhibits vascular inflammation and induces apoptosis via NFkb and AP1 signaling [218, 219].

1.5.5 Beneficial effects of ω-3 fatty acids

Animal models have demonstrated the beneficial effects of cyclooxygenase inhibition on mammary tumorigenesis by reducing cell migration, invasiveness, cell proliferation, and angiogenesis [220]. Similarly, reducing LA intake, an ω-6 fatty acid and precursor to AA, induced tumor apoptosis in mouse models [221]. Also, in the same study conducted by Connolly et al. increasing intake of DHA in combination with reduction of LA induced greater levels of apoptosis then reducing LA intake alone. Other animal studies regarding ω-3 supplementation (EPA, DHA, alone or combination of the two) have echoed these results regarding reduced tumor growth [222, 223], prevention of human breast cancer cell metastasis [224], and suppression of human breast cancer cell proliferation [225]. The beneficial effects of ω-3 intake within the cyclooxygenase pathway occur via the decreased production of the harmful COX-2 derived metabolite, PGE2 [68]. Thus, ω-3 fatty acids can competitively inhibit metabolism of AA via COX-2, and potentially reduce the tumorigenic effects of harmful COX-2 derived metabolites.

Laboratory studies have shown inhibition of the lipoxygenase pathway via

nordihydroguaiaretic acid (NDGA) can reduce 13-S-HODE production, and thus, reduce 13-S-HODE induced cellular proliferation in breast cells [226]. Specifically, ω-3 fatty acids have been shown to reduce 13-S-HODE production in liver cancer cells [227] and breast cancer cell lines [225]. Similarly, inhibition of the 5-LOX and 12-LOX enzymes has been shown to inhibit apoptosis and thereby reduce human mammary cancer growth [228-230]. DHA has been shown to inhibit linoleic acid-derived 12-S-HETE and 15-S-HETE production in mice [68]. Also, ω-3 fatty acids have been demonstrated to suppress human breast cancer cell line growth by reducing leukotriene B production *in vitro* [222]. Thus, ω-3 fatty acids have been shown to reduce production of harmful eicosanoids resulting from the lipoxygenase pathway, namely 13-S-HODE, 12-S-HETE, and 15-S-HETE.

Animal and laboratory studies have also examined the beneficial effects of fish oil, a major source of ω-3 PUFAs, on breast cancer tumorigenesis. Studies conducted in animals have shown that fish oil can have beneficial effects on breast cancer via multiple mechanisms, including: inhibition of breast cancer growth [231-233]; increased apoptosis [234]; down-regulation of anti-apoptotic gene activity [235]; increased expression of tumor suppressor molecule (syndecan-1) [216]; decreased cell proliferation [234]; and prevention of metastasis [232, 236, 237]. Other dietary supplements high in ω-3 fatty acid content (including walnuts, flaxseed oil, seal oil) have been shown to inhibit breast cancer growth, induce apoptosis, and prevent metastasis as well [238-241].

Animal and laboratory studies have also directly examined the effect of ω-3 fatty acids on breast cancer. Similar to the fish oil literature, these studies have demonstrated the ability for ω-3 fatty acids to reduce breast cancer growth [68, 223, 225, 242-249] and prevent metastasis [224, 250].

The beneficial effects of ω-3 fatty acids are not limited to the prostaglandin and leukotriene pathways. Omega-3 fatty acids have been shown to differentially activate PPARs in human breast cancer cells compared to ω-6 fatty acids. Omega-3 PUFAs were shown to inhibit transactivation of PPAR-γ, whereas ω-6 PUFAs were shown to stimulate activity in breast cancer cells [251]. Also, ω-3 fatty acids have been shown to inhibit progestin-driven invasiveness in human breast cancer cells [252]. Additionally, ω-3 supplementation was also shown to suppress the synthesis of interleukin-1 (IL-1) and TNF, inflammatory proteins that promote cell growth and differentiation [253].

1.5.6 Cytotoxic environment induced by ω-3 fatty acids

The beneficial effects of ω-3 fatty acids occur via a number of mechanisms mentioned previously, and they include the following: inhibition of cell proliferation; increased apoptosis; reduced angiogenesis; and prevention of metastasis. In addition to these mechanisms, lipid peroxidation has also been suggested to contribute to the beneficial effects of ω-3 fatty acids. Apoptosis in mammary cancer cells involves lipid peroxidation of both ω-3 and ω-6 fatty acids [254]. However, the level of lipid peroxidation and resulting cellular oxidative stress depends upon the number of double bonds within the fatty acids chain [255]. The number of double bonds found in ω-3 fatty acids, particularly in EPA and DHA, are greater than those found in ω-6 fatty acids, such as AA. This cytotoxic environment induced by lipid peroxidation of PUFAs can inhibit breast cancer growth [254, 256-258], and therefore could prove beneficial for breast cancer prevention and survival. DHA and fish oil supplementation has been suggested to increase ROS-initiated apoptosis in breast cancer cells *in vitro* and *in vivo* [259]. Genetic variation in oxidative stress enzymes conferring

greater reactive oxygen species (ROS) scavenging capabilities may lead to reduced levels of oxidative stress within the cell, and thus remove the potential benefit of this cytotoxic cellular environment on breast cancer [254, 256, 260].

The beneficial effect of ω-3 fatty acids on increasing apoptosis via lipid peroxidation may be limited to women with early stages of breast cancer. It has been previously reported that the ω-3 induced cytotoxicity was significantly less in normal cells compared to tumor cells [261]. It is possible that dietary supplementation of ω-3 fatty acids could increase lipid peroxidation resulting apoptosis in transformed or malignant mammary cells [262]. Thus, ω-3 fatty acid intake could have an impact on late stage promotion of breast cancer among women at high risk for breast cancer.

Additionally, the beneficial effects of inducing a cytotoxic environment could also prove beneficial for survival among women already diagnosed with breast cancer and who are receiving treatment. Studies have examined the effect of polyunsaturated fatty acid supplementation on enhancing treatment efficacy. DHA supplementation in addition to doxorubicin treatment significantly reduced cell viability in human breast cancer cell lines [255]. Another study examined the effect of fish oil supplementation on the cytotoxicity of breast cancer tumor cells in mice [263]. Fish oil supplementation was shown to increase the efficacy of doxorubicin therapy as evidenced by reduced tumor growth after initiation of doxorubicin treatment. Additionally, dietary DHA was able to sensitize resistant rodent malignant mammary tumors to chemotherapy [264] and radiation [265]. In addition to chemotherapy and radiation therapies, dietary EPA has also been demonstrated to restore tamoxifen sensitivity in breast cancer cell lines via inhibition of Akt signaling (involved in tamoxifen resistance) [266]. In addition to these laboratory studies, two clinical studies

suggested greater chemotherapy efficacy among those with high levels of DHA [267, 268]. Thus, lipid peroxidation via ω-3 fatty acids could potentially enhance the cytotoxic environment induced by breast cancer treatment, regardless of type of treatment (e.g., chemotherapy, radiation, and hormone therapy).

1.5.7 Summary

Metabolism of AA can occur via three different pathways, including cyclooxygenase, lipoxygenase, and cytochrome p450, as illustrated in **Figure 1.2** (see below). The resulting eicosanoids produced via AA metabolism have been demonstrated to influence breast carcinogenesis in experimental studies, and include: 13-S-HODE, 12-S-HETE, 15-S-HETE, PGE2, and 20-HETE. These AA-derived eicosanoids have been demonstrated to increase cell proliferation, metastatic potential, aromatase activity, angiogenesis, and cell proliferation. At the same time, these harmful eicosanoids can also reduce apoptosis and cell differentiation. Omega-3 fatty acids also bind to the same enzymes utilized in AA metabolism, however, the production of the harmful eicosanoids are reduced. In addition to the reduction of inflammatory eicosanoids, ω-3 fatty acids are also known to induce a cytotoxic environment within the cell by increasing levels of lipid peroxidation within the cell, and inducing apoptosis. Thus, intake of ω-3 fatty acids may provide a means for reducing breast carcinogenesis which could affect both incidence and survival.

1.6 PUFA Assessment in Epidemiologic Studies of Breast Cancer

1.6.1 Issues to Consider in the Evaluation of the PUFA-Breast Cancer Studies

A number of issues affect clear interpretation of findings drawn from epidemiologic

studies undertaken to address the potential link between PUFAs and breast cancer. Key issues include: (1) the distribution of PUFA classes varies by geography, and biologic effects are likely to vary by PUFA subtype – thus epidemiologic study results are likely to vary by geographic location; (2) methods used to assess PUFA subtype exposures (self-reports of dietary intake or biomarker levels) provide estimates of individual level exposures that reflect different time periods, and thus findings from epidemiologic studies are likely to vary by the PUFA assessment method; and (3) the epidemiologic study design employed affects the timing of the exposure assessment relative to the diagnosis of the disease, which in turn influences the underlying assumptions regarding the timing of the exposure –and thus epidemiologic findings are likely to vary by study type. These issues will be discussed in more detail below.

1.6.2 Geographic Variation in PUFA Intake

As previously discussed, the distribution of dietary intake of the classes of PUFA varies widely by country. Thus, the relative intake of ω-3 and ω-6 PUFA varies geographically, with western diets having an intake ratio of ω-3:ω-6 of 1:15-20, compared to the ratio of 1:5-6 in India and 1:4 in Japan [153]. Also, previous investigators have reported that serum levels of LC ω3 PUFA are lower among U.S. Whites when compared to other Asian populations [154]. Thus, if the association between PUFA subtypes and breast cancer varies by exposure dose, then studies conducted in different geographic populations with varying exposure doses could yield varying results. Thus, consideration of the geographic location of the population under study is critical.

Animal and laboratory evidence shows that ω-3 and ω-6 fatty acids have differential

effects on breast cancer. Thus, it is important to examine the different subtypes of PUFA intake since the laboratory evidence suggests that the association between PUFA and breast cancer is complex. A comprehensive examination of PUFA intake, including ω-3 subtypes, ω-6 subtypes, and the relative balance of ω-3 and ω-6 classes, is warranted and may help to capture this complexity and help elucidate the PUFA-breast cancer association. However, given this biologic complexity, few studies [269, 270] have comprehensively examined PUFA intake.

Therefore, consideration of the geographic location of the study population and whether assessment of PUFA intake was comprehensive (including ω-3 subtypes, ω-6 subtypes, and the relative balance of ω-3 and ω-6 fatty acids) are both important considerations when evaluating epidemiologic studies that address the PUFA-breast cancer association.

1.6.3 Self-reported Dietary Assessment of PUFAs

The majority of studies to date have relied upon self-reported measures of dietary PUFA intake. Among those studies using self-reported measures of dietary PUFA intake, the majority used food frequency questionnaires (FFQ) to measure usual dietary intake. Other alternative methods of dietary assessment include short-term recall (e.g., 24-hour recall) and diet records. Short-term 24-hour recall, which reflects foods eaten the day prior to the assessment only, has its limitation as it does not account for day-to-day variations, seasonality of dietary intake, or long-term patterns of intake. Multiple 24-hour recalls throughout the year could help deal with day-to-day variation in dietary intake; however, this approach is costly, inefficient, and a burden to the participants. While diet records consider

daily variations, this assessment method still fails to address seasonal intake, variations in intake that take place throughout the year, and long-term patterns of intake.

The FFQ is the most commonly used measure for assessment of usual dietary intake, since it provides an inexpensive method for assessing usual patterns of long-term intake. The FFQ typically assesses consumption of foods in the past year. This exposure assessment provides estimates of usual intake while accounting for seasonal dietary influences. Shortcomings of this approach, however, include difficulties in recalling patterns in the previous year or in accurately measuring energy intake. However, as reviewed by Willett [271], this instrument type is most useful for relative ranking of individual takes, rather than trying to estimate precise intake levels.

In addition to the type of dietary assessment, it is equally important to consider the potential for misclassification that can occur with self-reported dietary data. For example, in a case-control study of PUFA intake, it is possible that cases and controls may differentially recall dietary exposure, thus biasing effect estimates away from the null. Also, dietary exposures measured via questionnaires are subject to non-differential measurement error, which could attenuate effect estimates towards the null value. Dietary exposures measured via questionnaires fail to consider the impact of biologic processes involved in the body, and therefore, may not provide the estimates of bioavailability. Additionally, nutrient intakes derived from an FFQ may provide biased assessments due to subject assessments of portion size, frequency of intake, and differences in recipes and cooking methods.

The FFQ generally includes assessment of dietary intake in the past year, although for some instruments the assessment period can be for up to 5 to 10 year prior to the interview. Regardless of the number of years assessed, the intake estimates from the FFQ are assumed

to reflect usual diet – in other words, long-term patterns. The accuracy of this assumption has not been definitely demonstrated.

In sum, the FFQ is the most frequently used dietary intake assessment used in studies of PUFA and breast cancer. This approach is an inexpensive and efficient method for epidemiologists to estimate usual dietary intake in the past year, which possibly reflects long-term dietary intake.

1.6.4 Biochemical Markers of PUFAs

Biochemical markers, or biomarkers, of PUFAs provide an alternative way to measure dietary intake reflecting both dietary intake and the biologic processes involved in metabolism of the nutrient [272]. The hope is that these biomarkers would provide an alternative, unbiased assessment of dietary PUFA assessment. However, there are many limitations of using biomarker measurements of PUFAs that require consideration.

It is important to consider the type of biomarker measured and what exposure window is being measured. There are primarily three biomarkers for PUFA intake and include: serum, erythrocyte membrane, and adipose tissue biomarkers. Each of these different biomarkers reflects a different exposure window for PUFA intake. Serum fatty acid biomarkers reflect the shortest window of exposure of all the three fatty acid biomarkers. Most serum or plasma biomarkers reflect fatty acid intake over the past few meals or days [273]. The next biomarker level, erythrocyte-membrane bound biomarker tends to reflect a longer term of intake than serum and contains a high proportion of long-chain PUFAs. Erythrocyte-membrane bound PUFA biomarkers reflect dietary intake spanning the life cycle of a red blood cell, which is approximately 120 days [273]. Thus, erythrocyte-membrane

biomarkers will likely reflect PUFA intake over the past few months. However, it is important that samples for erythrocyte-membrane biomarker testing are stored pretreated, which would protect the membranes from degrading due to oxidation, and that samples are stored at -80°C in order to maintain stability up to five years [273]. Finally, adipose tissue biomarkers can be measured in order to reflect long-term dietary intake. The samples can be obtained from gluteal, abdominal, subscapular, pectoral, or from another site [273]. In general, fatty acids concentrations obtained from these different sites are similar [272]. However, adipose tissue biomarkers are subject to measurement error in the presence of weight loss [273].

There are numerous factors that are known to influence measured fatty acid biomarker levels and include the following: dietary intake, relative amounts of other fatty acids in media, supplement use, genetic polymorphisms of elongase and desaturase enzymes, tissue sampling site, tissue sampling procedure, amount sampled, handling and storage of samples, analytic method, lipolysis and fasting, nutritional status (e.g., iron, zinc, copper, and magnesium sufficiency), lipogenesis, diseases (e.g., cystic fibrosis, malabsorption, liver cirrhosis, diabetes, Zellweger Syndrome), and oxidation [273].

Therefore, there are many opportunities for measurement error even with a more objective measure of PUFA intake, and the time-period of the exposure reflected by the biomarker may not be relevant to the carcinogenic process, which is up to at least several decades [274].

1.6.5 Study Design and Timing of Exposure Assessment

The majority of studies that have examined the association between PUFAs and

breast cancer incidence have been case-control in study design [99, 104, 269, 275-287]. Some case-control studies were nested within a fully enumerated cohort [85, 288] and others were cohort studies [80, 100, 270, 289-298]. The issue of the type of study design is important because it often determines the timing of the PUFA assessment, which is likely to influence the observed associations that are reported in the different study designs found in the epidemiologic literature.

In case-control studies, subjects are typically interviewed to assess dietary intake at the time of diagnosis (for the cases) and at the time of interview (for the controls). In contrast, for cohort studies dietary assessment is made at the time of interview at the beginning of the study. It is possible that dietary assessment captured at diagnosis or interview (for case-control studies) and at enrollment (for cohort studies) does not adequately assess intake during the etiologically relevant time period. Thus, the estimated association for these study designs may be attenuated if the exposure does not reflect intake during the etiologically relevant time period [274].

Given a long enough period of follow-up (e.g., more than ten years), it is possible that assessment of dietary intake in cohort studies may capture intake closer to the etiologically relevant time period for breast cancer. If this is the case, then estimates derived from cohort studies may be stronger than those estimated from case-control designs. If, however, dietary intake patterns change over time, then neither the case-control nor the cohort study design will provide valid measures of association.

With regard to PUFA biomarkers, again effect estimates derived from case-control vs. cohort (or nested case-control studies) may not reflect the true PUFA breast cancer association. The PUFA biomarker obtained years prior to the outcome, as is the case for the

cohort or nested case-control study, may not reflect usual PUFA exposure levels in the time period relevant to carcinogenesis. The problem is similar for case-control studies – where the biomarker levels assessed closer to diagnosis also do not reflect usual exposure levels in the time period relevant to carcinogenesis.

In sum, regardless of the study design, the underlying assumption is that the dietary data estimate, or the biomarker levels, reflects long-term usual exposure levels, which may not be a valid assumption -- for any epidemiologic study design type or exposure assessment method used. Thus, in interpreting findings from epidemiologic studies, it is important to carefully consider the timing of the exposure assessments which is inherent to each of the different study designs when evaluating the epidemiologic literature.

1.6.6 Summary

A variety of issues could affect the estimated associations derived from previous epidemiologic studies undertaken to examine PUFA intake and breast cancer. The main issues include: geographic variation (e.g., study population); the assessment of PUFA intake (FFQ versus biomarker measurements); types of PUFA examined (e.g., ω-3 subtypes only, ω-6 subtypes only, or a more comprehensive measure including both classes and the relative balance); and study design (e.g., case-control versus cohort) as it affects the timing of the exposure assessment.

The evaluation of the epidemiologic literature is presented below for both incidence and survival. A summary of the literature examining the association between self-reported PUFA intake and breast cancer incidence is presented in **Tables 1.2 and 1.3** for case-control and cohort studies, respectively. The subsequent sections regarding the epidemiologic

literature examining self-reported PUFA intake is assessed separately for each study design, then by study population, and assessment of PUFA. A separate section is devoted to epidemiologic literature utilizing PUFA biomarkers and those studies examining dietary fish intake.

1.7 Epidemiology of PUFAs and Breast Cancer

1.7.1 PUFAs Assessed using Dietary Intake Measures and Incidence

As shown below in **Tables 1.4 and 1.5**, over two dozen observational studies have been published examining the relation between self-reported dietary PUFA intake and breast cancer incidence. However, as reviewed in more detail below, results remain inconclusive. The studies addressing the issue have utilized both case-control and cohort study designs, have been conducted among different international populations, and the PUFA measures assessed also vary across studies. Details of the existing evidence published to date, and the potential reasons for the lack of consistency across studies, are reviewed in more detail below. Results from the case-control studies are considered first, followed by those from the cohort studies.

1.7.1.1 Case-Control Studies

As shown in **Table 1.4**, sixteen case-control studies examining the effect of PUFA on breast cancer incidence have been conducted in populations across the globe, including Asian (n=3) [275-277], European (n=7) [99, 104, 269, 278-281], the U.S. (n=3) [285-287], Latin American (n=2) [283, 284], and Saudi Arabian (n=1) [282] populations.

Three case-control studies were conducted among Asian populations [275-277]. The

two earlier case-control studies conducted among Asians [275, 276] reported results for only total PUFA intake without considering the different classes (e.g., ω-3 and ω-6). Shun-Zhang et al. reported increased breast cancer risks for women with higher intakes of total polyunsaturated fatty acids, and the effect was stronger when polyunsaturated fatty acids were considered as a continuous variable (13 grams/day increase) versus tertiled [275]. Lee et al. reported increased breast cancer risk for total PUFA intake among postmenopausal women, but a risk reduction for premenopausal women [276]. However, both these earlier case-control studies included small sample sizes resulting in imprecise estimates of the PUFA and breast cancer association.

A third Asian case-control study, which was larger and conducted among a Korean population, examined the effects of total ω-3 fatty acid intake and LC ω-3 PUFAs [277]. The study reported risk reductions for all three exposures (e.g., total ω-3, DHA, and EPA), with stronger risk reductions seen among postmenopausal women consuming higher quantities of EPA and DHA. However, Kim et al. failed to estimate effects for ω-6 fatty acids and the relative intake of ω-3 and ω-6 PUFA intake in this Korean population. While Kim et al. had a larger sample size (N=718) than the earlier Asian studies, the effect estimates were still imprecise, even more so when stratified by menopausal status. Additionally, Kim et al. sampled controls from patients who attended the same hospital as the cases and received health check-ups and/or cancer screening [277]. This selection of hospital-based controls has the potential to bias the effect estimates if the distribution of ω-3 fatty acid intake differs between those attending the hospital versus the source population, and thus limiting the external validity of the study findings. Therefore, the conclusions from Asian case-control studies that examined the effects of PUFA intake and incidence of breast cancer are limited

due to a variety of reasons, including the lack of examination of all PUFAs, relative intake of ω-3 and ω-6 fatty acids, and issues with internal and external validity. However, among the few that have considered ω-3 alone, breast cancer risk reductions are apparent, but information on ω-6 and the relative balance are not well studied.

Seven European case-control studies have been conducted regarding PUFA intake and breast cancer incidence [99, 104, 269, 278-281]. Results for total PUFA intake are inconsistent, with some reporting risk reductions [99, 104, 279] and two reporting an increased risk [278, 280]. One European study examined the effect of only LA intake, and reported increased risk for breast cancer for women consuming high intakes of LA (Quartile 4 versus 1 OR = 1.22; 95% CI = 0.89, 1.68) [280]. The estimate was stronger among premenopausal women (Quartile 4 versus 1 OR = 1.46; 95% CI = (0.86, 2.49). Another European study reported a 20% breast cancer risk reduction for total ω-3 fatty acid intake (Quintile 5 versus 1 OR = 0.8; 95% CI = 0.7, 1.0) [281]. However, both of these studies did not examine the effect of the relative intake of ω-3 and ω-6 PUFAs within their respective European populations. These differences in reported results could reflect differences in intake of different polyunsaturated fatty acids across different populations (e.g., ω-3 versus ω-6), differences in control sampling methods (e.g., hospital-based versus population-based controls), and differences in covariate adjustment sets.

One European case-control study comprehensively examined (including ω-3 subtypes, ω-6 subtypes, and the relative balance of ω-3 and ω-6 fatty acids) the effect of PUFA on breast cancer incidence [269]. Another comprehensive case-control study examined the effect of ω-6 (LA, AA, total ω-6 intake) and ω-3 (ALA, DPA, EPA, DHA, total ω-3) fatty acids on breast cancer incidence among a French-Canadian population in

Montreal [269]. However, the reported effect estimates were not consistent with the biologic plausibility of ω-3 and ω-6 fatty acids. Nkondjock et al. reported increased risks for ω-3 fatty acids and risk reductions for ω-6 fatty acids [269]. Also, a 26% increase in breast cancer risk was observed for the highest quantile of ω-3:ω-6 intake (Quartile 4 versus 1 OR = 1.26; 95% CI = 0.86, 1.86) [269]. In comparison, a 58% increase in breast cancer risk was observed for the third quartile of intake ω-3:ω-6. It is possible that in this population the consumption of ω-3 fatty acids is not enough, and that the dietary habits of this French-Canadian population resemble those of other North American populations where intake of ω-6 fatty acid dominates. This may explain why the reported associations are not consistent with the animal and laboratory studies.

A few other case-control studies were conducted among Uruguayan [283], Saudi Arabian [282], and Mexican [284] populations. De Stefani et al. reported effects that are not consistent with the biology in their Urugauyan population, with increased risks for ALA, a long-chain ω-3 fatty acid and risk reduction for LA, a ω-6 fatty acid [283]. Aloithameen et al. reported only effects for total PUFA intake in a Saudi Arabian population [282]. The only comprehensive assessment of PUFA intake was conducted among a Mexican population and reported risk reduction for total ω-3 intake, increased risk for total ω-6 intake, and risk reduction for the favorable ratio of ω-3:ω-6 intake. The estimated increased risk and risk reductions reported were even stronger when examined among only premenopausal women [284].

In the U.S., only three studies examined the association between PUFAs assessed using dietary intake measures and breast cancer incidence [285-287]. However, two of these studies reported opposite effect estimates for LA intake, with one reporting increased risks

[287], and another reporting a risk reduction [285]. Another study conducted in Connecticut [286] reported risk reductions for LCω-3 fatty acids (EPA and DHA) among premenopausal women only. However, a nearly 20% risk reduction was observed when examining the effect of the ratio of ω-3:ω-6 fatty acids on breast cancer risk among all women, and this risk reduction for the ratio was more pronounced among premenopausal women (Quartile 4 versus 1 OR = 0.59; 95% CI = 0.29, 1.19). However, the study lacked a comprehensive measure of PUFA intake (including ω-3 subtypes, ω-6 subtypes, and ratio of ω-3:ω-6 fatty acids). Inconsistent effect estimates are observed among case-control studies conducted in the U.S. for the estimate of LA on breast cancer risk. Also, the studies conducted in the U.S. could benefit from a comprehensive assessment of PUFA intake utilizing a large population-based study.

In sum, numerous case-control studies have been conducted examining the association between PUFA and breast cancer, though inconsistencies in the estimated association exist. Among the sixteen case-control studies, only one examined the PUFA exposure comprehensively by subtypes and relative balance [269]. However, the reported associations are not consistent with the biology of ω-3 and ω-6 fatty acids [269]. Only three studies were conducted in the U.S. [285-287], of which only one examined the relative balance of ω-3 and ω-6 fatty acid intake [286].

1.7.1.2 Cohort Studies

As shown below in **Table 1.5**, fifteen cohort studies examining the effect of PUFA intake and breast cancer incidence have been conducted among various populations, including Asian [100, 289, 290], European [85, 291-294], and U.S. [80, 270, 288, 295-298].

Three Asian cohort studies [100, 289, 290] present consistent results with respect to long-chain ω-3 PUFAs and the relative balance of ω-3 and ω-6 fatty acid intake in relation to breast cancer risk. The Singapore Chinese Health Study reported the greatest breast cancer risk reduction for marine-derived ω-3 PUFA intake with a nearly 30% decrease in risk (Quartile 4 versus 1 RR = 0.72; 95% CI = 0.53, 0.98), and this effect was even greater among postmenopausal women (Quartile 4 versus 1 RR = 0.68; 95% CI = 0.47, 0.97) [289]. Also, the Singapore Chinese Health study reported an 87% increase in risk (OR = 1.87; 95% CI = 1.06, 3.27) when examining the joint effects of highest quartile of ω-6 PUFA intake and lowest quartile of ω-3 intake [289]. Similar effects were seen in the Japan Collaborative Cohort Study [100], where a 30% risk reduction for ω-3 fatty acid intake was reported and this risk reduction was more pronounced for long-chain ω-3 PUFA intake (Quartile 4 versus 1, RR = 0.50; 95% CI = 0.30, 0.85). Wakai et al. also reported an increase in breast cancer risk for the unfavorable ratio (ω-6:ω-3) of PUFAs (Quartile 4 versus 1, RR = 1.31; 95% CI = 0.78, 2.19). These findings were echoed in a recently published cohort study utilizing the Shanghai Women's Health Study [290]. Similar to the other Asian cohort studies, a 25% risk reduction was observed for long-chain ω-3 PUFAs (Quintile 5 versus 1, RR = 0.74; 95% CI = 0.52, 1.06) and a nearly 30% increase in risk was observed for unfavorable ratio of ω-6:ω3 fatty acid intake (Quintile 5 versus 1, RR = 1.29; 95% CI = 0.95, 1.75). Among Asian cohort studies, the effect of long-chain ω-3 fatty acids is consistently reported to reduce risk of breast cancer by approximately 30%. All three Asian cohorts also examined the ratio of ω-6 to ω-3 fatty acids and reported increases in risk ranging from 30% for all women [100, 290] to nearly 90% among postmenopausal women [289]. Thus, the Asian cohort studies are consistent with regard to breast cancer risk reduction conferred among those women

consuming long-chain ω-3 PUFA, and the increased breast cancer risks attributed to high intake of the unfavorable ratio of ω-6 to ω-3 PUFA.

While Asian cohort studies are consistent with regards to PUFAs assessed using self-reported dietary intake data and breast cancer risk, the associations reported among European and U.S. populations are not. A few European cohort studies reported inconsistent effects for total PUFA intake and breast cancer incidence, with one reporting an increased risk among a Finnish cohort [291] in comparison to other cohorts reporting risk reductions among Dutch and Swedish cohorts [292, 293]. This inconsistency between the studies could be due to differences in the model covariates. The Finnish cohort study reported effects adjusted for only age and total energy intake, the Dutch study reported effects adjusted for only age (energy did not influence the effect estimates), whereas the Swedish study reported effects that were adjusted for other potential confounders in addition to energy intake.

Another European study, utilizing a nested case-control study design within a well-defined Swedish cohort, comprehensively examined PUFA intake and reported the effect of total ω-3, total ω-6, and the effect of relative intake of ω-3 and ω-6 fatty acids (ω-3:ω-6 ratio). They reported increased risks for both total ω-3 intake (Quintile 5 versus 1 OR = 1.81; 95% CI = 1.09, 2.99; p for trend = 0.026) and total ω-6 intake (Quintile 5 versus 1 OR = 3.02; 95% CI = 1.78, 5.13; p for trend = 0.0002) [85]. However, a risk reduction was observed for the ω-3:ω-6 ratio on breast cancer incidence (Quintile 5 versus 1 OR = 0.66; 95% CI = 0.41, 1.08; p for trend = 0.137).

Other European cohort studies also examined the associations of PUFA subtypes and breast cancer incidence. In contrast to the Asian cohort studies, the results reported for LCω3 fatty acid intake and breast cancer risk are null. While a substantial risk reduction was

observed for ALA, null effects for were observed for LCω3 fatty acids in the Netherlands Cohort Study [292]. An essentially null effect was observed for LCω3 fatty acids intake and breast cancer incidence even though a slight risk reduction was reported among a French cohort [Quintile 5 versus 1, RR = 0.94; 95% CI = 0.80, 1.10) [294]. Only two European cohort studies examined the relative balance of ω-6 and ω-3 PUFAs [85, 294] and a null association was reported for the unfavorable ratio of ω-6 to ω-3 PUFA among the Swedish cohort (Quintile 5 versus 1, RR = 0.97; 95% CI = 0.83, 1.14) [294][299] and a stronger risk reduction was observed among the French cohort for the favorable ratio of ω-3 to ω-6 PUFA (Quintile 5 versus 1, RR = 0.66; 95% CI = 0.41, 1.08) [85].

Overall, the beneficial effects of long-chain ω-3 fatty acids and the deleterious effects of an unfavorable ratio of ω-6 to ω-3 fatty acids observed among Asian cohort studies was not replicated among European cohorts. Also, studies that assessed PUFA subtype intake primarily reported null estimates for subtypes of ω-3 and ω-6 fatty acids [292, 294], with the possible exception of ALA intake in the Netherlands Cohort. Thus, the estimates of PUFA intake and breast cancer risk among European populations are either inconsistent or null, and require further investigation.

Few cohort studies among U.S. populations have comprehensively examined the association of PUFA on breast cancer incidence. An early cohort study reported increasing age-adjusted breast cancer incidence rates incidence with each increasing tertile of total PUFA, LA, and ALA intake [295]. Two other cohort studies reported inconsistent effects for LA intake among postmenopausal women, with one reporting a nearly 30% increase breast cancer risk (Quintile 5 versus 1, RR = 1.29; 95% CI = 0.78, 2.13) [297] and the other reporting a slight risk reduction among Nurses' Health Study cohort (Quintile 5 versus 1, RR

= 0.93; 95% CI = 0.74, 1.16) [80]. This inconsistency between the two studies could be due to differences in exposure assessment methods. The Nurses' Health Study assessed changes in dietary intake via multiple assessments of the food frequency questionnaire during follow-up [80, 296]. While repeated questionnaires may help to provide changes in diet over time, it is possible that this cumulatively averaged LA intake used in the Nurses' Health Study may not accurately reflect the relevant exposure window for breast cancer etiology, and thus would result in a biased effect estimate towards the null [274]. Whereas, the Velie et al. paper assessed LA intake at baseline and reported increased risk for breast cancer which is concordant with the known biology of ω-6 fatty acids. The assessment made by Velie et al. may reflect LA intake during an etiologically relevant time period for breast cancer more accurately than cumulatively averaged intake presented in the Nurses' Health Cohort [80]. This may explain the differences in effect estimates obtained by the two studies.

Finally, only the Nurses' Health Study and the VITAL cohort study comprehensively examined the association between total PUFAs, ω-3 subtypes, and ω-6 subtypes on breast cancer risk among U.S. populations [270, 296]. Predominantly null effect estimates were reported among the Nurses' Health cohort, with the exception of ALA (RR for 1% increase in energy = 0.75; 95% CI = 0.54, 1.03) and total PUFA intake among postmenopausal women (RR for 5% increase in energy = 0.88; 95% CI = 0.74, 1.04) [296]. Risk reductions were observed among postmenopausal women in the VITAL cohort for LCω-3 PUFAs, including EPA (Quintile 5 versus Quintile 1 HR = 0.70; 95% CI = 0.54, 0.90) and DHA (Quintile 5 versus Quintile 1 HR = 0.67; 95% CI = 0.52, 0.87); whereas, a modest increase in breast cancer risk was reported for LA (Quintile 5 versus Quintile 1 HR = 1.18; 95% CI = 0.84, 1.66) [270]. In addition to the examination of ω-3 and ω-6 subtypes, the VITAL cohort

also reported modest risk reduction for relative intake of ω3 to ω6 (Quintile 5 versus Quintile 1 HR = 0.84; 95% CI = 0.65, 1.09) [270]. Therefore, the association between PUFAs and breast cancer risk is not clear among U.S. cohort studies, with studies reporting inconsistent results for ω-3 and ω-6 subtypes, and only one study [270] suggesting a modest risk reduction with favorable ω-3:ω-6 ratio.

In sum, fifteen cohort studies have estimated the potential association between PUFA and breast cancer incidence. Among the Asian cohort studies, consistency was observed with regard to LCω3 intake and breast cancer risk reduction [100, 289, 290]. Inconsistencies regarding the potential association between PUFAs and breast cancer incidence remain among the European and U.S. cohort studies. Among the five cohort studies conducted among U.S. populations, only one [270] examined the relative balance of ω-3 to ω-6 intake.

1.7.2 PUFAs Assessed using Dietary Intake Measures and Survival

Epidemiologic studies regarding self-reported PUFA intake and breast cancer risk are severely lacking, despite the demonstrated biologic plausibility in animal and laboratory studies. Only ecologic studies have been conducted regarding PUFA intake and survival after breast cancer diagnosis. One study [300] used age-specific breast cancer mortality rates from the World Health Organization (WHO) for 30 industrialized countries. PUFA intake was derived from 47 published dietary surveys from 17 different countries. The study reported high correlations between PUFA intake and breast cancer mortality rates, with high correlation coefficients from 0.53, 0.84, to 0.87 for age groups 50-54, 60-64, and 70-74 years, respectively. Another ecologic study was conducted to examine the relation between breast cancer mortality and dietary factors in Japanese women [301]. The study reported

high correlation coefficients for PUFAs and age-adjusted breast cancer mortality, with a correlation coefficient nearly 0.20. A higher correlation coefficient was reported for ω-3 fatty acids (0.22) than for ω-6 fatty acids (0.17). However, temporality between PUFAs and breast cancer mortality cannot be assessed since both these studies are ecological in design.

1.7.3 Summary

While there have been many studies that have examined the association between PUFAs and breast cancer risk, the studies have reported inconsistent results. Many of the earlier studies focused only on examination of total PUFA intake [99, 104, 275, 276, 278, 279, 282, 291], without considering the potentially opposite effects of ω-3 and ω-6 fatty acids. Other studies only considered one PUFA subtype, either ω-3 or ω-6 [80, 277, 280, 281, 287, 288, 297, 298, 302]. Few studies examined the relative intake of ω-3 and ω-6 fatty acids [85, 100, 269, 270, 284, 286, 289, 290, 294]. Among these studies examining the relative balance of ω-3 and ω-6 fatty acids, only two were conducted using a U.S. population [270, 286]. While Asian cohort studies have suggested a risk reduction for long-chain ω-3 PUFA and an increased risk with high ω-6 to ω-3 fatty acid ratio, the results from studies conducted among European and U.S. populations are still inconsistent. These latter populations could benefit from a comprehensive assessment of PUFA intake, including ω-3 and ω-6 subtypes and the relative intake of ω-3 and ω-6 fatty acids, and their effects on breast cancer incidence. Finally, the epidemiologic evidence on the association between PUFA intake and survival among women with breast cancer is limited to ecologic analyses, and thus additional research for this plausible association is needed.

1.8 PUFA Biomarkers

1.8.1 Epidemiology of PUFA Biomarkers and Breast Cancer

Several studies have been conducted examining PUFA biomarkers. Previous studies have been conducted regarding fatty acid biomarkers and breast cancer incidence [94, 303-317]. One examined the effect of fatty acid biomarkers on survival [318]. Many of these studies measured adipose tissue biomarkers [94, 303-305, 313, 314]. However, most of these studies were conducted among European populations, and interpretation of the results is limited by the reduced sample sizes or hospital-based study designs. Only three studies were conducted among U.S. populations [94, 317, 318]. One of the three studies [94] included only postmenopausal women diagnosed with stage 1 or 3 breast cancer in a hospital-based case-control study design. The study reported 40% risk reduction (95% CI = 0.4, 1.0) when comparing the third quartile of long-chain ω-3 fatty acids to the lowest quartile. No effects were seen for total PUFAs, and ω-6 fatty acids were not reported. The second study examined the effect of an adipose tissue biomarker on breast cancer survival [318]. However, only 16 of 161 women comprising the cohort died of breast cancer, thus, resulting in imprecise effect estimates and limiting the interpretation of the results. The third study examined adipose tissue levels of PUFAs and breast cancer incidence using a hospital-based case-control design [317]. The study included a total of 147 subjects and reported null estimates for ω-6 adipose tissue levels and breast cancer. However, the study reported approximately 8% risk reduction for long-chain ω-3 fatty acids (OR = 0.92; 95% CI = 0.84, 1.01), and 40% risk reduction for a unit increase in the ratio of long-chain ω-3 fatty acids to ω-6 fatty acids (OR = 0.60; 95% CI = 0.32, 1.10). However, the limited sample size for this study and the hospital-based design limits the inference of the study results to other

populations.

Although, there have been five studies that examined erythrocyte membrane fatty acids and breast cancer incidence, all of these studies were conducted among either Asian [306, 308] or European populations [307, 311, 312]. One of the studies selected the cases and controls from women who were formerly employed at the Shanghai Textile Industry Bureau, thus limiting inferences of the study results to other populations [308]. Among Japanese women, a higher ratio of ω-6 to ω-3 erythrocyte membrane biomarker resulted in increased risk for breast cancer (OR for highest tertile versus lowest = 1.51; 95% CI = 0.81, 2.81) [306]. Another study examining the ω-3 to ω-6 ratio reported a 4% risk reduction (95% CI = 0.78, 1.18) for postmenopausal breast cancer among a Swedish cohort [311].

Only four previous studies examined serum biomarkers of fatty acid intake on breast cancer incidence. A cohort study conducted among New York women reported nearly 30% reduction in breast cancer risk among those women with the highest quartile of serum ω-3 levels in fat compared to the lowest [309]. A similar risk reduction was observed for total ω-6, which is not consistent with the biologic mechanism of ω-6 fatty acid metabolism and breast carcinogenesis. Additionally, the study did not examine the relative levels of ω-3 to ω-6 fatty acids. The second study of serum biomarkers and breast cancer derived their study participants from a previous randomized trial and was comprised of women who were heavy smokers [310]. The final two European studies utilized a case-control study design conducted among Finnish [316] and French populations [315]. The Finnish study reported nearly 70% risk reduction for the highest tertile of total serum PUFAs to the lowest tertile [316]. The French study reported a slight breast cancer risk reduction for the highest quartile of serum ω-6 compared to the lowest (OR = 0.91; 95% CI = 0.40-2.06) and an approximately

40% risk reduction for the highest quartile of serum ω-3 compared to the lowest (OR = 0.58; 95% CI = 0.27, 1.28). In summary, few previous studies examined the relative balance of ω-3 and ω-6 PUFA biomarkers on breast cancer incidence in a large population-based sample.

1.8.2 Summary

Few studies have considered PUFA biomarkers using U.S. populations, but interpretation is limited due to small samples and failure to examine the relative balance of ω-3 and ω-6 fatty acids. Also, PUFA biomarkers, though an objective measurement of fatty acid intake, may not reflect a relevant time period of exposure for breast cancer etiology. Most importantly, PUFA biomarkers may reflect dietary intake from a few hours (serum), months (erythrocyte membrane), or years (adipose tissue) of intake. While adipose tissue biomarkers provide an appealing option for epidemiologic studies of diet and breast cancer, it is likely to result in selection bias due to the invasive procedure involved. Also, adipose tissue biomarkers may not accurately reflect exposure during the etiologically relevant time period due to changes in weight. In a situation with unlimited resources, repeated biomarker measurements would be ideal for examining the effects of PUFA levels on both breast cancer incidence and survival.

1.9 Epidemiology of Fish Intake and Breast Cancer

1.9.1 Fish Intake and Incidence

Twenty-two studies have been conducted regarding fish intake and breast cancer incidence. The majority of studies have been conducted among either European (n=9) [89, 279, 319-325] or Asian populations (n=7) [277, 326-331]. Comparatively, fewer studies

(n=5) have been conducted among U.S. populations [288, 298, 332-334], and one study was conducted among a Mexican population [335]. The literature regarding dietary fish intake and breast cancer incidence is assessed separately for case-control (n=15) and cohort studies (n=7), as the study design influences the timing of the exposure assessment.

1.9.1.1 Case-Control Studies

As shown below in **Table 1.6**, results from case-control studies of fish intake and breast cancer among European populations consistently show risk reduction for breast cancer incidence. The results are typically presented for total fish intake [89, 279, 321-323]. European case-control studies estimated associations for total fish intake and breast cancer ranging from approximately 30% risk reduction (when comparing highest quantile to lowest) [321-323] to a 65% risk reduction [279]. One study reported a more pronounced risk reduction for total fish intake among postmenopausal (OR = 0.76; 95% CI = 0.70, 0.90) than among premenopausal women (OR = 0.88; 95% CI = 0.70, 1.10) [89]. Another European case-control study presented results for types of fish in addition to total fish intake. Terry et al. [319] reported a modest risk reduction for total fish intake (OR = 0.88; 95% CI = 0.60, 1.29) among a Swedish population. However, when considering fatty fish intake, the primary source for LCω3 fatty acids, the association was stronger (OR = 0.70; 95% CI = 0.45, 1.10). Overall, European case-control studies tend to report risk reductions for fish intake. However, none of these studies took into account fish preparation methods, which could affect the PUFA content in the food, and potentially reduce its benefit.

Similar to European populations, Asian case-control studies are also consistent with respect to fish intake. The majority of Asian case-control studies report risk reductions for total fish intake [277, 326, 328, 330]. A Korean study reported stronger estimated

association between fatty fish and breast cancer incidence (OR=0.23; 95% CI = 0.13, 0.42) compared to total fish intake (OR=0.55; 95% CI = 0.32, 0.96), though estimates are imprecise [277]. More pronounced associations were also reported for total fish intake among postmenopausal women compared to premenopausal women [328, 329].

While the majority of Asian studies report a risk reduction for fish intake, the Shanghai Breast Cancer Study population seems to be an exception. Dai et al. [327] reported a nearly 50% increased risk of breast cancer for women in the highest quintile of intake for freshwater fish (OR = 1.48; 95% CI = 1.16, 1.89) and a modest increase in risk for marine fish (OR = 1.14; 95% CI = 0.90, 1.45). A recently published study using the Shanghai Breast Cancer Study population also reported similar results for freshwater (OR = 1.39; 95% CI = 1.23, 1.56; p for trend < 0.001) and marine fish (OR = 1.19; 95% CI = 1.02, 1.39) [331]. No differences were seen across ER/PR subtypes. The results from the Shanghai Breast Cancer studies are not consistent with other Asian studies with regard to fish intake. This increase in risk associated with freshwater and marine fish intake has been reported for other cancer sites as well, including endometrial [336] and colon [337] cancers. The authors suggest that this unexpected increase in risk for fish intake in this Shanghai population may be due to the high levels of chemical exposures, including methylmercury, dibenzofurans, and organochlorine residues [331].

Food science literature has demonstrated that different cooking methods could reduce the LCω-3 content in the food [147]. Also different food preparation methods (e.g., deep-fried fish) could introduce ω-6 PUFA, and thus reduce the potential benefits of fish intake by unfavorably tipping the relative balance of ω-3/ω-6 PUFAs towards high ω-6 intake [147]. Therefore, it is important to consider preparation methods when examining the potential

association between dietary fish intake and breast cancer. Cancer epidemiologic studies examining fish cooking method have reported differences in risk [338]; however, this topic remains understudied with respect to breast cancer. A few Asian studies also reported effect estimates for different type of fish cooking or preparation methods. One hospital-based case-control study conducted in Japan also attempted to examine the effect of cooking methods on breast cancer incidence [328]. However, the categorization of cooking methods was crude, where the authors had only two categories of preparation methods: (1) cooked/raw fish consumption; and (2) dried/salted fish consumption. Comparing the highest level of intake frequency (greater than 3 times per week) versus the lowest (almost never), the authors reported a risk reductions for cooked/raw fish intake and dried/salted fish among postmenopausal women only. The Shanghai Breast Cancer Study population [327] took into account different levels of deep-fried cooking (including never deep-fried, ever deep-fried, and well-done) and reported a 50% increased breast cancer risk for deep-fried freshwater fish and approximately 30% increased risk for deep-fried marine fish [327]. There was a suggestion of a potential U-shaped relation between consumption of ever deep-fried marine fish and well-done marine fish and breast cancer, with the second, third, and fourth quintiles of intake conferring a reduction in breast cancer risk. In comparison, an early Japanese hospital-based case-control study [326], examined the effects of boiled (or broiled) fish and sashimi intake on breast cancer incidence. Compared to those who consumed less than or equal to 3 servings per month, a 12% risk reduction was observed among premenopausal women who consumed 1-2 servings per week, and this effect was stronger among postmenopausal women (OR = 0.82; 95% CI = 0.63, 1.07).

Overall, Asian studies (with the exception of Shanghai populations) report consistent

risk reductions with respect to total fish intake and breast cancer incidence. Stronger associations were estimated for fatty fish intake and among postmenopausal women.

One Mexican study also examined the effect of total fish intake on breast cancer incidence [335], using a hospital-based case-control study of a total of approximately 400 subjects. Comparing highest consumption of fish intake (greater than 1.5 portions per week) to never, the authors reported a nearly 35% risk reduction (OR = 0.67; 95% CI = 0.26, 1.72). Risk reductions were also observed for those women consuming less than 1 portion per week (OR = 0.72; 95% CI = 0.29, 1.79) and 1-1.5 portions per week of total fish intake (OR = 0.89; 95% CI = 0.34, 2.30).

While many case-control studies regarding fish consumption and breast cancer incidence have been conducted among European and Asian populations, only one case-control study was conducted using a U.S. population. McElroy et al. conducted a population-based case-control study in Wisconsin, examining the effects of sport-caught fish on breast cancer risk [334]. The exposure was determined using the following questions: (1) "How often did you eat sport-caught fish?"; and (2) "Was any of this sport-caught fish from the Great Lakes?" In addition to these questions, recent consumption of trout or salmon from the Great Lakes was also assessed. The authors reported a modest risk reduction to null effects for any recent sport-caught fish consumption and any recent Great Lakes trout or salmon consumption.

In sum, several case-control studies have been conducted among both European and Asian populations examining dietary fish intake and breast cancer incidence. Consistent risk reductions were reported among both European and Asian populations for total fish intake and breast cancer risk. In both of these populations, more pronounced associations were

estimated for fatty fish intake and among postmenopausal women. While numerous case-control studies were conducted among both European and Asian populations, only one case-control study was conducted among a U.S. population. However, the exposure assessment utilized in this study is not ideal for considered dietary intake, thus limiting the interpretation of the study results.

1.9.1.2 Cohort Studies

As shown below in **Table 1.7**, all cohort studies examining the association of fish intake and breast cancer incidence have been conducted among either European [320, 324, 325] or U.S. populations [288, 298, 332, 333]. The European cohort studies examining the effect of fish intake and breast cancer report increased risks overall. However, among a Norwegian cohort of 14,500 women who were followed for 11 to 14 years, poached fish reduced breast cancer risk for both women who consumed 2-4 times per week (RR = 0.8; 95% CI = 0.5, 1.1) and those who consumed greater than or equal to 5 times per week (RR = 0.7; 95% CI = 0.4, 1.0), when compared to women consuming less than twice per month [320].

Cohort studies conducted among U.S. populations are also inconsistent, with most studies reporting a null [288, 332] or very modest reduction [298]. An increased incidence of breast cancer among premenopausal women was suggested among the Nurses' Health Study (RR = 1.17; 95% CI = 0.92, 1.50) [296]. In contrast, a modest risk reduction (approximately 10%) was reported for total fish intake on breast cancer incidence in the Iowa Women's Health Study, a cohort comprised of nearly 42,000 women aged 55-69 years [298]. While most U.S. studies report null results for fish intake and breast cancer, the VITamins And

Lifestyle (VITAL) Cohort reported a statistically significant 30% risk reduction for current users of fish oil supplements and breast cancer risk (HR = 0.68; 95% CI = 0.50, 0.98). Results on former users of fish oil supplements and breast cancer risk were null, and 10-year average use of fish oil supplements prior to baseline were reported to reduce risk by 20-25%, though no evidence of a trend was observed [333]. The authors suggest that current use may be a better measure of exposure during the etiologically relevant time period for breast cancer (0-7.3 years after baseline), and that former use of fish oil supplements may not represent a relevant exposure window for breast cancer etiology. While Brasky et al. reported a breast cancer risk reduction for current users of fish oil supplements, the effect of fish intake on breast cancer remains unresolved in the U.S. No recommendations regarding fish or fish oil supplementation should be made without further investigation into this topic.

In sum, European cohort studies examining fish intake and breast cancer incidence report increased risks, with the exception for poached fish, where a risk reduction was suggested. The potential association between fish intake and breast cancer incidence among U.S. cohort studies remains inconsistent across studies. However, a potential risk reduction was reported among the VITAL cohort for fish oil supplementation.

1.9.2 Fish Intake and Survival & Mortality

Unlike breast cancer incidence, the observational studies of dietary fish intake and breast cancer survival are limited. As shown in **Table 1.8**, three studies have been conducted examining the effect of fish intake on survival from breast cancer [339-341]. The earliest study was conducted in 1992 among a Japanese population following 213 breast cancer cases starting in 1975-1978 until 1987 [340]. A total of 47 breast cancer deaths occurred.

Compared to those in the lowest quartile of intake, women who had the highest quartile of fat consumption from fish origin (including fat from fresh fish, shell fish, and processed fish) were 40% more likely to die from breast cancer (HR = 1.4; 95% CI = 0.5, 1.7) [340]. Another study conducted among a Norwegian population followed 533, 276 women aged 35-54 from 1970 to 1985 [341]. Those women who were married to a fisherman were 33% less likely to die from breast cancer (RR = 0.67; 95% CI = 0.47, 0.94) compared to wives of unskilled workers. The results from this study are difficult to interpret with regards to fish intake and breast cancer mortality due to the exposure assessment for fish intake. It is assumed that wives of fisherman are likely to consume more fish than wives of unskilled workers, which may not be a valid assumption.

Only one of the three cohort studies on fish intake in relation to breast cancer survival was conducted using a U.S. population [339]. This study was conducted using the Women's Healthy Eating and Living (WHEL) study and followed more than 3,000 women with breast cancer for a median of 7.3 years. Marine sources of both EPA and DHA were assessed using multiple 24-hour dietary recalls. The study found that those women consuming the highest tertile of marine sources of EPA and DHA compared those women in the lowest tertile were less likely to have a breast cancer recurrence (HR = 0.72; 95% CI = 0.57, 0.90) and less likely to die (all-cause mortality HR = 0.59; 95% CI = 0.43, 0.82) [339]. An eligibility criterion for inclusion into the WHEL study was diagnosis of primary operable invasive stage breast carcinoma within the past 4 years. While Patterson et al. adjusted for time between diagnosis and study entry in their statistical models, it is still likely that assessment of fish intake occurred at different time periods after breast cancer diagnosis for different women. Thus, it is difficult to understand whether dietary intake early in diagnosis (versus later)

would prove to be more beneficial for survival. This information would be important for survivors regarding at what point to incorporate fish intake into dietary habits after breast cancer diagnosis and treatment.

1.9.3 Summary

The small number of studies, both case-control and cohort, that have examined the effects of fish intake on breast cancer incidence are not consistent among U.S. populations. No studies conducted among U.S. populations examined the effect of cooking or preparation methods. A consistent risk reduction for fish intake was observed among the majority of Asian case-control studies. However, an exception is the Shanghai Breast Cancer Study population, where studies have consistently reported increased risks for breast cancer for both freshwater and marine fish. European case-control studies reported a risk reduction for overall fish intake, whereas cohort studies reported increased risks for fish intake. Neither case-control nor cohort studies conducted among European populations examined cooking or preparation methods. The consistency observed among Asian populations may be due to differences in fish intake. Asian populations are known to consume large quantities of fish compared to other populations, and this may explain consistency observed among this population. Differences seen among European case-control and cohort studies may reflect differential recall of dietary intake. It is possible that differential recall was causing the European case-control studies to have risk reductions for overall fish intake. Overall, the effect of fish on breast cancer risk remains unresolved in Europe and the U.S.

1.10 Epidemiology of PUFA-Gene Interactions and Breast Cancer

Only two studies have been conducted examining the effect of PUFA-gene interaction and breast cancer incidence [287, 342]. First, using data from the Singapore Chinese Health Study, Gago-Dominguez et al. [342] examined the effect of marine ω-3 fatty acid intake, glutathione *S*-transferase polymorphisms and breast cancer incidence among postmenopausal women. Gago-Dominguez utilized a nested case-control study design including 258 women with incident breast cancer and a sample of 670 cohort controls. The study reported effect estimates stratified by genotype (e.g., *GSTM1 null*, *GSTM1 positive*, *GSTT1 null*, etc.) and comparing quartiles 2-4 to quartile 1 of dietary marine ω-3 intake. Large risk reductions were reported for postmenopausal women consuming dietary marine ω-3 fatty acid intake in quartiles 2-4 compared to quartile 1 when stratified by the following genotypes: *GSTM1 null* (OR = 0.66; 95% CI 0.37, 1.16); *GSTM1 positive* (OR = 0.83; 95% CI = 0.48, 1.42), *GSTT1 null* (OR = 0.54; 95% CI = 0.29, 1.00); *GSTP1* (A-to-G transition at base 1578 and C-to-T transition at base 2293) *AB/BB* genotypes (OR = 0.49; 95% CI = 0.26, 0.93), where *GSTP1*A* genotypes possess both AA (base 1578) and CC (base 2293), *GSTP1*B* genotypes possess both AG/GG (base 1578) and CC (base 2293) [342]. The strongest effects for marine ω-3 fatty acid intake were seen among those carrying genotypes conferring lower glutathione *S*-transferase activity, which is consistent with the biologic mechanism of PUFAs inducing a cytotoxic environment via lipid peroxidation.

The second PUFA-gene interaction study examined the effect of dietary LA and genetic polymorphisms in 5-lipoxygenase (*ALOX5*) and 5-lipoxygenase-activating protein (*ALOX5AP*) on breast cancer risk utilizing a the San Francisco Bay Area Breast Cancer Study, a population-based case-control study including Latina, African American, and White

women [287]. A total of three polymorphisms (one microsatellite and two SNPs) were examined for *ALOX5AP*, including: (1) -169 to -146 poly(A) microsatellite; (2) -4900 A>G rs4076128; and (3) -3472 A>G rs4073259. Three polymorphisms were also selected for *ALOX5*: (1) -176 to -147 Sp1-binding site 6-bp (-GGGCGG-) variable number of tandem repeat; (2) -1279 G>T rs6593482, and (3) 760 G>A rs2228065.

When examining the joint effects of the *ALOX5AP* -4900 A>G polymorphism and dietary LA intake, the largest risk for breast cancer was observed among women with the highest intake of LA (quartile 4) and AA genotype (OR = 1.8; 95% CI = 1.2, 2.9), and this increased risk among this grouping was consistent among Whites and Latinas [287]. However, the authors did note that the results from this nutrient-gene interaction paper were not consistent with the biology of ω-6 metabolism via the lipoxygenase pathway. They expected variant alleles for *ALOX5* transcription to have a reduced risk for breast cancer due to the reduced production of the inflammatory metabolite 5-hydroxy-6,8,11,14-eicosatetrenoic acid (5S-HpETE).

Only these two studies have examined the effect of genetic polymorphisms and dietary PUFA intake on breast cancer incidence. No studies have examined the effect of PUFA-gene interactions for breast cancer survival. Examining PUFA-gene interactions are important for identifying subgroups of women who may be susceptible to the beneficial effects of ω-3 fatty acids for both preventing breast cancer incidence and improving survival from breast cancer.

1.11 Background and Introduction Summary

Breast cancer is the second most common cancer among women and is the second

leading cause of cancer mortality in the U.S. [1, 2]. Despite this high burden of breast cancer in the U.S., few easily modifiable breast cancer risk reduction strategies or strategies to improve survival after diagnosis are available. Laboratory and animal evidence suggest that ω-3 fatty acids competitively inhibit ω-6 fatty acids, and thus help reduce inflammatory eicosanoids resulting from ω-6 metabolism. Despite this biologic plausibility, the results from epidemiologic studies of dietary PUFA intake and breast cancer incidence are inconsistent, especially among European and U.S. populations. Examination of the relative balance of ω-3 and ω-6 intake and the interaction of PUFAs with genes involved in biologically related pathways, may help to elucidate the potential association between dietary PUFA intake and breast cancer in the U.S. However, few studies have examined the association between the relative balance of ω-3 and ω-6 intake on breast cancer incidence [270, 286], and the interaction between PUFA intake and genes [287], among U.S. populations. Studies regarding dietary PUFA intake and survival from breast cancer are limited to ecologic analyses [300, 301], thus limiting inference regarding the potential benefit of ω-3 intake on improving survival from breast cancer. Therefore, comprehensive examination of dietary PUFA intake (including ω-3 subtypes, ω-6 subtypes, and the relative balance of ω-3 and ω-6), along with interaction with biologically plausible genes may help to elucidate the potential association between PUFA intake and breast cancer incidence and survival in the U.S.

Table 1.1 Polyunsaturated Fatty Acids and Sources

PUFA Category	Name	Carbon Chain length	# of double bonds	Examples of foods rich in PUFAs
ω-3	Alpha-linolenic acid (ALA)	18	3	Canola oil, linseed oil, mackerel, herring, salmon, trout, tuna, cod, flaxseed, soybeans
	Eicosapentaenoic acid (EPA)	20	5	
	Docosahexaenoic acid (DHA)	22	6	
	Docosapentaenoic acid (DPA)	22	5	
ω-6	Linoleic acid (LA)	18	2	Corn oil, sunflower oil, margarine, lard, egg, bacon, ham, maize, almond, brazil nut, peanut, walnut
	Arachidonic acid (AA)	20	4	

Source: [145, 146, 148]

Table 1.2 Summary of Case-Control Studies of Self-reported PUFA Intake and Breast Cancer Incidence

No.	Study, Year [Ref.]	Population	Study N		PUFA	ω3	Subtypes				ω6	Subtypes		ω3/ω6
			Case	Cont.			ALA	EPA	DHA	DPA		LA	AA	
1	Shun-Zhang, 1990 [275]	Shanghai	186	186	↑									
2	Lee, 1992 [276]	Singapore	200	420	↔									
3	Kim, 2009 [277]	Korea	358	360		↓		↓	↓					
4	Zaridze, 1991 [104]	Russia	139	139	↓									
5	Van't Veer, 1991 [99]	Netherlands	168	548	↓									
6	Katsouyanni, 1994 [278]	Greece	820	1547	↑									
7	Landa, 1994 [279]	Spain	100	100	↓									
8	Martin-Moreno, 1994 [280]	Spain	762	988	↑							↑		
9	Nkondjock, 2003 [269]	Montreal	414	429		↑	↑	↑	↔	↑	↔	↓	↓	↑
10	Tavani, 2003 [281]	Italy, Switz.	2900	3122		↓								
11	Alothaimeen, 2004 [282]	Saudi Arab.	499	498	↑									
12	De Stefani, 1998 [283]	Uruguay	365	397	↔		↑					↓		
13	Chajes, 2012 [284]	Mexico	1000	1074		↓					↑			↓
14	Witte, 1997 [285]	USA	140	220	↓							↓		
15	Goodstine, 2003 [286]	USA	565	554	↑			↓	↔					↓
16	Wang, 2008 [287]	USA	1788	2129								↑		

Note:
↑ indicates an effect estimate > 1. ↓ indicates an effect estimate < 1. ↔ indicates a null effect estimate.

Table 1.3 Summary of Cohort Studies of Self-reported PUFA Intake and Breast Cancer Incidence

No.	Study, Year [Ref.]	Population	N Events	PUFA	ω3	Subtypes					ω6	Subtypes		ω3/ω6	ω6/ω3	ω6/LCω3
						ALA	EPA	DHA	DPA	LC ω3		LA	AA			
1	Gago-Dominguez, 2003 [289]	Singapore	314	↑	↓					↓	↑			↔	↑	
2	Wakai, 2005 [100]	Japan	79	↑	↓					↓	↔				↑	
3	Murff, 2010 [290]	Shanghai	712			↑				↓		↑	↑		↔	↑[a]
4	Knekt, 1990 [291]	Finland	54	↑												
5	Voorrips, 2002 [292]	Netherlands	941	↓		↓	↔	↔				↓	↔			
6	Lof, 2007 [293]	Sweden	974	↓												
7	Wirfalt, 2002 [85]	Sweden	237[b]	↑	↑						↑			↓		
8	Thiebaut, 2009 [294]	France	1650		↔	↑				↓	↓	↓	↔		↔	
9	Toniolo, 1994 [288]	USA	180[c]									↑				
10	Barrett-Connor, 1993 [295]	USA	15	↑		↑						↑				
11	Holmes, 1999 [296]	USA	2956	↔	↑	↓	↑	↑				↓	↑			
12	Velie, 2000 [297]	USA	996									↑				
13	Byrne, 2002 [80]	USA	1071									↓				
14	Folsom, 2004 [298]	USA	1885		↓											
15	Sczaniecka, 2012 [270]	USA	772	↔		↔	↓	↓				↑	↔	↓		

Note:
↑ indicates an effect estimate > 1. ↓ indicates an effect estimate < 1. ↔ indicates a null effect estimate.
[a] Estimates presented as interactions (e.g., high ω-3 intake, low ω-6, etc.), not ratio of ω-3 and ω-6 intake.
[b] Nested case-control study of 237 cases and 673 controls.
[c] Nested case-control study of 180 cases and 900 controls.

Table 1.4 Case-Control Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR (95% CI), & Covariates
Shun-Zhang, et al. 1990 [275]	Shanghai	186/186 Population-based	68-item FFQ Continuous, Tertiles	PUFA 13g/day increase: 1.24 (0.75, 2.05) PUFA Q3 vs. Q1: 1.06 (95% CI Not reported) Covariates: Other sources of calories, education, BMI
Zaridze, et al. 1991 [104]	Russia	139/139 Clinic-based	145-item FFQ Quartiles	Q4 vs. Q1 PUFA: Post-menopausal women only Model 1: 0.28 (0.08, 0.87); p for trend = 0.045 Model 2: 0.14 (0.03, 0.69); p for trend = 0.008 Covariates: Model 1: Energy intake Model 2: Energy intake, age at menarche, education
Van't Veer, et al. 1991 [99]	Netherlands	168/548 Population-based	236-item FFQ Referent: PUFA ≤ 16% of total fat	PUFA high vs. low: Model 1: 0.85 (0.56, 1.29) Model 2: 0.84 (0.55, 1.27) Covariates: Model 1: Age Model 2: Age and fat
Lee, et al. 1992 [276]	Singapore	200/420 Hospital-based	FFQ Tertiles	<u>Premen.</u> <u>Postmen.</u> PUFA T3 vs. T1: 0.4 (0.2, 0.7) 1.7 (0.8, 3.6) p for trend: 0.004 0.05 Covariates: Pre: Age, Age at first birth Post: Age, nulliparity, height, education, family history
Katsouyanni, et al. 1994 [278]	Greece	820/(795, 753) Orthapaedic patients & hospital visitors	115-item FFQ Quintiles analyzed as continuous variable	PUFA unit-increase per quintile: 1.04 (0.97, 1.12) Covariates: Age, place of birth, age at first pregnancy, age at menarche, menopausal status, Quetelet's index, total energy intake

Table 1.4 (cont.) Case-Control Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR (95% CI), & Covariates
Landa, et al. 1994 [279]	Spain	100/100 Hospital-based	99-item FFQ Tertiles of monthly consumption	PUFA T3 vs. T1: 0.42 (0.1, 1.1) Covariates: calories
Martin-Moreno, et al. 1994 [280]	Spain	762/988 Population-based	118-item FFQ Quartiles	Q4 vs. Q1 Pre / Post / All women PUFA: 1.58 (0.93, 2.71) / 1.08 (0.73, 1.59) / 1.34 (0.98, 1.84) LA: 1.46 (0.86, 2.49) / 1.20 (0.81, 1.71) / 1.22 (0.89, 1.68) Covariates: Age, geographical region (province), SES, Quetelet's index, total energy intake
Witte, et al. 1997 [285]	USA & Canada: California, Connecticut, and Quebec	140: Premenopausal bilateral breast cancer 220: Unaffected sisters	Semi-quantitative FFQ Quartiles	Q4 vs. Q1 PUFA: 0.3 (0.1, 0.7); p for trend < 0.01 LA: 0.3 (0.1, 0.7); p for trend < 0.01 Covariates: Age, age at menarche, parity, oral contraceptive use, alcohol consumption, BMI, energy (residual method)
De Stefani, et al. 1998 [283]	Uruguay	365/397 Hospital-based	64-item FFQ Quartiles	Q4 vs. Q1 PUFA: 0.99 (0.59, 1.64); p for trend = 0.70 LA: 0.72 (0.44, 1.19); p for trend = 0.25 ALA: 3.24 (1.89, 5.58); p for trend = 0.01 Covariates: Age, residence, urban/rural status, family history, BMI, age at menarche, parity, alcohol, total energy, dietary fiber, folate
Goodstine, et al. 2003 [286]	USA: Connecticut	565/554 Hospital-based (New Haven) & Population-based (Tolland county)	Semi-quantitative FFQ Quartiles	Q4 vs. Q1 Overall / Pre / Post PUFA: 1.06 (0.68, 1.64) / n/a / n/a EPA: 0.94 (0.66, 1.34) / 0.79 (0.38, 1.64) / 0.97 (0.64, 1.47) DHA: 1.00 (0.70, 1.44) / 0.82 (0.40, 1.68) / 1.06 (0.70, 1.62) ω3/ω6: 0.82 (0.58, 1.15) / 0.59 (0.29, 1.19) / 0.89 (0.60, 1.34) Covariates: Age, age at menarche, age at first birth, number of live births, lactation history, BMI, menopausal status, race, family history, income

Table 1.4 (cont.) Case-Control Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR (95% CI), & Covariates
Nkondjock, et al. 2003 [269]	Montreal	414/429 Population-based	200-item FFQ Quartiles	Q4 vs. Q1 LA: 0.90 (0.61, 1.34) AA: 0.86 (0.58, 1.30) ω-6: 1.03 (0.70, 1.53) ALA: 1.27 (0.85, 1.89) ω-3: 1.11 (0.74, 1.65) DPA: 1.33 (0.89, 1.99) ω3/ω6: 1.26 (0.86, 1.86) EPA: 1.23 (0.82, 1.83) DHA: 0.98 (0.66, 1.46) Covariates: Age at first full-term pregnancy, history of breast cancer, history of benign breast disease, number of full-term pregnancies, smoking, marital status, and total energy intake
Tavani, et al. 2003 [281]	Italy, Switzerland	2,900/3,122 Hospital-based	78-item FFQ Quintiles, Continuous	ω-3 Q5 vs. Q1: 0.8 (0.7, 1.0) Continuous (1 gram/week increase): 0.90 (0.84, 0.95) Covariates: Age, study center, education, BMI, energy intake, parity
Alothaimeen, et al. 2004 [282]	Saudi Arabia	499/498 Hospital-based	FFQ Quartiles	PUFA Q4 vs. Q1: 2.12 (95% CI not reported) Covariates: Not reported
Wang, et al. 2008 [287]	USA: San Francisco	1,788/2,129 Population-based	106-item Block FFQ Quartiles	LA Q4 vs. Q1: Model 1: 1.27 (1.04, 1.54) Model 2: 1.10 (0.88, 1.37) Covariates: Model 1: Age, race/ethnicity, menopausal status, country of birth, education, family history, history of benign breast disease, age at menarche, parity, breast feeding, BMI, height, alcohol intake, total energy intake Model 2: Model 1 covariates plus saturated fat and oleic acid

Table 1.4 (cont.) Case-Control Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR (95% CI), & Covariates
Kim, et al. 2009 [277]	Korea	358/360 Hospital-based	103-item FFQ Quartiles	Q4 vs. Q1 Overall / Pre / Post ω-3: 0.47 (0.27, 0.80) / 0.46 (0.22, 0.96) / 0.51 (0.22, 1.13) EPA: 0.50 (0.28, 0.91) / 0.67 (0.54, 2.33) / 0.38 (0.15, 0.96) DHA: 0.44 (0.24, 0.79) / 0.54 (0.24, 1.20) / 0.32 (0.13, 0.82) All p for trend < 0.05, except for EPA (Pre) and DHA (Pre) Covariates: Age, BMI, family history, supplement use, education level, occupation, alcohol consumption, smoking status, physical activity, parity, total energy intake, menopausal status, age at menarche
Chajes, et al. 2012 [284]	Mexico	1,000/1,074 Population-based	FFQ Tertiles	T3 vs. T1 Overall / Pre / Post ω-3: 0.87 (0.68, 1.13) / 0.80 (0.54, 1.19) / 0.87 (0.61, 1.22) ω-6: 1.45 (1.03, 2.04) / 1.92 (1.13, 3.26) / 1.04 (0.65, 1.68) ω3/ω6: 0.82 (0.64, 1.05) / 0.71 (0.48, 1.05) / 0.89 (0.64, 1.25) Only ω-6 (overall) and ω-6 (pre) with p for trend < 0.05 Covariates: BMI, height, family history, age at first menses, age at first full-term pregnancy, number of full-term pregnancies, breast feeding, age at menopause, SES, hormone use, OC use, physical activity, energy intake, alcohol consumption

Table 1.5 Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR (95% CI), & Covariates
Knekt, et al. 1990 [291]	Finland	3,988 total cohort 54 outcomes 20 years (1967-1986)	Diet history at baseline (Tertiles)	Model 1 / Model 2 PUFA T3 vs. T1: 0.86 (0.44, 1.68) / 1.23 (0.55, 2.75) Covariates: age (Model 1), age and energy (Model 2)
Barrett-Connor, et al. 1993 [295]	USA: Rancho Bernardo, California	590 total cohort 15 outcomes	24-hour dietary recall (Tertiles)	Age-adjusted rates of incident breast cancer increase with increasing tertiles (values obtained from visual inspection of graphs) Tertile 1 / Tertile 2 / Tertile 3 PUFA: 5/1000py / 15/1000py / 55/1000py LA: 5/1000py / 15/1000py / 65/1000py ALA: 10/1000py / 20/1000py / 50/1000py
Toniolo, et al. 1994 [288]	USA: New York University Women's Health Study	180/900 Nested case-control study	71-item modified Block FFQ Quintiles	LA Q5 vs. Q1: 1.13 (0.65, 1.98) Higher risks seen for Q2 (1.21), Q3 (1.66), and Q4 (1.49) Covariates: energy
Holmes, et al. 1999 [296]	USA: Nurses' Health Study	88,795 total cohort 2,956 outcomes 14 years	Repeated FFQs, cumulatively averaged intake reported in 1980, 1984, 1986, and 1990 (continuous)	Overall / Pre / Post PUFA[a]: 0.91 (0.79, 1.04) / 0.99 (0.77, 1.27) / 0.88 (0.74, 1.04) ω-3[b]: 1.09 (1.03, 1.16) / 1.10 (0.96, 1.26) / 1.09 (1.02, 1.17) PUFA[c]: 0.97 (0.81, 1.16) LA[d]: 0.95 (0.92, 0.98) ALA[d]: 0.75 (0.54, 1.03) AA[d]: 1.05 (1.00, 1.10) EPA[d]: 1.06 (1.02, 1.10) DHA[d]: 1.04 (1.01, 1.06) [a]5% of energy, [b]ω-3 from fish (0.1% of energy), [c]5% of energy adjusted for other fats, [d]1% of energy Covariates: energy, age, energy-adjusted vitamin A intake, alcohol intake, time period, height, parity, age at first birth, weight change since age 18 years, BMI at 18 years, age at menopause, menopausal status, HRT use, family history, benign breast disease, age at menarche

Table 1.5 (cont.) Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Velie, et al. 2000 [297]	USA: Breast Cancer Detection Demonstration Project	40,022 post-menopausal women 996 breast cancer cases 5.3 years average follow-up	Block 60-item FFQ (Quintiles)	Postmenopausal women (N=40,022) LA Q5 vs. Q1: 1.05 (0.82, 1.34) Postmenopausal women with history of BBD (N=13,707) LA Q5 vs. Q1: 1.29 (0.78, 2.13) Covariates: total energy, BMI, height, family history, parity, age at first birth, educational level, alcohol use, age at menarche, history of BBD (for first model only)
Byrne, et al. 2002 [80]	USA: Nurses' Health Study	44,697 post-menopausal women 1,071 breast cancer cases	Repeated FFQs: 61-item FFQ (1980) 131-item FFQ (1984, 1986, 1990) Cumulative averaged dietary intake (Quintiles)	Replicate of Velie, et al., (2000) analysis Postmenopausal women (N=44,697) LA Q5 vs. Q1: 0.93 (0.74, 1.16) Q4, Q3, Q2 all consistent with RRs approx. 0.83 Covariates: age, height, age at menarche, age at menopause, use of postmenopausal hormones, parity, age at first birth, BMI, weight change since age 18, total energy, alcohol, family history, vitamin A intake.
Voorrips, et al. 2002 [292]	The Netherlands Cohort Study	Case-cohort study: 1,598 sub-cohort 941 cases 6.3 years average follow-up	150-item semi-quantitative FFQ (Quartiles)	Q5 vs. Q1 PUFA: 0.88 (0.67, 1.15) ALA: 0.68 (0.51, 0.91) LA: 0.95 (0.72, 1.24) EPA: 1.03 (0.78, 1.37) AA: 1.01 (0.77, 1.33) DHA: 1.02 (0.77, 1.36) Note: Q4 vs. Q1 (EPA) = 1.23 (0.93, 1.62) Q4 vs. Q1 (DHA) = 1.20 (0.91, 1.58) Age-adjusted (adjustment for other covariates including total energy intake didn't make a difference)

Table 1.5 (cont.) Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Wirfalt, et al. 2002 [85]	Sweden	237/673 Population-based nested case-control study	7-day diet history & FFQ Quintiles	Q5 vs. Q1 PUFA: 3.02 (1.75, 5.21); p for trend = 0.0007 ω-3: 1.81 (1.09, 2.99); p for trend = 0.026 ω-6: 3.02 (1.78, 5.13); p for trend = 0.0002 ω3/ω6: 0.66 (0.41, 1.08); p for trend = 0.137 Covariates: Past food habit change, energy intake, BMI, height, waist circumference, age at first birth, current hormone therapy, alcohol habits, and educational status
Gago-Dominguez, et al. 2003 [289]	The Singapore Chinese Health Study	35,298 women aged 45-74 years old 314 breast cancer cases 5.3 years average follow-up	165-item semi-quantitative FFQ Quartiles	Q4 vs. Q1 PUFA: 1.27 (0.92, 1.74) Note: Q3 0.83 (0.59, 1.18) ω-6: 1.22 (0.89, 1.67) Note: Q3 0.90 (0.64, 1.26) ω-3: 0.87 (0.64, 1.18) ω-3, marine: 0.72 (0.53, 0.98) ω-3, other: 1.00 (0.73, 1.36) Note: Q2 thru Q3 ↓ risk Pre / Post ω-3, marine: 0.90 (0.49, 1.65) / 0.68 (0.47, 0.97) High ω-6 intake (Q4), low ω-3 intake (Q1): 1.87 (1.06, 3.27) Low ω-6 intake (Q2), high ω-3 intake (Q2-4): 1.03 (0.69, 1.53) Covariates: age, year of recruitment, dialect group, education, daily alcohol drinker, family history, age at menarche, parity
Folsom, et al. 2004 [298]	USA: The Iowa Women's Health Study cohort	41,836 women aged 55-69 years 1,885 breast cancer cases	127-item FFQ Quintiles	ω-3 Q5 vs. Q1: 0.91 (0.77, 1.08) Covariates: age, energy intake, education, alcohol, smoking, pack-years of cigarette smoking, age at first birth, estrogen use, vitamin use, BMI, waist/hip ratio, diabetes, hypertension, intake of whole grains, fruit and vegetables, red meat, cholesterol, and saturated fat

Table 1.5 (cont.) Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Wakai, et al. 2005 [100]	Japan Collaborative Cohort Study (JACC)	26, 291 women aged 40-79 years 129 breast cancer cases	40-item FFQ Quartiles	Q5 vs. Q1 Age-adjusted / Multivariate-adjusted PUFA: 0.85 (0.52, 1.40) / 1.10 (0.63, 1.90) ω-3: 0.62 (0.37, 1.03) / 0.69 (0.40, 1.18) ω-6: 0.80 (0.49, 1.30) / 1.02 (0.59, 1.74) ω6/ω3: 1.20 (0.73, 1.99) / 1.31 (0.78, 2.19) LC ω-3: 0.50 (0.30, 0.83) / 0.50 (0.30, 0.85) Higher risks reported among postmenopausal women at baseline for total PUFA, ω-3, and ω-6. Covariates: age, study area, educational level, family history of breast cancer, age at menarche, age at menopause, age at first birth, parity, use of exogenous female hormones, alcohol consumption, smoking, consumption of green leafy vegetables, daily walking, height, body mass index, and total energy intake.
Lof, et al. 2007 [293]	Sweden	49,261 women 30-49 years of age 974 breast cancer cases	80-item FFQ Quintiles	Q5 vs. Q1 Overall / BC < 50 yrs / BC > 50 yrs PUFA: 0.72 (0.52, 1.00) / 1.06 (0.64, 1.75) / 0.54 (0.35, 0.85) Covariates: education, parity, age at menarche, use of oral contraceptives, age at first birth, first-degree relative with breast cancer, non-alcohol total energy intake, total fat intake, BMI, and alcohol intake

Table 1.5 (cont.) Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Thiebaut, et al. 2009 [294]	France	56,007 women 1,650 breast cancer cases	208-item diet history questionnaire Quintiles	Q5 vs. Q1 ω-6: 0.93 (0.80, 1.09) ω-3: 0.99 (0.84, 1.15) LA: 0.92 (0.79, 1.07) ALA: 1.05 (0.90, 1.23) AA: 0.99 (0.85, 1.16) LC ω-3: 0.94 (0.80, 1.10) ω6/ω3: 0.97 (0.83, 1.14) Covariates: age, non-alcohol energy, ethanol intake, smoking history, history of BBD, history of breast cancer in first-degree relative, age at menarche, parity, BMI, menopausal status, age at menopause, use of hormone treatment
Murff, et al. 2010 [290]	Shanghai Women's Health Study	72,571 women 712 breast cancer cases	FFQ Quintiles	Q5 vs. Q1 LA: 1.13 (0.82, 1.54) ALA: 1.07 (0.76, 1.50) AA: 1.06 (0.78, 1.45) LC ω-3: 0.74 (0.52, 1.06) ω6/ω3: 1.02 (0.77, 1.34) ω6/ LC ω3: 1.29 (0.95, 1.75) Covariates: age, BMI, total energy, family history of breast cancer, alcohol use, tobacco use, education, use of hormone replacement therapy, personal history of diabetes, menopausal status, age at menopause, age at menarche, parity, age at first pregnancy, level of physical activity, red meat intake, fish intake, vitamin E intake

Table 1.5 (cont.) Cohort Studies of PUFA and Breast Cancer Incidence

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Sczaniecka, et al. 2012 [270]	USA: VITamins And Lifestyle (VITAL) Cohort	30,252 postmenopausal women 772 invasive breast cancer cases Mean follow-up time = 6 years	FFQ Quintiles	Q5 vs. Q1 PUFA: 1.07 (0.76, 1.52) ALA: 0.97 (0.71, 1.32) LA: 1.18 (0.84, 1.66) EPA: 0.70 (0.54, 0.90) AA: 0.97 (0.74, 1.29) DHA: 0.67 (0.52, 0.87) ω3/ω6: 0.84 (0.65, 1.09) Covariates: age, race, education, height, BMI, age at menarche, age at first birth, age at menopause, history of hysterectomy, years of combined hormone therapy, years of estrogen hormone therapy, family history, mammography, history of benign breast biopsy, regular use of NSAIDs, exercise, alcohol consumption, vegetable intake, fruit intake, and total energy

Table 1.6 Case-Control Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR* (95% CI), & Covariates
Landa et al. 1994 [279]	Spain	100/100 Hospital-based	99-item FFQ Tertiles of monthly consumption	Fish: T2: 0.55, T3:0.34 (95% CI not reported) Covariates: Age
Franceschi et al. 1995 [321]	Italy	2,569/2,588 Hospital-based	79-item FFQ Quintiles	Fish: 0.69 (0.56, 0.84); p for trend < 0.01 Covariates: age, center, education, parity, energy, alcohol
Hirose et al. 1995 [326]	Japan	1,052/23,163 Hospital-based	FFQ ≤ 3/month 1-2/wk ≥ 3/wk	Boiled, or broiled fish, sashimi: ≤ 3/month (reference) Premenopausal / Postmenopausal 1-2/wk: 0.88 (0.71, 1.09) / 0.82 (0.63, 1.07) ≥ 3/wk: 0.98 (0.78, 1.24) / 0.75 (0.57, 0.98) Covariates: age, first-visit year
Braga et al. 1997 [89]	Italy	2,569/2,588 Hospital-based	78-item FFQ Quintiles	Fish (incl. boiled and roasted fish; fried fish, tinned tuna and sardines) Premenopausal / Postmenopausal OR: 0.88 (0.7, 1.1) / 0.76 (0.7, 0.9) Covariates: age, center, education, parity/age at 1st birth, and energy
Favero et al. 1998 [322]	Italy	2,569/2,588 Hospital-based	78-item FFQ Quintiles	Fish: 0.69 (0.6, 0.8); p for trend < 0.01 Covariates: age, center, education, parity, energy and alcohol

Table 1.6 (cont.) Case-Control Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR* (95% CI), & Covariates
Torres-Sanchez et al. 2000 [335]	Mexico	198/198 Hospital-based	95-item FFQ Portions per week	Fish portion/wk (never = reference) <1 0.72 (0.29, 1.79) 1-1.5 0.89 (0.34, 2.30) > 1.5 0.67 (0.26, 1.72) Covariates: total energy intake, age at menarche, number of children and age at first birth, lifetime lactation, family history of breast cancer, menopausal status, and Quetelet index
Dai et al. 2002 [327]	The Shanghai Breast Cancer Study	1,459/1,556 Population-based	76-item FFQ Quintiles	Freshwater fish: 1.48 (1.16, 1.89) Marine fish: 1.14 (0.90, 1.45) (F) Freshwater fish Deep-fried (Q5 vs. Q1; >20.7 g/d vs. ≤ 2.9) (M) Marine fish Deep-fried (Q5 vs. Q1) Never / Ever / Well-done F: 1.46 (1.03, 2.07) 1.50 (1.05, 2.13) 1.52 (1.05, 2.22) M: 0.94 (0.67, 1.33) 1.29 (0.91, 1.84)* 1.32 (0.91, 1.93)* *potential U-shape Covariates: age, education, family history of breast cancer, family history of breast fibroadenoma, WHR, age at menarche, physical activity, ever had live birth, age at first live birth, menopausal status, age at menopause, total energy
Terry et al. 2002 [319]	Sweden	2,085/2,000 Population-based	34-item FFQ Servings per week	>3.5 servings/week vs. 0 to ≤0.5 servings/week Age-adjusted / Multivariate Total fish: 0.99 (0.68, 1.43) 0.88 (0.60, 1.29) Fatty fish: 0.67 (0.44, 1.03) 0.70 (0.45, 1.10) Other fish: 0.81 (0.54, 1.21) 0.76 (0.49, 1.16) Covariates for multivariate model: age, BMI, height, smoking, leisure-time physical activity between 18 and 30 yrs of age, alcohol, consumption of Brassica vegetables, history of benign breast disease, menopause type (surgical or natural), parity, duration of hormone replacement therapy use, age at menarche, age at menopause, age at first birth

Table 1.6 (cont.) Case-Control Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR* (95% CI), & Covariates
Hirose et al. 2003 [328]	Japan	2,385/19,013 Hospital-based	18-item FFQ Categories	4 Categories (Almost never, 1-3 times/month, 1-2 times/week, 3+ times/week) ORs presented comparing highest vs. lowest category Premenopausal / Postmenopausal Cooked/raw fish: 0.95 (0.70, 1.28) / 0.75 (0.57, 0.98) Dried/salted fish: 1.15 (0.89, 1.49) / 0.78 (0.60, 1.01) Covariates: age, visit year, family history, age at menarche, parity, age at first full-term pregnancy, and BMI (for postmenopausal ORs)
McElroy et al. 2004 [334]	USA: Wisconsin	1,481/1,301 Population-based	2 questions: How often did you eat sport-caught fish? Was any of this sport-caught fish from the Great Lakes (GL)?	Recent sport-caught fish consumption: 1.00 (0.86, 1.17) Recent GL fish consumption: 1.06 (0.84, 1.33) Recent GL trout/salmon: 1.00 (0.78, 1.28) Recent meals per year (≥24 vs. 1-3): 0.94 (0.75, 1.18) Recent GL trout/salmon meals (≥7 vs. 1-2): 0.98 (0.62, 1.54) Stronger effects seen for premenopausal women for both questions. Covariates: age, family history of breast cancer, recent alcohol consumption, parity, age at first full-term pregnancy, lactation, menopausal status, age at menopause, weight at age 18, age at menarche, and education
Hirose et al. 2005 [329]	Japan	167/854 Hospital-based	119-item FFQ Tertiles	Fish/fish products: Premenopausal / Postmenopausal T2: 1.53 (0.74, 3.18) / 1.42 (0.75, 2.66) T3: 1.36 (0.65, 2.88) / 0.77 (0.39, 1.52) Covariates: age, motives for consultation, smoking, drinking, exercise, energy, family history, age at menarche, parity, age at first full-term pregnancy, BMI, and age at menopause (for postmenopausal women only)

Table 1.6 (cont.) Case-Control Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR* (95% CI), & Covariates
Bessaoud et al. 2008 [323]	France	437/922 Population-based	FFQ Quartiles	Age-adjusted / Multivariate Fish & seafood: 0.69 (0.49, 0.97) / 0.79 (0.54, 1.16) Covariates: total energy intake, education, parity, breast-feeding, age at first full-term pregnancy, duration of ovulatory activity, BMI, physical activity, and first-degree family history of breast cancer
Kim et al. 2009 [277]	Korea	358/360 Hospital-based	103-item FFQ Quartiles	Age-adjusted / Multivariate Total fish: 0.62 (0.41, 0.93) / 0.55 (0.32, 0.96)* Lean fish: 1.20 (0.81, 1.79) / 1.21 (0.72, 2.04)* Fatty fish: 0.27 (0.17, 0.44) / 0.23 (0.13, 0.42) *potential U-shape No difference by menopausal status for total fish and fatty fish. Increased risks (ORs = 1.34 – age-adjusted, 1.22 – multivariate) seen for lean fish intake in premenopausal women. Covariates: age, BMI, family history of breast cancer, supplement use, education level, occupation, alcohol consumption, smoking status, physical activity, parity, total energy intake, menopausal status, age at menarche
Zhang et al. 2009 [330]	China	438/438 Hospital-based	81-item FFQ Quartiles	Model 1 / Model 2 Fish: 0.56 (0.38, 0.85) / 0.72 (0.46, 1.10) Model 1 Covariates: age at menarche, live birth and age at first live birth, BMI, history of benign breast disease, mother/sister/daughter with breast cancer, physical activity, passive smoking, use of deep-fried cooking method, and total energy intake Model 2 Covariates: all Model 1 covariates, vegetable, fruit, and soy food intake

Table 1.6 (cont.) Case-Control Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Cases/Controls & Control Sampling	Exposure Assessment & Categorization	Results, Adjusted OR* (95% CI), & Covariates
Bao, et al. 2012 [331]	Shanghai Breast Cancer Study	3,443/3,474 Population-based	FFQ Tertiles (Freshwater) Quintiles (Marine)	Freshwater fish: T2: 1.17 (1.03, 1.32) T3: 1.39 (1.23, 1.56) Marine fish: Q5: 1.19 (1.02, 1.39) No differences seen when stratified by ER/PR status. Covariates: total energy, age, education, benign breast disease, family history, participation in regular exercise, body mass index, study phase, age at menarche, menopausal status, parity, total vegetable intake, total fruit intake

Table 1.7 Cohort Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Vatten et al. 1990 [320]	Norway	14,500 total cohort 152 breast cancer cases 11-14 years of follow-up	60-item FFQ Servings per week	Overall fish (> 2 times/week versus ≤ 2 times/week): Crude IRR = 1.2 (0.8, 1.7) Poached fish (< 2times/month = reference category) 2-4 times/week crude IRR: 0.8 (0.5, 1.1) ≥ 5 times/week crude IRR: 0.7 (0.4, 1.0)
Toniolo et al. 1994 [288]	USA: New York University Women's Health Study	14,291 women 180 breast cancer cases	71-item modified Block FFQ Quintiles	RR Q5: 1.02 (0.61, 1.71) Largest risk seen among Q2 (RR = 1.32) Covariates: energy
Holmes et al. 2003 [332]	USA: Nurses' Health Study	88,647 women 4,107 breast cancer cases 18 year follow-up	61-item FFQ answered in 1980 baseline Quintiles	Fish intake Overall RR: 1.04 (0.93, 1.14) Premenopausal: 1.17 (0.92, 1.50) Postmenopausal: 1.00 (0.89, 1.12) Covariates: age, 2 year time period, total energy intake, alcohol intake, parity and age at first birth, BMI at age 18, weight change since age 18, height, family history of breast cancer, history of benign breast disease, age at menarche, menopausal status, age at menopause, hormone replacement therapy, duration of menopause

Table 1.7 (cont.) Cohort Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Stripp et al. 2003 [324]	Denmark	23,693 postmenopausal women 424 breast cancer cases Median length of follow-up = 4.8 years	192-item FFQ (Quartiles and Continuous)	IRR Total fish intake (Continuous): 1.13 (1.03, 1.23) Fatty fish (Continuous): 1.11 (0.91, 1.34) Lean fish (Continuous): 1.13 (0.99, 1.29) Fried fish (Continuous): 1.09 (0.95, 1.25) Boiled fish (Continuous): 1.09 (0.85, 1.42) Processed fish (Continuous): 1.12 (0.93, 1.34) Total Fish (Q4 vs. Q1): 1.54 (1.18, 2.02) Approx. 14% increased incidence seen for total fish intake (continuous) for ER+ breast cancers. Covariates: parity (parous/nulliparous, number of births and age at first birth), benign breast tumor, years of school, use of hormone replacement therapy, duration of HRT use, BMI, and alcohol
Folsom et al. 2004 [298]	USA: The Iowa Women's Health Study	41,836 women aged 55-69 years 1,885 breast cancer cases 14 years of follow-up	127-item FFQ (Quintiles)	RR for breast cancer incidence = 0.92 (0.76, 1.12) Covariates: age, energy intake, educational level, physical activity, alcohol consumption, smoking status, pack-years of cigarette smoking, age at first live birth, estrogen use, vitamin use, body mass index, waist/hip ratio, diabetes, hypertension, intake of whole grains, fruit and vegetables, red meat, cholesterol, and saturated fat

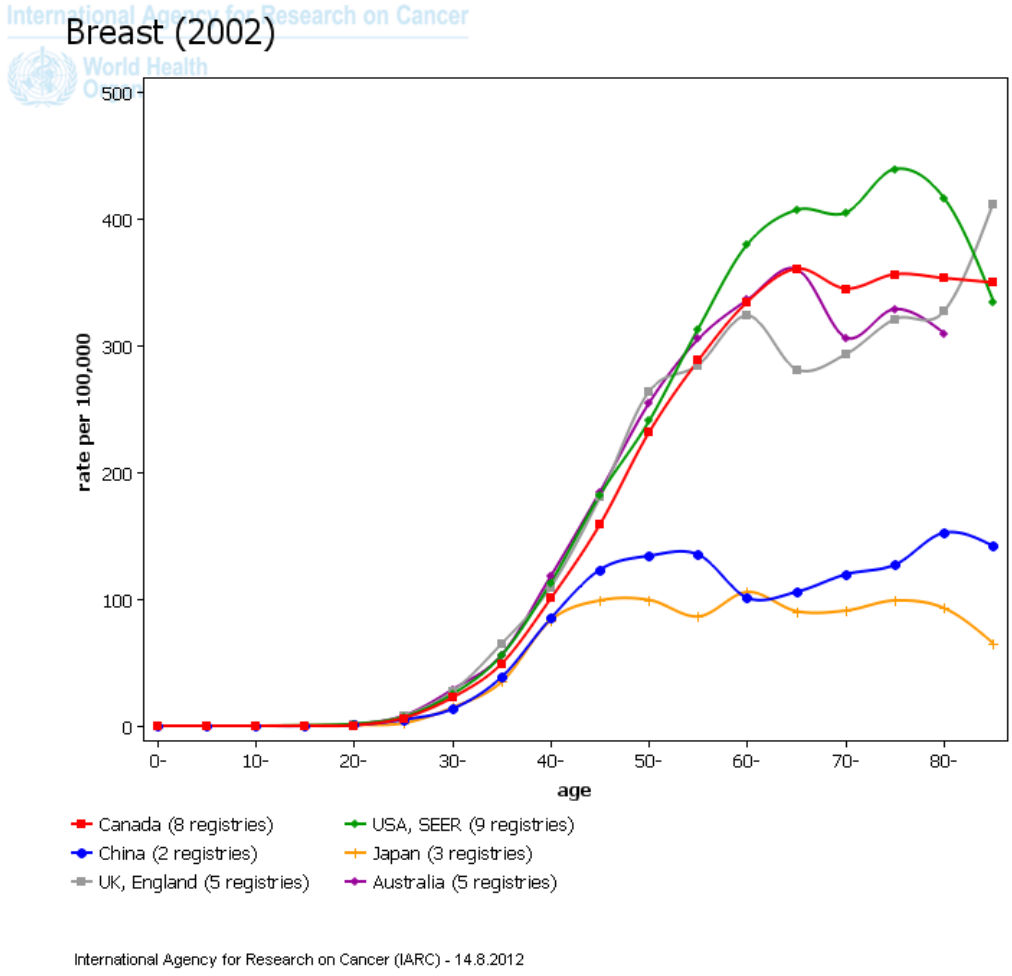
Table 1.7 (cont.) Cohort Studies of Dietary Fish Intake and Breast Cancer

Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Engeset et al. 2006 [325]	The European Prospective Investigation into Cancer and Nutrition (EPIC)	310,671 women aged 25-70 years 4,776 breast cancer cases Median follow-up = 6.4 years	FFQ Quintiles	Total fish: 1.07 (0.95, 1.20) Total fish (premenopausal): 1.11 (0.84, 1.45) Total fish (postmenopausal): 1.10 (0.95, 1.28) Lean fish: 1.07 (0.95, 1.21) Fatty fish: 1.13 (1.01, 1.26) Covariates: center, adjusted for time of follow-up, energy intake, height, weight, age at menarche, number of full-term pregnancies, age at first full-term pregnancy, current use of hormone replacement therapy, current use of oral contraceptives, menopausal status (excluded for pre- and post RRs)
Brasky et al. 2010 [333]	USA: VITamins And Lifestyle (VITAL) Cohort	35,016 women aged 50-76 years 880 breast cancer cases Mean follow-up time = 6 years	Detailed questionnaire of supplement use Fish oil supplements (Nonuser, former, current)	HR Current fish oil users (compared to nonusers): 0.68 (0.50, 0.92) Covariates: age, race, education, BMI, height, fruit consumption, vegetable consumption, alcohol consumption, physical activity, age at menarche, age at menopause, age at first birth, history of hysterectomy, years of combined hormone therapy, family history, benign breast biopsy, mammography, low-dose aspirin use, regular aspirin use, ibuprofen use, naproxen use, and use of multivitamins

Table 1.8 Studies of Fish Intake and Breast Cancer Survival and Mortality

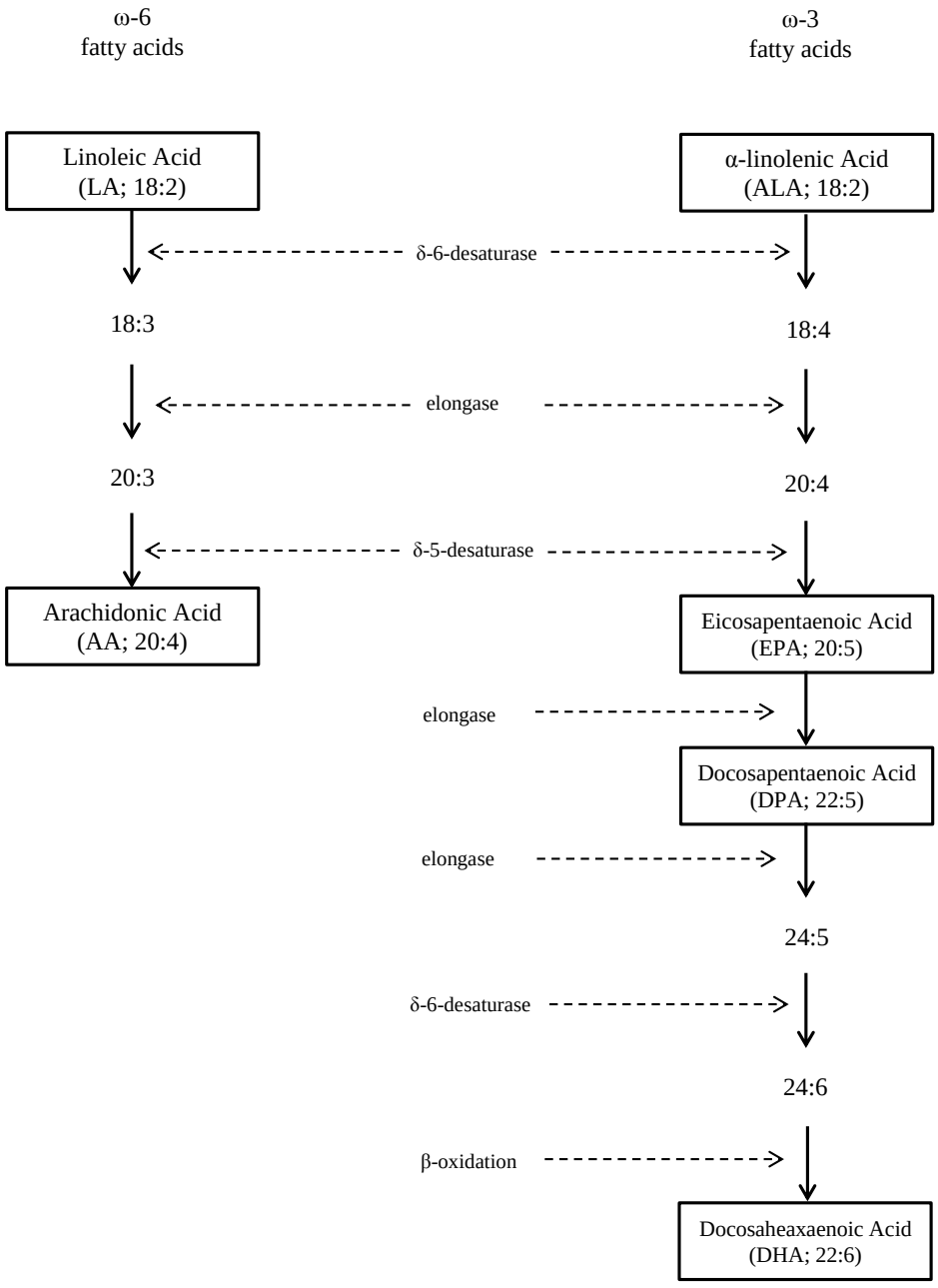
Author, Year	Population	Sample size, Median Length of Follow-up	Exposure Assessment (Categorization)	Results, Adjusted HR, RR* (95% CI), & Covariates
Kyogoku et al. 1992 [340]	Japan	213 breast cancer cases (from case-control study) 47 breast cancer deaths Follow-up from 1975-1978 through 1987	FFQ (Quartiles)	Fat from fish origin (includes fresh fish, shell fish, and processed fish) HR = 1.4 (0.5, 4.3) Covariates: clinical stage, BMI, age at menarche, age at first birth, age at operation, radiation therapy, chemotherapy, endocrine therapy, operative procedure, and each of the nutrients
Lund et al. 1993 [341]	Norway	533,276 women aged 35-54 years Follow-up from 1970 through 1985	Married to a fisherman	RR for breast cancer mortality = 0.67 (0.47, 0.94) compared to wives of unskilled workers Covariates: age and number of children
Patterson et al. 2010 [339]	USA: Women's Healthy Eating and Living (WHEL) Study	3,081 breast cancer cases Median follow-up time = 7.3 years	Repeated 24-hour recalls Multiple dietary assessments (Tertiles)	HR for additional breast cancer events Marine sources of EPA/DHA: 0.72 (0.57. 0.90) No differences for food adjusted for supplements, food plus supplements HR for all-cause mortality Marine sources of EPA/DHA: 0.59 (0.43, 0.82) Reduced hazard (HR = 0.68) for food plus supplements Covariates: tumor stage, grade, and time between diagnosis and study entry (additional adjustments for obesity, age, physical activity, intervention group and entry cohort did not change the statistical significance of results)

Figure 1.1 Age-specific breast cancer incidence rates (per 100,000) for Western and Asian populations in 2002



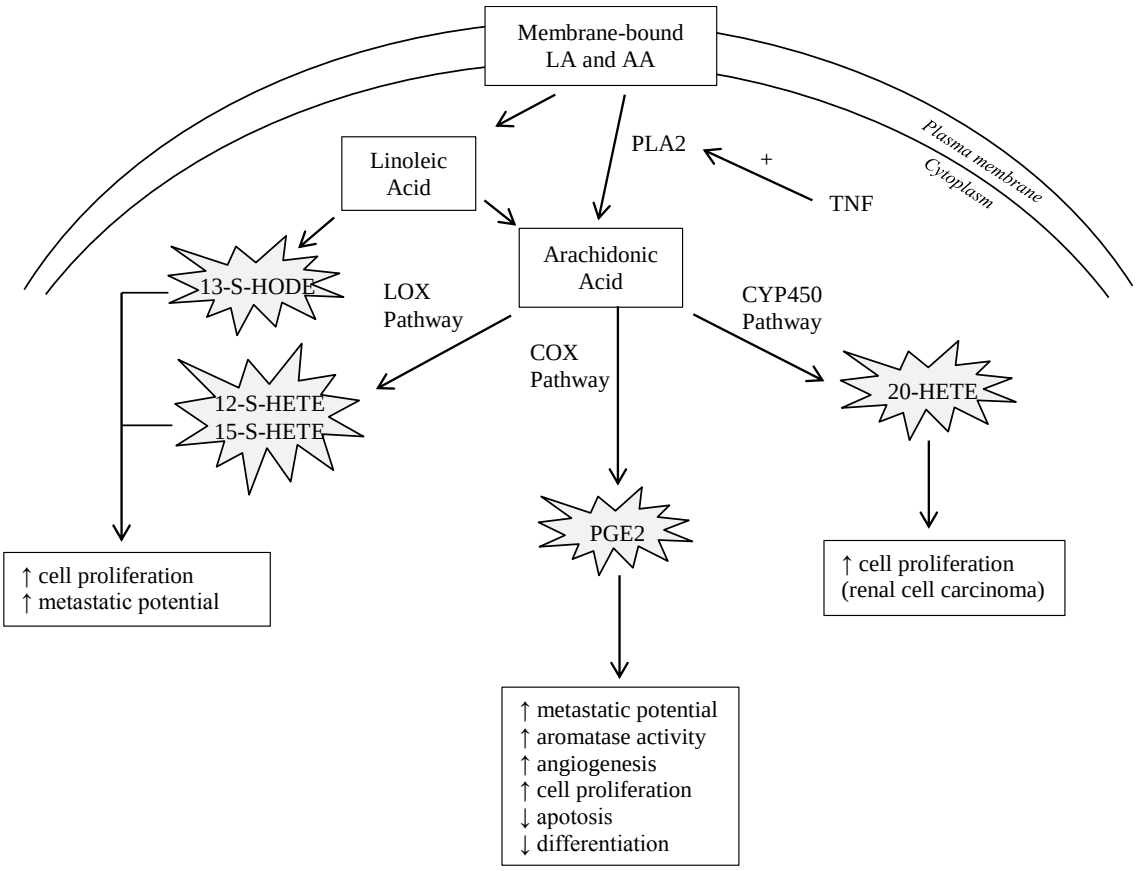
Graph generated from online resource Ferlay et al. [343]

Figure 1.2 PUFA Biosynthesis



Adapted from [148]

Figure 1.3 Metabolism of Arachidonic Acid



CHAPTER 2:
METHODS

2.1 Study Overview

2.1.1 Specific Aims

Among American women, breast cancer is the leading incident cancer, with an estimated 207,100 incident cases, and the second leading cause of cancer mortality, with an estimated 40,000 breast cancer deaths in 2013 [1]. Laboratory studies indicate that high intakes of ω-6 fatty acids increase cancer risk whereas intakes of ω-3 fatty acids reduce risk via the differential production of inflammatory eicosanoids resulting from arachidonic acid metabolism [53]. However, the epidemiologic studies regarding dietary intake of PUFAs and breast cancer are inconsistent.

Both ω-6 and ω-3 fatty acids are metabolized via the cyclooxygenase and lipoxygenase pathways [163, 344]. However, the eicosanoids resulting from ω-6 and ω-3 fatty acid metabolism differ, with ω-3 fatty acids resulting in fewer inflammatory eicosanoids than ω-6 fatty acids [345]. PUFAs have also been implicated in the regulation of additional inflammation and oxidative stress factors involved in carcinogenesis, including cyclooxygenase (*PTGS-2*) [53], peroxisome proliferator-activated receptors (*PPARs*) [345, 346], tumor-necrosis factor-alpha (*TNF-α*) [347], *FAS/FASL* [212, 348], and cytochrome aromatase enzymes (*CYP17*, *CYP19*) [199]. Additionally, PUFAs can induce a cytotoxic environment via lipid peroxidation, consequently inhibiting breast cancer growth [254, 256, 257].

Previous population studies on prostate [349, 350] and colorectal cancer [351-354] have considered potential interactions between PUFA intake and genetic polymorphisms associated with PUFA metabolism via the inflammation pathway. Despite evidence from animal studies, only two previous epidemiologic studies of breast cancer have considered potential PUFA-gene interactions, and examined only genetic variants in the leukotriene pathway [287] and with glutathione *S*-transferases (*GST*s) [342].

The hypotheses examined in this dissertation are that higher intake of ω-6 will interact with genetic polymorphisms in the inflammation, oxidative stress, and estrogen metabolism pathways to increase the risk of developing breast cancer, which may be less pronounced when combined with higher intakes of ω-3 fatty acids. Further, among women diagnosed with breast cancer, higher intakes of ω-3 PUFA will enhance survival, but among those with variant polymorphisms in oxidative stress enzymes (conferring greater efficiency in ROS scavenging) will have reduced survival.

To evaluate these hypotheses, existing Long Island Breast Cancer Study Project (LIBCSP) data were used, including: self-reported dietary intake of PUFA food sources; other potential breast cancer risk factors; and select functional genetic polymorphisms in pathways biologically related to PUFA metabolism. The LIBCSP is a population-based study, which includes a case-control component (cases = 1,508; controls = 1,556) and a follow-up cohort of the case women to determine vital status. This dissertation addresses the following **specific aims**.

Aim 1: **Investigate the potential association of dietary PUFA and fish intake, genetic susceptibility and breast cancer incidence**.

1a. Estimate ω-3 and ω-6 intake among LIBCSP participants in order to estimate potential association between PUFA intake [ω-3, ω-6, and (ω-3/ω-6) ratio; PUFA subtypes] and fish intake [by cooking method] and breast cancer incidence.

1b. Utilize existing data on genetic variants in the cyclooxygenase (*PTGS-2*), inflammation (*PPAR-α, TNF- α,* and *FAS/FASL*), oxidative stress (*MnSOD, MPO, CAT, GPx, COMT, GSTs*), and estrogen metabolism (*CYP17*) pathways to estimate the joint effects of PUFAs (mentioned in Aim 1a) and genetic polymorphisms on breast cancer incidence.

Aim 2: **Investigate the potential association of dietary PUFA intake and fish intake, genetic susceptibility and breast cancer survival.**

2a. Estimate all-cause and breast cancer specific mortality associated with PUFA intake [ω-3, ω-6, and (ω-3/ω-6) ratio; PUFA subtypes] and fish intake [by cooking method].

2b. Estimate the effects of genetic variants (mentioned in Aim 1b) in inflammation, estrogen, and oxidative stress pathways on breast cancer survival.

2c. Explore joint effects of PUFAs (mentioned in Aim 2a) and genetic variants (mentioned in Aim 1b) on breast cancer survival.

2.1.2 Importance of Knowledge to be gained

Despite recent downward trends in breast cancer rates, the burden of breast cancer in the U.S. remains high [1]. Thus, identification of an individually tailored, low-cost chemoprevention strategy to reduce the burden of breast cancer will potentially benefit large numbers of women. Findings from this dissertation will enhance our understanding of the relation between dietary PUFA and breast carcinogenesis, by identifying potential subgroups

of women who may be genetically susceptible to their effects. This analysis will be the first to examine the role of genetic variations in the inflammation, estrogen metabolism, and oxidative stress pathways and PUFA measures (ω-3, ω-6, PUFA subtypes, and relative balance) in relation to breast cancer incidence and survival. Finally, the analysis is cost- and time- efficient, because it builds upon the existing resources of a large, population-based study of breast cancer.

2.2. Long Island Breast Cancer Study Project (LIBCSP)

2.2.1 Case-Control Study

The LIBCSP was a federally mandated, population-based study that was initiated as a case-control study to determine whether breast cancer risk was associated with environmental exposures [355]. The case-control study was conducted among adult women of Nassau and Suffolk counties on Long Island, New York. Cases and controls were identified over a 12-month period starting in 1996. Cases were English-speaking adult women with a first primary *in situ* or invasive breast cancer between August 1, 1996 and July 31, 1997. Potential cases were identified from 28 hospitals on Long Island as well as three large tertiary care hospitals in New York City. Eligible controls were sampled at a continuous rate using 8 waves of random digit dialing for those less than 65 years of age, and twice using the Health Care Finance Administration (HCFA) rosters to identify those older than 65 years. Controls were frequency matched to cases by 5-year age group. There were no race or age restrictions. The final LIBCSP study sample includes a total of 1,508 cases (83%) and 1,556 controls (68%) of eligible subjects who completed the case-control interview.

The 100-minute case-control interviewer-administered, in-person structured questionnaires were administered shortly after diagnosis for cases (average within 3 months). The main case-control interview obtained information regarding various environmental and occupational histories; medical history, including co-morbidities, medication use, and family history of cancer; life course changes in body size, physical activity, smoking, alcohol use, and hormone use; menstrual and reproductive history; and demographic characteristics. Immediately following the main questionnaire, a validated 101-item Block food frequency questionnaire (FFQ) was self-completed by 98% of respondents to assess usual eating habits in the year prior to diagnosis for cases and the previous year for controls. Blood samples were obtained by 1,102 cases and 1,141 controls (73% of respondents) among those completing the main questionnaire, with a lower proportion of older control women donating blood. As part of the case-control study, cases were asked to sign a medical record release form and medical records were abstracted to determine stage of disease and hormone receptor status.

2.2.2 Follow-up Study

The LIBCSP was later expanded to follow-up the cohort of case women to determine vital status and first course of treatment for the first primary breast cancer [127, 356]. Potentially eligible subjects for the LIBCSP follow-up study were breast cancer cases who gave permission to be re-contacted at the end of the LIBCSP case-control interview (N = 1,414). The eligible cases were first re-contacted via mail five years post-diagnosis, and then a second time via telephone to schedule an interviewer-administered telephone interview. A total of 1,098 interviews (93 of which were proxies) were conducted. The follow-up

interview was used to determine first course of treatment for the first primary breast cancer diagnosis. Cases were asked about procedures that they may have completed, including: surgery (needle biopsies, tumor biopsies, modified mastectomy, radical mastectomy, node removal); radiation; chemotherapy; and hormonal treatments. Medical records were re-abstracted as part of the follow-up study to ascertain first course of treatment for the first primary breast cancer. Complete medical records were abstracted for 598 breast cancer cases. Comparison of the self-reported responses versus information recorded in the medical records showed excellent correspondence between the two sources of information (kappa coefficients were 0.97 for radiation, 0.96 for chemotherapy, and 0.92 for hormonal therapy) [127].

The National Death Index (NDI) was utilized for ascertaining vital status for all cases. The NDI includes death records from 1979 to the present and is updated yearly [357]. The NDI is a gold standard for vital statistics in the U.S., providing a high sensitivity cause of death field [358]. The following case information was created, encrypted, and sent to NDI via for potential matches: first and last name; city; state; date of birth; Social Security number; gender; race; and marital status. Previous NDI linkages were conducted in 2002, 2005, and 2009. The latest NDI linkage was conducted for follow-up through 2011.

2.2.3 Characteristics of LIBCSP Population

The LIBSCP study population was predominantly Caucasian (cases = 94%, controls = 92%), which reflects the underlying racial distribution of the two study counties on Long Island [355]. Women were more likely to be 45-74 years old of age and postmenopausal (67%), which reflects the age- and menopausal-distribution of breast cancer among white

women in the U.S [355]. Also, the population was well educated with more than half of cases (52%) and controls (56%) attended college, graduating from college, or received any post college education. The population was also less likely to be poor with approximately two-thirds of both cases (66%) and controls (69%) reporting a household income of greater than $35,000. This primarily white, well-educated older-age sample of women is not generalizable to all women in the U.S., but is generalizable to those who are at highest risk of developing breast cancer in the U.S. Also, the geographic location of this American study population offers a unique opportunity for higher fish consumption, the largest source of ω-3 fatty acids.

2.3 Outcome and Exposure Assessments

2.3.1 Outcome Assessment

For the follow-up cohort of the 1,508 women newly diagnosed with breast cancer (participant cases from the LIBCSP parent case-control study) (required for Aim 2), identification of deaths, both all-cause and breast-cancer specific, were obtained via the NDI, as described above. Participants were followed from diagnosis until December 31, 2011, for a median follow-up of 14.7 years (range 0.2 to 15.4 years). Among the 1,508 women diagnosed with breast cancer, 506 died (34%), of which 219 were due to breast cancer (43%). Women who died from all-causes (death from any cause), and those whose deaths were breast cancer-related (breast cancer-specific mortality) were identified. Breast cancer-related deaths were determined using the International Classification of Disease (codes 174.9 or C-50.9).

2.3.2 Dietary Assessment

The majority of epidemiologic studies estimating the association between dietary factors and breast cancer utilize dietary assessment via FFQ. Dietary intake assessed by FFQ is thought to reflect usual dietary intake, which is more likely to reflect intake during the relevant etiologic window of exposure for breast cancer. In the LIBCSP, diet history was assessed using a self-administered modified 101-item Block FFQ which had been previously validated [359]. The FFQ was completed by approximately 98% of cases and controls who completed the main questionnaire. The Block FFQ was used to assess usual dietary intake in the previous year for the controls and the year prior to diagnosis in cases. To help reduce misclassification of exposure assessments, including usual diet, cases were interviewed on average three months after diagnosis. To facilitate completion of the interview as quickly as possible after diagnosis, a "super-rapid" identification was implemented [355]. This included contacting pathology departments of 28 hospitals on Long Island and three tertiary care hospitals in New York City two to three times a week, of which seven hospitals (those servicing the largest number of Long Island residents with breast cancer) were contacted daily.

While use of dietary food records and recall (i.e. 7-day food records, 24-hour dietary recall) may be considered the "gold standard" for dietary assessment, it is difficult for food records and recalls to assess usual intake, unless applied multiple times in order to account for variation in food intake [360]. Additionally, food records and recalls may induce reporting error among participants due to high burden. FFQs are subject to both systematic and random error. Nevertheless, individual nutrient intake obtained from FFQs can be ranked using quantiles allowing comparisons across different levels of nutrient intake [360].

Assessment of usual diet from FFQs will better represent diet during the etiologically relevant induction period for breast cancer, whereas dietary intake assessed using food records and recalls will likely reflect highly variable current diet, which may be an inappropriate window of exposure for a case-control study of breast cancer incidence.

2.3.3 PUFA Exposure Assessment

Dietary PUFA intake -- both ω-3 and ω-6 fatty acids -- were estimated for this ancillary study using responses from the modified FFQ and the nutrient quantities from the U.S. Department of Agriculture (USDA) database (Aim 1a). The modified Block FFQ used in the LIBCSP assessed both frequency and portion size. For example, participants were asked: "Over the last 12 months, how often did you eat the following foods? (Ignore any recent changes)". Frequency of intake during the past 12 months was categorized into the following groups: never; <1 per month; 1 per month; 2-3 per month; 1-2 per week; 3-4 per week; 5-6 per week; 1 per day; 2+ per day. Portion size was assessed in comparison to the average portion size of a particular food item. For example, the average serving size for shell fish on the FFQ is 5 pieces, ¼ cup, or 3 ounces, and participants were asked if they consumed less than, equal to, or more than the average intake for shell fish. PUFA intake was estimated using FFQ responses of food intake (grams per day) over the past 12 months, and the average nutrient composition of PUFAs in foods ascertained from the USDA National Nutrient Database for Standard Reference, Release 23 (example calculation provided in **Table 2.1**). In addition to total ω-3 and ω-6 PUFAs, specific ω-3 and ω-6 subtypes were also estimated for this ancillary study, including ALA, EPA, DHA, DPA, LA, and AA.

Fish intake and cooking methods were also examined in addition to the derived

PUFA exposure assessment explained above. Fish intake was assessed for: (1) tuna, tuna salad, tuna casserole; (2) shell fish (e.g., shrimp, lobster, crab, oysters, etc.); and (3) other fish (broiled or baked). A more detailed examination of consumption of different cooking methods of fish was also examined and included the following: (1) grilled or barbequed; (2) pan-fried (not deep-fat-fried); (3) oven-broiled; (4) oven-baked; and (5) microwaved.

2.3.4 Genotyping

The interaction between PUFA intake and genetic variants involved in relevant biologic pathways were examined for incidence (Aim 1a) and survival (Aim 2c). The following biologically relevant putatively functional genetic variants were selected: *PTGS-2* (rs20417 and rs5275); *PPAR-α* (rs1800206); *FAS* (rs2234767); *FASL* (rs763110); *TNF-α* (rs1800629); *MnSOD* (rs4880); *MPO* (rs2333227); *CAT* (rs1001179); *COMT* (rs4680 and rs737865); *GPX1* (rs1050450); *GSTM1* (deletion); *GSTP1* (rs1695); *GSTT1* (deletion); *GSTA1* (rs3957356); *CYP17* (rs743572). Proposed functionality for each variant was identified using NIEHS SNPInfo web server [361]. Variants affecting polyphen prediction (*GPX1*), transcription factor binding prediction (*PTGS-2* rs20417, *FAS*, *FASL*, *TNF-α*, *MPO*, *CAT*, *GSTA1*, *COMT* rs737865, *CYP17*), miRNA binding (*PTGS-2* rs5275, *GPX1*), 3D conformation (*PPAR-α*, *COMT* rs4680), or splicing regulation (*PPAR-α*, *FAS* rs2234767, *GPX1*, *GSTP1*, *COMT* rs4680) were considered as putatively functional variants. Additionally, variants resulting in base pair changes that were non-synonymous were also classified as potentially functional (*PPAR-α*, *MnSOD*, *GPX1*, *GSTP1*, *COMT* rs4680). Please refer to **Table 2.2** for a detailed summary regarding the chosen putatively functional genetic variants.

Blood samples were collected from subjects at the time of case-control interview, which occurred shortly after the time of diagnosis. Study interviewers for the LIBCSP, who were also certified nurses or phlebotomists, obtained non-fasting 40ml blood samples from participants. A pre-chemotherapy blood sample was obtained from approximately 77% of the cases who donated a blood sample. Blood samples were shipped overnight at room temperature to a single laboratory, and processed within 24 hours of collection by lab personnel who were masked regarding the case-control status of the samples. DNA was extracted in the laboratory of Dr. Regina Santella at Columbia University. Processed and aliquoted samples were stored at -80°C. Using the banked DNA, the LIBCSP has previously genotyped several variants involved in inflammation (*PTGS-2, PPARs, TNF-α, FAS, FASL*), oxidative stress (*MnSOD, MPO, CAT, GPx, GSTs, COMT*), and estrogen metabolism (*CYP17*).

Genotyping methods for existing oxidative stress genes (*CAT, MPO, MnSOD, GPx, GSTs, COMT*) have been published elsewhere [362-366], but briefly, DNA was extracted from mononuclear cells in whole blood which was separated by Ficoll (Sigma Chemical Co., St. Louis, Missouri). DNA was isolated using standard phenol, and chloroform isoamyl alcohol extraction and RNase treatment. BioServe Biotechnologies (Laurel, Maryland) performed the genotyping using high-throughput, matrix assisted, laser desorption/ionization time-of-flight mass spectrometry of Sequenom, Inc. (San Diego, California). Gene deletions for *GSTM1* and *GSTT1* were determined by a multiplex polymerase chain reaction method, with the constitutively present gene β-globulin as an internal positive control [366]. For inflammation genes, namely *FAS, FASL, PPARs, COX-2*, the following assays were used: Taqman 5'-Nuclease Assay (Applied Biosystems, Foster City, CA) and AcycloPrime™-FP

SNP Detection Kit obtained from Perkin Elmer Life Sciences (Boston, Massachusetts, USA) [65, 367, 368]. For the aromatase enzyme (*CYP17*), the following assay was used: AcycloPrime™-FP SNP Detection Kit obtained from Perkin Elmer Life Sciences (Boston, Massachusetts, USA) with 10 µM probe [369, 370]. The following procedures were used for quality control: (i) genotyping results were reviewed manually; (ii) cases and two nontemplate controls were included on each plate; (iii) 8% of blinded duplications were distributed throughout the DNA samples; and (iv) laboratory personnel were blinded to the case/control status of the samples.

2.4 Results from Previous Analyses

2.4.1 Inflammation genes and breast cancer risk in LIBCSP

Investigators of the LIBCSP have previously conducted a study regarding genetic polymorphisms in *PTGS-2* (also known as *COX-2*) and breast cancer risk [65]. In addition to the main effects of polymorphisms in *PTGS-2*, the study authors also examined the interactive effects with non-steroidal anti-inflammatory drug use (NSAIDs). No associations were seen with regard to variant alleles in *PTGS-2* and breast cancer risk, nor was there any evidence for an interaction with NSAID use. However, a slight decrease in breast cancer risk was seen among hormone receptor positive women using NSAIDs and possessing a *PTGS-2* variant allele (OR = 0.7, 95% CI = 0.5, 1.0). In 2007, Crew et al. examined the effects of genetic polymorphisms in *FAS* and *FAS* ligand (*FASL*) on breast cancer risk [367]. Main effects of variant alleles in *FAS* and *FASL* did not show any association with breast cancer risk. However, an increased risk in breast cancer was observed among those carrying the variant alleles in *FAS* when considering effect measure modification by lactation history

(Ever lactating: OR = 1.46, 95% CI = 1.04, 2.06; Never lactating: OR = 0.82, 95% CI = 0.64, 1.06). Golembesky et al. also found nearly double the risk for breast cancer among women with a variant *PPAR-α* allele (OR = 1.97; 95% CI = 1.14, 3.43) [368]. These initial studies conducted on inflammation genes using the LIBCSP have shown plausibility for differences in breast cancer risk by genetic variants in inflammation genes, and this genetic variability may be further modified by biologically plausible environmental exposures.

2.4.2 Oxidative stress genes and breast cancer risk in LIBCSP

LIBCSP investigators have also examined numerous oxidative stress genes in relation to breast cancer risk. Steck et al. [366] examined the effects of glutathione *S*-transferases (GST), specifically *GSTM1*, *GSTT1*, *GSTP1*, on breast cancer risk. Null genotypes, or those conferring reduced activity in *GSTM1*, *GSTT1*, and *GSTP1*, did not show substantial effects on breast cancer when examined individually. However, when main effects of genes were examined in combination (i.e. *GSTM1* and *GSTT1*, *GSTM1* and *GSTP1*, *GSTP1* and *GSTT1*) those with polymorphisms conferring reduced activity in both *GSTM1* and *GSTT1* were at increased risk for breast cancer (OR = 1.38; 95% CI = 1.09, 1.31). Effects of other genetic polymorphisms in antioxidant enzymes, such as *MnSOD*, *MPO*, *CAT*, and *GPx*, were also previously examined by LIBCSP investigators [362-366]. No associations were reported for the main effects of *MnSOD* and *GPx* polymorphisms and breast cancer risk. A variant allele conferring reduced *MPO* transcriptional activity was associated with a reduced risk for breast cancer (OR = 0.87; 95% CI = 0.73, 1.04). The variant allele conferring greater *CAT* enzyme ROS-scavenging capability reduced breast cancer risk by 17% (OR = 0.83; 95% CI = 0.69, 1.00). Risk reductions were also observed for variant alleles for both *COMT* rs4680 and

COMT rs737865 [371].

In the LIBCSP, joint effects of dietary exposures and oxidative stress genes on breast cancer incidence were also examined. For example, risk reductions were observed for the interactive effects of high fruit and vegetable intake and variant alleles in *MnSOD* [365], *MPO* [362], *CAT* [363], *GPX1* [364], *GSTM1* [366], *GSTP1* [366], *GSTT1* [366], and *GSTA1* [372]. Overall, polymorphisms in oxidative stress genes conferring reduced enzyme activity seem to increase breast cancer risk in the LIBCSP. It is possible that PUFA intake, in combination with polymorphisms in oxidative stress genes may modify breast cancer risk.

2.4.3 Aromatase genes and breast cancer risk in LIBCSP

LIBCSP investigators have previously examined the effects of aromatase genes on breast cancer incidence. Chen et al. [369] examined the interaction between the estrogen-biosynthesis gene *CYP17* and reproductive, hormonal, and lifestyle factors on breast cancer incidence. Overall, no associations were reported between the *CYP17* genotype and breast cancer risk. A risk reduction (OR = 0.81; 95% CI = 0.52, 1.25) was observed for homozygotes for the variant allele (resulting in an increased level of serum estrogen) among premenopausal women, whereas a 25% (95% CI = 0.90, 1.73) increased risk was observed for homozygotes for the variant *CYP17* allele among postmenopausal women. The joint effects of higher BMI (25+) and possessing the variant allele increased breast cancer risk among postmenopausal women (OR = 1.93; 95% CI = 1.32, 2.84), whereas a risk reduction was reported among premenopausal women (OR = 0.65; 95% CI = 0.39, 1.08). An increase in breast cancer risk was suggested for joint effects of the variant allele and nulliparity, ever alcohol drinkers, and oral contraceptive use (ever and ≥6 months) among postmenopausal

women. Talbott et al. [370] reported increased risks for the synonymous *CYP19* SNPs among premenopausal women. Strongest effects were suggested for the variant *CYP19* allele for ER- (OR = 3.89; 95% CI = 1.74, 8.70) and PR- (OR = 2.52; 95% CI = 1.26, 5.05), though estimates were imprecise. Although CYP19 is part of the estrogen metabolism pathway the proposed analysis will not examine *CYP19* variants, due to the potential lack of functionality as determined by the NIEHS SNPInfo web server [361].

2.5 Data Analysis

2.5.1 Statistical Methods

All data analysis was conducted using SAS version 9.2 (Cary, NC). For all analyses considering dietary exposures, cases and controls with implausible values for total energy intake (those with log-transformed caloric intake three standard deviations above or below the mean) were excluded.

For Aim 1a, standard unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CIs) for the association between breast cancer incidence and multiple measures of intake of PUFAs (ω-3, ω-6, ratio, and subtypes) and fish (by cooking method) [373]. Different exposure variable types (e.g., continuous, quadratic, trinomial, splines, and quantiles) were considered, and quartiles were chosen based upon the shape of the relation between PUFA intake and the log-odds of breast cancer (**Figures A.1-A.3**). There was no evidence for a monotonic relation between intake and breast cancer incidence, thus a formal linear trend test was not conducted. The most appropriate model will be determined using model fit and the likelihood ratio test (LRT) using nested models.

Interaction between dietary PUFA intake and genotype (Aim 1b) was assessed on both multiplicative (**Tables 3.7, 3.8**) and additive scales (**Tables 3.4, 3.5**). For the former, a multiplicative interaction term was included in the logistic regression model (e.g., ω-3*gene). Additionally, PUFA-gene interactions for total PUFA, total ω-3, total ω-6, and subtypes were also conducted (**Tables A.3-A.11**). In order to maximize the sample sizes within subgroups of the interaction, PUFA intake was dichotomized at the median (based upon distribution among control) and genes were dichotomized using a dominant model (homozygous wild type versus variant allele). Statistically significant multiplicative interactions were assessed using LRT using nested models for the interaction term. Interactions on the additive scale were assessed using single-referent coding. Departures from additivity were assessed using Relative Excess Risk due to Interaction ($RERI = OR_{high\ PUFA,variant} - OR_{high\ PUFA} - OR_{variant} + 1$) and corresponding 95% CI for RERI were estimated using Hosmer and Lemeshow's method [374].

For Aim 2, Cox proportional hazards regression were conducted to estimate hazard ratios (HR) and corresponding 95% CIs for all-cause and breast cancer-specific mortality. The proportional hazards assumption was assessed using exposure interactions with time, and also using Martingale residuals [375]. Kaplan-Meier survival curves constructed and log-rank tests were conducted to test for differences between the survivor functions (**Figures A.4-A.6**). Hazard ratios and corresponding 95% CIs were estimated for PUFA intake (Aim 2a), main effects of genes (Aim 2b) and the PUFA-gene interaction (Aim 2c) with respect to all-cause and breast-cancer specific mortality. Appropriate exposure variable categorization was determined by examining the PUFA exposure in relation to log-hazard of mortality (**Figures A.7-A.9**) using different variables to represent dietary intake (e.g., continuous,

quadratic, trinomial, splines, and quantiles). The main effects of genetic polymorphisms (Aim 2b) were examined using both dominant and additive models. For the final aim (Aim 2c), the joint association between PUFA intake and polymorphisms in biologically relevant genes on mortality was assessed similar to methods used for Aim 1b, on both multiplicative (**Tables A.12-A.13)** and additive scales (**Tables 4.6, 4.8**). Similar to Aim 1, PUFA-gene interactions for total PUFA, total ω-3, total ω-6, and subtypes were also conducted in relation to all-cause mortality (**Tables A.14-A.22**).

2.5.2 Multiple Comparisons

Basing study conclusions solely on statistically significant p-values remains controversial in the field of epidemiology. It is possible to obtain statistically significant p-values for nearly null associations that are precise (based upon the width of the 95% confidence interval), and for strong associations that are imprecise [376]. However, it is difficult to convince peer-reviewers and journals alike of the potential harm of making conclusions solely based upon p-values, and researchers are often expected to provide p-values for all estimated associations.

The issue of p-values becomes more complicated when conducting multiple comparisons, since the number of false positive results increases with increasing number of statistical comparisons. Therefore, in order to prevent large numbers of false-positives in studies conducting multiple comparisons, further control of the type 1 error rate is required. Since all genetic variants for this analysis were chosen based upon strong biologic rationale it is possible that no correction is required, compared to more agnostic approaches (e.g., genome-wide association studies). However, the issue of adjustment for multiple

comparisons remains controversial and adjustment for multiple comparisons were considered for this analysis even though conclusions were not based solely upon statistically significant findings.

A number of methods have been proposed for addressing issues concerned with multiple comparisons, including: Bonferroni correction; false discovery rate (FDR); and empirical methods (false positive report probability (FPRP), Bayesian false discovery probability (BFDP), and hierarchical modeling) [302, 377-379].

Bonferroni correction is a commonly used method to adjust for multiple comparisons. However, it is possible that many genetic polymorphisms are likely correlated (e.g., polymorphisms belonging to the same gene) and thus large test statistics are not as likely [377]. Therefore, Bonferroni correction for multiple testing is known to be overly conservative [377] and conclusions made based solely on statistically significant Bonferroni correction could overlook important associations. The FDR provides an improvement compared to the overly conservative Bonferroni correction since the FDR is based on the average value of the proportion of false positive results among all statistically significant results [380, 381]. However, the FDR has its limitations since it fails to account for study power [381].

Bayesian approaches for controlling multiple comparisons have also been suggested. FPRP provides another method and is the probability that the null hypothesis is true given a statistically significant result. The magnitude of the FPRP is based upon the p-value, prior probability of alternative hypothesis, and statistical power to detect the alternative hypothesis [378]. However, a major limitation of the FPRP approach is the difficulty in assigning a range of prior probabilities for the alternative hypothesis [378]. Also, some have suggested

that the derivation of the FPRP approach is flawed due to its incorrect probabilistic representation of an observation [382]. Similar to the FPRP, the BFDP is used to identify statistically significant findings within the context of multiple comparisons. However, in comparison to the FPRP, the BFDP incorporates additional information and defines a threshold in terms of false discovery and nondiscovery [379]. Hierarchical modeling has also been proposed as another Bayesian approach for control of multiple comparisons by incorporating prior knowledge regarding the individual comparisons and how they may be correlated [302]. However, the utility of this approach over other methods may be questionable for datasets with uncorrelated exposures. Overall, the advantages of employing Bayesian approaches for the multiple comparisons may be hindered by the limited availability of well-defined priors.

In sum, a variety of methods have been proposed for control of multiple comparisons. Each of these methods has their own strengths and limitations, and some are more computationally rigorous than others. Adjustment for multiple comparisons was considered using the FDR approach, since it is easily implemented and is suggested to be less conservative than standard Bonferroni correction. A p-value for statistical significance was identified when the following expression was met:

$$\text{raw p-value}(i) \leq (0.05i)/m$$

where, i = p-value rank, and m = total number of statistical comparisons [381]. The FDR-adjusted p-value to for statistical significance for each Aim was calculated using the expression above. However, none of the comparisons were found to be statistically significant after adjusting for multiple comparisons (**Tables A.23-A.29**). Also, given all the genetic variants for this analysis were chosen based upon biologic plausibility, conclusions

were based primarily upon the precision of the estimated associations (95% CIs).

2.5.3 Confounding and Effect Measure Modification

All analyses were adjusted for the matching factor, five-year age group [373]. Potential covariates were included in the Directed Acylic Graph (DAG) constructed using prior knowledge and existing literature (**Figures 2.1-2.2**), and confounders were identified using DAG rules, and adjustments were not made for variables considered colliders or on the causal path due to the potential for biased effect estimates [373]. Confounders, previously identified from DAG analysis, were included in the statistical model if inclusion of the confounder changed the effect estimate by more than 10% [373].

Risk and prognostic factors for breast cancer often vary across different subgroups of women. Breast cancer incidence rates continue to increase after menopause among Western women, whereas the incidence rates tend to stabilize after menopause among Asian women (**Figure 1.1**). The ER+/PR+ breast cancers are the predominant subtype among postmenopausal Caucasian women. Therefore, it is possible that potential associations between PUFA and breast cancer could vary according to both menopausal and hormone receptor status. For example, in the LIBCSP the effect estimates for physical activity and obesity differ by menopausal status and by hormone receptor status [128, 129]. Effect measure modification by menopausal status and hormone receptor status was also observed for dietary exposures in the LIBCSP [383]. Thus, it is possible that similar variations in the effect estimates will be observed in relation to the PUFA and/or fish intake measures considered. Therefore, effect modification of the main effect (Aims 1a and 2a) of dietary intake-breast cancer association by breast cancer subtype (ER+/PR+ versus other subtypes)

and menopausal status (pre- versus post-menopausal) were evaluated by conducting a stratified analysis for each proposed effect measure modifier (**Tables A.30-A.34**). Additionally, effect measure modification by each individual treatment (e.g., chemotherapy, radiation, and hormone therapy) was explored for survival for each treatment separately (**Tables A.35-A.37**).

2.5.4 Energy Adjustment

Energy adjustment is common practice in all nutritional epidemiology studies. The majority of epidemiologic studies of PUFA and/or fish intake and breast cancer incidence have controlled for energy intake in the statistical model. Willett has proposed many reasons for energy adjustment in nutritional epidemiologic studies, including: (1) control for confounding; (2) removal of extraneous variation; and (3) simulation of a dietary intervention [384]. However, it is known that PUFA contributes to 7% of total energy intake [146] and thus energy intake could be considered on the causal path between PUFA intake and breast cancer incidence. Animal studies support inhibition of mammary tumor development with caloric restriction [385]. However for humans, the balance between energy intake and energy expenditure (considering energy intake, physical activity, and body size) may be more important for breast cancer prevention than caloric restriction itself [386].

Adjustment for total energy intake when examining the potential association between intake of PUFAs and breast cancer could lead to biased effect estimates [373], if we consider total energy intake as a causal intermediate. Interestingly, no differences in effect estimates were observed when examining different methods of energy adjustment (e.g., standard multivariate model without energy adjustment, standard multivariate model with energy-

adjustment, nutrient residual model, energy-partition model, multivariate nutrient density model) when examining the effect of fat on coronary heart disease [387].

Nevertheless, the energy adjustment for this analysis could affect the interpretation of the effect estimates and has the potential to reflect very different public health messages. For example, including energy adjustment in the model for main effect could reflect a *substitution* of food sources of PUFA for other components of the diet, which may be important when examining the effect of relative balance of PUFA (ω-3:ω-6 ratio) which would constitute a change composition of the diet. However, if effect estimates are presented without energy-adjustment, then the estimates could be interpreted as the effect of *adding* food sources of PUFA to the diet (e.g., supplementation of the diet) on breast cancer. Therefore, main and interactive effects for Aims 1 and 2 were analyzed and are presented with and without energy adjustment since they reflect different public health messages. Sensitivity analyses were conducted examining different methods of energy adjustment (**Table A.38**), thus reflecting different interpretations for the estimate of PUFA intake in relation to either incidence (**Tables A.39-A.40**) or survival (**Tables A.41-A.42**). Furthermore, sensitivity analysis examining adjustment for the other PUFA class (either ω-3 or ω-6) was also conducted for both incidence (**Table A.43**) and survival (**Table A.44**). However, the results from the sensitivity analyses did not alter our conclusions.

2.6 Study Power

Power was calculated for incidence (Aim 1) using National Cancer Institute (NCI) Power Version 3.0 [388, 389]. Power was calculated considering the main effect of PUFA/fish intake (Aim 1a) as categorical variables (e.g., quartiles, tertiles, dichotomized).

The detectable OR represented the comparison of the highest to the lowest category (e.g., quartile 4 versus quartile 1, tertile 3 versus tertile 1, etc). This was accomplished by assuming a dichotomous exposure variable with prevalence of exposure 50% (highest quartile) and varying the study sample sizes (e.g., ½ total sample for quartiles, ⅔ total sample for tertiles, and full sample size for dichotomized exposure). Interactions (Aim 1b) were considered for both super-multiplicative and sub-multiplicative interactions. The following values for departure from multiplicative interactions (θ) were considered: 0.32, 0.53, 2, and 3. Power calculations for Aim 1 are presented in **Tables A.45-A.46**.

A similar approach was employed for survival (Aim 2), except using PROC POWER in SAS (Cary, NC). Among the 1,508 LIBCSP cases, there were 444 total deaths, of which 203 were due to breast cancer. The number of deaths used for the power calculations were proportional to the total sample size for the exposure. For example, PUFA intake is available for 98% of the cases (n=1,481), therefore only 98% of total deaths (n=436) were used in the power calculations. The event rate was calculated by dividing the estimated number of deaths (e.g., 436 deaths) by the total person-years (e.g., 1,481 persons * 12.7 years = 18,800 person-years). A similar procedure was used for calculating power related to breast-cancer specific mortality, using only 203 breast cancer deaths. Power calculations for Aim 2 are presented in **Tables A.47-A.48**.

2.7 Data Interpretation Issues

For Aims 1a and 2a, a decreased breast cancer incidence and improved survival among those consuming a higher ratio of ω-3 fatty acids relative to ω-6 fatty acids were expected. Risk reductions were also expected for fish intake, and these estimates were

thought to vary by cooking method. However, differences by cooking methods were not observed in this population (**Tables A.49-A.50**) and may be largely due to low fish intake and resulting small cell sizes. This decreased risk of breast cancer incidence among those consuming more ω-3 fatty acids may be modified by inflammation, oxidative stress, or aromatase genes. For genetic polymorphisms, effect estimates were expected to vary by genotype; those variants conferring greater enzyme activity may increase mortality – although possible risk reductions were evident, depending upon the biologic function of the gene (e.g., increasing versus lowering levels of oxidative stress) and the variant allele's impact on gene function (Aim 2b). The findings for the polymorphisms are more likely to be favorable when their effects are considered in an environment rich with ω-3 fatty acids. Thus, for women with a higher ω-3 to ω-6 ratio and genetic variants conferring greater enzyme activity pronounced risk reductions in incidence and mortality were expected (Aims 1b and 2c).

Due to the improved survival observed among women diagnosed with breast cancer in the U.S. [1], the number of deaths observed in the LIBCSP cohort is lower than anticipated. Thus, the results were expected to be imprecise, especially for the interaction between genes and survival (Aim 2c). Therefore, results from the PUFA-gene interaction analyses, especially with respect to survival were interpreted with caution.

Exposure assessment of dietary intake of both PUFAs and fish may not be accurate, because of incomplete assessment by the FFQ. For the case-control analyses, differential recall between cases and controls is also a possibility, although this concern would not affect the case-only cohort analysis. It is also possible that dietary assessment may not represent the actual diet intake during the induction period for development and/or progression of

breast cancer, consequently resulting in underestimation of the effect estimates in relation to breast cancer incidence and mortality.

The LIBCSP is a population-based study, representing women with breast cancer in a proscribed geographic area, Nassau and Suffolk counties on Long Island, NY. Thus, findings are applicable to this group of Long Island women, who are primarily white postmenopausal women. This is the specific sub-population that is at highest risk for breast cancer in the U.S. However, the study is not generalizable to other races or ethnicities, although the underlying associations observed in the proposed study are not expected to vary widely from other populations with similar intake of PUFA-rich foods and similar genetic profiles.

2.8 Study Purpose

To the best of my knowledge, this study is the first to comprehensively examine the potential interaction between PUFA intake and genetic susceptibility in inflammation, oxidative stress, and aromatase enzymes on breast cancer incidence and survival.

Table 2.1 Example calculation of PUFA intake using USDA

Food	Daily grams of intake*	ω-3 (per 100g)**				ω-6 (per 100g)**	
		ALA	**EPA**	**DHA**	**DPA**	**LA**	**AA**
Tuna	200	na	0.363	1.141	0.160	0.068	0.055
		na	0.726†	2.282	0.320	0.136	0.110
Total ω-3 intake		= 0.726 + 2.282 + 0.320 = 3.328					
Total ω-6 intake						= 0.136 + 0.110 = 0.246	

Note:

*Generated from participant responses to modified Block FFQ

** Available from USDA National Nutrient Database for Standard Reference, Release 23

† 200 grams of tuna * (0.363 grams of EPA per 100 grams of tuna) = 0.726 grams of EPA

Table 2.2 Summary of putatively functional SNPs and LIBCSP results

Gene [Ref.]	rs#	Function*	Allele substitution	Main Effects OR (95% CI)	Interaction Effects with Diet OR (95% CI)
Inflammation					
PTGS-2 [65]	rs20417	TFBS	G > C	GC/CC genotype: 0.9 (0.8, 1.1)	GC/CC genotype & ever aspirin use: 0.8 (0.6, 1.1)
	rs5275	miRNA	T > C	TC/CC genotype: 0.9 (0.8, 1.1)	TC/CC genotype & ever aspirin use: 0.7 (0.5, 0.9)
PPAR-α [368]	rs1800206	splicing nsSNP 3D	C > G	None	G variant allele & aspirin use: ICR = 0.16 (-0.59, 0.91)
FAS [367]	rs2234767	TFBS splicing	G > A	None	None
	rs1800682	TFBS	G > A	AA genotype: 1.13 (0.89, 1.43)	None
FASL [367]	rs763110	TFBS	C > T	None	None
TNF-α	rs1800629	TFBS	G > A	None	None
Oxidative Stress					
MnSOD [365]	rs4880	splicing nsSNP	C > T	None	0.63 (0.41, 0.95) variant T allele & high total fruit/vegetable
MPO [362]	rs2333227	TFBS	G > A	AA genotype: 0.83 (0.58, 1.19)	0.75 (0.58, 0.97) AA genotype & high total fruit/vegetable
CAT [363]	rs1001179	TFBS	C > T	CC genotype: 0.77 (0.49, 1.22)	0.69 (0.53, 0.90) CC genotype & high total fruit/vegetable
GPX1 [364]	rs1050450	splicing miRNA nsSNP polyphen	C > T	TT genotype: 1.06 (0.79, 1.42)	0.73 (0.53, 1.01) CC genotype & high total fruit/vegetable

*Defined as functional in HapMap CEU population using SNPinfo web server; Abbreviations: transcription factor binding site prediction (TFBS), non-synonymous single nucleotide polymorphism (nsSNP), micro RNA binding (miRNA), splicing regulation (splicing), polyphen prediction (polyphen), 3D conformation (3D)

Table 2.2 (cont.) Summary of putatively functional SNPs and LIBCSP results

Gene [Ref.]	rs#	Function*	Allele substitution	Main Effects OR (95% CI)	Interaction Effects with Diet OR (95% CI)
Oxidative Stress					
GSTM1 [366]	deletion	deletion	deletion	None	0.68 (0.45, 1.03) GSMT1 present & high cruciferous vegetable intake among postmenopausal women
GSTP1 [366]	rs1695	splicing nsSNP	A > G	None	0.89 (0.64, 1.25) Variant G allele & high cruciferous vegetable
GSTT1 [366]	deletion	deletion	deletion	None	0.74 (0.53, 1.05) GSTT1 present & high cruciferous vegetable intake among postmenopausal women
GSTA1 [372]	rs3957356	TFBS	G > A	AA genotype: 1.20 (0.94, 1.54)	0.83 (0.51, 1.37) AA genotype & high total vegetable intake
COMT [371]	rs4680	splicing nsSNP 3D	G > A	AA genotype: 0.88 (0.69, 1.12)	None
	rs737865	TFBS	C > T	CC genotype: Premenopausal: 0.84 (0.49, 1.45)	None
Estrogen Metabolism					
CYP17 [369]	rs743572	TFBS	T > C	CC genotype: Premenopausal: 0.81 (0.52, 1.25) Postmenopausal: 1.25 (0.90, 1.73)	None

*Defined as functional in HapMap CEU population using SNPinfo web server; Abbreviations: transcription factor binding site prediction (TFBS), non-synonymous single nucleotide polymorphism (nsSNP), micro RNA binding (miRNA), splicing regulation (splicing), polyphen prediction (polyphen), 3D conformation (3D)

Figure 2.1 DAG of potential confounders of the PUFA/Fish intake and breast cancer incidence association

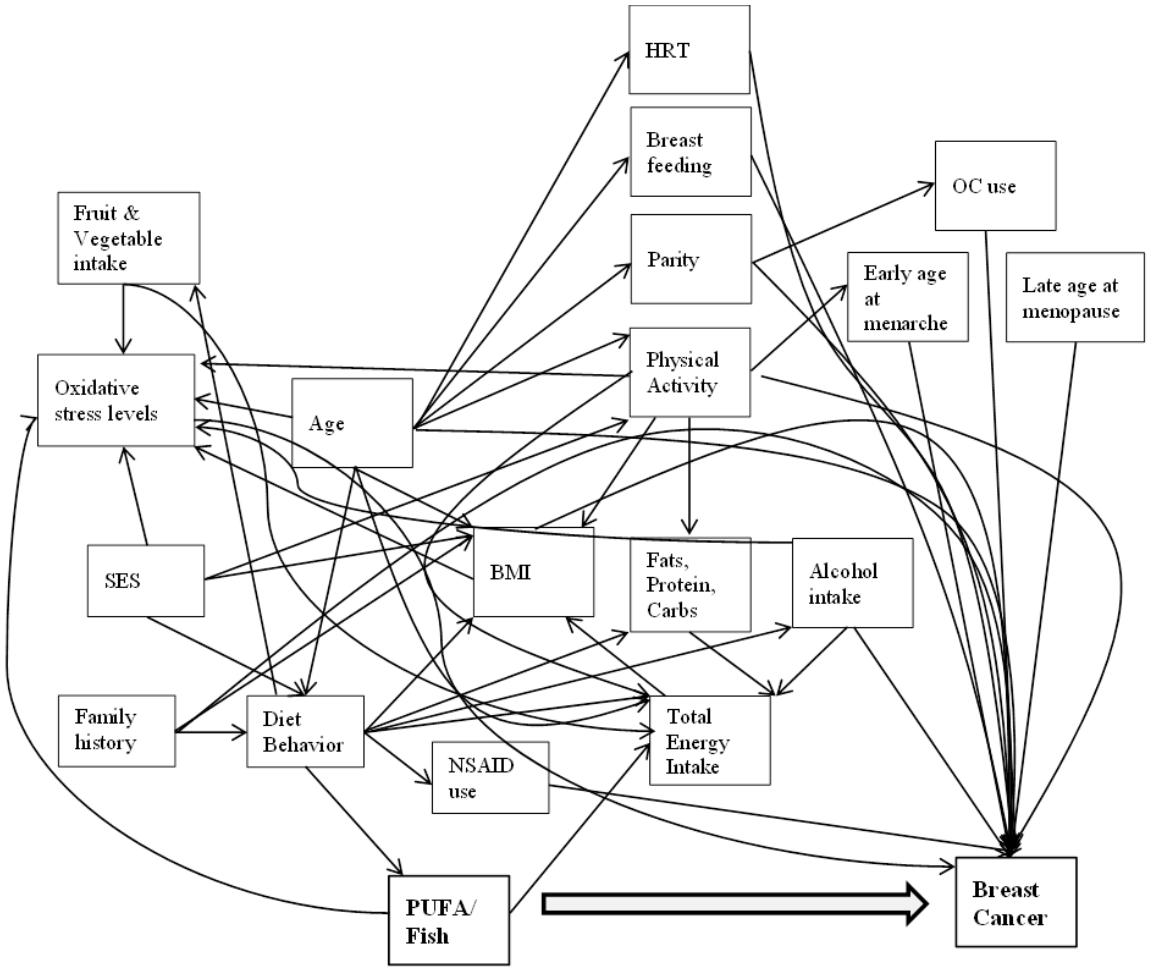
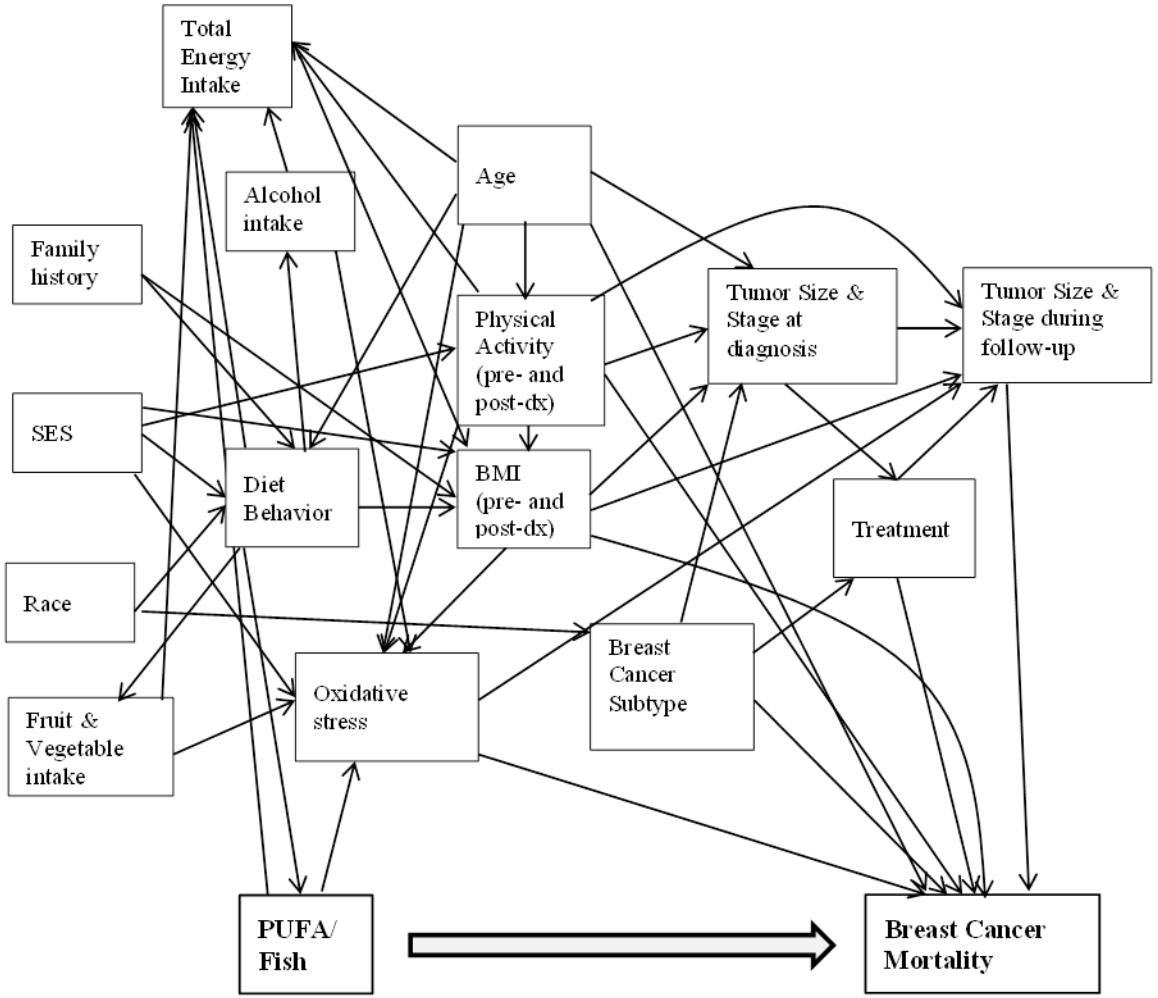


Figure 2.2 DAG of potential confounders of the PUFA/Fish intake and breast cancer mortality association



CHAPTER 3:
INTERACTION BETWEEN PUFAs, GENETIC POLYMORPHISMS, AND BREAST CANCER RISK: A POPULATION-BASED, CASE-CONTROL STUDY ON LONG ISLAND, NEW YORK

3.1 Introduction

Breast cancer incidence rates are almost two and a half times higher in the United States (U.S.) and European countries than in Asian countries, such as China or Japan [2, 390]. Migration studies conducted among Asian immigrants have shown that the breast cancer incidence patterns begin to reach those of Western countries a few generations after migration [4-7], suggesting that environmental factors may play a role in the geographic variation in incidence rates observed in Asian and Western countries.

One potential environmental exposure is polyunsaturated fatty acids (PUFAs), of which there are two primary classes, ω-3 and ω-6 fatty acids. Laboratory studies show that ω-3 PUFAs competitively inhibit ω-6 fatty acids, thus lowering levels of inflammatory eicosanoids resulting from ω-6 metabolism [155]. Asian populations have a substantially higher ratio of ω-3/ω-6 intake compared to European and U.S. populations [153, 154]. Fish is a major source of long-chain ω-3 PUFAs and is commonly consumed among Asian populations [146, 391, 392], which may partially explain the lower risk seen in these populations.

Experimental evidence suggests that higher ω-3 relative to ω-6 could reduce breast cancer through mechanisms related to inflammation, oxidative stress, and estrogen metabolism [155, 199, 202, 256]. Despite this biologic plausibility, previous epidemiologic

studies that have examined the association between PUFAs and the occurrence of breast cancer remain inconsistent among U.S. and European populations [80, 85, 99, 104, 269, 270, 278-281, 285-288, 292-298]. However, in Asian studies, consistent risk reductions have been observed for long-chain ω-3 PUFAs and breast cancer incidence [100, 277, 289, 290]. One potential reason for this observed inconsistency could be related to low fish intake. The biologic influence of PUFAs is unlikely to differ across populations; however, low fish intake in Western countries may mask important risk reductions. Also, Western populations consume higher levels of ω-6 PUFAs than Asian populations [152]. Thus, we hypothesized that consideration of both ω-3 and ω-6 intake, as an interaction or as the relative balance (ω-3/ω-6 ratio), may help to clarify the potential benefit of ω-3 intake among populations with low fish intake.

PUFAs affect carcinogenesis via multiple biologic pathways [155, 199, 202, 256], thus, it may also be important to examine interaction with biologically relevant genetic polymorphisms in relation to breast cancer. However, only two previous studies [287, 342] examined interactions between ω-3 or ω-6 intake and genetic polymorphisms involved in PUFA metabolism, and only the glutathione *S*-transferase (GST) [342] or lipoxygenase enzymes [287] were examined. Given the multitude of biologic pathways through which PUFAs could potentially affect carcinogenesis, we hypothesized that examination of the potential interaction with genetic polymorphisms involved in inflammation, as well as oxidative stress, and estrogen metabolism pathways, may help to further clarify the impact of PUFA intake on breast cancer development.

In the study reported here, we examined the interaction between dietary ω-3 and ω-6 PUFA classes, and the interactions between the ω-3/ω-6 ratio and genetic polymorphisms in

three related pathways, in association with breast cancer risk among women on Long Island, New York (NY).

3.2 Materials and Methods

This study utilizes the population-based case-control component of the Long Island Breast Cancer Study Project (LIBCSP). Details of the parent study have been published previously [355]. Institutional Review Board approval was obtained from all participating institutions.

Study population. Cases and controls were English-speaking residents of Long Island, NY (Nassau and Suffolk counties). Cases were adult women newly diagnosed with a first primary in situ or invasive breast cancer between August 1, 1996 and July 31, 1997, and were identified using a "super-rapid" network where study personnel contacted (either 2-3 times per week or daily) hospital pathology departments. Controls were identified using Waksberg's method of random digit dialing [393] for women under 65 years of age, and the Health Care Finance Administration rosters for women 65 years and older. Controls were frequency matched to the expected age-distribution of the case women. There were no upper age or race restrictions for subject eligibility.

The parent LIBCSP respondents included 1,508 cases and 1,556 controls. Respondents ranged in age from 20 to 98 years of age, 67% were postmenopausal, and the majority self-reported their race as white (94%), followed by black or African American (4%), or other (2%), which is consistent with the racial population distribution of these two counties at the time of data collection [355].

Assessment of PUFAs and other covariates. All LIBCSP participants were

administered a main questionnaire by a trained interviewer about 3 months after diagnosis for cases and 5.5 months after identification for controls. The questionnaire asked about demographic characteristics, pregnancy history, menstrual history, hormone use, medical history, family history of cancer, body size changes, alcohol use, active and passive cigarette smoking, physical activity, occupational history, and other potential risk factors for breast cancer [355]. LIBCSP researchers have previously reported that breast cancer risk in this population is associated with known reproductive risk factors (increasing age at first birth, few or no children, little or no breastfeeding, late age at menarche) [21], and lifestyle risk factors (increasing alcohol intake and, for postmenopausal breast cancer, decreased physical activity and increased body size) [52, 394].

Approximately 98% of participants (1,479 cases and 1,520 controls) also completed the validated [359, 395, 396] self-administered 101-item modified Block food frequency questionnaire (FFQ). Participants with implausible total energy intake (± 3 standard deviations from the mean) were excluded (n = 36). Thus, 1,463 cases and 1,500 controls were included in our examination of the association between PUFA intake and breast cancer risk.

We estimated PUFA intake by linking responses from the FFQ (i.e., grams per day for each line item) with nutrient values available in the U.S. Department of Agriculture databases for ω-3 and ω-6 PUFAs [397]. The following PUFAs were estimated: (1) ω-3 fatty acids, including alpha-linolenic acid (ALA), docosapentanoic acid (DPA), docosahexanoic acid (DHA), eicosapentanoic acid (EPA); and (2) ω-6 fatty acids, including linoleic acid (LA) and arachidonic acid (AA). An estimate of total PUFA intake was calculated by combining all individual fatty acids. Additionally, an estimate of total ω-3 and ω-6 fatty

acids was obtained by summing each individual fatty acid within category (e.g., total ω-3 = ALA + DPA + DHA + EPA).

Fish and/or seafood intakes were assessed according to the following items recorded in the FFQ: (1) tuna, tuna salad, tuna casserole; (2) shell fish (shrimp, lobster, crab, oysters, etc.); and (3) other fish (either broiled/baked).

Genotyping. Eighteen polymorphisms (in fifteen genes) were selected for this analysis spanning three biologically plausible pathways for PUFA metabolism, including inflammation, oxidative stress, and estrogen metabolism pathways. Variants affecting polyphen prediction (*GPX1*), transcription factor binding prediction (*PTGS-2* rs20417, *FAS*, *FASL*, *TNF-α*, *MPO*, *CAT*, *GSTA1*, *COMT* rs737865, *CYP17*), miRNA binding (*PTGS-2* rs5275, *GPX1*), 3D conformation (*PPAR-α*, *COMT* rs4680), or splicing regulation (*PPAR-α*, *FAS* rs2234767, *GPX1*, *GSTP1*, *COMT* rs4680) were considered as putatively functional variants as defined in the NIEHS SNPInfo WebServer [361].

Blood samples collected from subjects at the time of the case-control interview were used as the source of DNA for the genotyping. Genotyping methods have been previously described [65, 366-370, 398]. Briefly, DNA was isolated from mononuclear cells in whole blood which was separated by Ficoll (Sigma Chemical Co., St. Louis, Missouri) in the laboratory of Dr. Regina Santella at Columbia University using standard phenol and chloroform-isoamyl alcohol extraction and RNase treatment [398]. Genotyping for inflammation genes (*PTGS-2*, *FAS*, *FASL*, *PPAR-α*, *TNF-α*), used the following assays: Taqman 5'-Nuclease Assay (Applied Biosystems, Foster City, CA) and AcycloPrime^{TM}-FP SNP Detection Kit obtained from Perkin Elmer Life Sciences (Boston, Massachusetts, USA) [65, 367, 368]. The same assay was used for aromatase gene (*CYP17*) with a 10 μM probe

[369, 370]. For oxidative stress genes (*CAT*, *MPO*, *MnSOD*, *GPX*, *GSTA1*, *GSTP1*, *COMT*), BioServe Biotechnologies (Laurel, Maryland) performed the genotyping using high-throughput, matrix assisted, laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of Sequenom, Inc. (San Diego, California). Gene deletions for *GSTM1* and *GSTT1* were determined by a multiplex polymerase chain reaction method, with the constitutively present gene β-globulin as an internal positive control [366].

Data were missing for some genetic polymorphisms, primarily due to laboratory failures. Thus, the final sample sizes for our examination of gene-environment interactions are *PTGS-2* rs20417 and rs5275 (n = 2,106), *PPAR-α* rs1800206 (n = 1,815), *FAS* rs2234767 (n = 2,106), *FAS* rs1800682 (n = 2,095) *FASL* rs763110 (n = 2,110), *TNF-α* rs1800629 (n = 2,088), *MnSOD* rs4880 (n = 2,063), *MPO* rs2333227 (n = 2,078), CAT rs1001179 (n = 2,068), *GPX1* rs1050450 (n = 2,074), *GSTM1* deletion (n = 1,925), *GSTP1* rs1695 (n = 2,040), *GSTT1* deletion (n = 1,946), *GSTA1* rs3957356 (n = 2,075), *COMT* rs4680 (n = 2,084), *COMT* rs737865 (n = 2,064), and *CYP17* rs743572 (n = 2,044).

Tests for Hardy-Weinberg equilibrium (HWE) among the controls were conducted. Only *PTGS-2* rs20417 and *MPO* polymorphisms deviated significantly from HWE ($p < 0.05$). However, the observer agreement in 8% of the randomly selected was high (*PTGS-2* rs20417 kappa statistic = 0.99, *MPO* kappa statistic = 0.91), and the failure rate of the assay was less than 1% for both polymorphisms. Also, the genotype frequencies for both *PTGS-2* rs20417 and *MPO* polymorphisms were reported to be similar to those observed in other studies [362, 399].

Statistical analyses. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC). Unconditional logistic regression was used to estimate odds ratios

(ORs) and 95% confidence intervals (95% CI) for the association between PUFA intake and breast cancer risk. All PUFA intake estimates (i.e., total PUFA, total ω-3, ALA, DPA, DHA, EPA, total ω-6, LA, AA, ratio of ω-3/ω-6) were categorized as quartiles, according to the distribution among controls. Quartiles were selected over other possible methods of categorization (e.g., tertiles, quintiles, linear, splines) because the shape of the dose-response between PUFAs and breast cancer risk was best captured using these cut-points. Similarly, fish intake was categorized using quartiles according to the distribution among those controls who reported consuming fish (i.e., tuna, shell fish, other fish); non-consumers of fish were considered the referent group. Tests for linear trend were not conducted, since the relation between any of the PUFA measures and breast cancer risk was not strictly monotonic [373].

Interactions between total ω-3 and total ω-6 intake, and between the ω-3/ω-6 ratio and the eighteen genetic polymorphisms, in association with breast cancer risk were assessed on the additive (common referent) and multiplicative scales. Additive interaction was evaluated using relative excess risk due to interaction (RERI), with 95% CI estimated using the Hosmer and Lemeshow method [374]. Multiplicative interactions were evaluated by comparing nested models using the Likelihood Ratio Test (LRT) [373]. Total ω-3, total ω-6, and ratio of ω-3/ω-6 were dichotomized at the median for use in the interaction models. Similarly, in order to maximize cell sample sizes, genotypes were dichotomized according to a dominant model and categorized into "high" and "low" risk groups based upon the function of the variant allele, which was determined using the existing literature (see Supplemental **Table 3.6**) [219, 399-412].

We also considered effect modification of the association between PUFA intake and breast cancer risk by: menopausal status (post- vs. pre-menopausal status); and dietary

supplement use (yes/no). However, little or no heterogeneity was observed with either of these covariates, and thus the results are not shown. We also considered potential heterogeneity across breast cancer subtypes, defined by hormone receptor status (any hormone receptor positive breast cancer vs. no hormone receptor positive breast cancer), by constructing polytomous regression models; however, no differences in the association with PUFA intake were observed across hormone receptor subtype, and thus the results are not shown.

All models were adjusted for the frequency matching factor five-year age group. Other potential confounders (including total energy intake, non-steroidal anti-inflammatory drugs (NSAID), family history of breast cancer, income, body mass index, alcohol use, fruit and vegetable intake, and physical activity) were identified using directed acyclic graph (DAG) [373]. The only covariates that changed the estimates by more than 10% were total energy intake for PUFA intake, and energy intake and NSAID use for fish intake. It is possible that chronic NSAID users experience gastrointestinal problems (e.g., stomach ulcers, reflux) which may subsequently influence diet, including fish consumption [413]. Thus, all PUFA models were adjusted for age and energy intake, and all fish intake models included age, energy intake and NSAID use.

3.3 Results

As presented in **Table 3.1,** the average intake of total ω-3 fatty acids (1.01 grams per day (SD = 0.74)) was lower relative to ω-6 intake [7.66 grams per day (SD = 5.68)] among the 1,500 control women in this population-based sample of Long Island residents without breast cancer. The highest contributor to total ω-3 intake was ALA with an average intake of

0.86 grams per day (SD = 0.71), whereas LA was the highest contributor to total ω-6 intake with an average intake of 7.59 grams per day (SD = 5.66). Tuna intake was reported at higher levels [11.92 grams per day (SD = 15.09)] in our control population compared to shell fish intake [7.28 gram per day (SD = 11.88)].

As also shown in **Table 3.1,** fish was a large contributor to high intake of long-chain ω-3 PUFAs, including DPA, DHA, and EPA. In contrast, foods that contributed to high ALA intake were biscuits/muffins and other fried foods, which was similar to what was observed for LA intake. High AA intake appeared to be predominantly driven by eggs and meats, including fish, chicken, and ham.

As presented in **Table 3.2**, the age-adjusted and multivariate-adjusted odds ratios for the associations between all measures of PUFA intake and breast cancer risk were imprecise. For example, elevated odds ratios were observed for high intake of total PUFA, total ω-3, ALA, ω-6, and LA intakes, but CIs were wide. No associations were observed for the long-chain ω-3 PUFA (DPA, DHA, EPA), or AA intake. Similarly, no associations were observed for high intakes of the ratio of ω-3/ω-6 intake and breast cancer risk.

As shown in **Table 3.3,** higher intake of tuna, shell fish, or other fish (broiled/baked) was not associated with breast cancer risk in our study.

As shown in **Table 3.4**, we observed an interaction between ω-3 and ω-6 intake, which was statistically significant on the additive scale [multivariate-adjusted RERI = 0.41 (95% CI = 0.06, 0.76)]. The risk reductions for breast cancer were modest for women who consumed low levels of both ω-3 and ω-6 [multivariate-adjusted OR = 0.83 (95% CI = 0.63, 1.09)], compared to women who consumed high ω-3 and low ω-6. For women who consumed high levels of ω-3 and ω-6, the odds ratios were close to the null value

[multivariate-adjusted OR = 0.95 (95% CI = 0.72, 1.26)]. In contrast, higher intakes of ω-6 fatty acids in conjunction with lower intake of ω-3 fatty acids was associated with an approximately 20% increased risk of breast cancer [multivariate-adjusted OR = 1.20 (95% CI = 0.85, 1.69)]. The increased risk observed for this group was super-additive (41% greater) compared to the 22% (= 5% + 17%) expected risk reduction, derived from the individual ORs for those consuming either high levels (≥ median; 5% risk reduction), or low levels (< median; 17% risk reduction) of both ω-3 and ω-6 fatty acids. Similar results were observed when we considered interactions between ω-3 and ω-6 on a multiplicative scale (see Supplemental **Table 3.7**).

As presented in **Table 3.5**, when we explored interactions between the ratio of ω-3/ω-6 intake and genetic polymorphisms in the inflammatory, oxidative stress, and estrogen metabolism pathway, we found little evidence to support an additive interaction for most of the genes considered. The strongest and most precise risk estimate for the hypothesized highest risk group (low ω-3/ω-6 intake and high risk genotype) was observed for *PTGS-2* rs5275 [OR = 1.29 (95% CI = 1.00, 1.66)] in comparison to the hypothesized lowest risk group (high intake of ω-3/ω-6 and low risk genotype). We observed similar, but less precise, elevations for the corresponding interactions with *GSTP1* [OR = 1.23 (95% CI = 0.86, 1.57)], and *COMT* rs737865 [OR = 1.35 (95% CI = 0.87, 2.11)]. For the majority of interactions we examined, breast cancer risk remained elevated for low intake of ω-3/ω-6 ratio regardless of genotype, with the exceptions of the *FASL* rs763110 and *COMT* rs4680 genes. Similar results were observed when we considered interactions between the ω-3/ω-6 ratio and polymorphisms on a multiplicative scale (see Supplemental **Table 3.8**).

3.4 Discussion

We are the first to report an additive interaction between ω-3 intake and ω-6 intake in relation to breast cancer risk in a population-based sample of U.S women. Specifically, we observed a 20% increase in the odds of breast cancer among consumers of high levels of ω-6 and low levels of ω-3 compared to those who consumed low levels of ω-6 and high levels of ω-3. The odds ratio for women consuming high ω-6 and low ω-3 was increased, whereas the corresponding estimates for intake of either high levels or low levels, of either PUFA class, were reduced. This interaction underscores the importance of considering intake of ω-3 and ω-6 simultaneously when examining associations with breast cancer in the U.S.

No previous studies in the U.S. have examined the potential interaction between ω-3 and ω-6 PUFAs and breast cancer risk, and only two have examined the ratio of ω-3 and ω-6 intakes [270, 286]. Consideration of an interaction may be preferable, given that a ratio measure permits only one type of relation between two exposures, whereas an interaction is more flexible. One previous study has reported on the interaction between ω-3 and ω-6 intake on breast cancer risk among women in Shanghai [290]; the investigators observed a significant two-fold increased risk for high ω-6 in combination with low marine-derived ω-3 intake. The LIBCSP results presented here for the interaction between ω-3 and ω-6 intake are in the same direction as those reported in the Shanghai study, but less pronounced. Importantly, daily fish consumption in the Shanghai population was almost five times greater than the frequency reported among our population-based sample of Long Island residents, which could partially explain the weaker association observed in our study.

The slight positive association between ω-6 PUFA (total ω-6, LA, AA) and breast cancer risk observed here is similar to associations reported in other studies conducted

among Western populations [270, 280, 289, 290, 297]. However, our findings for the association between long-chain ω-3 (DPA, DHA, EPA) PUFAs are not consistent with previous studies conducted among Asian or some European populations [100, 277, 289, 290, 294], where risk reductions were reported. Additionally, the increased risk for ALA intake we observed is inconsistent with the laboratory evidence for inhibition of breast cancer growth [238, 240]. However, in other epidemiologic studies, the association between ALA intake and breast cancer risk remains unclear, with some studies reporting increased risks [269, 283, 290, 294, 295], and others reporting risk reductions [292, 296]. The variation in results across studies may be associated with the different dietary assessment methods used, consumption of different food sources of ALA (e.g., biscuits/muffins and fried foods were major contributors in our population), or with potential recall bias that can occur in case-control studies. It is also possible that ALA reduces breast cancer growth only after conversion to long-chain ω-3 PUFAs. The *in vivo* conversion of ALA to long-chain ω-3 PUFAs is inefficient in the presence of high ω-6 [150]. Thus, it is possible that in populations with high ω-6 intake, benefits of ALA intake are less evident.

The slight breast cancer risk reductions we observed in relation to a high ω-3/ω-6 ratio in our study were modest compared to the estimates reported in several other studies in European [85], Mexican [284], and U.S. populations [270, 286]. However, this may be a reflection of the relatively low intake of ω-3 and ω-6 in our study population. Very low intake of both ω-3 and ω-6 PUFAs could result in a high ratio value for ω-3/ω-6 intake. Thus, a high ratio of ω-3/ω-6 derived from low intakes of ω-3 and ω-6 PUFAs may not represent a sufficient dose for ω-3 to exert a beneficial response *in vivo*. In the U.S., only one previous population-based study has considered the PUFA ratio in association with

breast cancer risk; utilizing data from the Vitamins and Lifestyle Cohort (VITAL) [270], a 16% risk reduction was observed in association with high ω-3/ω-6 intake ratio. This Western Washington-based study included marine-derived ω-3 intake from both dietary sources and supplements, and thus levels of ω-3 intake were higher than the dietary-derived intake estimates observed in our Long Island-based study. Nonetheless, given the weak results reported for the PUFA ratio in both our study and the Western Washington study, examining the interaction between ω-3 and ω-6 intake, rather than the ratio, may be a more favorable strategy in populations where PUFA intake is relatively low.

We are the first study to examine PUFA-gene interactions when considering multiple genes in several biologically plausible pathways, including inflammation. Nonetheless, we found little evidence for an interaction between PUFA intake and the eighteen genetic polymorphisms in the three pathways considered, despite the biologic plausibility for these interactions to impact breast cancer development. Instead, for all of the interactions with genetic polymorphisms, more pronounced associations were observed for those consuming low ratio of ω-3/ω-6, regardless of genotype.

Previously, only two epidemiologic studies have considered potential interactions between PUFA intake, genetic polymorphisms, and breast cancer [287, 342]. Given the examination of PUFA-gene interactions in previous studies is limited to only one or two exposures and one or two genetic polymorphisms, comparison of our results to previous studies is challenging. Additionally, both of these previous studies focused only on one PUFA exposure (either marine-derived ω-3 or LA) in isolation, without concurrently considering ω-6 PUFAs. Considering ω-3 and ω-6 PUFA simultaneously is likely to better reflect the complexity of this dietary exposure, given the competitive inhibition of ω-3 and

ω-6 in PUFA metabolism.

The strengths of our population-based, case-control study include the examination of the interaction between ω-3 and ω-6 on breast cancer development, which has not been previously assessed in a U.S. population. Additionally, we also examined associations with fish intake (a dietary source rich in ω-3 fatty acids) among this sample of women who reside in a geographic area that is surrounded by water, and for whom the variability of fish intake would presumably be greater than for others who reside in more land-locked areas of the U.S [391]. In fact, women living in New York City [414] have been reported to consume fish greater than the national estimates from NHANES [415], which our study corroborates. Finally, we are the first to examine PUFA-gene interactions with consideration given to genetic polymorphisms spanning multiple biological pathways relevant to PUFA metabolism.

This study also has limitations that should be considered. The LIBCSP study population includes predominantly Caucasian women, which reflects the racial distribution of the residents of the two source counties on Long Island, NY. Consequently, examination of racial differences in our study was not possible. Our results are therefore generalizable to only Caucasian-American women, for whom breast cancer risk remains high [1]. Though we examined PUFA interactions with multiple polymorphisms spanning several biologic pathways, our selected genes are not exhaustive. For studies with larger sample sizes, it may be beneficial to examine additional genetic polymorphisms involved in relevant pathways, such as genes involved in the *in vivo* metabolism of ω-3 and ω-6. Humans do not possess the enzymes necessary to desaturate LA to ALA, thus conversion from ω-6 to ω-3 is impossible [148]. However, through a series of desaturations and elongations, formation of long-chain

ω-3 PUFAs from ALA, and AA from LA, is possible *in vivo*. A recent dietary intervention, reported increased levels of EPA, DHA, and DPA, among women who lowered their dietary LA intake, suggesting increased efficiency in the conversion of ALA to long-chain ω-3 PUFAs *in vivo* [416] among this subgroup. Therefore, consideration of polymorphisms in genes involved in this conversion, in combination with PUFA intake, may be helpful for understanding the bioavailability of different PUFA subtypes *in vivo*.

Future studies may also be warranted to evaluate the timing of exposure relative to breast cancer development. FFQ responses are assumed to reflect usual adult diet [271], although recent changes due to a disease diagnosis or treatment regimens could influence those responses. The LIBCSP questionnaires were administered within months of diagnosis, and for two-thirds of women this was prior to the onset of chemotherapy [355], which is likely to reduce the impact of dietary changes and perhaps recall of diet on the FFQ. Estimating PUFA intake via FFQ linkage with the USDA databases, however, could result in measurement error. For example, it is possible that the PUFA content measured in the foods reported in the USDA database [397] differ from those actually consumed by LIBCSP participants due to differences in harvesting, storage, processing, and cooking methods [147, 417, 418]. Additionally, we were unable to assess the relation between breast cancer and consumption of different fish varieties in this study. This is important given the amount of long-chain ω-3 content found in fish differs by species [419]. However, we assessed tuna intake, which is the most frequently consumed fish variety in the U.S. and is also a major source of dietary ω-3s [391]. Biomarkers could provide an objective measure of PUFA intake. However, PUFA biomarkers may reflect different time periods of exposure, ranging from a few days to one year (depending on the type of biomarker used) [273]. Therefore, use

of the PUFA biomarker measurements in a case-control study may not reflect the etiologically relevant time period for breast cancer development.

In conclusion, we found that among a population-based sample of Long Island residents, women who consume high levels of ω-6 and low levels of ω-3 had an increased risk for breast cancer, compared to women who consume low levels of ω-6 and high levels of ω-3. For interactions with genetic polymorphisms, increased risks were observed for those consuming a low ratio of ω-3/ω-6, regardless of genotype. Our results suggest that high intake of ω-3 PUFA, coupled with low intake of ω-6, may be a potential risk reduction strategy for breast cancer among U.S. women.

Table 3.1 Characteristics of polyunsaturated fatty acid intake (PUFA) and fish intake among the population-based sample of control women (N=1500) in the LIBCSP, 1996-1997

Nutrient/Food	Mean	SD	25th Pct	50th Pct	75th Pct	Major PUFA-rich foods contributing to high nutrient intake in the LIBCSP
Nutrient (g/day)						
Total PUFA[a]	8.67	6.31	4.21	7.27	11.25	Butter, Mayonnaise/salad dressings, safflower/corn oil, margarine, peanuts/peanut butter
Total ω-3[b]	1.01	0.74	0.49	0.83	1.30	Biscuits/muffins, butter, mayonnaise/salad dressings, fish, safflower/corn oil
ALA	0.86	0.71	0.35	0.68	1.14	Biscuits/muffins, French fries/fried potatoes, butter, cookies, mayonnaise/salad dressings
DPA	0.01	0.01	0.01	0.01	0.02	Tuna, fish, chicken, shellfish, beef
DHA	0.09	0.09	0.03	0.06	0.12	Tuna, fish, eggs, shellfish, chicken
EPA	0.04	0.05	0.01	0.03	0.06	Fish, tuna, shellfish, chicken
Total ω-6[c]	7.66	5.68	3.68	6.31	10.10	Biscuits/muffins, French fries/fried potatoes, butter, chips/popcorn, mayonnaise/salad dressings
LA	7.59	5.66	3.65	6.23	9.99	Biscuits/muffins, French fries/fried potatoes, butter, chips/popcorn, mayonnaise/salad dressings
AA	0.07	0.06	0.04	0.06	0.09	Eggs, Fish, chicken, ham/lunch meats, shellfish
ω-3/ω-6	0.15	0.14	0.10	0.14	0.17	N/A
Fish (g/day)[d]						
Tuna	11.92	15.09	4.77	7.85	12.40	N/A
Shell fish	7.28	11.88	2.00	4.62	9.23	N/A
Other (broiled/baked)	11.98	11.35	4.67	6.46	16.80	N/A

Note:
[a] Total PUFA = ALA + DPA + DHA + EPA + LA + AA
[b] Total ω-3 = ALA + DPA + DHA + EPA
[c] Total ω-6 = LA + AA
[d] Controls with null values for tuna (N=393), shell fish (N=765), and other (N=592) are included in calculations.
LIBCSP = Long Island Breast Cancer Study Project
SD = standard deviation
N/A = not applicable

Table 3.2 Age- and multivariate[a]-adjusted ORs and 95% CI for the association between dietary PUFA intake and the risk of breast cancer in the LIBCSP, 1996-1997

PUFA	Covariates	Q1			Q2				Q3				Q4			
		Co	Ca	OR	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI
Total PUFA	Age	375	342	1.00	375	392	1.19	0.97,1.46	375	386	1.19	0.97,1.46	375	343	1.08	0.87,1.33
	Multivariate			1.00			1.23	1.00,1.52			1.27	1.02,1.59			1.25	0.95,1.63
Total ω-3	Age	375	340	1.00	375	403	1.20	0.98,1.47	375	377	1.15	0.93,1.41	375	343	1.05	0.85,1.29
	Multivariate			1.00			1.25	1.01,1.54			1.23	0.98,1.54			1.20	0.92,1.58
ALA	Age	375	335	1.00	375	390	1.18	0.96,1.45	375	389	1.21	0.98,1.48	375	349	1.09	0.88,1.34
	Multivariate			1.00			1.23	0.99,1.51			1.29	1.04,1.61			1.25	0.96,1.62
DPA	Age	375	365	1.00	375	354	0.97	0.79,1.20	375	375	1.05	0.85,1.28	375	369	1.04	0.84,1.27
	Multivariate			1.00			0.99	0.81,1.22			1.08	0.88,1.33			1.09	0.88,1.36
DHA	Age	375	372	1.00	375	336	0.90	0.74,1.11	375	369	1.00	0.82,1.23	375	386	1.03	0.84,1.26
	Multivariate			1.00			0.91	0.74,1.13			1.02	0.83,1.26			1.06	0.86,1.31
EPA	Age	375	350	1.00	375	359	1.01	0.82,1.24	375	365	1.05	0.86,1.29	375	389	1.10	0.90,1.35
	Multivariate			1.00			1.02	0.83,1.25			1.08	0.88,1.33			1.14	0.92,1.40
Total ω-6	Age	375	347	1.00	375	374	1.11	0.91,1.37	375	405	1.23	1.00,1.51	375	337	1.04	0.84,1.28
	Multivariate			1.00			1.15	0.93,1.42			1.31	1.05,1.63			1.18	0.91,1.55
LA	Age	375	351	1.00	375	367	1.08	0.88,1.33	375	407	1.22	1.00,1.50	375	338	1.03	0.83,1.27
	Multivariate			1.00			1.12	0.91,1.38			1.30	1.05,1.62			1.18	0.90,1.54
AA	Age	375	371	1.00	375	378	1.03	0.84,1.26	375	367	1.00	0.81,1.22	375	347	0.97	0.79,1.19
	Multivariate			1.00			1.05	0.85,1.29			1.03	0.83,1.27			1.03	0.81,1.29
ω-3/ω-6	Age	375	360	1.00	375	384	1.08	0.88,1.32	375	346	0.94	0.76,1.15	375	373	0.99	0.80,1.21
	Multivariate			1.00			1.09	0.89,1.34			0.95	0.77,1.17			0.99	0.80,1.21

Note:
Co=Controls, Ca=Cases, LIBCSP=Long Island Breast Cancer Study Project
[a] Multivariate-adjusted ORs and 95% CI adjusted for matching factor (5-year age group) and total energy intake (kcal/day)

Table 3.3 Age- and multivariate[a]-adjusted ORs and 95% CI for the association between fish intake and the risk of breast cancer in the LIBCSP, 1996-1997

PUFA	Covariates	Never			Q1				Q2				Q3				Q4			
		Co	Ca	OR	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI
Tuna	Age			1.00			1.08	0.86,1.36			1.29	1.04,1.60			1.16	0.88,1.52			1.14	0.94,1.39
	Multivariate	393	343	1.00	256	241	1.08	0.85,1.37	285	320	1.24	0.99,1.55	141	141	1.12	0.84,1.49	425	418	1.15	0.93,1.42
Shell fish	Age			1.00			0.87	0.66,1.15			1.12	0.91,1.38			0.99	0.78,1.25			1.04	0.83,1.30
	Multivariate	765	750	1.00	126	102	0.78	0.58,1.05	227	245	1.10	0.89,1.38	178	169	0.99	0.78,1.26	204	197	1.09	0.86,1.38
Other fish	Age			1.00			1.32	1.07,1.64			1.40	1.03,1.91			1.15	0.95,1.39			1.18	0.96,1.46
(broiled/baked)	Multivariate	592	505	1.00	224	253	1.26	1.00,1.58	87	104	1.38	1.00,1.91	346	341	1.08	0.88,1.32	251	260	1.18	0.94,1.47

Note:
Co=Controls, Ca=Cases, LIBCSP = Long Island Breast Cancer Study Project
[a] Multivariate-adjusted ORs and 95% CI adjusted for matching factor (5-year age group), total energy intake (kcal/day), and NSAID use.

Table 3.4 Age- and multivariate[a]-adjusted ORs and 95% CI for the additive interaction between dietary ω-3 and ω-6 (high and low intake) and the risk of breast cancer in the LIBCSP, 1996-1997

Model	Low ω-6 (< median)			High ω-6 (≥ median)			RERI[b]	95% CI[c]
	N	OR	95% CI	N	OR	95% CI		
Age-adjusted								
High ω-3 (≥ median)	256	1.00		1,214	0.90	0.69, 1.19		
Low ω-3 (< median)	1,215	0.87	0.66, 1.14	278	1.21	0.86, 1.70	0.43	0.09, 0.78
Multivariate[a]								
High ω-3 (≥ median)	256	1.00		1,214	0.95	0.72, 1.26		
Low ω-3 (< median)	1,215	0.83	0.63, 1.09	278	1.20	0.85, 1.69	0.41	0.06, 0.76

Note:
Co=Controls, Ca=Cases, LIBCSP=Long Island Breast Cancer Study Project
[a] Multivariate ORs and 95% CI adjusted for matching factor (5-year age group) and total energy intake (kcal/day)
[b] RERI (Relative Excess Risk due to Interaction) = OR_{11} - OR_{10} - OR_{01} +1
[c] 95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table 3.5 Multivariate[a]-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the risk of breast cancer for the hypothesized highest risk additive interaction between ω-3/ω-6 ratio and putatively functional genetic polymorphisms[b] in the LIBCSP, 1996-1997

Gene	rs#	Allele Substitution	Variant allele function[g]	High risk genotype	High ω-3/ω-6 (≥ median)			Low ω-3/ω-6 (< median)				RERI[e]	95% CI[f]
					Low risk	High risk		Low risk		High risk			
					OR[c]	OR	95% CI	OR	95% CI	OR[d]	95% CI		
PTGS-2	rs20417	G > C	↓ Inf	GG	1.00	1.09	0.84, 1.41	1.26	0.95, 1.67	1.23	0.95, 1.59	-0.12	-0.54, 0.30
PTGS-2	rs5275	T > C	↓ Inf	TT	1.00	1.15	0.89, 1.47	1.23	0.98, 1.55	1.30	1.02, 1.66	-0.08	-0.49, 0.33
PPAR-α	rs1800206	C > G	↓ Inf	CC	1.00	0.77	0.51, 1.18	1.12	0.63, 1.98	0.96	0.63, 1.46	0.06	-0.55, 0.68
FAS	rs2234767	G > A	↓ Inf	GG	1.00	1.19	0.89, 1.58	1.57	1.09, 2.24	1.29	0.97, 1.71	-0.46	-1.06, 0.14
FAS	rs1800682	G > A	↑ Inf	GA/AA	1.00	1.22	0.91, 1.62	1.39	0.99, 1.94	1.39	1.04, 1.84	-0.22	-0.73, 0.28
FASL	rs763110	C > T	↓ Inf	CC	1.00	1.18	0.90, 1.53	1.35	1.09, 1.66	1.06	0.81, 1.38	-0.47	-0.92,-0.02
TNF-α	rs1800629	G > A	↑ Inf	GA/AA	1.00	0.96	0.73, 1.27	1.18	0.96, 1.44	1.12	0.85, 1.48	-0.02	-0.43, 0.40
MnSOD	rs4880	C > T	↓ Os	CT/TT	1.00	0.92	0.68, 1.23	1.07	0.75, 1.51	1.12	0.83, 1.50	0.14	-0.26, 0.53
MPO	rs2333227	G > A	↓ Os	GA/AA	1.00	0.87	0.68, 1.13	1.14	0.91, 1.43	1.08	0.83, 1.39	0.06	-0.30, 0.41
CAT	rs1001179	C > T	↑ Os	CC	1.00	0.80	0.62, 1.04	1.06	0.80, 1.41	1.00	0.77, 1.29	0.14	-0.20, 0.47
GPX1	rs1050450	C > T	↑ Os	CC	1.00	1.05	0.82, 1.35	1.34	1.05, 1.70	1.08	0.85, 1.37	-0.31	-0.73, 0.10
GSTM1	deletion	deletion	↑ Os	present	1.00	0.77	0.59, 1.00	0.99	0.76, 1.29	1.02	0.79, 1.32	0.26	-0.06, 0.58
GSTP1	rs1695	A > G	↑ Os	AA	1.00	1.03	0.80, 1.33	1.14	0.89, 1.46	1.23	0.96, 1.57	0.05	-0.33, 0.43
GSTT1	deletion	deletion	↑ Os	present	1.00	0.94	0.68, 1.28	1.02	0.69, 1.50	1.11	0.82, 1.52	0.16	-0.26, 0.57
GSTA1	rs3957356	G > A	↑ Os	GG	1.00	0.83	0.63, 1.08	1.10	0.89, 1.37	1.10	0.86, 1.42	0.17	-0.18, 0.53
COMT	rs4680	G > A	↑ Os	GG	1.00	1.37	1.02, 1.83	1.28	1.05, 1.57	1.20	0.92, 1.56	-0.45	-0.97, 0.07
COMT	rs737865	C > T	↓ Os	CT/TT	1.00	1.16	0.74, 1.81	1.34	0.77, 2.34	1.35	0.87, 2.11	-0.15	-0.87, 0.58
CYP17	rs743572	T > C	↑ Es	TC/CC	1.00	1.16	0.89, 1.50	1.34	1.00, 1.81	1.28	1.00, 1.65	-0.22	-0.68, 0.24

Note:
Inf = inflammation, Os = oxidative stress, Es = estrogen, LIBCSP = Long Island Breast Cancer Study Project
[a] All models adjusted for matching factor, 5-year age group, and total energy intake (kcal/day).
[b] Genotypes dichotomized using dominant genetic model.
[c] Hypothesized lowest risk group (referent group) - low risk genotype for PUFA-gene interaction, high ω-3/ω-6 ratio.
[d] Hypothesized highest risk group - high risk genotype for PUFA-gene interaction, low ω-3/ω-6 ratio.
[e] RERI (Relative Excess Risk Due to Interaction) = OR_{11} - OR_{10} - OR_{01} + OR_{00} (e.g., RERI for PTGS-2 rs20417 = 1.23 - 1.26 - 1.09 + 1.00 = -0.12).
[f] 95% CI for RERI estimated using Hosmer & Lemeshow [374]
[g] Variant allele function is based upon previous literature. Please refer to Table 3.6 for references.

Table 3.6 Assumed variant allele function based on previous literature

Gene	rs#	Allele Substitution	Variant allele function	Reference
PTGS-2	rs20417	G > C	↓ Inf	[399]
PTGS-2	rs5275	T > C	↓ Inf	[399]
PPAR-α	rs1800206	C > G	↓ Inf	[219]
FAS	rs2234767	G > A	↓ Inf	[400]
FAS	rs1800682	G > A	↑ Inf	[400]
FASL	rs763110	C > T	↓ Inf	[401]
TNF-α	rs1800629	G > A	↑ Inf	[402]
MnSOD	rs4880	C > T	↓ Os	[403]
MPO	rs2333227	G > A	↓ Os	[404]
CAT	rs1001179	C > T	↑ Os	[405]
GPX1	rs1050450	C > T	↑ Os	[406]
GSTM1	deletion	deletion	↑ Os	[407]
GSTP1	rs1695	A > G	↑ Os	[408]
GSTT1	deletion	deletion	↑ Os	[407]
GSTA1	rs3957356	G > A	↑ Os	[409]
COMT	rs4680	G > A	↑ Os	[412]
COMT	rs737865	C > T	↓ Os	[410]
CYP17	rs743572	T > C	↑ Es	[411]

Note:
Inf = inflammation, Os = oxidative stress, Es = estrogen

Table 3.7 Age- and multivariate[a]-adjusted ORs and 95% CI for the multiplicative interaction between dietary ω-3 and ω-6 (high and low intake) and the risk of breast cancer in the LIBCSP, 1996-1997

Model	Low ω-6 (< median)			High ω-6 (≥ median)			LRT $\chi 2^{b}$	p value
	N	OR	95% CI	N	OR	95% CI		
Age-adjusted								
High ω-3 (≥ median)	256	1.00		1,214	1.00			
Low ω-3 (< median)	1,215	0.87	0.66, 1.14	278	1.33	1.02, 1.73	5.01	0.03
Multivariate[a]								
High ω-3 (≥ median)	256	1.00		1,214	1.00			
Low ω-3 (< median)	1,215	0.83	0.63, 1.09	278	1.26	0.96, 1.65	4.61	0.03

Note:
Co=Controls, Ca=Cases, LIBCSP=Long Island Breast Cancer Study Project, LRT=likelihood ratio test
[a] Multivariate ORs and 95% CI adjusted for matching factor (5-year age group) and total energy intake (kcal/day)
[b] LRT $\chi 2$ calculated using nested models for the multiplicative interaction.

Table 3.8 Multivariate[a]-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for the risk of breast cancer for the hypothesized highest risk multiplicative interaction between ω-3/ω-6 ratio and putatively functional genetic polymorphisms[b] in the LIBCSP, 1996-1997

Gene	rs#	Allele Substitution	Variant allele function[d]	High risk genotype	High ω-3/ω-6 (≥ median)			Low ω-3/ω-6 (< median)			LRT $\chi 2^c$	p value
					Low risk	High risk		Low risk	High risk			
					OR	OR	95% CI	OR	OR	95% CI		
PTGS-2	rs20417	G > C	↓ Inf	GG	1.00	1.09	0.84, 1.41	1.00	0.98	0.77, 1.25	0.36	0.55
PTGS-2	rs5275	T > C	↓ Inf	TT	1.00	1.15	0.89, 1.47	1.00	1.05	0.83, 1.34	0.23	0.64
PPAR-α	rs1800206	C > G	↓ Inf	CC	1.00	0.77	0.51, 1.18	1.00	0.86	0.56, 1.32	0.11	0.74
FAS	rs2234767	G > A	↓ Inf	GG	1.00	1.19	0.89, 1.58	1.00	0.82	0.61, 1.11	3.03	0.08
FAS	rs1800682	G > A	↑ Inf	GA/AA	1.00	1.22	0.91, 1.62	1.00	1.00	0.77, 1.30	1.02	0.31
FASL	rs763110	C > T	↓ Inf	CC	1.00	1.18	0.90, 1.53	1.00	0.79	0.61, 1.02	4.63	0.03
TNF-α	rs1800629	G > A	↑ Inf	GA/AA	1.00	0.96	0.73, 1.27	1.00	0.95	0.72, 1.26	0.00	0.97
MnSOD	rs4880	C > T	↓ Os	CT/TT	1.00	0.92	0.68, 1.23	1.00	1.05	0.80, 1.38	0.42	0.51
MPO	rs2333227	G > A	↓ Os	GA/AA	1.00	0.87	0.68, 1.13	1.00	0.94	0.74, 1.21	0.17	0.68
CAT	rs1001179	C > T	↑ Os	CC	1.00	0.80	0.62, 1.04	1.00	0.94	0.73, 1.20	0.77	0.38
GPX1	rs1050450	C > T	↑ Os	CC	1.00	1.05	0.82, 1.35	1.00	0.80	0.63, 1.02	2.24	0.13
GSTM1	deletion	deletion	↑ Os	present	1.00	0.77	0.59, 1.00	1.00	1.03	0.80, 1.32	2.52	0.11
GSTP1	rs1695	A > G	↑ Os	AA	1.00	1.03	0.80, 1.33	1.00	1.07	0.84, 1.37	0.05	0.82
GSTT1	deletion	deletion	↑ Os	present	1.00	0.94	0.68, 1.28	1.00	1.09	0.81, 1.48	0.49	0.49
GSTA1	rs3957356	G > A	↑ Os	GG	1.00	0.83	0.63, 1.08	1.00	1.00	0.78, 1.29	1.03	0.31
COMT	rs4680	G > A	↑ Os	GG	1.00	1.37	1.02, 1.83	1.00	0.93	0.71, 1.22	3.60	0.06
COMT	rs737865	C > T	↓ Os	CT/TT	1.00	1.16	0.74, 1.81	1.00	1.01	0.69, 1.48	0.21	0.64
CYP17	rs743572	T > C	↑ Es	TC/CC	1.00	1.16	0.89, 1.50	1.00	0.95	0.74, 1.23	1.05	0.31

Note:
Inf = inflammation, Os = oxidative stress, Es = estrogen, LIBCSP = Long Island Breast Cancer Study Project, LRT=likelihood ratio test
[a] All models adjusted for matching factor, 5-year age group, and total energy intake (kcal/day).
[b] Genotypes dichotomized using dominant genetic model.
[c] LRT $\chi 2$ calculated using nested models for the multiplicative interaction .
[d] Variant allele function is based upon previous literature. Please refer to Table 3.6 for references.

CHAPTER 4:
DIETARY INTAKE OF FISH, PUFAs, AND SURVIVAL AFTER BREAST CANCER: A POPULATION-BASED, FOLLOW-UP STUDY ON LONG ISLAND, NEW YORK

4.1 Introduction

Breast cancer is the second cause of cancer death among women in the U.S. with approximately 40,000 estimated new deaths in 2013 [1]. Clinical and demographic indicators of breast cancer prognosis include large tumor size, lymph node involvement, hormone receptor-negative subtype, early age at diagnosis, and low socioeconomic status [9]. Weight maintenance and physical activity may improve survival following breast cancer [128, 129]. Because breast cancer is a multi-factorial disease, it is plausible that additional strategies, including intake of specific nutritional factors, could also improve survival among women diagnosed with breast cancer.

One potential nutritional risk reduction strategy is polyunsaturated fatty acids (PUFAs), of which ω-3 and ω-6 are the two primary classes. Inflammatory eicosanoids of arachidonic acid (AA), an ω-6 PUFA, have been shown in laboratory studies to: increase cell proliferation, metastatic potential, aromatase activity, and angiogenesis; and reduce apoptosis, and cellular differentiation [155]. ω-3 fatty acids bind to the same enzymes utilized in AA metabolism, thus, potentially lowering levels of inflammatory eicosanoids generated by ω-6 metabolism [155]. Also, the cytotoxic environment induced by ω-3 has been reported to increase apoptosis and reduce cell growth in transformed and malignant breast cancer cells [155, 256]. Long-chain ω-3 PUFA have demonstrated ability to chemo-

sensitize breast cancer tumors and, thus, potentially improve treatment efficacy [268]. Thus, it is plausible that intake of ω-3, for which fish is the major dietary source, could provide an opportunity for improving survival among women with breast cancer.

Despite this laboratory evidence, few epidemiologic studies have examined the association between dietary PUFA intake and survival after breast cancer [300, 301, 318]. Two [300, 301] utilized cross-sectional study designs, thus, limiting inferences regarding the potential association between PUFA intake and breast cancer survival. Another study examined the association between adipose tissue biomarkers of PUFA on survival after breast cancer [318] and reported no associations; however, interpretation of results was limited due to the small number of deaths. Thus, the epidemiologic evidence for the potential association between dietary PUFA intake and survival among women with breast cancer is limited.

Previous studies examining fish intake and mortality among breast cancer survivors are inconsistent [339, 340]. One Japanese investigation followed cases for 9 to 12 years, and reported increased breast cancer mortality with high fish consumption [340]; however, the study population was based on a small number of deaths. The second study utilized participants from the Women's Healthy Eating and Living (WHEL) study, a dietary intervention aimed to reduce total fat intake, and found that higher intake of ω-3 fatty acids (eicosapentanoic acid, EPA; and docosahexanoic acid, DHA) from fish was associated with reduced breast cancer recurrence and all-cause mortality [339]. This U.S.-based study, however, did not assess short-chain ω-3 PUFA (i.e., alpha-linolenic acid, ALA), which are readily obtained in the diet of Western populations.

For the study reported here, we examined whether higher intake of fish, as well as

any other dietary sources of ω-3 PUFAs, are associated with improved survival among women diagnosed with breast cancer on Long Island, New York. We also considered associations with ω-6 PUFAs and the balance between ω-3 and ω-6 intake. Given that PUFAs affect tumor initiation and promotion via multiple biologic pathways, we also explored interactions with genes involved in inflammation, oxidative stress, and estrogen metabolism pathways.

4.2 Methods

This follow-up study utilizes resources from the Long Island Breast Cancer Study Project (LIBCSP), a population-based study [128, 129, 355]. Institutional Review Board approval was obtained from all participating institutions.

Study Population. Women eligible for the LIBCSP follow-up study were English-speaking residents of Long Island, New York (Nassau and Suffolk counties) who were newly diagnosed with a first primary *in situ* or invasive breast cancer between August 1, 1996 and July 31, 1997. After obtaining physician approval, study personnel contacted pathology departments from participating hospitals (2-3 times per week or daily, for the hospitals with large numbers of newly diagnosed cases) to identify potentially eligible subjects. The final LIBCSP follow-up sample consisted of 1,508 women with breast cancer. Within approximately three months of diagnosis, 98% (n=1,479) completed a validated self-administered 101-item modified Block food frequency questionnaire (FFQ) [359, 395, 396]. Subjects with implausible total energy intake (±3 standard deviations from the mean) were excluded (n=16). Thus, the final analytic cohort for this ancillary study included 1,463 women with newly diagnosed breast cancer.

At diagnosis (baseline) with the first primary breast cancer, participants ranged in age from 20-98 years, 67% were postmenopausal, and 94% reported their race as white, 4% as black, and 2% as other, which reflects the underlying racial distribution of Nassau and Suffolk counties at the time of data collection [128, 129, 355].

Outcome Assessment. Vital status through December 31, 2011 for all LIBCSP participants was determined via linkage with the National Death Index, a standard epidemiologic resource for ascertaining mortality data in the U.S. [358]. We identified women who died from all-causes (death from any cause), and those whose deaths were breast cancer-related (breast cancer-specific mortality). Breast cancer-related deaths were determined using the International Classification of Disease (codes 174.9 or C-50.9). Among the 1,463 participants included in this study, the median follow-up time was 14.7 years after breast cancer diagnosis (range=0.2-15.4 years), and we identified 485 total deaths, of which 210 were breast cancer-specific.

Assessment of PUFA Intake and Other Prognostic Factors. LIBCSP participants self-completed the FFQ, and were administered a baseline, structured questionnaire by a trained interviewer, within three months, on average, after diagnosis. The FFQ assessed dietary intake in the year prior to the interview. Other factors assessed included: demographic characteristics; reproductive and menstrual history; exogenous hormone use, family history of breast cancer, and other medical history; body size, physical activity, and alcohol use; active and passive cigarette smoking; occupational history and other environmental exposures [355]. Medical records were abstracted to determine tumor characteristics of the first primary breast cancer and the first course of treatment for the first primary breast cancer. Concordance between the medical record and the self-reported treatment data was high

(kappa>90%), and thus, the self-reported data are used here.

PUFA intake from any dietary source was estimated by linking participant responses from the FFQ (i.e., grams per day for each line item) with nutrient values available in the U.S. Department of Agriculture databases for ω-3 and ω-6 PUFAs [397]. The following PUFA subtypes were estimated: (1) ω-3 PUFA including, alpha-linolenic acid (ALA), docosapentanoic acid (DPA), DHA, EPA; and (2) ω-6 PUFA including, linoleic acid (LA) and AA. An estimate of total ω-3 and ω-6 PUFA intake (henceforth, total PUFA intake) was calculated by combining all individual PUFA subtypes. Additionally, an estimate of total ω-3 and ω-6 PUFA was obtained by summing each individual fatty acid within category (e.g., total ω-3=ALA+DPA+DHA+EPA).

Fish and/or seafood consumption were also assessed by FFQ as: (1) tuna, tuna salad, tuna casserole; (2) shell fish (shrimp, lobster, crab, oysters, etc.); and (3) other fish (broiled/baked).

Genotyping. Eighteen variants in 15 genes were selected for this analysis representing three biologically plausible pathways for PUFA metabolism, including inflammation, oxidative stress, and estrogen metabolism pathways. Variants affecting polyphen prediction (*GPX1*), transcription factor binding prediction (*PTGS-2* rs20417, *FAS*, *FASL*, *TNF-α*, *MPO*, *CAT*, *GSTA1*, *COMT* rs737865, *CYP17*), miRNA binding (*PTGS-2* rs5275, *GPX1*), 3D conformation (*PPAR-α*, *COMT* rs4680), or splicing regulation (*PPAR-α*, *FAS* rs2234767, *GPX1*, *GSTP1*, *COMT* rs4680) were considered as putatively functional variants as identified using the NIEHS SNPInfo WebServer [361].

Blood samples were collected from participants at the baseline interview. Genotyping methods have been previously published [65, 366-370, 398]. Briefly, DNA was

isolated from mononuclear cells in whole blood which was separated by Ficoll (Sigma Chemical Co., St. Louis, MO) using standard phenol and chloroform-isoamyl alcohol extraction and RNase treatment [398]. Genotyping for inflammation genes (*PTGS-2*, *FAS*, *FASL*, *PPAR-α*), were conducted using: Taqman 5'-Nuclease Assay (Applied Biosystems, Foster City, CA) and AcycloPrime^{TM}-FP SNP Detection Kit obtained from Perkin Elmer Life Sciences (Boston, MA) [65, 367, 368]. For *CYP17*, the same assay was used with a 10 μM probe [369, 370]. For oxidative stress genes (*CAT*, *MPO*, *MnSOD*, *GPX*, *GSTA1*, *GSTP1*), BioServe Biotechnologies (Laurel, MD) performed the genotyping using high-throughput, matrix assisted, laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of Sequenom, Inc. (San Diego, CA). Deletions for *GSTM1* and *GSTT1* genes were determined by a multiplex polymerase chain reaction method, with the constitutively present gene β-globulin as an internal positive control [366].

Tests for Hardy-Weinberg equilibrium (HWE) indicated that only *PTGS-2* rs20417 and *MPO* polymorphisms deviated significantly from HWE ($p<0.05$). However, for both polymorphisms, the observer agreement in 8% of randomly selected samples was high (*PTGS-2* rs20417 kappa statistic=0.99, *MPO* kappa statistic=0.91), and the failure rate of the assay was low (<1%). Missing data for the genetic polymorphisms reduced the sample size for the gene-PUFA interaction analyses, resulting in total sample sizes ranging from 950 to 1,035 women with breast cancer.

Statistical analyses. Kaplan-Meier survival curves were constructed, and Cox-proportional hazards regression [375] models were used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI), to assess the associations between intake of fish, as well as PUFAs from any dietary source, and all-cause and breast cancer-specific mortality for the

full 15-years of follow-up. As a sensitivity analysis, we also calculated PUFA effect estimates for 5-years of follow-up.

For regression analyses, the proportional hazards assumption was assessed using exposure interactions with time, and also using Martingale residuals [375]. Quartiles were created for each PUFA exposure (total PUFA, total ω-3, ALA, DPA, DHA, EPA, total ω-6, LA, AA, ratio of ω-3/ω-6) based on the distributions among the 1,463 women with breast cancer included in our study. Other exposure cut-points were considered (e.g., tertiles, quintiles, linear, splines); however, the shape of the dose-response between PUFAs and the log-hazard of mortality was best captured with quartiles. Similarly, quartiles were created for exposures related to fish intake (i.e., tuna, shell fish, other fish). Tests for linear trend were not conducted, given the relation between continuous PUFA and log-hazard of all-cause mortality was not strictly monotonic [373].

We also utilized Cox-proportional hazards models to examine the associations between genotypes in three biologically plausible pathways and mortality. To maximize the number of events per exposure category, genes were dichotomized according to a dominant model, and categorized into "high" and "low" risk groups based upon the function of the variant allele, which was determined using the existing literature [219, 399-412].

In the Cox proportional regression models, we also considered interactions between total ω-3 and total ω-6, and between the ω-3/ω-6 ratio and genotypes in association with mortality. For these interaction analyses, PUFAs were dichotomized at the median, and a dominant genetic model was assumed. Interaction was evaluated on the additive scale and measured using relative excess risk due to interaction (RERI), with corresponding 95% CI calculated using the Hosmer and Lemeshow method [374]. Multiplicative interactions were

also assessed using the Likelihood Ratio Test; however, the conclusions did not change (data not shown). The relatively low number of breast-cancer specific deaths resulted in imprecise estimates for the ω-3 and ω-6 interaction, and the PUFA-genotype interactions with this outcome. Thus, only the interaction results for all-cause mortality are shown.

Effect modification of the PUFA-mortality associations by menopausal status (post- vs. pre-menopausal); hormone receptor status (hormone receptor-positive breast cancer vs. negative), dietary supplement use (yes/no), and treatment (chemotherapy, radiation, hormone therapy) were also examined in the PUFA regression models. After stratification, however, little or no heterogeneity was observed, thus these results are not shown.

Potential confounders for the PUFA-mortality regression models were identified using a directed acyclic graph (DAG) [373], and included age (5-year age group), total energy intake (kcal/day), non-steroidal anti-inflammatory drugs (NSAID), family history of breast cancer, income, body mass index, alcohol use, fruit and vegetable intake, physical activity, and race. However, only age and total energy intake changed the HR estimates by more than 10%, and thus all PUFA-mortality regression models were adjusted for these two confounders only.

All statistical analyses were conducted using PROC PHREG (Cox proportional hazards regression models) and PROC LIFETEST (Kaplan-Meier survival curves) in SAS version 9.2 (SAS Institute, Cary, NC).

4.3 Results

PUFA and Fish Intake. As shown in **Table 4.1**, among our population-based sample of women with breast cancer (n = 1,463) intake at baseline of total ω-3 fatty acids from any

dietary source (average total ω-3 intake 0.99 grams per day, SD = 0.69) was lower relative to ω-6 intake (average total ω-6 intake of 7.51 grams per day, SD = 5.26). ALA intake was the highest contributor to total ω-3 intake with an average intake of 0.85 grams per day (SD = 0.67), whereas LA was the highest contributor to total ω-6 intake with an average intake of 7.44 grams per day (SD = 5.24). Tuna intake was higher (8.13 grams per day, SD = 10.61) compared to shell fish intake (3.30 grams per day, SD = 6.01). As also shown in **Table 4.1,** fish was a primary contributor to high intake of long-chain ω-3 PUFAs, including DPA, DHA, and EPA. In contrast, foods that contributed to high ALA intake were biscuits/muffins and other fried foods. Additionally, high AA intake appeared to be driven by eggs and meats, including fish, chicken, and ham.

PUFAs and Mortality. The Kaplan-Meir curves shown in **Figure 4.1** indicate survival was improved among our population-based sample of women with breast cancer reporting higher intake (quartiles (Q) 3 and 4) of the long-chain ω-3 fatty acids DPA, DHA, and EPA. As presented in **Table 4.2**, reductions of 16-34% in all-cause mortality were observed for higher intake of long-chain ω-3 fatty acids. Specifically, lower death rates were observed for the highest two quartiles of intake (Q3 or Q4), compared to the lowest quartile (Q1), of DHA ($HR_{Q3vs.Q1}$=0.73, 95%CI=0.56,0.94; and $HR_{Q4vs.Q1}$=0.71, 95% CI=0.55,0.92), EPA ($HR_{Q3vs.Q1}$=0.70, 95% CI=0.54,0.91; and $HR_{Q4vs.Q1}$=0.75, 95% CI=0.58,0.97); and DPA ($HR_{Q3vs.Q1}$=0.66, 95%CI=0.51,0.86; and $HR_{Q4vs.Q1}$=0.84, 95%CI=0.64,1.10). The corresponding hazard for ω-3/ω-6 ratio was modestly decreased by 15%, but the confidence interval included the null value. As also shown in **Table 4.2**, adjusted hazards for all-cause mortality were increased by 14-30% for higher intakes of total ω-6, LA, AA, and ALA but confidence intervals were wide. Patterns were similar, but less precise, for PUFA intake

from all dietary sources in relation to breast cancer-specific mortality after 15 years of follow-up (Supplemental **Table 4.4**), and when we considered all-cause mortality after 5-years of follow-up (Supplemental **Table 4.5**).

We also considered an interaction between ω-3 and ω-6, and all-cause mortality; however, as shown in Supplemental **Table 4.6**, no interaction on an additive scale was observed.

Fish and Mortality. As shown in **Table 4.3,** fish intake was associated with a 25-34% reduction in all-cause mortality. Specifically, lower rates of death were observed for: the highest quartile of intake for those in the highest quartile of tuna intake, compared to the lowest quartile (HR = 0.71, 95% CI = 0.55, 0.92); and the highest two quartiles for other fish (broiled/baked) ($HR_{Q3vs.Q1}$ = 0.66, 95% CI = 0.51, 0.85; and $HR_{Q4vs.Q1}$ = 0.75, 95% CI = 0.58, 0.97). There was little or no evidence of an association between all-cause mortality and shell fish intake. Adjusted estimates for breast cancer-specific mortality showed pronounced but imprecise reductions when we considered tuna and other fish in relation to 5 years of follow-up (Supplemental **Table 4.5**). Estimates, however, were closer to the null when we considered 15 years of follow up [tuna ($HR_{Q4vs.Q1}$ = 0.81, 95% CI = 0.54, 1.21) and other baked/broiled fish ($HR_{Q4vs.Q1}$ = 1.04, 95% CI = 0.71, 1.52)].

Genotypes and Mortality. As presented in Supplemental **Table 4.7**, the association between polymorphisms involved in inflammation, oxidative stress, and estrogen metabolism and all-cause mortality using a dominant model were largely consistent with no association. When we explored the interactions on an additive scale between ω-3/ω-6 ratio and polymorphisms in multiple biologic pathways on overall mortality (Supplemental **Table 4.8**), we observed a 19% increase in the rate of death (HR = 1.19, 95% CI = 0.85, 1.68) for low

intake of the favorable ratio of ω-3/ω-6 and high risk *TNF-α* rs1800682 genotype. This interaction was statistically significant on the additive scale (RERI = 0.49; 95% CI = 0.03, 0.96), suggesting antagonism between low intake of ω-3/ω-6 and high risk *TNF-α* genotype.

4.4 Discussion

In this population-based follow-up study of women with breast cancer, we observed reduced hazards of 16-34% for all-cause mortality after 15 years of follow-up with consumption of higher levels, compared to the lowest level, of long-chain ω-3 fatty acids (DPA, DHA, and EPA). Similarly, higher intake of tuna and other baked/broiled fish was associated with 25-34% decreased all-cause mortality (and an imprecise 19% reduction in breast cancer-specific mortality in association with tuna intake only). We found little evidence for interaction between ω-3 and ω-6, or between PUFAs and most genotypes considered in three related pathways.

Our population-based results are consistent with one previous study on PUFA and/or fish intake in relation to survival among women with breast cancer that is relatively comparable to our own [339]. Patterson and colleagues [339] examined marine food sources of EPA and DHA in the WHEL study, and reported reductions of 28% for recurrence and 41% for all-cause mortality. The study methods for the WHEL study differ from ours in that only marine ω-3 sources were assessed using repeated 24-hour recalls after varying lengths of time since diagnosis among a convenience sample of breast cancer survivors; whereas, we considered marine and other dietary PUFA sources that were assessed using a 101-item FFQ administered within three months of diagnosis to a population-based sample of women newly diagnosed with breast cancer. The robustness of the results across studies, despite the

methodological differences in our LIBCSP study and the WHEL study, support the possibility of long-chain ω-3 as a potential risk reduction strategy for breast cancer survivors. Further research is needed to confirm these findings.

Our reported results for long-chain ω-3 fatty acids (DPA, DHA, EPA), total ω-6, LA, AA, and the ratio of ω-3/ω-6 are consistent with the biologic mechanism of a PUFA-induced cytotoxic environment via lipid peroxidation, and the potential benefit of this environment on reducing apoptosis and cell growth in cancer cells [155, 256]. Long-chain ω-3 fatty acids contain more double bonds within the fatty acid chain compared to ALA and ω-6. These double bonds provide additional opportunities for lipid peroxidation and thus could help to better promote a cytotoxic environment within the cell. Consequently, this cytotoxic environment could provide a beneficial environment for women with breast cancer.

Our results for ALA intake, which suggest a modest increase in the rate of overall death, are not consistent with a biologic hypothesis via inflammatory pathways. This discrepancy may reflect the foods that are contributing to high ALA intake in our Long Island population; namely, we observed that the foods containing butter and fried foods are contributing to high ALA intake. This similarity between ALA and ω-6 intake in terms of foods contributing to high intake, may explain the potentially spurious increased rate of overall death observed for greater intake of ALA. Additionally, the *in vivo* conversion of ALA into long-chain ω-3 PUFAs is inefficient in populations with high ω-6 [150], which may possibly explain the modest increase in the overall rate of death observed here.

We observed an additive interaction for low intake of ω-3/ω-6 and high risk *TNF-α* genotype with all-cause mortality, which could be due to chance. However, the variant *TNF-α* allele is thought to increase activity [402], and *TNF-α* indirectly increases cellular levels of

AA by inducing phospholipase A_2 (PLA2) activity in human tumor cells [205, 206]. Thus, the antagonism observed for low intake of ω-3/ω-6 in combination with potential for increased *TNF-α* activity (high risk genotype) is consistent with biology, but requires confirmation by others.

This prospective, population-based study has multiple strengths. We are the first to examine the potential relation between PUFA intake and breast cancer survival, while simultaneously considering both ω-3 and ω-6 PUFAs. Additionally, we examined multiple ω-3 (e.g., ALA, DPA, DHA, and EPA) and ω-6 (e.g., LA, AA) subtypes in relation to all-cause mortality. Consideration of non-marine sources of PUFAs may be critical for some U.S. populations that consume low amounts of fish [146]. We are also the first to examine the interaction between ω-3/ω-6 ratio and genetic susceptibility in inflammation, oxidative stress, and estrogen metabolism in relation to all-cause mortality; identification of high risk subgroups defined by genotype could aid in tailoring risk reduction strategies.

However, our follow-up study also has limitations. Despite our relatively large sample size, effect estimates for the associations with breast cancer-specific mortality were imprecise for both the 5-year and 15-year follow-up periods. We were able to capture dietary intake close to the time of diagnosis, and thus this exposure window may be more relevant for the 5-year follow period, given the more pronounced effect estimates observed for this time period. However, the imprecision for all breast cancer-specific estimates, regardless of the time period, is likely to be due to PUFA measurement error. For example, it is possible that a one-time baseline FFQ measurement of diet may not accurately reflect dietary intake throughout the 15-year period following diagnosis. One recent study compared pre-diagnosis versus post-diagnosis dietary intake, and reported dietary increases in oily fish and fish oil

consumption post breast cancer diagnosis [420]. However, this repeated measure study enrolled women 9-15 months after diagnosis, and asked them to concurrently recall dietary intake one year prior to diagnosis as well as changes in diet following diagnosis; thus, the reported changes in dietary intake are subject to errors in recall. Nonetheless, it is possible that the estimates of long-chain ω-3 PUFA intake in our study population are conservative. Further, long-chain ω-3 PUFA levels differ by fish species, with tuna being among the highest [419]. Thus, future, larger studies should consider repeated PUFA measurements (self-reported intake of specific fish species, fish oil, and/or biomarkers) throughout follow-up to enhance our examination of the potential association between long-chain ω-3 PUFAs and breast cancer survival.

Although we are the first to explore PUFA interactions with multiple genotypes from multiple biologic pathways, future studies may want to consider assessing interactions with additional genes from these relevant pathways. In addition, although our study is population-based and reflects the racial distribution of the target study population on Long Island, which improves external validity, the LIBCSP population includes predominantly Caucasian women; therefore, examination of racial differences is not possible. However, our results are generalizable to Caucasian-American women for whom the rates of breast cancer remain high [1]. Future studies may consider exploring possible heterogeneity between PUFA and survival by race, or by breast cancer tumor subtypes.

In conclusion, in our population-based follow-up study of women with breast cancer on Long Island, NY, we observed 16-34% reductions in all-cause mortality after 15 years of follow-up for high intake of fish, and long-chain ω-3 (DPA, DHA, and EPA), which is consistent with laboratory evidence and the one other U.S.-based epidemiologic study

considering this issue [339]. Thus, pending additional replication, dietary intake of fish and other sources of long-chain ω-3 fatty acids may provide an additional strategy to improve survival following breast cancer.

Table 4.1 Distributions of intakes of polyunsaturated fatty acid (PUFA) and fish at baseline among a population-based sample of women newly diagnosed with breast cancer (N=1463), LIBCSP, 1996-1997

Nutrient/Food	Mean	SD	25th Pct	50th Pct	75th Pct	Major PUFA-rich foods contributing to high nutrient intake in the LIBCSP
Nutrient (g/day)						
Total PUFA[a]	8.50	5.83	4.38	7.24	10.92	Butter, mayonnaise/salad dressings, safflower/corn oil, margarine, peanuts/peanut butter
Total ω-3[b]	0.99	0.69	0.52	0.82	1.26	Biscuits/muffins, butter, fish, mayonnaise/salad dressings, safflower/corn oil
ALA	0.85	0.67	0.38	0.68	1.10	Biscuits/muffins, French fries/fried potatoes, butter, cookies, mayonnaise/salad dressings, safflower/corn oil
DPA	0.01	0.01	0.01	0.01	0.02	Fish, tuna, chicken, shellfish, beef
DHA	0.09	0.10	0.03	0.06	0.12	Fish, tuna, eggs, chicken, shellfish
EPA	0.05	0.05	0.01	0.03	0.06	Fish, tuna, shellfish, chicken
Total ω-6[c]	7.51	5.26	3.83	6.43	9.73	Biscuits/muffins, French fries/fried potatoes, butter, mayonnaise/salad dressings
LA	7.44	5.24	3.80	6.35	9.66	Biscuits/muffins, French fries/fried potatoes, butter, mayonnaise/salad dressings, safflower/corn oil
AA	0.07	0.05	0.04	0.06	0.09	Eggs, fish, chicken, ham/lunch meats, shellfish
ω-3/ω-6	0.15	0.11	0.11	0.13	0.17	N/A
Fish (g/day)[d]						
Tuna	8.13	10.61	1.40	4.77	12.40	N/A
Shell fish	3.30	6.01	0.00	0.00	4.62	N/A
Other (broiled/baked)	7.88	14.70	0.00	3.85	10.77	N/A

Note:
[a] Total PUFA = ALA + DPA + DHA + EPA + LA + AA
[b] Total ω-3 = ALA + DPA + DHA + EPA
[c] Total ω-6 = LA + AA
[d] Cases with null values for tuna (N=343), shell fish (N=750), and other (N=505) are included in calculations.
SD = standard deviation
N/A = not applicable
LIBCSP = Long Island Breast Cancer Study Project

Table 4.2 Age- and multivariate[a]-adjusted HRs and 95% CI for the association between dietary PUFA intake and all-cause mortality among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

PUFA	Covariates	Q1			Q2				Q3				Q4			
		D	Co	HR	D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI
Total PUFA	Age	124	241	1.00	128	238	1.15	0.90,1.47	110	256	1.01	0.78,1.31	123	243	1.28	0.99,1.64
	Multivariate			1.00			1.12	0.87,1.44			0.97	0.73,1.27			1.16	0.84,1.61
Total ω-3	Age	124	242	1.00	116	249	0.97	0.75,1.25	124	243	1.15	0.90,1.48	121	244	1.14	0.89,1.46
	Multivariate			1.00			0.93	0.71,1.21			1.07	0.82,1.41			1.00	0.72,1.38
ALA	Age	114	252	1.00	129	237	1.21	0.94,1.55	121	244	1.24	0.96,1.61	121	245	1.25	0.97,1.62
	Multivariate			1.00			1.18	0.91,1.52			1.18	0.90,1.55			1.14	0.83,1.57
DPA	Age	136	230	1.00	131	235	0.98	0.77,1.25	99	266	0.71	0.55,0.92	119	247	0.94	0.74,1.21
	Multivariate			1.00			0.93	0.73,1.19			0.66	0.51,0.86			0.84	0.64,1.10
DHA	Age	139	227	1.00	130	235	0.95	0.75,1.21	105	261	0.76	0.59,0.98	111	255	0.76	0.60,0.98
	Multivariate			1.00			0.93	0.73,1.18			0.73	0.56,0.94			0.71	0.55,0.92
EPA	Age	136	230	1.00	131	234	0.95	0.75,1.21	102	264	0.74	0.57,0.96	116	250	0.81	0.63,1.04
	Multivariate			1.00			0.93	0.73,1.18			0.70	0.54,0.91			0.75	0.58,0.97
Total ω-6	Age	122	243	1.00	124	242	1.12	0.87,1.43	112	254	1.07	0.83,1.38	127	239	1.34	1.04,1.72
	Multivariate			1.00			1.10	0.85,1.42			1.04	0.79,1.38			1.27	0.92,1.76
LA	Age	121	244	1.00	126	240	1.15	0.90,1.48	111	255	1.07	0.83,1.39	127	239	1.36	1.06,1.74
	Multivariate			1.00			1.14	0.88,1.47			1.05	0.79,1.39			1.30	0.94,1.79
AA	Age	122	243	1.00	120	247	1.03	0.80,1.32	113	252	0.93	0.72,1.20	130	236	1.24	0.97,1.59
	Multivariate			1.00			1.00	0.78,1.30			0.89	0.68,1.16			1.15	0.87,1.52
ω-3/ω-6	Age	124	242	1.00	113	252	0.90	0.70,1.17	128	238	0.95	0.75,1.22	120	246	0.84	0.66,1.08
	Multivariate			1.00			0.89	0.69,1.14			0.93	0.73,1.19			0.85	0.66,1.09

Note:
Co=Cohort N, D=Deaths N, LIBCSP=Long Island Breast Cancer Study Project
[a] Multivariate-adjusted HRs and 95% CI adjusted age (5-year age group) and total energy intake (kcal/day)

Table 4.3 Age- and multivariate[a]-adjusted HRs and 95% CI for the association between fish intake and all-cause mortality among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

PUFA	Covariates	Never			Q1				Q2				Q3				Q4			
		D	Co	HR	D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI
Tuna	Age			1.00			0.98	0.75,1.29			0.95	0.72,1.26			1.08	0.82,1.44			0.74	0.58,0.96
	Multivariate	125	218	1.00	86	155	0.98	0.74,1.29	84	162	0.93	0.70,1.22	78	137	1.06	0.80,1.41	112	306	0.71	0.55,0.92
Shell fish	Age			1.00			0.72	0.47,1.10			1.00	0.78,1.27			0.80	0.59,1.10			1.10	0.84,1.45
	Multivariate	268	482	1.00	23	79	0.71	0.46,1.09	84	161	0.98	0.76,1.25	46	123	0.79	0.57,1.08	64	133	1.05	0.79,1.39
Other fish (broiled/baked)	Age			1.00			0.95	0.73,1.24			0.98	0.72,1.34			0.68	0.53,0.87			0.77	0.60,1.00
	Multivariate	319	186	1.00	145	75	0.95	0.72,1.24	87	50	0.97	0.71,1.33	91	250	0.66	0.51,0.85	83	177	0.75	0.58,0.97

Note:
Co=Cohort N, D=Deaths N, LIBCSP = Long Island Breast Cancer Study Project
[a] Multivariate-adjusted HRs and 95% CI adjusted for age (5-year age group), total energy intake (kcal/day).

Figure 4.1 Kaplan-Meier survival curves for dietary intake (quartiles) of long-chain ω-3 fatty acids DPA, DHA, and EPA, among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

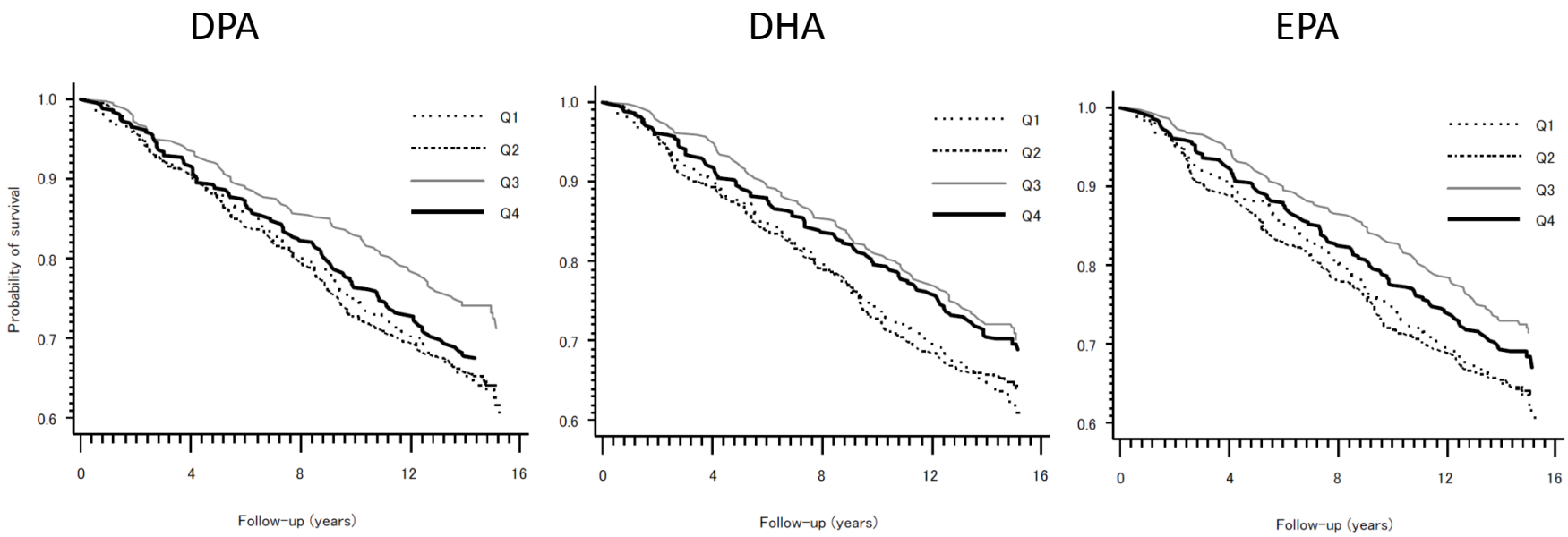


Table 4.4 Age- and multivariate[a]-adjusted HRs and 95% CI for the association between dietary PUFA intake and breast cancer-specific mortality among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

PUFA	Covariates	Q1			Q2				Q3				Q4			
		D	Co	HR	D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI
Total PUFA	Age	38	327	1.00	62	304	1.67	1.12,2.50	53	313	1.40	0.92,2.13	57	309	1.59	1.05,2.41
	Multivariate			1.00			1.56	1.03,2.36			1.25	0.80,1.95			1.25	0.74,2.11
Total ω-3	Age	43	323	1.00	51	314	1.18	0.79,1.77	63	304	1.53	1.04,2.26	53	312	1.29	0.86,1.93
	Multivariate			1.00			1.08	0.71,1.64			1.32	0.87,2.01			0.96	0.57,1.61
ALA	Age	46	320	1.00	50	316	1.13	0.76,1.69	57	308	1.32	0.89,1.95	57	309	1.32	0.89,1.94
	Multivariate			1.00			1.06	0.71,1.60			1.17	0.77,1.77			1.03	0.64,1.68
DPA	Age	55	311	1.00	50	316	0.91	0.62,1.34	44	321	0.76	0.51,1.13	61	305	1.10	0.76,1.58
	Multivariate			1.00			0.85	0.58,1.25			0.69	0.46,1.03			0.93	0.63,1.38
DHA	Age	55	311	1.00	50	315	0.92	0.62,1.34	50	316	0.86	0.59,1.27	55	311	0.96	0.66,1.40
	Multivariate			1.00			0.88	0.58,1.25			0.81	0.55,1.19			0.86	0.59,1.27
EPA	Age	53	313	1.00	50	315	0.95	0.65,1.40	51	315	0.91	0.62,1.34	56	310	1.02	0.70,1.49
	Multivariate			1.00			0.92	0.62,1.35			0.84	0.57,1.25			0.92	0.63,1.36
Total ω-6	Age	37	328	1.00	61	305	1.68	1.12,2.53	54	312	1.48	0.98,2.26	58	308	1.67	1.10,2.53
	Multivariate			1.00			1.59	1.05,2.42			1.34	0.86,2.11			1.36	0.81,2.30
LA	Age	37	328	1.00	61	305	1.69	1.12,2.55	54	312	1.49	0.98,2.26	58	308	1.67	1.10,2.54
	Multivariate			1.00			1.60	1.05,2.43			1.35	0.86,2.11			1.36	0.81,2.29
AA	Age	47	318	1.00	48	319	1.02	0.68,1.53	52	313	1.09	0.74,1.62	63	303	1.41	0.96,2.06
	Multivariate			1.00			0.98	0.65,1.47			1.01	0.67,1.52			1.22	0.80,1.86
ω-3/ω-6	Age	63	303	1.00	44	321	0.68	0.46,1.00	53	313	0.84	0.58,1.21	50	316	0.78	0.54,1.13
	Multivariate			1.00			0.65	0.44,0.95			0.79	0.55,1.15			0.79	0.54,1.14

Note:
Co=Cohort N, D=Deaths N, LIBCSP=Long Island Breast Cancer Study Project
[a] Multivariate-adjusted HRs and 95% CI adjusted age (5-year age group) and total energy intake (kcal/day)

Table 4.5 Age- and energy-adjusted HRs and 95% CI for competing risks analysis for all-cause, breast cancer-specific, other cause mortality, among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 (within 5 years of diagnosis)

LC ω-3 and Fish intake (g/day)		All-cause mortality				Breast cancer-specific mortality				Other cause mortality			
		D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI
DPA													
	Q1	44	322	1.00		27	322	1.00		17	322	1.00	
	Q2	45	321	0.95	0.62,1.44	26	321	0.86	0.50,1.47	19	321	1.16	0.60,2.26
	Q3	30	335	0.61	0.38,0.99	23	335	0.70	0.40,1.24	7	335	0.46	0.19,1.13
	Q4	41	325	0.81	0.51,1.28	29	325	0.80	0.45,1.41	12	325	0.91	0.41,2.01
DHA													
	Q1	46	320	1.00		29	320	1.00		17	320	1.00	
	Q2	47	318	0.99	0.66,1.48	29	318	0.94	0.56,1.58	18	318	1.06	0.55,2.06
	Q3	28	338	0.57	0.35,0.92	22	338	0.66	0.37,1.15	6	338	0.39	0.15,0.99
	Q4	39	327	0.74	0.48,1.15	25	327	0.70	0.40,1.21	14	327	0.90	0.44,1.86
EPA													
	Q1	43	323	1.00		28	323	1.00		15	323	1.00	
	Q2	50	315	1.10	0.73,1.65	30	315	1.01	0.60,1.69	18	315	1.27	0.65,2.49
	Q3	29	337	0.63	0.39,1.01	22	337	0.67	0.38,1.17	6	337	0.53	0.21,1.30
	Q4	38	328	0.76	0.49,1.20	25	328	0.72	0.41,1.26	12	328	0.91	0.43,1.95
Tuna													
	Never	40	303	1.00		23	303	1.00		17	303	1.00	
	Q1	27	214	0.95	0.58,1.55	16	214	0.98	0.52,1.85	11	214	0.91	0.43,1.94
	Q2	32	214	1.10	0.69,1.76	24	214	1.38	0.78,2.45	8	214	0.71	0.30,1.64
	Q3	29	186	1.17	0.72,1.89	20	186	1.31	0.72,2.39	9	186	0.99	0.44,2.24
	Q4	32	386	0.64	0.40,1.02	22	386	0.69	0.38,1.24	10	386	0.56	0.26,1.23
Other Fish (boiled/baked)													
	Never	62	443	1.00		41	443	1.00		21	443	1.00	
	Q1	32	188	1.22	0.80,1.87	20	188	1.12	0.65,1.91	12	188	1.45	0.71,2.94
	Q2	13	124	0.74	0.41,1.34	10	124	0.85	0.43,1.70	3	124	0.50	0.15,1.67
	Q3	24	317	0.54	0.34,0.86	17	317	0.56	0.32,0.98	7	317	0.52	0.22,1.22
	Q4	29	231	0.81	0.52,1.26	17	231	0.73	0.42,1.29	12	231	1.02	0.50,2.08

Note: Co=Cohort, D=Deaths, LIBCSP = Long Island Breast Cancer Study Project, LC = long-chain

Table 4.6 Age- and multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CI) for all-cause mortality among a population-based sample of women with breast cancer for the interaction between dietary ω-3 and ω-6 intake, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

Model	Low ω-6 (< median)		High ω-6 (≥ median)		RERI[b]	95% CI[c]
	HR	95% CI	HR	95% CI		
Age-adjusted						
High ω-3 (≥ median)	1.00		0.97	0.71, 1.32		
Low ω-3 (< median)	0.82	0.60, 1.11	0.94	0.63, 1.40	0.16	-0.24, 0.55
Multivariate[a]						
High ω-3 (≥ median)	1.00		0.93	0.68, 1.28		
Low ω-3 (< median)	0.85	0.62, 1.16	0.94	0.63, 1.41	0.17	-0.23, 0.56

Note:

[a] Multivariate HRs and 95% CI adjusted for age (5-year age group) and total energy intake (kcal/day)

[b] RERI (Relative Excess Risk due to Interaction) = HR_{11} - HR_{10} - HR_{01} +1

[c] 95% CI for RERI estimated using Hosmer & Lemeshow [374]

LIBCSP = Long Island Breast Cancer Study Project

Table 4.7 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for all-cause mortality for the association with putatively functional genetic polymorphisms using dominant model (N=1463) among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

Gene	rs#	Allele Substitution.	No Copies of Variant Allele		≥ 1 Copy of Variant Allele		No Copies of Variant Allele		≥ 1 Copy of Variant Allele		
			N		N		D	HR	D	HR	95% CI
PTGS-2	rs20417	G > C	GG	653	GC/CC	377	218	1.00	109	0.85	0.68, 1.07
PTGS-2	rs5275	T > C	TT	463	TC/CC	570	154	1.00	175	0.94	0.76, 1.17
PPAR-α	rs1800206	C > G	CC	777	CG/GG	101	258	1.00	30	0.91	0.62, 1.32
FAS	rs2234767	G > A	GG	792	GA/AA	238	255	1.00	71	0.91	0.70, 1.18
FAS	rs1800682	G > A	GG	274	GA/AA	750	87	1.00	237	1.00	0.78, 1.28
FASL	rs763110	C > T	CC	327	CT/TT	708	110	1.00	217	0.89	0.71, 1.12
TNF-α	rs1800629	G > A	GG	758	GA/AA	261	236	1.00	85	1.05	0.82, 1.34
MnSOD	rs4880	C > T	CC	250	CT/TT	756	76	1.00	240	1.06	0.82, 1.37
MPO	rs2333227	G > A	GG	629	GA/AA	386	199	1.00	121	1.00	0.80, 1.26
CAT	rs1001179	C > T	CC	614	CT/TT	395	174	1.00	144	1.39	1.11, 1.73
GPX1	rs1050450	C > T	CC	463	CT/TT	550	147	1.00	173	0.98	0.79, 1.22
GSTM1	deletion	deletion	present	493	deletion	457	161	1.00	140	0.93	0.74, 1.16
GSTP1	rs1695	A > G	AA	500	AG/GG	499	155	1.00	161	1.06	0.85, 1.32
GSTT1	deletion	deletion	present	754	deletion	203	246	1.00	60	0.90	0.68, 1.19
GSTA1	rs3957356	G > A	GG	334	GA/AA	679	108	1.00	213	0.96	0.76, 1.21
COMT	rs4680	G > A	GG	280	GA/AA	740	96	1.00	237	0.87	0.68, 1.10
COMT	rs737865	C > T	CC	99	CT/TT	903	24	1.00	292	1.40	0.92, 2.12
CYP17	rs743572	T > C	TT	345	TC/CC	656	113	1.00	206	0.94	0.75, 1.19

LIBCSP = Long Island Breast Cancer Study Population, D=deaths

Table 4.8 Age- and energy-adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for all-cause mortality for the hypothesized highest risk interaction between ω-3/ω-6 ratio and putatively functional genetic polymorphisms among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

Gene	rs#	Allele Substitution	Variant allele function	High risk genotype	High ω-3/ω-6 (> median)			Low ω-3/ω-6 (< median)				RERI[e]	95% CI[f]
					Low risk	High risk		Low risk		High risk			
					HR[a]	HR	95% CI	HR	95% CI	HR[b]	95% CI		
PTGS-2	rs20417	G > C	↓ Inf [399]	GG	1.00	1.03	0.74, 1.43	0.87	0.60, 1.26	1.08	0.78, 1.49	0.19	-0.23, 0.61
PTGS-2	rs5275	T > C	↓ Inf [399]	TT	1.00	0.90	0.66, 1.23	0.89	0.66, 1.20	1.02	0.76, 1.37	0.23	-0.17, 0.62
PPAR-α	rs1800206	C > G	↓ Inf [219]	CC	1.00	1.64	0.91, 2.97	2.05	0.99, 4.25	1.42	0.79, 2.58	-1.26	-2.90, 0.37
FAS	rs2234767	G > A	↓ Inf [400]	GG	1.00	1.35	0.92, 1.98	1.24	0.78, 1.97	1.26	0.86, 1.85	-0.32	-1.00, 0.36
FAS	rs1800682	G > A	↑ Inf [400]	GA/AA	1.00	0.92	0.65, 1.32	0.92	0.60, 1.40	0.96	0.67, 1.38	0.12	-0.33, 0.57
FASL	rs763110	C > T	↓ Inf [401]	CC	1.00	1.01	0.73, 1.39	0.92	0.70, 1.20	1.22	0.88, 1.70	0.30	-0.17, 0.77
TNF-α	rs1800629	G > A	↑ Inf [402]	GA/AA	1.00	0.81	0.56, 1.16	0.89	0.69, 1.15	1.19	0.85, 1.68	0.49	0.03, 0.96
MnSOD	rs4880	C > T	↓ Os [403]	CT/TT	1.00	1.41	0.95, 2.10	1.45	0.92, 2.30	1.27	0.85, 1.90	-0.59	-1.37, 0.20
MPO	rs2333227	G > A	↓ Os [404]	GA/AA	1.00	1.14	0.83, 1.56	1.12	0.84, 1.47	0.95	0.68, 1.32	-0.30	-1.37, 0.20
CAT	rs1001179	C > T	↑ Os [405]	CC	1.00	0.65	0.47, 0.89	0.93	0.67, 1.29	0.70	0.51, 0.95	0.12	-0.24, 0.48
GPX1	rs1050450	C > T	↑ Os [406]	CC	1.00	1.35	0.98, 1.85	1.27	0.94, 1.71	1.05	0.76, 1.44	-0.56	-1.14, 0.02
GSTM1	deletion	deletion	↑ Os [407]	present	1.00	1.06	0.77, 1.47	1.00	0.71, 1.39	1.09	0.80, 1.48	0.03	-0.44, 0.49
GSTP1	rs1695	A > G	↑ Os [408]	AA	1.00	0.88	0.64, 1.20	0.93	0.68, 1.26	0.94	0.69, 1.28	0.14	-0.26, 0.54
GSTT1	deletion	deletion	↑ Os [407]	present	1.00	1.34	0.90, 2.01	1.43	0.86, 2.38	1.22	0.81, 1.82	-0.56	-1.38, 0.27
GSTA1	rs3957356	G > A	↑ Os [409]	GG	1.00	0.89	0.63, 1.26	0.92	0.71, 1.21	1.09	0.80, 1.49	0.27	-0.16, 0.71
COMT	rs4680	G > A	↑ Os [412]	GG	1.00	1.25	0.89, 1.75	1.07	0.83, 1.39	1.14	0.81, 1.61	-0.18	-0.74, 0.38
COMT	rs737865	C > T	↓ Os [410]	CT/TT	1.00	1.30	0.68, 2.47	0.90	0.40, 2.02	1.40	0.74, 2.65	0.20	-0.55, 0.95
CYP17	rs743572	T > C	↑ Es [411]	TC/CC	1.00	0.86	0.62, 1.18	0.93	0.64, 1.35	0.88	0.65, 1.20	0.10	-0.32, 0.51

Note:
Inf = inflammation, Os = oxidative stress, Es = estrogen, LIBCSP = Long Island Breast Cancer Study Project
[a] Hypothesized lowest risk group (referent group) - low risk genotype for PUFA-gene interaction, high ω-3/ω-6 ratio.
[b] Hypothesized highest risk group - high risk genotype for PUFA-gene interaction, low ω-3/ω-6 ratio.
[c] RERI (Relative Excess Risk Due to Interaction) = HR_{11} - HR_{10} - HR_{01} + HR_{00} (e.g., RERI for PTGS-2 rs20417 = 1.08 - 0.87 - 1.03 + 1.00 = 0.19).
[d] 95% CI for RERI estimated using Hosmer & Lemeshow [374]

CHAPTER 5:
CONCLUSIONS

5.1 Summary of Study Aims and Results

The purpose of this dissertation was to examine the impact of dietary intake of polyunsaturated fatty acids (PUFAs) and fish (a major source of beneficial PUFAs) and, genetic susceptibility in biologically relevant genetic pathways (i.e., inflammation, oxidative stress, and estrogen metabolism) on: the risk of developing breast cancer (Aim 1); and survival following a new breast cancer diagnosis (Aim 2). To address these aims, data from a population-based sample of women residing on Long Island, New York were utilized.

Previous studies undertaken to address whether PUFAs are associated with the risk of developing and dying from breast cancer were limited and inconsistent, when focused on populations in the U.S. and other western countries [270, 296, 298, 339]. Thus, the *a priori* study hypothesis was, given that experimental studies suggest that ω-3 PUFAs inhibit production of inflammatory eicosanoids from ω-6 PUFA metabolism [155], simultaneous consideration of both ω-3 and ω-6 PUFA intake, or interactions with genetic polymorphisms in biologically relevant pathways, would facilitate identification of PUFA-breast cancer associations in a U.S. population.

Key dissertation results were as follows. For Aim 1, a 43% excess risk for developing breast cancer was observed among women who consumed high ω-6 and low ω-3 PUFAs compared to those consuming low ω-6 and high ω-3. No interactions were observed with polymorphisms considered, but odds were elevated for low ω-3/ω-6 ratio across

genotypes. Additionally, notable associations with dietary intake of fish or individual PUFA subtypes in relation to breast cancer incidence were not observed.

For Aim 2, key dissertation results included the following. Among a population-based sample of women who were newly-diagnosed with their first primary breast cancer, reductions in the hazard for all-cause mortality (16-34%) were observed among those consuming highest levels of long-chain (LC) ω-3 PUFA (i.e., DPA, DHA, and EPA). Additionally, similar hazard reductions were noted among those with high dietary intakes of tuna and other fish (broiled or baked). Interactions between PUFAs and the select genotypes considered in relation to all-cause mortality were not observed.

5.2 Summary of Public Health Impact

The burden of breast cancer in the U.S. remains high, with approximately 232,000 new diagnoses and nearly 40,000 deaths per year [1]. Despite this high burden, few easily modifiable strategies exist for reducing risk of breast cancer development, or improving survival after diagnosis. Thus, the results from this ancillary study suggest that dietary intake of ω-3 may provide an opportunity to reduce breast cancer risk and mortality following diagnosis. Specifically, women who consume unfavorable diets of high ω-6 and low ω-3 may be at higher risk for developing breast cancer compared to those consuming a more favorable diet of low ω-6 and high ω-3. Additionally, women diagnosed with breast cancer may want to consider increasing dietary intake of fish, and consuming more foods that are high in LC ω-3 PUFAs. If confirmed by future investigations, interventions targeted towards increasing consumption of LC ω-3 PUFA may be warranted.

5.3 Study Strengths

This study has a number of strengths. First, the study aims are innovative. Regarding Aim 1, this study is the first to consider an interaction between dietary intake of ω-3 and ω-6 PUFAs and breast cancer incidence among a U.S. population. Regarding Aim 2, this study is the first to identify risk reductions for long-chain ω-3 PUFAs in relation to all-cause mortality using a population-based sample in the U.S. Additionally, this study is also the first to examine the interaction between PUFA intake and genetic polymorphisms in multiple relevant biologic pathways in a population-based sample for both incidence and survival.

Another strength of this dissertation is that the major classes of PUFAs (ω-3 and ω-6), and their individual subtypes (ω-3: ALA, EPA, DPA, DHA; and ω-6: LA, AA) were estimated. This approach maximizes the likelihood of improved exposure assessment of the PUFA classes, thus facilitating examination of both ω-3 and ω-6 simultaneously. Fish and other marine sources are considered the predominant source of the LC ω-3 [146]. However, for many Americans foods other than fish are important contributors to their PUFA intake (**Tables 3.1 and 4.1**). Thus, for this dissertation multiple PUFA subtypes were derived from marine and other food sources, to improve measurement of PUFA intake in the LIBCSP population.

To enhance the likelihood of PUFA intake variability in a U.S. population, this ancillary study was conducted among women residing on Long Island, New York, a population that is reported to have higher fish intake compared to the national estimates from the National Health and Nutrition Examination Survey (NHANES) [391]. These NHANES findings were confirmed by the LIBCSP data; the LIBCSP reported mean consumption of 8.1 g/day among cases and 11.9 g/day among controls was higher than the reported mean tuna

intake of 5.9 g/day in the general NHANES population sample of the U.S. [392]. It is possible, however, that the LIBCSP FFQ estimate are inaccurate, given that the FFQ is designed to provide relative rather than absolute intake. Nevertheless, the LIBCSP may be one of the ideal populations to examine PUFAs and fish intake in relation to breast cancer incidence and mortality in the U.S. Despite the relatively higher fish consumption in the Long Island population compared to the general U.S. population, fish consumption in the LIBCSP population was much lower than the fish intake reported among Asian populations [146, 391, 392]. However, in the LIBCSP, intake of ω-3 was low relative to ω-6 intake, which is consistent with PUFA intake estimates reported for other Western populations [153, 154].

An additional benefit of the approach used in this dissertation is the population-based design of the LIBCSP, where cases in a circumscribed geographic area were ascertained within three months of diagnosis ("super-rapid identification"), and controls were incidence-density sampled. This population-based approach allows for stronger inferences regarding breast cancer incidence [373]. Also, to ascertain outcomes for the follow-up study (Aim 2), vital status for the population-based cases was determined using the NDI, a standard resource for assessing mortality in the U.S., with high sensitivity [358].

Finally, the LIBCSP is a rich resource with which to address the study aims. Existing LIBCSP data available for this dissertation included: FFQ data, which was assessed using a validated 101-item Block FFQ [359, 395, 396]; genetic polymorphisms in biologically relevant genes; and 15 years post-diagnosis vital status of the population-based cohort of women with breast cancer. Thus, the study design was cost-efficient and utilized extant resources from a population-based study.

5.4 Study Limitations

There are several limitations to this study. The LIBCSP study population includes primarily Caucasian and postmenopausal women; therefore examination of potential racial differences was not possible. However, the results are generalizable to the subgroup of women who are at high risk for developing breast cancer in the U.S. [1].

For the case-control approach utilized in Aim 1, it is not possible to rule out recall bias, where it is possible that cases and controls may differentially recall foods high PUFA content. Also, a single dietary assessment via FFQ may not necessarily reflect diet during the etiologically relevant time period for breast cancer development. Similarly with regard to possible measurement error for Aim 2 follow-up approach, a one-time dietary assessment via FFQ is unable to assess changes in diet that may have occurred following breast cancer diagnosis. Although, a recent study has reported that intake of oily fish and fish oil increases post breast cancer diagnosis [420], thus suggesting that the estimate reported in this dissertation for PUFA intake near time of diagnosis may be conservative.

Another limitation, relevant for both Aims 1 and 2, is the potential errors associated with dietary PUFA assessment via linkage with the USDA database. It is possible that the PUFA content in foods available in the USDA database differs from those actually consumed by LIBCSP participants. This could be due to a variety of reasons, including geographic differences in harvesting, storage, processing, and cooking methods [147, 417, 418]. For example, the nutrient composition of wild versus farmed fish of the same species differs substantially, where the farmed fish tend to contain lower amounts of LC ω-3 PUFAs [421]. Furthermore, the food sources for various ω-3 PUFA subtypes differ, and thus ω-3 content

obtained from these different sources could vary due to differences in food storage. For example, ALA is primarily obtained via vegetable and plant-derived oils, which are prone to oxidation due to prolonged storage [417]. Any observed benefit of ALA may be masked due to oxidation-induced ALA loss, thus further lowering formation of downstream LC ω-3 PUFAs resulting from ALA metabolism. However, absolute PUFA measurement error may be less of a concern since this dissertation considered PUFA via relative ranking of individuals, using quartiles.

Another concern regarding PUFA measurement for Aims 1 and 2 is that the parent LIBCSP did not include assessment of consumption of different fish species, other than tuna. Levels of LC ω-3 PUFA differ by fish species [419]. Tuna, however, is the most common fish consumed in the U.S. and is a major food source of LC ω-3 PUFA [391]. Nonetheless, exposure assessment would have been improved if the LIBCSP participants had been also asked about their consumption of other specific fish species that are also high in LC ω-3 and may be commonly consumed in the U.S., such as salmon (rather than grouping all other fish species together). Additionally, although LIBCSP participants were queried about their cooking practices, the prevalence of fish intake was relatively low, which limits inferences regarding the impact of cooking methods due to small sample sizes. However, even with a larger sample, more detailed information would be required on factors affecting PUFA content (e.g., cooking time, type of oil used if fried, type of fish consumed) in order to more accurately assess the impact of different cooking methods on PUFA content, and its subsequent relation with breast cancer risk and mortality.

Another limitation of this dissertation is the potential for inadequate coverage of genes involved in related biologic pathways. Although key putatively functional SNPs

involved in relevant pathways were considered (i.e., inflammation, oxidative stress, estrogen metabolism), it remains possible that some PUFA-gene interactions may have been missed due to failure to consider other relevant SNPs. For example, genes involved in the *in vivo* metabolism of PUFA, namely LA and ALA (**Figure 1.2**) may interact with PUFAs to influence breast carcinogenesis, given their role in PUFA bioavailability. The efficiency of enzymes involved in this metabolism, in combination with dietary intake of PUFAs could influence consequent eicosanoid production. For example, it has been reported that the conversion of ALA into LC ω-3 is highly inefficient in populations consuming high ω-6 [150], thus further hindering the potential benefit derived from ALA consumption. However, a recent dietary intervention conducted among subjects with high ω-6 intake at baseline, observed increases in LC ω-3 PUFA plasma concentrations among subjects who lowered their ω-6 intake, thus suggesting improved enzyme efficiency in ALA to LC ω-3 PUFA metabolism in populations consuming high ω-6 [416]. Thus, consideration of these additional genes, in concert with dietary intake of PUFAs, may further elucidate the relation between PUFAs and breast cancer.

Finally, this dissertation had limited study power to make inferences regarding breast-cancer specific mortality, because of the low number of deaths due to breast cancer in the LIBCSP study population even after 15 years of follow-up. However, the magnitude of the effect estimates for breast cancer-specific mortality was similar to those for all-cause mortality, for both 5-year survival as well as the entire 15-year follow-up. Thus, these findings are consistent with the proposed biology of PUFAs when considering the relation with breast-cancer specific mortality, though estimates were imprecise.

5.5 Future Directions

Ideally, future studies, utilizing a cohort approach, should consider measuring repeated PUFA exposure assessments, via multiple FFQs, 24-hour recalls, or biomarkers, throughout the etiologically relevant periods in order to better capture dietary changes both before and after a diagnosis of breast cancer. However, each method of PUFA assessment has its strengths and limitations. Repeated FFQs may provide a benefit over repeated 24-hour recalls since FFQs typically assess usual intake and have the potential to capture long-term changes in dietary habits. Whereas, repeated 24-hour recalls are subject to day-to-day variations in intake, and may not accurately assess long-term patterns of intake. In comparison, biomarkers provide an objective measure of PUFA intake. However, they may reflect very different exposure windows depending on the type of biomarker measured (adipose tissue, red-blood cells, or serum) [272]. Similar to 24-hour recalls, serum PUFA biomarkers provide an objective measure of short-term intake. Adipose tissue biomarkers could reflect fat intake up to one year post-biopsy. However, the possibility for selection bias due to the invasive procedure of obtaining an adipose tissue sample via needle aspiration needs to be considered. Also, adipose tissue biomarkers are also subject to measurement error in the presence of weight changes. Red-blood cell biomarkers reflect up to three months of intake, however, issues with membrane degradation due to oxidation during storage have been reported [273]. The window of exposure reflected by each PUFA assessment varies; thus, consideration needs to be given in order to identify relevant PUFA exposures in relation to breast cancer development and prognosis.

Another consideration of the approach used in this dissertation is the potential limitations of a ratio measure of ω-3 and ω-6 in populations with low intake. Future studies

with improved power may also want to consider three-level interactions between ω-6, ω-3, and genetic polymorphisms in relation to both the risk of developing breast cancer and the risk of dying from breast cancer. This alternative approach would better reflect the interaction between PUFAs and breast cancer in Aim 1, and perhaps improve the likelihood of detecting an interaction with biologically relevant genetic polymorphisms. With regards to Aim 2, given the low prevalence of ω-3-rich foods in the U.S., future follow-up studies should increase the study sample size to facilitate examination of interactions between the exposure for which the effect estimates were strongest in this dissertation (e.g., fish, or LC ω-3 fatty acids), and genetic polymorphisms. Finally, future studies should include a larger sample population to enhance the likelihood of confirming the exploratory findings regarding LC ω-3 and breast cancer-specific mortality observed in this dissertation. Also, a larger sample size would facilitate examination of: additional genes involved in PUFA metabolism; a more detailed consideration of fish cooking methods; and modification by breast cancer subtypes and/or race.

5.6 Conclusion

Findings from this dissertation indicate that higher consumption of ω-6 fatty acids in combination with low ω-3 may increase breast cancer risk compared to those women consuming lower ω-6 and higher ω-3. Increased risks for breast cancer were evident regardless of polymorphisms in relevant genes. Additionally, higher consumption of LC ω-3 PUFAs following a first-primary diagnosis of breast cancer may reduce risk of mortality. Strategies aimed to increase dietary intake of ω-3 relative to ω-6 (e.g., via fish consumption or fish oil supplementation) may be warranted, pending confirmation from future studies.

APPENDIX: TABLES & FIGURES

Table A.1 Pearson correlation between PUFA subtypes among 1,500 LIBCSP controls

	ALA	**DPA**	**DHA**	**EPA**	**LA**	**AA**
ALA	1					
DPA	0.1816 p<0.0001	1				
DHA	0.0745 p=0.0039	0.9348 p<0.0001	1			
EPA	0.0645 p=0.0125	0.9162 p<0.0001	0.9515 p<0.0001	1		
LA	0.8441 p<0.0001	0.19488 p<0.0001	0.0811 p=0.0017	0.0697 p=0.0069	1	
AA	0.3756 p<0.001	0.7135 p<0.0001	0.5798 p<0.0001	0.52051 p<0.0001	0.3588 p<0.0001	1

Table A.2 Pearson correlation between PUFA subtypes among 1,463 LIBCSP cases

	ALA	**DPA**	**DHA**	**EPA**	**LA**	**AA**
ALA	1					
DPA	0.1014 p=0.0001	1				
DHA	0.0135 p=0.6061	0.9480 p<0.0001	1			
EPA	0.01501 p=0.5662	0.9344 p<0.0001	0.9730 p<0.0001	1		
LA	0.8237 p<0.0001	0.1251 p<0.0001	0.0305 p=0.2431	0.0324 p=0.2160	1	
AA	0.2948 p<0.001	0.7551 p<0.0001	0.6307 p<0.0001	0.6016 p<0.0001	0.3076 p<0.0001	1

Table A.3 Age- and energy-adjusted ORs and 95% CI for the interaction between total PUFA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	Low intake				High intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	196	196	1.00		206	181	1.01	0.74, 1.36		
rs20417	GG	345	315	0.91	0.71, 1.17	329	338	1.17	0.89, 1.54	0.25	-0.08, 0.59
PTGS-2	TC/CC	305	293	1.00		314	277	1.06	0.82, 1.37		
rs5275	TT	235	221	0.98	0.77, 1.26	219	242	1.30	0.99, 1.70	0.25	-0.11, 0.62
PPAR-α	GC/GG	39	48	1.00		53	53	0.96	0.54, 1.74		
rs18000206	CC	432	383	0.73	0.47, 1.14	413	394	0.88	0.55, 1.40	0.19	-0.34, 0.72
FAS	GA/AA	133	123	1.00		118	114	1.19	0.81, 1.73		
rs2234767	GG	408	390	1.01	0.76, 1.34	417	402	1.17	0.87, 1.59	-0.02	-0.47, 0.43
FAS	GG	138	124	1.00		163	150	1.17	0.83, 1.66		
rs1800682	GA/AA	398	384	1.09	0.82, 1.44	372	366	1.29	0.95, 1.75	0.03	-0.40, 0.46
FASL	CT/TT	376	351	1.00		348	357	1.25	0.99, 1.59		
rs763110	CC	164	165	1.07	0.82, 1.39	187	162	1.07	0.80, 1.42	-0.25	-0.67, 0.16
TNF-α	GG	388	379	1.00		396	379	1.13	0.90, 1.43		
rs1800629	GA/AA	146	122	0.86	0.65, 1.14	139	139	1.19	0.88, 1.60	0.20	-0.21, 0.60
MnSOD	CC	129	112	1.00		130	138	1.39	0.96, 2.01		
rs4880	CT/TT	400	382	1.09	0.82, 1.46	398	374	1.25	0.91, 1.72	-0.23	-0.75, 0.28
MPO	GG	328	317	1.00		305	312	1.24	0.96, 1.59		
rs2333227	GA/AA	206	183	0.93	0.72, 1.19	224	203	1.08	0.83, 1.42	-0.08	-0.46, 0.30
CAT	CT/TT	196	200	1.00		184	195	1.19	0.88, 1.62		
rs1001179	CC	335	298	0.86	0.67, 1.11	344	316	1.04	0.79, 1.37	-0.01	-0.39, 0.37
GPX1	CT/TT	292	278	1.00		260	272	1.28	0.99, 1.67		
rs1050450	CC	242	223	0.98	0.76, 1.25	267	240	1.10	0.85, 1.44	-0.16	-0.55, 0.24
GSTM1	Null	208	243	1.00		235	214	0.89	0.67, 1.18		
deletion	Present	275	222	0.68	0.53, 0.88	257	271	1.04	0.78, 1.38	0.47	0.18, 0.76
GSTP1	AG/GG	260	226	1.00		272	273	1.33	1.02, 1.75		
rs1695	AA	258	270	1.22	0.95, 1.57	251	230	1.23	0.93, 1.63	-0.33	-0.77, 0.12
GSTT1	Null	110	104	1.00		104	99	1.17	0.78, 1.77		
deletion	Present	381	364	1.01	0.74, 1.37	394	390	1.19	0.86, 1.65	0.01	-0.47, 0.49
GSTA1	GA/AA	340	345	1.00		350	334	1.10	0.86, 1.39		
rs3957356	GG	191	156	0.81	0.63, 1.06	181	178	1.13	0.86, 1.50	0.22	-0.13, 0.58
COMT	AG/AA	392	358	1.00		405	382	1.21	0.96, 1.53		
rs4680	GG	140	146	1.16	0.88, 1.52	127	134	1.32	0.97, 1.79	-0.05	-0.53, 0.43
COMT	CC	60	53	1.00		50	46	1.17	0.67, 2.05		
rs737865	CT/TT	471	445	1.06	0.71, 1.57	481	458	1.22	0.81, 1.84	0.00	-0.64, 0.63
CYP17	TT	185	168	1.00		190	177	1.17	0.85, 1.61		
rs743572	TC/CC	339	330	1.07	0.82, 1.38	329	326	1.24	0.93, 1.65	0.00	-0.41, 0.41

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.4 Age- and energy-adjusted ORs and 95% CI for the interaction between total ω-3 intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	High intake				Low intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	207	181	1.00		195	196	1.08	0.79, 1.46		
rs20417	GG	337	323	1.10	0.85, 1.42	337	330	1.04	0.79, 1.36	-0.14	-0.53, 0.25
PTGS-2	TC/CC	317	274	1.00		302	296	1.05	0.81, 1.35		
rs5275	TT	225	229	1.16	0.91, 1.49	229	234	1.09	0.83, 1.42	-0.12	-0.51, 0.26
PPAR-α	GC/GG	48	57	1.00		44	44	0.76	0.42, 1.37		
rs18000206	CC	426	377	0.72	0.48, 1.09	419	400	0.73	0.47, 1.12	0.24	-0.22, 0.70
FAS	GA/AA	127	113	1.00		124	125	1.07	0.73, 1.55		
rs2234767	GG	418	385	1.04	0.78, 1.39	407	407	1.04	0.76, 1.42	-0.07	-0.50, 0.37
FAS	GG	165	145	1.00		136	129	1.02	0.72, 1.44		
rs1800682	GA/AA	380	352	1.08	0.83, 1.42	390	398	1.10	0.83, 1.46	0.00	-0.40, 0.40
FASL	CT/TT	351	329	1.00		373	379	1.01	0.79, 1.27		
rs763110	CC	194	172	0.94	0.73, 1.22	157	155	0.97	0.72, 1.30	0.02	-0.33, 0.38
TNF-α	GG	391	358	1.00		393	400	1.03	0.81, 1.29		
rs1800629	GA/AA	153	142	1.02	0.78, 1.34	132	119	0.91	0.66, 1.23	-0.14	-0.53, 0.25
MnSOD	CC	133	125	1.00		126	125	0.99	0.69, 1.43		
rs4880	CT/TT	404	369	0.99	0.74, 1.31	394	387	0.97	0.72, 1.31	-0.01	-0.41, 0.39
MPO	GG	305	304	1.00		328	325	0.91	0.71, 1.17		
rs2333227	GA/AA	233	194	0.84	0.65, 1.07	197	192	0.89	0.68, 1.18	0.15	-0.16, 0.46
CAT	CT/TT	349	296	1.00		193	195	0.87	0.64, 1.18		
rs1001179	CC	187	200	0.80	0.62, 1.03	330	318	0.82	0.62, 1.09	0.15	-0.15, 0.46
GPX1	CT/TT	262	231	1.00		278	285	0.97	0.74, 1.26		
rs1050450	CC	274	265	0.92	0.72, 1.18	247	232	0.89	0.68, 1.18	0.01	-0.32, 0.33
GSTM1	Null	239	214	1.00		204	243	1.25	0.94, 1.67		
deletion	Present	259	249	1.08	0.84, 1.40	273	244	0.93	0.70, 1.22	-0.41	-0.84, 0.02
GSTP1	AG/GG	275	257	1.00		257	242	0.93	0.71, 1.22		
rs1695	AA	256	231	0.97	0.76, 1.25	253	269	1.06	0.81, 1.38	0.16	-0.18, 0.49
GSTT1	Null	107	101	1.00		107	102	0.94	0.63, 1.42		
deletion	Present	397	367	0.98	0.72, 1.33	378	387	1.00	0.72, 1.40	0.08	-0.33, 0.49
GSTA1	GA/AA	355	323	1.00		335	356	1.07	0.84, 1.36		
rs3957356	GG	185	172	1.03	0.80, 1.34	187	162	0.88	0.66, 1.16	-0.23	-0.60, 0.15
COMT	AG/AA	418	365	1.00		379	375	1.02	0.81, 1.29		
rs4680	GG	124	134	1.20	0.90, 1.59	143	146	1.07	0.80, 1.44	-0.15	-0.59, 0.29
COMT	CC	56	46	1.00		54	53	1.11	0.64, 1.95		
rs737865	CT/TT	484	443	1.12	0.74, 1.70	468	460	1.11	0.72, 1.71	-0.12	-0.77, 0.52
CYP17	TT	192	175	1.00		183	170	0.95	0.69, 1.30		
rs743572	TC/CC	336	311	1.01	0.78, 1.30	332	345	1.06	0.80, 1.40	0.10	-0.25, 0.46

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.5 Age- and energy-adjusted ORs and 95% CI for the interaction between ALA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	High intake				Low intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	209	182	1.00		193	195	1.06	0.78, 1.43		
rs20417	GG	335	337	1.15	0.90, 1.48	339	316	0.97	0.74, 1.27	-0.24	-0.64, 0.16
PTGS-2	TC/CC	316	280	1.00		303	290	0.97	0.76, 1.25		
rs5275	TT	226	238	1.17	0.91, 1.49	228	225	1.01	0.77, 1.32	-0.13	-0.51, 0.24
PPAR-α	GC/GG	48	59	1.00		44	42	0.67	0.37, 1.21		
rs18000206	CC	424	383	0.70	0.47, 1.06	421	394	0.67	0.44, 1.03	0.30	-0.12, 0.71
FAS	GA/AA	125	119	1.00		126	119	0.91	0.62, 1.32		
rs2234767	GG	419	395	0.99	0.74, 1.32	406	397	0.93	0.68, 1.26	0.03	-0.35, 0.42
FAS	GG	163	143	1.00		138	131	1.00	0.71, 1.41		
rs1800682	GA/AA	382	367	1.12	0.86, 1.47	388	383	1.05	0.79, 1.39	-0.08	-0.49, 0.33
FASL	CT/TT	354	351	1.00		370	357	0.89	0.70, 1.12		
rs763110	CC	190	166	0.88	0.68, 1.14	161	161	0.91	0.69, 1.22	0.14	-0.19, 0.47
TNF-α	GG	390	366	1.00		394	392	0.96	0.76, 1.21		
rs1800629	GA/AA	154	148	1.02	0.78, 1.34	131	113	0.83	0.61, 1.14	-0.15	-0.53, 0.23
MnSOD	CC	132	129	1.00		127	121	0.91	0.63, 1.31		
rs4880	CT/TT	403	379	0.99	0.74, 1.31	395	377	0.89	0.66, 1.20	0.00	-0.39, 0.38
MPO	GG	311	314	1.00		322	315	0.87	0.68, 1.12		
rs2333227	GA/AA	227	198	0.86	0.67, 1.10	203	188	0.83	0.63, 1.09	0.09	-0.21, 0.40
CAT	CT/TT	185	206	1.00		195	189	0.78	0.58, 1.06		
rs1001179	CC	350	304	0.78	0.61, 1.01	329	310	0.76	0.57, 1.00	0.19	-0.09, 0.47
GPX1	CT/TT	270	271	1.00		282	279	0.88	0.68, 1.14		
rs1050450	CC	265	239	0.90	0.70, 1.15	244	224	0.83	0.63, 1.09	0.05	-0.26, 0.36
GSTM1	Null	236	218	1.00		207	239	1.14	0.86, 1.52		
deletion	Present	260	260	1.09	0.85, 1.40	272	233	0.84	0.63, 1.11	-0.39	-0.80, 0.02
GSTP1	AG/GG	277	264	1.00		255	255	0.87	0.66, 1.13		
rs1695	AA	252	240	1.01	0.79, 1.29	257	260	0.96	0.74, 1.26	0.09	-0.24, 0.42
GSTT1	Null	109	104	1.00		105	99	0.90	0.60, 1.35		
deletion	Present	392	377	1.00	0.73, 1.35	383	377	0.93	0.67, 1.29	0.04	-0.37, 0.44
GSTA1	GA/AA	354	338	1.00		336	341	0.95	0.75, 1.21		
rs3957356	GG	185	171	0.97	0.75, 1.26	187	163	0.83	0.63, 1.10	-0.09	-0.44, 0.25
COMT	AG/AA	415	380	1.00		382	360	0.92	0.73, 1.15		
rs4680	GG	127	133	1.11	0.84, 1.47	140	147	1.04	0.77, 1.39	0.01	-0.40, 0.42
COMT	CC	55	45	1.00		55	54	1.08	0.62, 1.89		
rs737865	CT/TT	485	458	1.15	0.76, 1.75	467	445	1.06	0.69, 1.63	-0.18	-0.83, 0.47
CYP17	TT	191	187	1.00		184	158	0.79	0.57, 1.08		
rs743572	TC/CC	334	315	0.95	0.74, 1.23	334	341	0.94	0.71, 1.24	0.20	-0.11, 0.51

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.6 Age- and energy-adjusted ORs and 95% CI for the interaction between DPA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	High intake				Low intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	208	200	1.00		194	177	0.89	0.67, 1.20		
rs20417	GG	321	328	1.06	0.82, 1.35	353	325	0.90	0.70, 1.17	-0.05	-0.39, 0.30
PTGS-2	TC/CC	303	291	1.00		316	279	0.87	0.68, 1.10		
rs5275	TT	224	238	1.09	0.86, 1.40	230	225	0.96	0.74, 1.23	-0.01	-0.35, 0.34
PPAR-α	GC/GG	40	57	1.00		52	44	0.58	0.33, 1.03		
rs18000206	CC	425	395	0.65	0.42, 1.00	420	382	0.60	0.39, 0.93	0.37	0.01, 0.74
FAS	GA/AA	135	107	1.00		116	131	1.34	0.93, 1.93		
rs2234767	GG	395	420	1.33	1.00, 1.78	430	372	1.02	0.76, 1.37	-0.65	-1.24, -0.06
FAS	GG	395	420	1.00		430	372	0.83	0.59, 1.16		
rs1800682	GA/AA	135	107	1.05	0.80, 1.37	116	131	0.93	0.70, 1.23	0.06	-0.30, 0.41
FASL	CT/TT	361	354	1.00		363	354	0.95	0.76, 1.18		
rs763110	CC	169	175	1.07	0.83, 1.39	182	152	0.80	0.61, 1.05	-0.22	-0.58, 0.15
TNF-α	GG	407	392	1.00		377	366	0.95	0.77, 1.17		
rs1800629	GA/AA	118	131	1.18	0.88, 1.57	167	130	0.76	0.58, 1.01	-0.36	-0.78, 0.05
MnSOD	CC	118	126	1.00		141	124	0.79	0.55, 1.12		
rs4880	CT/TT	404	388	0.90	0.68, 1.21	394	368	0.83	0.62, 1.11	0.14	-0.20, 0.48
MPO	GG	299	318	1.00		334	311	0.83	0.66, 1.05		
rs2333227	GA/AA	225	204	0.86	0.67, 1.10	205	182	0.78	0.60, 1.02	0.09	-0.21, 0.39
CAT	CT/TT	176	191	1.00		204	204	0.87	0.65, 1.16		
rs1001179	CC	346	326	0.87	0.67, 1.12	333	288	0.75	0.57, 0.98	0.01	-0.31, 0.33
GPX1	CT/TT	289	292	1.00		263	258	0.92	0.72, 1.17		
rs1050450	CC	236	227	0.97	0.76, 1.24	273	236	0.82	0.64, 1.04	-0.07	-0.39, 0.26
GSTM1	Null	203	243	1.00		240	214	0.71	0.54, 0.93		
deletion	Present	281	249	0.75	0.58, 0.97	251	244	0.76	0.59, 0.99	0.30	0.04, 0.57
GSTP1	AG/GG	263	237	1.00		269	262	1.02	0.79, 1.32		
rs1695	AA	249	278	1.25	0.98, 1.60	260	222	0.89	0.69, 1.16	-0.38	-0.78, 0.02
GSTT1	Null	108	112	1.00		106	91	0.79	0.53, 1.17		
deletion	Present	384	387	0.97	0.72, 1.31	391	367	0.85	0.62, 1.16	0.09	-0.29, 0.46
GSTA1	GA/AA	349	347	1.00		341	332	0.93	0.74, 1.15		
rs3957356	GG	173	171	1.02	0.78, 1.32	199	163	0.78	0.60, 1.01	-0.17	-0.52, 0.18
COMT	AG/AA	401	390	1.00		396	350	0.85	0.69, 1.05		
rs4680	GG	123	133	1.10	0.83, 1.46	144	147	0.98	0.74, 1.29	0.03	-0.37, 0.43
COMT	CC	58	45	1.00		52	54	1.30	0.75, 2.26		
rs737865	CT/TT	466	470	1.33	0.88, 2.01	486	433	1.10	0.73, 1.67	-0.53	-1.35, 0.28
CYP17	TT	198	174	1.00		177	171	1.05	0.77, 1.41		
rs743572	TC/CC	316	339	1.22	0.94, 1.58	352	317	0.96	0.74, 1.25	-0.30	-0.72, 0.12

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.7 Age- and energy-adjusted ORs and 95% CI for the interaction between DHA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	High intake				Low intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	205	204	1.00		197	173	0.87	0.65, 1.16		
rs20417	GG	325	324	0.99	0.77, 1.27	349	329	0.93	0.73, 1.20	0.07	-0.26, 0.40
PTGS-2	TC/CC	303	304	1.00		316	266	0.82	0.65, 1.04		
rs5275	TT	225	225	0.98	0.77, 1.25	229	238	1.02	0.80, 1.30	0.21	-0.10, 0.53
PPAR-α	GC/GG	39	53	1.00		53	48	0.66	0.37, 1.17		
rs18000206	CC	428	398	0.67	0.43, 1.04	417	379	0.65	0.42, 1.01	0.32	-0.08, 0.73
FAS	GA/AA	134	108	1.00		117	130	1.35	0.94, 1.94		
rs2234767	GG	397	418	1.29	0.97, 1.73	428	374	1.06	0.79, 1.42	-0.59	-1.16, -0.01
FAS	GG	144	152	1.00		157	122	0.73	0.52, 1.01		
rs1800682	GA/AA	386	375	0.93	0.71, 1.22	384	375	0.92	0.70, 1.21	0.27	-0.04, 0.57
FASL	CT/TT	352	360	1.00		372	348	0.90	0.73, 1.12		
rs763110	CC	178	169	0.93	0.72, 1.20	173	158	0.88	0.68, 1.15	0.05	-0.29, 0.38
TNF-α	GG	408	397	1.00		376	361	0.98	0.80, 1.20		
rs1800629	GA/AA	120	125	1.09	0.82, 1.45	165	136	0.84	0.64, 1.09	-0.23	-0.63, 0.17
MnSOD	CC	119	126	1.00		140	124	0.84	0.59, 1.20		
rs4880	CT/TT	404	388	0.92	0.69, 1.23	394	368	0.88	0.65, 1.17	0.12	-0.23, 0.47
MPO	GG	299	320	1.00		334	309	0.86	0.69, 1.08		
rs2333227	GA/AA	266	200	0.84	0.66, 1.08	204	186	0.84	0.65, 1.08	0.14	-0.17, 0.44
CAT	CT/TT	178	195	1.00		202	200	0.89	0.67, 1.18		
rs1001179	CC	346	320	0.84	0.65, 1.09	333	294	0.79	0.61, 1.03	0.06	-0.25, 0.38
GPX1	CT/TT	287	292	1.00		265	258	0.94	0.74, 1.20		
rs1050450	CC	238	224	0.94	0.73, 1.20	271	239	0.86	0.68, 1.10	-0.02	-0.34, 0.31
GSTM1	Null	204	231	1.00		239	226	0.82	0.63, 1.07		
deletion	Present	281	253	0.79	0.61, 1.02	251	240	0.83	0.64, 1.08	0.23	-0.06, 0.52
GSTP1	AG/GG	262	236	1.00		270	263	1.07	0.84, 1.38		
rs1695	AA	249	276	1.25	0.98, 1.60	260	224	0.94	0.73, 1.22	-0.38	-0.79, 0.03
GSTT1	Null	106	111	1.00		108	92	0.82	0.56, 1.21		
deletion	Present	387	380	0.94	0.70, 1.27	388	374	0.91	0.67, 1.24	0.15	-0.23, 0.52
GSTA1	GA/AA	349	345	1.00		341	334	0.98	0.79, 1.22		
rs3957356	GG	174	170	1.00	0.77, 1.30	198	164	0.83	0.64, 1.07	-0.16	-0.51, 0.20
COMT	AG/AA	400	389	1.00		397	351	0.90	0.73, 1.10		
rs4680	GG	127	133	1.07	0.81, 1.42	140	147	1.05	0.80, 1.38	0.08	-0.32, 0.48
COMT	CC	60	43	1.00		50	56	1.54	0.89, 2.67		
rs737865	CT/TT	465	471	1.42	0.94, 2.15	487	432	1.22	0.81, 1.86	-0.74	-1.67, 0.20
CYP17	TT	211	170	1.00		164	175	1.32	0.98, 1.78		
rs743572	TC/CC	304	344	1.40	1.09, 1.81	364	312	1.05	0.81, 1.35	-0.68	-1.19, -0.16

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.8 Age- and energy-adjusted ORs and 95% CI for the interaction between EPA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	High intake				Low intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	208	205	1.00		194	172	0.88	0.66, 1.18		
rs20417	GG	324	323	1.01	0.79, 1.30	350	330	0.93	0.73, 1.20	0.04	-0.30, 0.37
PTGS-2	TC/CC	304	301	1.00		315	269	0.84	0.66, 1.06		
rs5275	TT	227	228	1.00	0.78, 1.28	227	235	1.02	0.79, 1.30	0.18	-0.15, 0.50
PPAR-α	GC/GG	39	55	1.00		53	46	0.62	0.35, 1.10		
rs18000206	CC	427	396	0.65	0.42, 1.01	418	381	0.63	0.40, 0.97	0.36	-0.02, 0.74
FAS	GA/AA	131	110	1.00		120	128	1.23	0.86, 1.77		
rs2234767	GG	402	416	1.22	0.91, 1.63	423	376	1.02	0.76, 1.37	-0.43	-0.96, 0.10
FAS	GG	152	145	1.00		149	129	0.88	0.63, 1.23		
rs1800682	GA/AA	380	381	1.06	0.81, 1.39	390	369	0.98	0.75, 1.29	0.04	-0.33, 0.41
FASL	CT/TT	366	361	1.00		358	347	0.96	0.78, 1.19		
rs763110	CC	167	168	1.03	0.79, 1.33	184	159	0.86	0.66, 1.11	-0.13	-0.49, 0.23
TNF-α	GG	407	396	1.00		377	362	0.96	0.78, 1.18		
rs1800629	GA/AA	123	124	1.04	0.78, 1.39	162	137	0.85	0.65, 1.12	-0.15	-0.54, 0.24
MnSOD	CC	117	123	1.00		142	127	0.85	0.60, 1.21		
rs4880	CT/TT	408	389	0.92	0.69, 1.23	390	367	0.88	0.66, 1.18	0.11	-0.24, 0.47
MPO	GG	295	315	1.00		338	314	0.86	0.69, 1.08		
rs2333227	GA/AA	234	204	0.83	0.65, 1.06	196	182	0.85	0.65, 1.10	0.16	-0.14, 0.46
CAT	CT/TT	190	191	1.00		190	204	1.04	0.78, 1.38		
rs1001179	CC	336	323	0.95	0.74, 1.23	343	291	0.82	0.63, 1.06	-0.17	-0.54, 0.20
GPX1	CT/TT	292	288	1.00		260	262	1.00	0.79, 1.27		
rs1050450	CC	237	227	0.98	0.77, 1.26	272	236	0.87	0.68, 1.11	-0.12	-0.46, 0.23
GSTM1	Null	210	231	1.00		233	226	0.87	0.66, 1.13		
deletion	Present	276	252	0.83	0.64, 1.07	256	241	0.84	0.64, 1.09	0.14	-0.17, 0.45
GSTP1	AG/GG	259	239	1.00		273	260	1.02	0.79, 1.31		
rs1695	AA	254	272	1.18	0.92, 1.51	255	228	0.95	0.73, 1.22	-0.26	-0.64, 0.13
GSTT1	Null	108	110	1.00		106	93	0.84	0.57, 1.25		
deletion	Present	386	379	0.96	0.71, 1.29	389	375	0.92	0.68, 1.25	0.12	-0.26, 0.50
GSTA1	GA/AA	356	351	1.00		334	328	0.98	0.79, 1.21		
rs3957356	GG	170	162	0.99	0.76, 1.28	202	172	0.85	0.66, 1.09	-0.12	-0.47, 0.24
COMT	AG/AA	398	389	1.00		399	351	0.88	0.71, 1.08		
rs4680	GG	130	130	1.01	0.76, 1.34	137	150	1.08	0.82, 1.42	0.19	-0.20, 0.59
COMT	CC	61	46	1.00		49	53	1.43	0.83, 2.48		
rs737865	CT/TT	466	464	1.34	0.90, 2.02	486	439	1.19	0.79, 1.78	-0.59	-1.45, 0.27
CYP17	TT	201	172	1.00		174	173	1.15	0.85, 1.55		
rs743572	TC/CC	317	341	1.26	0.97, 1.63	351	315	1.02	0.79, 1.32	-0.39	-0.83, 0.06

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.9 Age- and energy-adjusted ORs and 95% CI for the interaction between total ω-6 intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	Low intake				High intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	197	192	1.00		205	185	1.08	0.80, 1.46		
rs20417	GG	343	308	0.92	0.71, 1.18	331	345	1.24	0.94, 1.63	0.24	-0.11, 0.59
PTGS-2	TC/CC	302	287	1.00		317	283	1.11	0.86, 1.42		
rs5275	TT	237	216	0.97	0.75, 1.24	217	247	1.38	1.05, 1.80	0.31	-0.07, 0.68
PPAR-α	GC/GG	42	45	1.00		50	56	1.24	0.69, 2.24		
rs18000206	CC	427	375	0.82	0.53, 1.28	418	402	1.03	0.65, 1.63	-0.03	-0.71, 0.64
FAS	GA/AA	134	124	1.00		117	114	1.23	0.85, 1.79		
rs2234767	GG	406	379	0.99	0.75, 1.32	419	413	1.23	0.91, 1.66	0.00	-0.46, 0.46
FAS	GG	136	117	1.00		165	157	1.28	0.90, 1.81		
rs1800682	GA/AA	399	380	1.12	0.84, 1.49	371	370	1.38	1.01, 1.89	-0.01	-0.47, 0.44
FASL	CT/TT	374	347	1.00		350	361	1.29	1.02, 1.63		
rs763110	CC	165	158	1.03	0.79, 1.34	186	169	1.14	0.86, 1.51	-0.17	-0.59, 0.24
TNF-α	GG	389	371	1.00		395	387	1.20	0.96, 1.51		
rs1800629	GA/AA	144	119	0.87	0.66, 1.15	141	142	1.24	0.93, 1.67	0.17	-0.25, 0.59
MnSOD	CC	127	110	1.00		132	140	1.41	0.98, 2.04		
rs4880	CT/TT	401	373	1.07	0.80, 1.43	397	383	1.31	0.96, 1.80	-0.17	-0.68, 0.34
MPO	GG	329	306	1.00		304	323	1.35	1.05, 1.73		
rs2333227	GA/AA	204	183	0.97	0.75, 1.25	226	203	1.13	0.86, 1.48	-0.19	-0.60, 0.22
CAT	CT/TT	198	193	1.00		182	202	1.32	0.97, 1.78		
rs1001179	CC	332	294	0.89	0.69, 1.15	347	320	1.11	0.84, 1.45	-0.10	-0.51, 0.31
GPX1	CT/TT	297	272	1.00		255	278	1.41	1.08, 1.83		
rs1050450	CC	236	218	1.01	0.79, 1.30	273	245	1.16	0.89, 1.50	-0.26	-0.68, 0.16
GSTM1	Null	205	236	1.00		238	221	0.93	0.70, 1.24		
deletion	Present	277	219	0.68	0.52, 0.88	255	274	1.09	0.82, 1.45	0.49	0.19, 0.79
GSTP1	AG/GG	261	224	1.00		271	275	1.38	1.05, 1.80		
rs1695	AA	256	261	1.20	0.94, 1.54	253	239	1.30	0.99, 1.73	-0.27	-0.73, 0.18
GSTT1	Null	108	101	1.00		106	102	1.22	0.81, 1.83		
deletion	Present	382	357	1.00	0.73, 1.36	393	397	1.25	0.90, 1.73	0.03	-0.45, 0.51
GSTA1	GA/AA	338	336	1.00		352	343	1.16	0.91, 1.47		
rs3957356	GG	192	154	0.81	0.63, 1.06	180	180	1.20	0.90, 1.58	0.23	-0.14, 0.59
COMT	AG/AA	392	348	1.00		405	392	1.30	1.03, 1.63		
rs4680	GG	139	145	1.19	0.90, 1.57	128	135	1.37	1.01, 1.86	-0.11	-0.61, 0.39
COMT	CC	59	52	1.00		51	47	1.18	0.67, 2.06		
rs737865	CT/TT	471	435	1.03	0.69, 1.53	481	468	1.26	0.84, 1.91	0.06	-0.56, 0.68
CYP17	TT	186	163	1.00		189	182	1.28	0.93, 1.76		
rs743572	TC/CC	337	324	1.09	0.84, 1.42	331	332	1.32	0.99, 1.75	-0.05	-0.48, 0.38

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.10 Age- and energy-adjusted ORs and 95% CI for the interaction between LA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	Low intake				High intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	197	191	1.00		205	186	1.09	0.81, 1.48		
rs20417	GG	344	307	0.92	0.71, 1.18	330	346	1.26	0.95, 1.65	0.24	-0.11, 0.60
PTGS-2	TC/CC	302	286	1.00		317	284	1.12	0.87, 1.44		
rs5275	TT	238	215	0.96	0.75, 1.23	216	248	1.40	1.07, 1.83	0.32	-0.06, 0.70
PPAR-α	GC/GG	41	45	1.00		51	56	1.20	0.67, 2.17		
rs18000206	CC	429	373	0.80	0.51, 1.25	416	404	1.02	0.64, 1.63	0.03	-0.62, 0.67
FAS	GA/AA	133	124	1.00		118	114	1.22	0.84, 1.77		
rs2234767	GG	408	377	0.98	0.74, 1.30	417	415	1.24	0.92, 1.67	0.04	-0.41, 0.49
FAS	GG	137	117	1.00		164	157	1.30	0.92, 1.84		
rs1800682	GA/AA	399	378	1.12	0.85, 1.50	371	372	1.41	1.03, 1.92	-0.02	-0.48, 0.45
FASL	CT/TT	374	345	1.00		350	363	1.31	1.04, 1.66		
rs763110	CC	166	158	1.03	0.79, 1.34	185	169	1.16	0.88, 1.54	-0.18	-0.60, 0.25
TNF-α	GG	389	370	1.00		395	388	1.22	0.97, 1.53		
rs1800629	GA/AA	145	118	0.86	0.65, 1.14	140	143	1.27	0.95, 1.71	0.20	-0.22, 0.62
MnSOD	CC	128	110	1.00		131	140	1.45	1.00, 2.09		
rs4880	CT/TT	401	371	1.07	0.80, 1.44	397	385	1.34	0.97, 1.83	-0.19	-0.71, 0.34
MPO	GG	330	306	1.00		303	323	1.37	1.06, 1.76		
rs2333227	GA/AA	204	181	0.96	0.75, 1.24	226	205	1.15	0.88, 1.51	-0.18	-0.59, 0.23
CAT	CT/TT	199	192	1.00		181	203	1.35	1.00, 1.83		
rs1001179	CC	332	293	0.90	0.70, 1.16	347	321	1.13	0.86, 1.48	-0.12	-0.54, 0.30
GPX1	CT/TT	297	272	1.00		255	278	1.42	1.09, 1.84		
rs1050450	CC	237	216	1.00	0.78, 1.28	272	247	1.18	0.91, 1.53	-0.24	-0.66, 0.18
GSTM1	Null	206	236	1.00		237	221	0.94	0.71, 1.26		
deletion	Present	277	218	0.68	0.52, 0.88	255	275	1.11	0.84, 1.47	0.49	0.19, 0.79
GSTP1	AG/GG	262	222	1.00		270	277	1.42	1.08, 1.86		
rs1695	AA	257	261	1.21	0.94, 1.56	252	239	1.34	1.01, 1.77	-0.29	-0.76, 0.17
GSTT1	Null	108	100	1.00		106	103	1.25	0.83, 1.88		
deletion	Present	383	357	1.00	0.74, 1.37	392	397	1.27	0.92, 1.76	0.02	-0.48, 0.51
GSTA1	GA/AA	338	335	1.00		352	344	1.17	0.92, 1.48		
rs3957356	GG	193	153	0.81	0.62, 1.05	179	181	1.22	0.92, 1.62	0.24	-0.13, 0.61
COMT	AG/AA	393	347	1.00		404	393	1.32	1.05, 1.66		
rs4680	GG	139	144	1.19	0.90, 1.57	128	136	1.40	1.03, 1.89	-0.11	-0.61, 0.40
COMT	CC	59	52	1.00		51	47	1.18	0.68, 2.07		
rs737865	CT/TT	472	433	1.02	0.69, 1.52	480	470	1.28	0.85, 1.93	0.08	-0.55, 0.70
CYP17	TT	186	161	1.00		189	184	1.32	0.96, 1.81		
rs743572	TC/CC	338	324	1.10	0.85, 1.43	330	332	1.35	1.02, 1.79	-0.07	-0.51, 0.37

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.11 Age- and energy-adjusted ORs and 95% CI for the interaction between AA intake (low or high) and genetic variants on breast cancer incidence

Gene	Genotype	Low intake				High intake				RERI	95% CI
		Co	Ca	OR	95% CI	Co	Ca	OR	95% CI		
PTGS-2	GC/CC	184	188	1.00		218	189	0.89	0.67, 1.20		
rs20417	GG	351	338	0.95	0.74, 1.22	323	315	1.00	0.76, 1.30	0.15	-0.17, 0.48
PTGS-2	TC/CC	298	306	1.00		321	264	0.83	0.65, 1.06		
rs5275	TT	236	223	0.91	0.71, 1.16	218	240	1.11	0.86, 1.43	0.37	0.05, 0.68
PPAR-α	GC/GG	48	46	1.00		44	55	1.31	0.74, 2.33		
rs18000206	CC	418	403	0.97	0.63, 1.49	427	374	0.92	0.59, 1.42	-0.36	-1.14, 0.42
FAS	GA/AA	125	135	1.00		126	103	0.79	0.55, 1.14		
rs2234767	GG	410	393	0.88	0.66, 1.16	415	399	0.92	0.69, 1.24	0.26	-0.07, 0.59
FAS	GG	134	130	1.00		167	144	0.92	0.66, 1.30		
rs1800682	GA/AA	397	395	1.04	0.78, 1.37	373	355	1.04	0.78, 1.40	0.08	-0.29, 0.45
FASL	CT/TT	361	373	1.00		363	335	0.93	0.74, 1.16		
rs763110	CC	174	157	0.86	0.66, 1.12	177	170	0.98	0.75, 1.28	0.19	-0.14, 0.53
TNF-α	GG	379	389	1.00		405	369	0.93	0.75, 1.15		
rs1800629	GA/AA	151	128	0.83	0.63, 1.09	134	133	1.02	0.77, 1.37	0.27	-0.09, 0.62
MnSOD	CC	132	127	1.00		127	123	1.05	0.73, 1.50		
rs4880	CT/TT	395	382	1.00	0.75, 1.32	403	374	1.01	0.75, 1.36	-0.03	-0.45, 0.38
MPO	GG	329	306	1.00		304	323	1.21	0.95, 1.53		
rs2333227	GA/AA	199	206	1.12	0.87, 1.44	231	180	0.88	0.68, 1.15	-0.44	-0.85, -0.03
CAT	CT/TT	187	208	1.00		193	187	0.90	0.67, 1.21		
rs1001179	CC	338	304	0.80	0.62, 1.02	341	310	0.86	0.66, 1.12	0.16	-0.15, 0.47
GPX1	CT/TT	261	278	1.00		291	272	0.93	0.72, 1.19		
rs1050450	CC	265	237	0.85	0.67, 1.09	244	226	0.93	0.71, 1.20	0.14	-0.17, 0.45
GSTM1	Null	223	232	1.00		220	225	1.04	0.79, 1.36		
deletion	Present	267	247	0.88	0.68, 1.13	265	246	0.95	0.72, 1.24	0.03	-0.32, 0.38
GSTP1	AG/GG	259	268	1.00		273	231	0.86	0.66, 1.11		
rs1695	AA	258	239	0.89	0.70, 1.14	251	261	1.06	0.82, 1.38	0.31	0.00, 0.62
GSTT1	Null	100	90	1.00		114	113	1.17	0.79, 1.75		
deletion	Present	396	391	1.10	0.80, 1.51	379	363	1.12	0.81, 1.56	-0.15	-0.65, 0.36
GSTA1	GA/AA	330	350	1.00		360	329	0.91	0.73, 1.14		
rs3957356	GG	195	167	0.81	0.63, 1.05	177	167	0.95	0.72, 1.24	0.23	-0.09, 0.54
COMT	AG/AA	384	369	1.00		413	371	0.99	0.80, 1.23		
rs4680	GG	143	149	1.09	0.83, 1.43	124	131	1.15	0.85, 1.54	0.07	-0.36, 0.49
COMT	CC	49	55	1.00		61	44	0.68	0.39, 1.18		
rs737865	CT/TT	478	454	0.85	0.57, 1.28	474	449	0.89	0.59, 1.35	0.37	-0.03, 0.76
CYP17	TT	189	177	1.00		186	168	1.01	0.74, 1.37		
rs743572	TC/CC	327	336	1.09	0.85, 1.41	341	320	1.04	0.79, 1.36	-0.06	-0.45, 0.32

Co=Controls, Ca=Cases, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.12 Age- and multivariate-adjusted hazard ratios (HRs) and 95% confidence intervals (CI) for all-cause mortality among a population-based sample of women with breast cancer for the multiplicative interaction between dietary ω-3 and ω-6 intake, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

Model	Low ω-6 (< median)		High ω-6 (> median)		LRT χ^{2c}	p value
	HR	95% CI	HR	95% CI		
Age-adjusted						
High ω-3 (> median)	1.00		1.00			
Low ω-3 (< median)	0.82	0.60, 1.11	0.97	0.71, 1.34	0.58	0.45
Multivariate[a]						
High ω-3 (> median)	1.00		1.00			
Low ω-3 (< median)	0.85	0.62, 1.16	1.01	0.73, 1.41	0.61	0.43

Note:
[a] Multivariate ORs and 95% CI adjusted for age (5-year age group) and total energy intake (kcal/day)
[b] RERI (Relative Excess Risk due to Interaction) = HR_{11} - HR_{10} - HR_{01} +1
[c] 95% CI for RERI estimated using Hosmer & Lemeshow [374]
LIBCSP = Long Island Breast Cancer Study Project

Table A.13 Age- and energy-adjusted hazard ratios (HRs) and 95% confidence intervals (95% CIs) for all-cause mortality for the hypothesized highest risk multiplicative interaction between ω-3/ω-6 ratio and putatively functional genetic polymorphisms among a population-based sample of women with breast cancer, LIBCSP, 1996/1997 through 2011(an average of 14.7 years of follow-up)

Gene	rs#	Allele Substitution	Variant allele function	High risk genotype	High ω-3/ω-6 (≥ median)			Low ω-3/ω-6 (< median)			LRT $\chi 2^c$	p value
					Low risk	High risk		Low risk	High risk			
					HR[a]	HR	95% CI	HR	HR	95% CI		
PTGS-2	rs20417	G > C	↓ Inf [399]	GG	1.00	1.03	0.74, 1.43	1.00	1.25	0.90, 1.73	0.70	0.40
PTGS-2	rs5275	T > C	↓ Inf [399]	TT	1.00	0.90	0.66, 1.23	1.00	1.14	0.84, 1.55	1.16	0.28
PPAR-α	rs1800206	C > G	↓ Inf [219]	CC	1.00	1.64	0.91, 2.97	1.00	0.70	0.42, 1.14	4.86	0.03
FAS	rs2234767	G > A	↓ Inf [400]	GG	1.00	1.35	0.92, 1.98	1.00	1.02	0.71, 1.46	1.09	0.30
FAS	rs1800682	G > A	↑ Inf [400]	GA/AA	1.00	0.92	0.65, 1.32	1.00	1.05	0.75, 1.47	0.25	0.62
FASL	rs763110	C > T	↓ Inf [401]	CC	1.00	1.01	0.73, 1.39	1.00	1.34	0.97, 1.85	1.47	0.23
TNF-α	rs1800629	G > A	↑ Inf [402]	GA/AA	1.00	0.81	0.56, 1.16	1.00	1.33	0.95, 1.88	3.94	0.05
MnSOD	rs4880	C > T	↓ Os [403]	CT/TT	1.00	1.41	0.95, 2.10	1.00	0.88	0.62, 1.23	3.19	0.07
MPO	rs2333227	G > A	↓ Os [404]	GA/AA	1.00	1.14	0.83, 1.56	1.00	0.85	0.62, 1.18	1.56	0.21
CAT	rs1001179	C > T	↑ Os [405]	CC	1.00	0.65	0.47, 0.89	1.00	0.75	0.55, 1.02	0.43	0.51
GPX1	rs1050450	C > T	↑ Os [406]	CC	1.00	1.35	0.98, 1.85	1.00	0.83	0.61, 1.13	4.68	0.03
GSTM1	deletion	deletion	↑ Os [407]	present	1.00	1.06	0.77, 1.47	1.00	1.09	0.79, 1.50	0.01	0.91
GSTP1	rs1695	A > G	↑ Os [408]	AA	1.00	0.88	0.64, 1.20	1.00	1.02	0.75, 1.39	0.43	0.51
GSTT1	deletion	deletion	↑ Os [407]	present	1.00	1.34	0.90, 2.01	1.00	0.85	0.57, 1.26	2.50	0.11
GSTA1	rs3957356	G > A	↑ Os [409]	GG	1.00	0.89	0.63, 1.26	1.00	1.18	0.86, 1.62	1.38	0.24
COMT	rs4680	G > A	↑ Os [412]	GG	1.00	1.25	0.89, 1.75	1.00	1.06	0.76, 1.49	0.44	0.51
COMT	rs737865	C > T	↓ Os [410]	CT/TT	1.00	1.30	0.68, 2.47	1.00	1.56	0.90, 2.69	0.18	0.67
CYP17	rs743572	T > C	↑ Es [411]	TC/CC	1.00	0.86	0.62, 1.18	1.00	0.95	0.68, 1.32	0.19	0.66

Note:
Inf = inflammation, Os = oxidative stress, Es = estrogen, LIBCSP = Long Island Breast Cancer Study Project
[a] Hypothesized lowest risk group (referent group) - low risk genotype for PUFA-gene interaction, high ω-3/ω-6 ratio.
[b] Hypothesized highest risk group - high risk genotype for PUFA-gene interaction, low ω-3/ω-6 ratio.
[c] LRT $\chi 2$ calculated using nested models for the multiplicative interaction .

Table A.14 Age- and energy-adjusted HRs and 95% CI for the interaction between PUFA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	Low intake			High intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	69	1.00		40	0.63	0.42, 0.96		
rs20417	GG	100	0.86	0.63, 1.18	118	1.00	0.72, 1.39	0.51	0.17, 0.85
PTGS-2	TC/CC	99	1.00		76	0.89	0.64, 1.23		
rs5275	TT	71	0.93	0.68, 1.26	83	1.00	0.72, 1.38	0.19	-0.21, 0.58
PPAR-α	GC/GG	17	1.00		13	0.66	0.31, 1.40		
rs18000206	CC	131	0.88	0.53, 1.46	127	0.86	0.51, 1.45	0.32	-0.21, 0.85
FAS	GA/AA	37	1.00		34	0.96	0.59, 1.58		
rs2234767	GG	131	1.17	0.81, 1.69	124	1.12	0.76, 1.66	-0.01	-0.55, 0.53
FAS	GG	41	1.00		46	0.97	0.62, 1.51		
rs1800682	GA/AA	125	0.98	0.69, 1.39	112	0.96	0.65, 1.41	0.02	-0.46, 0.50
FASL	CT/TT	113	1.00		104	0.92	0.69, 1.25		
rs763110	CC	55	1.06	0.77, 1.47	55	1.16	0.81, 1.65	0.17	-0.31, 0.65
TNF-α	GG	124	1.00		112	0.92	0.68, 1.23		
rs1800629	GA/AA	39	0.90	0.63, 1.29	46	1.09	0.76, 1.58	0.28	-0.19, 0.75
MnSOD	CC	33	1.00		43	0.97	0.61, 1.56		
rs4880	CT/TT	128	1.07	0.73, 1.57	112	1.07	0.71, 1.61	0.02	-0.50, 0.54
MPO	GG	104	1.00		95	0.98	0.71, 1.35		
rs2333227	GA/AA	59	0.97	0.70, 1.33	62	0.98	0.70, 1.37	0.03	-0.41, 0.47
CAT	CT/TT	70	1.00		74	1.08	0.76, 1.54		
rs1001179	CC	92	0.75	0.55, 1.03	82	0.70	0.49, 0.98	-0.14	-0.56, 0.29
GPX1	CT/TT	89	1.00		84	1.05	0.76, 1.46		
rs1050450	CC	74	1.12	0.82, 1.52	73	1.04	0.74, 1.45	-0.13	-0.61, 0.35
GSTM1	Null	74	1.00		66	1.07	0.75, 1.52		
deletion	Present	78	1.11	0.80, 1.52	83	1.12	0.79, 1.58	-0.06	-0.55, 0.44
GSTP1	AG/GG	75	1.00		86	1.08	0.76, 1.52		
rs1695	AA	88	1.05	0.77, 1.43	67	0.91	0.63, 1.30	-0.22	-0.70, 0.25
GSTT1	Null	32	1.00		28	0.93	0.54, 1.58		
deletion	Present	123	1.03	0.70, 1.52	123	1.05	0.70, 1.58	0.09	-0.44, 0.62
GSTA1	GA/AA	111	1.00		102	0.99	0.73, 1.34		
rs3957356	GG	53	1.05	0.76, 1.46	55	1.02	0.72, 1.45	-0.03	-0.50, 0.45
COMT	AG/AA	110	1.00		117	1.11	0.82, 1.48		
rs4680	GG	55	1.37	0.99, 1.89	41	1.05	0.71, 1.55	-0.42	-1.01, 0.16
COMT	CC	15	1.00		9	0.72	0.31, 1.67		
rs737865	CT/TT	147	1.26	0.74, 2.14	145	1.26	0.73, 2.17	0.28	-0.38, 0.94
CYP17	TT	59	1.00		54	0.82	0.55, 1.22		
rs743572	TC/CC	104	0.79	0.58, 1.09	102	0.84	0.59, 1.18	0.22	-0.15, 0.59

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.15 Age- and energy-adjusted HRs and 95% CI for the interaction between ω-3 intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	High intake			Low intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	46	1.00		63	1.29	0.86, 1.94		
rs20417	GG	117	1.48	1.06, 2.09	101	1.15	0.79, 1.67	-0.64	-1.31, 0.03
PTGS-2	TC/CC	80	1.00		95	1.04	0.74, 1.44		
rs5275	TT	83	1.16	0.85, 1.58	71	0.93	0.65, 1.31	-0.27	-0.76, 0.21
PPAR-α	GC/GG	15	1.00		15	1.31	0.62, 2.75		
rs18000206	CC	130	1.30	0.76, 2.22	128	1.10	0.62, 1.94	-0.51	-1.56, 0.55
FAS	GA/AA	29	1.00		42	1.48	0.90, 2.45		
rs2234767	GG	131	1.58	1.06, 2.37	124	1.33	0.86, 2.05	-0.73	-1.59, 0.12
FAS	GG	51	1.00		36	0.83	0.53, 1.30		
rs1800682	GA/AA	109	0.93	0.67, 1.30	128	0.89	0.63, 1.27	0.13	-0.30, 0.56
FASL	CT/TT	101	1.00		116	0.99	0.74, 1.34		
rs763110	CC	60	1.24	0.90, 1.71	50	1.06	0.73, 1.53	-0.18	-0.70, 0.34
TNF-α	GG	114	1.00		122	0.95	0.71, 1.28		
rs1800629	GA/AA	45	1.06	0.75, 1.49	40	0.96	0.66, 1.41	-0.04	-0.54, 0.46
MnSOD	CC	39	1.00		37	0.96	0.61, 1.54		
rs4880	CT/TT	117	1.11	0.77, 1.59	123	1.03	0.70, 1.51	-0.04	-0.57, 0.49
MPO	GG	96	1.00		103	0.98	0.71, 1.34		
rs2333227	GA/AA	64	1.08	0.78, 1.48	57	0.87	0.61, 1.25	-0.18	-0.64, 0.28
CAT	CT/TT	77	1.00		67	0.85	0.59, 1.21		
rs1001179	CC	80	0.63	0.46, 0.86	94	0.66	0.47, 0.92	0.18	-0.15, 0.52
GPX1	CT/TT	81	1.00		92	0.98	0.71, 1.36		
rs1050450	CC	78	1.12	0.82, 1.53	69	0.96	0.67, 1.37	-0.14	-0.60, 0.33
GSTM1	Null	71	1.00		69	0.82	0.57, 1.17		
deletion	Present	77	1.00	0.72, 1.38	84	0.95	0.68, 1.34	0.14	-0.28, 0.55
GSTP1	AG/GG	86	1.00		75	0.83	0.59, 1.16		
rs1695	AA	70	0.82	0.60, 1.13	85	0.90	0.64, 1.25	0.25	-0.12, 0.61
GSTT1	Null	32	1.00		28	0.85	0.50, 1.46		
deletion	Present	119	1.04	0.70, 1.54	127	0.95	0.62, 1.46	0.06	-0.46, 0.58
GSTA1	GA/AA	108	1.00		105	0.85	0.63, 1.15		
rs3957356	GG	51	0.90	0.65, 1.26	57	1.02	0.71, 1.45	0.26	-0.16, 0.69
COMT	AG/AA	112	1.00		115	0.90	0.67, 1.21		
rs4680	GG	48	1.10	0.78, 1.54	48	1.09	0.75, 1.56	0.09	-0.41, 0.59
COMT	CC	11	1.00		13	0.90	0.39, 2.04		
rs737865	CT/TT	145	1.39	0.75, 2.57	147	1.32	0.70, 2.49	0.03	-0.79, 0.84
CYP17	TT	58	1.00		55	1.00	0.68, 1.48		
rs743572	TC/CC	101	0.95	0.69, 1.32	105	0.85	0.60, 1.21	-0.11	-0.57, 0.35

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.16 Age- and energy-adjusted HRs and 95% CI for the interaction between ALA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	High intake			Low intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	47	1.00		62	1.17	0.78, 1.75		
rs20417	GG	118	1.37	0.98, 1.93	100	1.11	0.76, 1.60	-0.44	-1.04, 0.16
PTGS-2	TC/CC	80	1.00		95	1.01	0.73, 1.40		
rs5275	TT	85	1.14	0.84, 1.55	69	0.91	0.64, 1.29	-0.24	-0.72, 0.23
PPAR-α	GC/GG	16	1.00		14	1.08	0.51, 2.28		
rs18000206	CC	129	1.17	0.70, 1.98	129	1.02	0.58, 1.76	-0.24	-1.11, 0.64
FAS	GA/AA	32	1.00		39	1.34	0.82, 2.20		
rs2234767	GG	130	1.47	1.00, 2.17	125	1.25	0.83, 1.90	-0.56	-1.32, 0.21
FAS	GG	48	1.00		39	0.80	0.51, 1.24		
rs1800682	GA/AA	112	0.89	0.63, 1.25	125	0.88	0.62, 1.27	0.20	-0.21, 0.60
FASL	CT/TT	108	1.00		109	0.93	0.69, 1.25		
rs763110	CC	55	1.15	0.83, 1.59	55	1.08	0.76, 1.53	0.00	-0.49, 0.49
TNF-α	GG	114	1.00		122	0.93	0.69, 1.24		
rs1800629	GA/AA	48	1.06	0.76, 1.48	37	0.93	0.62, 1.37	-0.06	-0.55, 0.44
MnSOD	CC	43	1.00		33	0.80	0.50, 1.27		
rs4880	CT/TT	116	1.00	0.71, 1.42	124	0.96	0.66, 1.38	0.15	-0.30, 0.61
MPO	GG	97	1.00		102	0.96	0.70, 1.31		
rs2333227	GA/AA	65	1.07	0.78, 1.47	56	0.86	0.60, 1.23	-0.18	-0.63, 0.28
CAT	CT/TT	77	1.00		67	0.86	0.60, 1.22		
rs1001179	CC	83	0.65	0.48, 0.89	91	0.64	0.46, 0.89	0.13	-0.21, 0.48
GPX1	CT/TT	83	1.00		69	0.95	0.68, 1.31		
rs1050450	CC	78	1.09	0.80, 1.49	90	0.95	0.67, 1.35	-0.09	-0.54, 0.36
GSTM1	Null	72	1.00		68	0.77	0.54, 1.10		
deletion	Present	78	0.95	0.69, 1.31	83	0.94	0.66, 1.32	0.21	-0.18, 0.61
GSTP1	AG/GG	89	1.00		72	0.77	0.55, 1.08		
rs1695	AA	71	0.81	0.59, 1.10	84	0.86	0.62, 1.19	0.28	-0.07, 0.64
GSTT1	Null	33	1.00		27	0.82	0.48, 1.40		
deletion	Present	119	1.02	0.69, 1.50	127	0.94	0.62, 1.43	0.10	-0.40, 0.60
GSTA1	GA/AA	106	1.00		107	0.91	0.67, 1.23		
rs3957356	GG	55	1.02	0.74, 1.42	53	0.96	0.67, 1.37	0.03	-0.42, 0.48
COMT	AG/AA	114	1.00		113	0.92	0.68, 1.23		
rs4680	GG	48	1.18	0.84, 1.66	48	1.04	0.72, 1.48	-0.06	-0.58, 0.45
COMT	CC	10	1.00		14	1.02	0.44, 2.33		
rs737865	CT/TT	148	1.51	0.79, 2.87	144	1.39	0.72, 2.71	-0.13	-1.07, 0.81
CYP17	TT	61	1.00		52	1.00	0.67, 1.48		
rs743572	TC/CC	100	0.96	0.70, 1.32	106	0.84	0.60, 1.19	-0.11	-0.57, 0.35

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.17 Age- and energy-adjusted HRs and 95% CI for the interaction between DPA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	High intake			Low intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	46	1.00		63	1.70	1.15, 2.52		
rs20417	GG	101	1.41	1.00, 2.00	117	1.56	1.10, 2.21	-0.56	-1.29, 0.18
PTGS-2	TC/CC	76	1.00		99	1.47	1.07, 2.02		
rs5275	TT	71	1.16	0.84, 1.61	83	1.32	0.96, 1.81	-0.31	-0.89, 0.26
PPAR-α	GC/GG	14	1.00		16	1.69	0.82, 3.49		
rs18000206	CC	117	1.23	0.71, 2.15	141	1.50	0.86, 2.64	-0.42	-1.58, 0.75
FAS	GA/AA	24	1.00		47	1.68	1.02, 2.78		
rs2234767	GG	123	1.47	0.95, 2.28	132	1.75	1.12, 2.74	-0.40	-1.24, 0.44
FAS	GG	40	1.00		47	1.24	0.81, 1.91		
rs1800682	GA/AA	107	0.98	0.68, 1.41	130	1.24	0.86, 1.79	0.02	-0.52, 0.57
FASL	CT/TT	97	1.00		120	1.26	0.95, 1.67		
rs763110	CC	50	1.15	0.81, 1.61	60	1.47	1.06, 2.04	0.06	-0.51, 0.63
TNF-α	GG	105	1.00		131	1.30	0.99, 1.70		
rs1800629	GA/AA	39	1.07	0.74, 1.55	46	1.31	0.92, 1.87	-0.06	-0.65, 0.53
MnSOD	CC	27	1.00		49	1.94	1.21, 3.12		
rs4880	CT/TT	114	1.49	0.98, 2.27	126	1.67	1.10, 2.55	-0.76	-1.71, 0.19
MPO	GG	89	1.00		110	1.29	0.97, 1.73		
rs2333227	GA/AA	55	1.01	0.72, 1.41	66	1.26	0.91, 1.75	-0.04	-0.56, 0.48
CAT	CT/TT	58	1.00		86	1.47	1.04, 2.06		
rs1001179	CC	85	0.82	0.59, 1.14	89	0.91	0.64, 1.28	-0.38	-0.92, 0.16
GPX1	CT/TT	83	1.00		90	1.18	0.87, 1.61		
rs1050450	CC	60	0.95	0.68, 1.32	87	1.34	0.98, 1.82	0.20	-0.27, 0.68
GSTM1	Null	72	1.00		68	1.03	0.74, 1.44		
deletion	Present	63	0.84	0.60, 1.18	98	1.33	0.98, 1.82	0.46	0.02, 0.90
GSTP1	AG/GG	68	1.00		93	1.18	0.85, 1.63		
rs1695	AA	76	0.90	0.65, 1.25	79	1.21	0.87, 1.69	0.13	-0.33, 0.59
GSTT1	Null	28	1.00		32	1.61	0.97, 2.70		
deletion	Present	111	1.23	0.81, 1.87	135	1.50	0.98, 2.28	-0.35	-1.18, 0.48
GSTA1	GA/AA	100	1.00		113	1.23	0.93, 1.62		
rs3957356	GG	44	0.97	0.68, 1.38	64	1.33	0.96, 1.83	0.13	-0.39, 0.65
COMT	AG/AA	110	1.00		117	1.14	0.87, 1.49		
rs4680	GG	36	0.93	0.64, 1.36	60	1.50	1.09, 2.07	0.43	-0.11, 0.97
COMT	CC	9	1.00		15	1.32	0.58, 3.02		
rs737865	CT/TT	130	1.45	0.74, 2.86	162	1.98	1.00, 3.89	0.20	-0.73, 1.14
CYP17	TT	51	1.00		62	1.32	0.90, 1.93		
rs743572	TC/CC	90	0.89	0.63, 1.26	116	1.20	0.85, 1.69	-0.01	-0.53, 0.51

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.18 Age- and energy-adjusted HRs and 95% CI for the interaction between DHA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	High intake			Low intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	49	1.00		60	1.48	1.01, 2.18		
rs20417	GG	96	1.27	0.90, 1.80	122	1.47	1.05, 2.06	-0.28	-0.92, 0.35
PTGS-2	TC/CC	83	1.00		92	1.26	0.93, 1.71		
rs5275	TT	62	0.98	0.71, 1.37	92	1.28	0.95, 1.73	0.04	-0.45, 0.53
PPAR-α	GC/GG	14	1.00		16	1.48	0.71, 3.05		
rs18000206	CC	115	1.16	0.67, 2.03	143	1.41	0.81, 2.46	-0.23	-1.25, 0.79
FAS	GA/AA	21	1.00		50	1.88	1.13, 3.16		
rs2234767	GG	124	1.64	1.03, 2.61	131	1.87	1.17, 2.98	-0.66	-1.64, 0.33
FAS	GG	42	1.00		45	1.16	0.76, 1.78		
rs1800682	GA/AA	103	0.93	0.65, 1.34	134	1.20	0.84, 1.71	0.11	-0.40, 0.62
FASL	CT/TT	100	1.00		117	1.16	0.88, 1.52		
rs763110	CC	45	1.01	0.71, 1.44	65	1.48	1.08, 2.03	0.31	-0.22, 0.84
TNF-α	GG	105	1.00		131	1.29	0.99, 1.67		
rs1800629	GA/AA	36	1.06	0.72, 1.54	49	1.30	0.92, 1.82	-0.05	-0.63, 0.54
MnSOD	CC	34	1.00		42	1.34	0.85, 2.11		
rs4880	CT/TT	104	1.12	0.76, 1.65	136	1.40	0.96, 2.05	-0.06	-0.69, 0.57
MPO	GG	87	1.00		112	1.30	0.98, 1.73		
rs2333227	GA/AA	54	1.03	0.73, 1.44	67	1.24	0.90, 1.71	-0.09	-0.61, 0.44
CAT	CT/TT	56	1.00		88	1.48	1.06, 2.08		
rs1001179	CC	84	0.83	0.59, 1.17	90	0.91	0.65, 1.27	-0.41	-0.96, 0.14
GPX1	CT/TT	80	1.00		93	1.18	0.87, 1.60		
rs1050450	CC	60	0.96	0.69, 1.34	87	1.32	0.97, 1.80	0.18	-0.29, 0.66
GSTM1	Null	67	1.00		73	1.05	0.75, 1.47		
deletion	Present	63	0.86	0.61, 1.22	98	1.34	0.98, 1.84	0.43	-0.01, 0.87
GSTP1	AG/GG	67	1.00		94	1.20	0.87, 1.65		
rs1695	AA	74	0.93	0.67, 1.29	81	1.19	0.86, 1.65	0.06	-0.41, 0.53
GSTT1	Null	25	1.00		35	1.76	1.05, 2.95		
deletion	Present	109	1.32	0.86, 2.04	137	1.58	1.02, 2.43	-0.51	-1.42, 0.40
GSTA1	GA/AA	99	1.00		114	1.17	0.89, 1.54		
rs3957356	GG	42	0.90	0.63, 1.30	66	1.33	0.97, 1.83	0.26	-0.23, 0.76
COMT	AG/AA	109	1.00		118	1.10	0.84, 1.43		
rs4680	GG	34	0.87	0.59, 1.28	62	1.52	1.11, 2.08	0.55	0.02, 1.07
COMT	CC	9	1.00		15	1.11	0.48, 2.54		
rs737865	CT/TT	128	1.31	0.67, 2.57	164	1.78	0.91, 3.50	0.36	-0.42, 1.15
CYP17	TT	52	1.00		61	1.19	0.82, 1.72		
rs743572	TC/CC	88	0.83	0.59, 1.17	118	1.14	0.82, 1.59	0.12	-0.35, 0.59

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.19 Age- and energy-adjusted HRs and 95% CI for the interaction between EPA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	High intake			Low intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	51	1.00		58	1.41	0.96, 2.07		
rs20417	GG	92	1.17	0.83, 1.64	126	1.50	1.08, 2.09	-0.07	-0.66, 0.51
PTGS-2	TC/CC	82	1.00		93	1.31	0.96, 1.77		
rs5275	TT	61	0.96	0.69, 1.33	93	1.35	1.00, 1.83	0.09	-0.40, 0.58
PPAR-α	GC/GG	13	1.00		17	1.87	0.90, 3.87		
rs18000206	CC	116	1.30	0.73, 2.30	142	1.61	0.90, 2.86	-0.55	-1.83, 0.72
FAS	GA/AA	23	1.00		48	1.97	1.19, 3.26		
rs2234767	GG	120	1.61	1.03, 2.52	135	1.92	1.22, 3.01	-0.66	-1.64, 0.32
FAS	GG	40	1.00		47	1.23	0.80, 1.89		
rs1800682	GA/AA	102	0.93	0.65, 1.34	135	1.28	0.89, 1.84	0.12	-0.41, 0.65
FASL	CT/TT	95	1.00		122	1.29	0.98, 1.70		
rs763110	CC	48	1.11	0.78, 1.57	62	1.55	1.12, 2.14	0.15	-0.42, 0.72
TNF-α	GG	103	1.00		133	1.34	1.03, 1.74		
rs1800629	GA/AA	36	1.03	0.71, 1.51	49	1.38	0.98, 1.95	0.01	-0.58, 0.60
MnSOD	CC	29	1.00		47	1.68	1.05, 2.67		
rs4880	CT/TT	107	1.28	0.85, 1.93	133	1.62	1.08, 2.43	-0.34	-1.12, 0.44
MPO	GG	85	1.00		114	1.39	1.04, 1.86		
rs2333227	GA/AA	54	1.05	0.74, 1.47	67	1.31	0.94, 1.82	-0.13	-0.68, 0.42
CAT	CT/TT	57	1.00		87	1.44	1.03, 2.02		
rs1001179	CC	80	0.77	0.54, 1.07	94	0.95	0.68, 1.33	-0.25	-0.77, 0.26
GPX1	CT/TT	80	1.00		93	1.18	0.87, 1.59		
rs1050450	CC	58	0.89	0.64, 1.25	89	1.40	1.03, 1.91	0.34	-0.13, 0.80
GSTM1	Null	64	1.00		76	1.18	0.85, 1.66		
deletion	Present	64	0.92	0.65, 1.31	97	1.42	1.04, 1.96	0.32	-0.16, 0.80
GSTP1	AG/GG	69	1.00		92	1.16	0.85, 1.60		
rs1695	AA	70	0.85	0.61, 1.18	85	1.26	0.91, 1.74	0.25	-0.20, 0.69
GSTT1	Null	26	1.00		34	1.64	0.98, 2.74		
deletion	Present	106	1.22	0.80, 1.88	140	1.56	1.02, 2.40	-0.30	-1.12, 0.52
GSTA1	GA/AA	97	1.00		116	1.32	1.00, 1.74		
rs3957356	GG	41	1.00	0.69, 1.44	67	1.37	1.00, 1.87	0.05	-0.49, 0.59
COMT	AG/AA	107	1.00		120	1.19	0.91, 1.55		
rs4680	GG	34	0.93	0.63, 1.37	62	1.54	1.12, 2.12	0.42	-0.13, 0.97
COMT	CC	11	1.00		13	1.04	0.46, 2.33		
rs737865	CT/TT	123	1.22	0.66, 2.26	169	1.74	0.94, 3.22	0.48	-0.24, 1.21
CYP17	TT	50	1.00		63	1.29	0.89, 1.88		
rs743572	TC/CC	88	0.86	0.61, 1.22	118	1.21	0.86, 1.70	0.06	-0.44, 0.56

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.20 Age- and energy-adjusted HRs and 95% CI for the interaction between ω-6 intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	Low intake			High intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	65	1.00		44	1.27	0.85, 1.91		
rs20417	GG	99	1.43	1.01, 2.02	119	1.17	0.80, 1.71	-0.53	-1.18, 0.12
PTGS-2	TC/CC	94	1.00		81	0.95	0.69, 1.32		
rs5275	TT	71	1.04	0.77, 1.42	83	0.94	0.66, 1.33	-0.05	-0.49, 0.38
PPAR-α	GC/GG	15	1.00		15	1.18	0.56, 2.48		
rs18000206	CC	128	1.19	0.69, 2.03	130	1.10	0.62, 1.94	-0.27	-1.19, 0.66
FAS	GA/AA	36	1.00		35	0.89	0.55, 1.46		
rs2234767	GG	127	1.13	0.77, 1.64	128	1.07	0.71, 1.61	0.05	-0.45, 0.56
FAS	GG	38	1.00		49	0.90	0.57, 1.40		
rs1800682	GA/AA	123	0.98	0.70, 1.36	114	0.91	0.63, 1.30	0.04	-0.42, 0.50
FASL	CT/TT	110	1.00		107	0.97	0.72, 1.30		
rs763110	CC	53	1.23	0.89, 1.70	57	1.05	0.73, 1.49	-0.15	-0.67, 0.36
TNF-α	GG	120	1.00		116	0.98	0.73, 1.31		
rs1800629	GA/AA	38	1.17	0.83, 1.64	47	0.89	0.60, 1.31	-0.26	-0.79, 0.27
MnSOD	CC	33	1.00		43	0.95	0.59, 1.52		
rs4880	CT/TT	123	1.12	0.79, 1.59	117	1.01	0.70, 1.46	-0.06	-0.59, 0.47
MPO	GG	100	1.00		99	0.93	0.68, 1.27		
rs2333227	GA/AA	58	1.01	0.73, 1.38	63	0.88	0.62, 1.26	-0.05	-0.49, 0.39
CAT	CT/TT	65	1.00		79	0.80	0.56, 1.13		
rs1001179	CC	92	0.62	0.45, 0.84	82	0.63	0.45, 0.88	0.22	-0.10, 0.54
GPX1	CT/TT	85	1.00		88	0.83	0.60, 1.15		
rs1050450	CC	73	0.95	0.70, 1.30	74	0.96	0.68, 1.35	0.17	-0.22, 0.57
GSTM1	Null	71	1.00		69	0.84	0.59, 1.20		
deletion	Present	77	1.04	0.75, 1.43	84	0.94	0.66, 1.33	0.06	-0.37, 0.49
GSTP1	AG/GG	74	1.00		87	0.87	0.62, 1.23		
rs1695	AA	84	0.89	0.65, 1.22	71	0.89	0.64, 1.23	0.13	-0.26, 0.52
GSTT1	Null	32	1.00		28	0.99	0.58, 1.69		
deletion	Present	119	1.13	0.75, 1.71	127	1.01	0.64, 1.58	-0.11	-0.71, 0.49
GSTA1	GA/AA	106	1.00		107	0.89	0.66, 1.20		
rs3957356	GG	53	1.00	0.72, 1.38	55	0.97	0.68, 1.38	0.07	-0.37, 0.52
COMT	AG/AA	106	1.00		121	0.80	0.60, 1.08		
rs4680	GG	54	0.93	0.66, 1.32	42	1.13	0.80, 1.60	0.40	-0.06, 0.86
COMT	CC	14	1.00		10	1.18	0.52, 2.69		
rs737865	CT/TT	143	1.66	0.88, 3.15	149	1.50	0.77, 2.89	-0.34	-1.44, 0.76
CYP17	TT	56	1.00		57	1.06	0.72, 1.57		
rs743572	TC/CC	102	0.99	0.72, 1.37	104	0.86	0.60, 1.23	-0.19	-0.68, 0.30

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.21 Age- and energy-adjusted HRs and 95% CI for the interaction between LA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	Low intake			High intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	66	1.00		43	1.32	0.88, 1.99		
rs20417	GG	99	1.46	1.03, 2.07	119	1.20	0.82, 1.76	-0.58	-1.25, 0.09
PTGS-2	TC/CC	95	1.00		80	0.97	0.70, 1.35		
rs5275	TT	71	1.05	0.77, 1.43	83	0.95	0.67, 1.35	-0.07	-0.52, 0.37
PPAR-α	GC/GG	15	1.00		15	1.19	0.56, 2.49		
rs18000206	CC	129	1.18	0.69, 2.03	129	1.11	0.63, 1.96	-0.26	-1.18, 0.67
FAS	GA/AA	37	1.00		34	0.93	0.57, 1.53		
rs2234767	GG	127	1.15	0.78, 1.68	128	1.10	0.73, 1.67	0.02	-0.50, 0.55
FAS	GG	38	1.00		49	0.91	0.58, 1.42		
rs1800682	GA/AA	124	0.97	0.70, 1.36	113	0.92	0.64, 1.32	0.04	-0.43, 0.50
FASL	CT/TT	110	1.00		107	0.97	0.72, 1.31		
rs763110	CC	54	1.22	0.88, 1.69	56	1.07	0.75, 1.52	-0.13	-0.64, 0.38
TNF-α	GG	120	1.00		116	0.98	0.73, 1.31		
rs1800629	GA/AA	39	1.16	0.82, 1.63	46	0.91	0.62, 1.33	-0.23	-0.76, 0.29
MnSOD	CC	33	1.00		43	0.95	0.59, 1.52		
rs4880	CT/TT	124	1.11	0.78, 1.58	116	1.02	0.70, 1.47	-0.05	-0.57, 0.48
MPO	GG	101	1.00		98	0.94	0.69, 1.29		
rs2333227	GA/AA	58	1.01	0.74, 1.39	63	0.90	0.63, 1.28	-0.06	-0.50, 0.38
CAT	CT/TT	66	1.00		78	0.81	0.57, 1.15		
rs1001179	CC	92	0.62	0.45, 0.84	82	0.64	0.46, 0.89	0.21	-0.12, 0.53
GPX1	CT/TT	85	1.00		88	0.84	0.61, 1.16		
rs1050450	CC	74	0.95	0.70, 1.29	73	0.97	0.69, 1.37	0.19	-0.21, 0.58
GSTM1	Null	72	1.00		68	0.87	0.61, 1.23		
deletion	Present	77	1.05	0.76, 1.45	84	0.95	0.67, 1.35	0.04	-0.40, 0.47
GSTP1	AG/GG	75	1.00		86	0.89	0.64, 1.26		
rs1695	AA	84	0.90	0.66, 1.23	71	0.90	0.65, 1.25	0.11	-0.29, 0.50
GSTT1	Null	32	1.00		28	0.99	0.58, 1.69		
deletion	Present	120	1.12	0.74, 1.70	126	1.02	0.65, 1.60	-0.10	-0.70, 0.50
GSTA1	GA/AA	107	1.00		106	0.91	0.68, 1.23		
rs3957356	GG	53	1.01	0.73, 1.39	55	0.98	0.68, 1.40	0.06	-0.39, 0.51
COMT	AG/AA	106	1.00		121	0.81	0.60, 1.08		
rs4680	GG	55	0.92	0.64, 1.31	41	1.15	0.82, 1.62	0.42	-0.03, 0.88
COMT	CC	15	1.00		9	1.35	0.58, 3.13		
rs737865	CT/TT	143	1.80	0.92, 3.53	149	1.64	0.82, 3.27	-0.52	-1.78, 0.75
CYP17	TT	56	1.00		57	1.06	0.72, 1.58		
rs743572	TC/CC	103	0.98	0.71, 1.36	103	0.87	0.61, 1.24	-0.17	-0.66, 0.31

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.22 Age- and energy-adjusted HRs and 95% CI for the interaction between AA intake (low or high) and genetic variants on all-cause mortality

Gene	Genotype	Low intake			High intake			RERI	95% CI
		D	HR	95% CI	D	HR	95% CI		
PTGS-2	GC/CC	60	1.00		49	1.33	0.90, 1.96		
rs20417	GG	97	1.54	1.11, 2.15	121	1.11	0.78, 1.58	-0.76	-1.46, -0.07
PTGS-2	TC/CC	92	1.00		83	0.98	0.72, 1.35		
rs5275	TT	67	1.12	0.83, 1.51	87	0.90	0.64, 1.25	-0.21	-0.66, 0.25
PPAR-α	GC/GG	13	1.00		17	0.89	0.43, 1.84		
rs18000206	CC	129	1.03	0.62, 1.72	129	0.97	0.57, 1.63	0.05	-0.66, 0.75
FAS	GA/AA	42	1.00		29	1.34	0.82, 2.17		
rs2234767	GG	114	1.53	1.03, 2.29	141	1.21	0.79, 1.85	-0.66	-1.46, 0.14
FAS	GG	34	1.00		53	0.66	0.42, 1.02		
rs1800682	GA/AA	121	0.83	0.60, 1.15	116	0.80	0.57, 1.13	0.31	-0.04, 0.67
FASL	CT/TT	103	1.00		114	0.83	0.62, 1.10		
rs763110	CC	53	1.06	0.77, 1.46	57	1.04	0.74, 1.47	0.15	-0.30, 0.60
TNF-α	GG	110	1.00		126	0.80	0.61, 1.06		
rs1800629	GA/AA	45	0.85	0.59, 1.21	40	1.02	0.72, 1.45	0.38	-0.06, 0.81
MnSOD	CC	40	1.00		36	1.24	0.79, 1.97		
rs4880	CT/TT	113	1.33	0.92, 1.93	127	1.10	0.74, 1.61	-0.48	-1.17, 0.21
MPO	GG	91	1.00		108	0.90	0.67, 1.21		
rs2333227	GA/AA	62	1.00	0.72, 1.37	59	0.88	0.63, 1.22	-0.01	-0.44, 0.42
CAT	CT/TT	67	1.00		77	0.78	0.55, 1.10		
rs1001179	CC	85	0.63	0.47, 0.86	89	0.59	0.43, 0.83	0.18	-0.14, 0.50
GPX1	CT/TT	87	1.00		86	1.00	0.73, 1.37		
rs1050450	CC	68	1.17	0.87, 1.59	79	0.94	0.67, 1.31	-0.24	-0.72, 0.24
GSTM1	Null	61	1.00		79	0.76	0.54, 1.07		
deletion	Present	84	0.94	0.69, 1.29	77	0.95	0.69, 1.31	0.25	-0.14, 0.64
GSTP1	AG/GG	75	1.00		86	0.72	0.52, 1.00		
rs1695	AA	76	0.77	0.57, 1.04	79	0.85	0.61, 1.17	0.36	0.02, 0.69
GSTT1	Null	24	1.00		36	0.82	0.48, 1.39		
deletion	Present	122	1.05	0.72, 1.52	124	0.93	0.63, 1.38	0.07	-0.45, 0.58
GSTA1	GA/AA	100	1.00		113	0.83	0.63, 1.11		
rs3957356	GG	55	0.93	0.67, 1.29	53	0.97	0.69, 1.36	0.21	-0.21, 0.63
COMT	AG/AA	106	1.00		121	0.88	0.67, 1.17		
rs4680	GG	49	1.15	0.82, 1.61	47	1.03	0.73, 1.45	0.00	-0.51, 0.50
COMT	CC	13	1.00		11	0.80	0.36, 1.81		
rs737865	CT/TT	141	1.31	0.71, 2.42	151	1.23	0.66, 2.30	0.12	-0.63, 0.86
CYP17	TT	51	1.00		62	0.84	0.57, 1.24		
rs743572	TC/CC	104	0.84	0.61, 1.15	102	0.82	0.58, 1.14	0.14	-0.25, 0.52

D=deaths, RERI=Relative Excess Risk due to Interaction
95% CI for RERI estimated using Hosmer & Lemeshow [374]

Table A.23 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) multivariate-adjusted associations (Aim 1a)

PUFA/ Fish	Comparison	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
ω-6	Q3 vs Q1	0.0154	1	0.01754	0.000877193	no
LA	Q3 vs Q1	0.0179	2	0.03509	0.001754386	no
ALA	Q3 vs Q1	0.0226	3	0.05263	0.002631579	no
PUFA	Q3 vs Q1	0.0324	4	0.07018	0.003508772	no
ω-3	Q2 vs Q1	0.0377	5	0.08772	0.004385965	no
Other fish	Q2 vs never	0.0483	6	0.10526	0.005263158	no

Note:
[a] Weight = rank/57, where 57 represents the total number of comparisons for Aim 1a
[b] FDR p = weight*0.05
[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.24 Multiple comparisons adjustment via FDR for statistically significant (p<0.05) multivariate-adjusted associations for individual PUFA-gene interactions (Aim 1b)

PUFA	Gene	Comparison	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
ω-6	*GSTM1*	Low, high risk	0.003	1	0.00185	9.25926E-05	no
LA	*GSTM1*	Low, high risk	0.003	2	0.0037	0.000185185	no
PUFA	*GSTM1*	Low, high risk	0.0033	3	0.00556	0.000277778	no
ratio	*FASL*	Low, high risk	0.0057	4	0.00741	0.00037037	no
DHA	*CYP17*	High, high risk	0.0096	5	0.00926	0.000462963	no
LA	*GPX1*	High, low risk	0.0096	6	0.01111	0.000555556	no
ω-6	*GPX1*	High, low risk	0.011	7	0.01296	0.000648148	no
LA	*GSTP1*	High, low risk	0.011	8	0.01481	0.000740741	no
DPA	*GSTM1*	Low, low risk	0.0141	9	0.01667	0.000833333	no
LA	*MPO*	High, low risk	0.0142	10	0.01852	0.000925926	no
ratio	*FAS* rs2234767	Low, low risk	0.0147	11	0.02037	0.001018519	no
ratio	*COMT* rs4680	Low, low risk	0.0154	12	0.02222	0.001111111	no
LA	*PTGS2* rs5275	High, high risk	0.0165	13	0.02407	0.001203704	no
ratio	*GPX1*	Low, low risk	0.0174	14	0.02593	0.001296296	no
ω-6	*MPO*	High, low risk	0.0183	15	0.02778	0.001388889	no
LA	*COMT* rs4680	High, low risk	0.0183	16	0.02963	0.001481481	no
ω-6	*GSTP1*	High, low risk	0.0197	17	0.03148	0.001574074	no
DPA	*PPAR*	Low, high risk	0.0227	18	0.03333	0.001666667	no
LA	*FASL*	High, low risk	0.024	19	0.03519	0.001759259	no
ω-6	*PTGS2* rs5275	High, high risk	0.0249	20	0.03704	0.001851852	no
DPA	*GSTM1*	High, high risk	0.0257	21	0.03889	0.001944444	no
ω-6	*COMT* rs4680	High, low risk	0.0262	22	0.04074	0.002037037	no
ratio	*FAS* rs1800682	Low, high risk	0.0264	23	0.04259	0.00212963	no
LA	*COMT* rs4680	High, high risk	0.0301	24	0.04444	0.002222222	no
LA	*FAS* rs1800682	High, high risk	0.0303	25	0.0463	0.002314815	no
DPA	*CAT*	Low, high risk	0.0329	26	0.04815	0.002407407	no
ω-6	*FASL*	High, low risk	0.0338	27	0.05	0.0025	no
ratio	*COMT* rs4680	High, high risk	0.0365	28	0.05185	0.002592593	no
EPA	*PPAR*	Low, high risk	0.0376	29	0.0537	0.002685185	no
PUFA	*GSTP1*	High, low risk	0.0377	30	0.05556	0.002777778	no
LA	*CYP17*	High, high risk	0.0391	31	0.05741	0.00287037	no
ω-6	*COMT* rs4680	High, high risk	0.04	32	0.05926	0.002962963	no
ω-6	*FAS* rs1800682	High, high risk	0.0404	33	0.06111	0.003055556	no
LA	*GSTP1*	High, high risk	0.0432	34	0.06296	0.003148148	no
DPA	*GSTM1*	Low, high risk	0.0455	35	0.06481	0.003240741	no
DPA	*PPAR*	High, high risk	0.0474	36	0.06667	0.003333333	no
ratio	*GSTM1*	High, high risk	0.0475	37	0.06852	0.003425926	no
ALA	*CAT*	Low, high risk	0.0486	38	0.07037	0.003518519	no
LA	*MnSOD*	High, low risk	0.0486	39	0.07222	0.003611111	no

Note:

[a] Weight = rank/540, where 540 represents the total number of comparisons for Aim 1b interactions

[b] FDR p = weight*0.05

[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.25 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) additive interactions (RERI) for PUFA-gene interactions (Aim 1b)

PUFA	Gene	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
ω-6	*GSTM1*	0.0014	1	0.0056	0.000277778	no
PUFA	*GSTM1*	0.0015	2	0.0111	0.000555556	no
LA	*GSTM1*	0.0015	3	0.0167	0.000833333	no
DHA	*CYP17*	0.0107	4	0.0222	0.001111111	no
DPA	*GSTM1*	0.0247	5	0.0278	0.001388889	no
DPA	*FAS* rs2234767	0.0296	6	0.0333	0.001666667	no
AA	*MPO*	0.0340	7	0.0389	0.001944444	no
ratio	*FASL*	0.0425	8	0.0444	0.002222222	no
DPA	*PPAR*	0.0462	9	0.0500	0.0025	no
DHA	*FAS* rs2234767	0.0462	10	0.0556	0.002777778	no
AA	*GSTP1*	0.0476	11	0.0611	0.003055556	no

Note:
[a] Weight = rank/180, where 180 represents the total number of RERIs for Aim 1b
[b] FDR p = weight*0.05
[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.26 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) multivariate-adjusted associations (Aim 2a)

PUFA/ Fish	Comparison	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
Other fish	Q3 vs never	0.0012	1	0.01754	0.00088	no
DPA	Q3 vs Q1	0.0023	2	0.03509	0.00175	no
EPA	Q3 vs Q1	0.007	3	0.05263	0.00263	no
DHA	Q4 vs Q1	0.0086	4	0.07018	0.00351	no
Tuna	Q4 vs never	0.0106	5	0.08772	0.00439	no
DHA	Q3 vs Q1	0.0141	6	0.10526	0.00526	no
Other fish	Q4 vs never	0.0289	7	0.12281	0.00614	no
EPA	Q4 vs Q1	0.0294	8	0.14035	0.00702	no

Note:
[a] Weight = rank/57, where 57 represents the total number of comparisons for Aim 2a
[b] FDR p = weight*0.05
[c] If $p \leq$ FDR p-value then "yes", otherwise "no"

Table A.27 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) gene associations according to a dominant model (Aim 2b)

Gene	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
CAT	0.0035	1	0.0556	0.0028	no

Note:
[a] Weight = rank/18, where 18 represents the total number of comparisons for Aim 2b
[b] FDR p = weight*0.05
[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.28 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) multivariate-adjusted associations for individual PUFA-gene interactions (Aim 2c)

PUFA	Gene	Comparison	p	Rank	Weight[a]	FDR p-value[b]	Significant[c]
LA	*GSTT1*	High, low risk	0.0006	1	0.00185	9.25926E-05	no
AA	*CAT*	High, high risk	0.0019	2	0.0037	0.000185185	no
ω-6	*CAT*	Low, high risk	0.0022	3	0.00556	0.000277778	no
LA	*CAT*	Low, high risk	0.0025	4	0.00741	0.00037037	no
ω-3	*CAT*	High, high risk	0.0033	5	0.00926	0.000462963	no
AA	*CAT*	Low, high risk	0.0033	6	0.01111	0.000555556	no
EPA	*FAS* rs2234767	Low, high risk	0.0046	7	0.01296	0.000648148	no
DPA	*MnSOD*	Low, low risk	0.0061	8	0.01481	0.000740741	no
LA	*GSTT1*	High, high risk	0.0063	9	0.01667	0.000833333	no
ω-6	*CAT*	High, high risk	0.0064	10	0.01852	0.000925926	no
ALA	*CAT*	High, high risk	0.0065	11	0.02037	0.001018519	no
ratio	*CAT*	High, high risk	0.0066	12	0.02222	0.001111111	no
EPA	*COMT* rs4680	Low, high risk	0.0075	13	0.02407	0.001203704	no
LA	*CAT*	High, high risk	0.0081	14	0.02593	0.001296296	no
EPA	*FAS* rs2234767	Low, low risk	0.0083	15	0.02778	0.001388889	no
EPA	*FASL*	Low, high risk	0.0083	16	0.02963	0.001481481	no
ALA	*CAT*	Low, high risk	0.0088	17	0.03148	0.001574074	no
DHA	*FAS* rs2234767	Low, high risk	0.0088	18	0.03333	0.001666667	no
DHA	*COMT* rs4680	Low, high risk	0.0096	19	0.03519	0.001759259	no
DPA	*COMT* rs4680	Low, high risk	0.0135	20	0.03704	0.001851852	no
ω-3	*CAT*	Low, high risk	0.014	21	0.03889	0.001944444	no
DPA	*FAS* rs2234767	Low, high risk	0.014	22	0.04074	0.002037037	no
DPA	*PTGS2* rs20417	Low, low risk	0.0141	23	0.04259	0.00212963	no
DHA	*FASL*	Low, high risk	0.0148	24	0.04444	0.002222222	no
AA	*PTGS2* rs20417	Low, high risk	0.0151	25	0.0463	0.002314815	no
DHA	*FAS* rs2234767	Low, low risk	0.016	26	0.04815	0.002407407	no
DPA	*MnSOD*	Low, high risk	0.0168	27	0.05	0.0025	no
EPA	*MnSOD*	Low, high risk	0.0202	28	0.05185	0.002592593	no

Note:

[a] Weight = rank/540, where 540 represents the total number of comparisons for Aim 2c interactions

[b] FDR p = weight*0.05

[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.28 (cont.) Multiple comparisons adjustment via FDR for statistically significant (p<0.05) multivariate-adjusted associations for individual PUFA-gene interactions (Aim 2c)

PUFA	Gene	Comparison	p	Rank	Weight[a]	FDR p-value[b]	Significant[c]
DPA	*FASL*	Low,high risk	0.0221	29	0.0537	0.002685185	no
ratio	*CAT*	Low,high risk	0.0223	30	0.05556	0.002777778	no
DHA	*CAT*	Low, low risk	0.0227	31	0.05741	0.00287037	no
EPA	*MPO*	Low, low risk	0.0248	32	0.05926	0.002962963	no
ω-3	*FAS* rs2234767	High,high risk	0.0264	33	0.06111	0.003055556	no
DPA	*CAT*	Low, low risk	0.0278	34	0.06296	0.003148148	no
EPA	*PTGS2* rs20417	Low,high risk	0.0288	35	0.06481	0.003240741	no
EPA	*MnSOD*	Low, low risk	0.0297	36	0.06667	0.003333333	no
EPA	*GSTM1*	Low,high risk	0.0299	37	0.06852	0.003425926	no
EPA	*TNFa*	Low, low risk	0.0304	38	0.07037	0.003518519	no
DHA	*GSTT1*	Low, low risk	0.031	39	0.07222	0.003611111	no
EPA	*GPX1*	Low,high risk	0.0315	40	0.07407	0.003703704	no
ω-3	*PTGS2* rs20417	High,high risk	0.0337	41	0.07593	0.003796296	no
EPA	*CAT*	Low, low risk	0.0346	42	0.07778	0.003888889	no
DPA	*PTGS2* rs20417	Low,high risk	0.0365	43	0.07963	0.003981481	no
DHA	*FAS* rs2234767	High,high risk	0.0367	44	0.08148	0.004074074	no
AA	*FAS* rs2234767	Low,high risk	0.0368	45	0.08333	0.004166667	no
PUFA	*CAT*	High,high risk	0.0375	46	0.08519	0.004259259	no
EPA	*FAS* rs2234767	High,high risk	0.0376	47	0.08704	0.004351852	no
DPA	*PTGS2* rs5275	Low, low risk	0.0382	48	0.08889	0.004444444	no
DHA	*GSTT1*	Low,high risk	0.0397	49	0.09074	0.004537037	no
EPA	*GSTT1*	Low,high risk	0.0407	50	0.09259	0.00462963	no
DPA	*FAS* rs2234767	Low, low risk	0.0414	51	0.09444	0.004722222	no
EPA	*GSTA1*	Low, low risk	0.0471	52	0.0963	0.004814815	no
AA	*GSTP1*	Low, low risk	0.0488	53	0.09815	0.004907407	no
DPA	*COMT* rs737865	Low,high risk	0.0491	54	0.1	0.005	no

Note:
[a] Weight = rank/540, where 540 represents the total number of comparisons for Aim 2c interactions
[b] FDR p = weight*0.05
[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.29 Multiple comparisons adjustment via FDR for statistically significant ($p<0.05$) additive interactions (RERI) for PUFA-gene interactions (Aim 2c)

PUFA	Gene	p	Rank	Weight[a]	FDR p value[b]	Significant[c]
PUFA	*PTGS2* rs20417	0.00755	1	0.00556	0.00028	no
AA	*PTGS2* rs20417	0.03335	2	0.01111	0.00056	no
AA	*GSTP1*	0.03519	3	0.01667	0.00083	no
DPA	*GSTM1*	0.03848	4	0.02222	0.00111	no
ratio	*TNFa*	0.03859	5	0.02778	0.00139	no
DHA	*COMT* rs4680	0.04166	6	0.03333	0.00167	no

Note:
[a] Weight = rank/180, where 180 represents the total number of RERIs for Aim 2c
[b] FDR p = weight*0.05
[c] If p ≤ FDR p-value then "yes", otherwise "no"

Table A.30 Age-adjusted ORs and 95% CIs for the potential association between PUFA and breast cancer incidence stratified by supplement use

PUFA	Supplement use	Q1			Q2				Q3				Q4				LRT p
		Co	Ca	OR	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	
PUFA	Yes	144	124	1.00	142	158	1.32	0.95, 1.84	121	133	1.34	0.95, 1.89	133	116	1.07	0.75, 1.51	0.49
	No	86	96	1.00	102	106	0.99	0.66, 1.48	134	134	0.98	0.67, 1.43	128	129	1.02	0.69, 1.50	
ω-3	Yes	131	117	1.00	146	162	1.26	0.90, 1.76	138	140	1.16	0.82, 1.64	125	112	1.02	0.71, 1.46	0.79
	No	95	103	1.00	104	110	0.97	0.66, 1.43	124	131	1.02	0.70, 1.48	127	121	0.94	0.65, 1.38	
ALA	Yes	141	129	1.00	135	144	1.17	0.83, 1.63	134	147	1.22	0.87, 1.71	130	111	0.96	0.67, 1.35	0.81
	No	87	89	1.00	106	119	1.12	0.75, 1.66	133	134	1.04	0.71, 1.53	124	123	1.04	0.70, 1.54	
DPA	Yes	119	132	1.00	126	127	0.90	0.63, 1.28	147	127	0.79	0.56, 1.11	148	145	0.89	0.63, 1.24	0.25
	No	115	123	1.00	126	108	0.79	0.55, 1.13	103	122	1.15	0.79, 1.66	106	112	1.04	0.72, 1.51	
DHA	Yes	125	123	1.00	118	122	1.04	0.73, 1.49	145	125	0.88	0.62, 1.24	152	161	1.05	0.75, 1.46	0.21
	No	114	133	1.00	134	110	0.71	0.50, 1.02	103	120	1.02	0.70, 1.47	99	102	0.89	0.61, 1.30	
EPA	Yes	128	111	1.00	125	131	1.19	0.84, 1.70	129	133	1.19	0.84, 1.70	158	156	1.11	0.79, 1.56	0.44
	No	115	125	1.00	122	111	0.79	0.55, 1.15	117	122	0.99	0.69, 1.42	96	107	1.03	0.71, 1.51	
ω-6	Yes	144	126	1.00	141	153	1.26	0.90, 1.76	126	140	1.33	0.95, 1.87	129	112	1.04	0.73, 1.47	0.49
	No	85	97	1.00	104	97	0.87	0.58, 1.30	131	145	1.06	0.73, 1.55	130	126	0.96	0.65, 1.41	
LA	Yes	144	127	1.00	142	151	1.23	0.88, 1.71	124	141	1.35	0.96, 1.91	130	112	1.02	0.72, 1.45	0.50
	No	85	97	1.00	104	96	0.86	0.57, 1.29	131	146	1.07	0.73, 1.57	130	126	0.96	0.65, 1.41	
AA	Yes	133	133	1.00	130	140	1.07	0.76, 1.50	141	147	1.04	0.74, 1.45	136	111	0.83	0.59, 1.18	0.77
	No	118	110	1.00	107	120	1.27	0.88, 1.84	99	114	1.27	0.87, 1.86	126	121	1.10	0.76, 1.58	
ω-3/ω-6	Yes	132	124	1.00	132	142	1.15	0.82, 1.62	135	119	0.90	0.63, 1.28	141	146	1.02	0.72, 1.44	0.93
	No	117	128	1.00	116	125	0.99	0.69, 1.42	122	114	0.83	0.58, 1.20	95	98	0.88	0.60, 1.29	

Table A.31 Age-adjusted ORs and 95% CIs for the potential association between PUFA and breast cancer incidence stratified by menopausal status

PUFA	Model	Q1			Q2				Q3				Q4				LRT
		Co	Ca	OR	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	p
PUFA	Premenopausal	90	79	1.00	118	114	1.15	0.77, 1.72	136	130	1.13	0.76, 1.66	143	134	1.16	0.79, 1.71	0.95
	Postmenopausal	274	252	1.00	239	271	1.26	0.99, 1.61	224	248	1.25	0.97, 1.60	216	206	1.08	0.84, 1.40	
ω-3	Premenopausal	98	80	1.00	114	125	1.35	0.91, 2.00	142	130	1.16	0.79, 1.70	133	122	1.17	0.79, 1.72	0.85
	Postmenopausal	262	249	1.00	248	273	1.16	0.91, 1.48	217	238	1.17	0.90, 1.50	226	217	1.03	0.80, 1.33	
ALA	Premenopausal	91	82	1.00	122	110	1.04	0.70, 1.55	134	140	1.22	0.83, 1.79	140	125	1.07	0.72, 1.57	0.76
	Postmenopausal	267	241	1.00	240	275	1.27	0.99, 1.62	227	241	1.19	0.93, 1.53	219	220	1.13	0.87, 1.45	
DPA	Premenopausal	111	110	1.00	129	97	0.75	0.51, 1.09	124	126	1.02	0.71, 1.46	124	123	1.00	0.70, 1.45	0.41
	Postmenopausal	247	249	1.00	234	246	1.05	0.82, 1.35	233	243	1.05	0.82, 1.36	239	239	1.02	0.80, 1.32	
DHA	Premenopausal	126	115	1.00	132	101	0.86	0.60, 1.24	128	128	1.09	0.76, 1.55	101	113	1.19	0.82, 1.72	0.53
	Postmenopausal	228	249	1.00	227	231	0.90	0.70, 1.16	233	236	0.94	0.73, 1.21	265	261	0.94	0.73, 1.21	
EPA	Premenopausal	131	116	1.00	118	95	0.93	0.64, 1.35	133	133	1.13	0.80, 1.60	105	113	1.19	0.82, 1.72	0.72
	Postmenopausal	225	227	1.00	244	254	1.02	0.79, 1.32	227	228	1.01	0.78, 1.31	257	268	1.05	0.81, 1.35	
ω-6	Premenopausal	89	83	1.00	117	105	1.01	0.68, 1.51	139	138	1.10	0.75, 1.61	142	131	1.07	0.73, 1.58	0.65
	Postmenopausal	275	253	1.00	239	263	1.22	0.96, 1.56	220	259	1.33	1.04, 1.71	219	202	1.05	0.81, 1.35	
LA	Premenopausal	88	83	1.00	117	104	0.99	0.66, 1.48	140	139	1.08	0.74, 1.59	142	131	1.06	0.72, 1.57	0.66
	Postmenopausal	276	257	1.00	239	256	1.17	0.92, 1.50	219	261	1.33	1.04, 1.71	219	203	1.04	0.80, 1.34	
AA	Premenopausal	102	100	1.00	132	115	0.87	0.59, 1.26	113	117	1.04	0.71, 1.53	140	125	0.91	0.63, 1.32	0.47
	Postmenopausal	255	259	1.00	227	258	1.12	0.88, 1.44	253	244	0.96	0.75, 1.22	218	216	1.00	0.78, 1.30	
ω-3/ω-6	Premenopausal	133	126	1.00	134	143	1.15	0.82, 1.62	129	89	0.72	0.50, 1.04	91	99	1.09	0.74, 1.59	0.08
	Postmenopausal	222	226	1.00	231	235	1.01	0.78, 1.31	230	251	1.05	0.81, 1.36	270	265	0.93	0.73, 1.20	

Table A.32 Age-adjusted ORs and 95% CI for potential association between PUFA intake and breast cancer according to breast cancer tumor subtype

PUFA		Controls N	ER+ or PR+ vs. all controls			ER-/PR- vs. all controls		
			N	OR	95% CI	N	OR	95% CI
PUFA	Q1	375	185	1.00		42	1.00	
	Q2	375	202	1.17	0.91, 1.49	57	1.35	0.89, 2.07
	Q3	375	193	1.13	0.88, 1.46	66	1.57	1.03, 2.38
	Q4	375	180	1.09	0.85, 1.41	40	0.95	0.60, 1.51
ω-3	Q1	375	168	1.00		42	1.00	
	Q2	375	227	1.37	1.07, 1.75	57	1.36	0.89, 2.07
	Q3	375	189	1.19	0.92, 1.54	60	1.43	0.94, 2.17
	Q4	375	176	1.11	0.86, 1.44	46	1.09	0.70, 1.70
ALA	Q1	375	173	1.00		36	1.00	
	Q2	375	207	1.22	0.95, 1.56	62	1.72	1.11, 2.66
	Q3	375	203	1.24	0.97, 1.60	59	1.64	1.06, 2.54
	Q4	375	177	1.09	0.84, 1.41	48	1.33	0.84, 2.11
DPA	Q1	375	188	1.00		61	1.00	
	Q2	375	184	1.01	0.78, 1.29	47	0.77	0.51, 1.16
	Q3	375	184	1.02	0.79, 1.31	48	0.79	0.52, 1.18
	Q4	375	204	1.15	0.90, 1.47	49	0.80	0.54, 1.20
DHA	Q1	375	191	1.00		58	1.00	
	Q2	375	179	0.94	0.73, 1.21	44	0.76	0.50, 1.15
	Q3	375	191	1.01	0.78, 1.29	57	0.98	0.66, 1.46
	Q4	375	199	1.03	0.80, 1.32	46	0.79	0.53, 1.20
EPA	Q1	375	181	1.00		60	1.00	
	Q2	375	188	1.02	0.79, 1.31	41	0.68	0.45, 1.04
	Q3	375	181	1.01	0.78, 1.30	56	0.93	0.63, 1.38
	Q4	375	210	1.15	0.90, 1.47	48	0.80	0.53, 1.20
ω-3	Q1	375	189	1.00		42	1.00	
	Q2	375	188	1.06	0.82, 1.36	55	1.31	0.85, 2.01
	Q3	375	206	1.20	0.93, 1.53	68	1.62	1.07, 2.45
	Q4	375	177	1.05	0.81, 1.35	40	0.95	0.60, 1.51
LA	Q1	375	191	1.00		42	1.00	
	Q2	375	185	1.03	0.80, 1.33	55	1.31	0.85, 2.01
	Q3	375	207	1.19	0.93, 1.52	68	1.62	1.07, 2.45
	Q4	375	177	1.04	0.80, 1.34	40	0.95	0.60, 1.51
AA	Q1	375	188	1.00		51	1.00	
	Q2	375	201	1.09	0.85, 1.39	57	1.12	0.74, 1.67
	Q3	375	189	1.03	0.80, 1.32	47	0.92	0.60, 1.40
	Q4	375	182	1.04	0.81, 1.33	50	0.98	0.64, 1.48
ω3/ω6	Q1	375	172	1.00		55	1.00	
	Q2	375	218	1.30	1.02, 1.67	46	0.84	0.55, 1.27
	Q3	375	180	1.01	0.78, 1.30	49	0.89	0.59, 1.35
	Q4	375	190	1.01	0.79, 1.31	55	1.01	0.67, 1.51

Table A.33 Age-adjusted HRs and 95% CIs for the potential association between PUFA and all-cause mortality stratified by supplement use

PUFA	Supplement Use	Q1	Q2		Q3		Q4		LRT p
		HR	HR	95% CI	HR	95% CI	HR	95% CI	
PUFA	No	1.00	1.31	0.83, 2.07	1.17	0.75, 1.83	1.24	0.80, 1.92	0.22
	Yes	1.00	1.08	0.69, 1.68	1.06	0.67, 1.69	1.48	0.94, 2.32	
ω-3	No	1.00	0.90	0.57, 1.42	1.11	0.72, 1.71	1.09	0.72, 1.65	0.26
	Yes	1.00	1.28	0.82, 2.01	1.23	0.77, 1.95	1.22	0.75, 1.98	
ALA	No	1.00	1.03	0.65, 1.61	1.24	0.80, 1.93	1.06	0.69, 1.64	0.43
	Yes	1.00	1.23	0.78, 1.94	1.32	0.84, 2.07	1.31	0.81, 2.10	
DPA	No	1.00	1.51	1.02, 2.24	0.64	0.39, 1.03	0.96	0.62, 1.50	0.001
	Yes	1.00	0.63	0.41, 0.97	0.65	0.41, 1.01	0.68	0.44, 1.04	
DHA	No	1.00	1.35	0.92, 1.99	0.75	0.48, 1.19	0.78	0.50, 1.24	0.01
	Yes	1.00	0.64	0.41, 1.00	0.74	0.48, 1.14	0.57	0.37, 0.88	
EPA	No	1.00	1.13	0.77, 1.67	0.64	0.41, 1.00	0.73	0.46, 1.14	0.02
	Yes	1.00	0.68	0.43, 1.08	0.82	0.53, 1.26	0.69	0.45, 1.07	
ω-6	No	1.00	1.22	0.77, 1.95	1.28	0.82, 2.00	1.30	0.84, 2.01	0.19
	Yes	1.00	1.13	0.72, 1.76	1.06	0.66, 1.70	1.60	1.02, 2.50	
LA	No	1.00	1.23	0.77, 1.95	1.28	0.82, 1.99	1.30	0.84, 2.01	0.19
	Yes	1.00	1.22	0.78, 1.91	1.10	0.68, 1.77	1.67	1.07, 2.63	
AA	No	1.00	1.02	0.66, 1.59	0.95	0.61, 1.50	1.24	0.81, 1.89	0.29
	Yes	1.00	1.04	0.67, 1.60	0.77	0.49, 1.21	0.95	0.60, 1.51	
ω-3/ω-6	No	1.00	1.02	0.67, 1.54	1.00	0.66, 1.50	0.76	0.48, 1.21	0.14
	Yes	1.00	0.63	0.40, 1.00	0.75	0.48, 1.17	0.60	0.38, 0.93	

Table A.34 Age-adjusted HRs and 95% CIs for the potential association between PUFA and all-cause mortality stratified by menopausal status

PUFA	Model	Q1	Q2		Q3		Q4		LRT
		HR	HR	95% CI	HR	95% CI	HR	95% CI	p
PUFA	Premenopausal	1.00	1.33	0.68, 2.60	1.52	0.80, 2.90	1.60	0.85, 3.00	0.08
	Postmenopausal	1.00	1.12	0.86, 1.47	0.93	0.70, 1.24	1.20	0.90, 1.59	
ω-3	Premenopausal	1.00	1.56	0.78, 3.13	1.98	1.02, 3.87	1.97	1.01, 3.84	0.02
	Postmenopausal	1.00	0.91	0.69, 1.19	1.03	0.78, 1.36	1.01	0.77, 1.34	
ALA	Premenopausal	1.00	1.44	0.72, 2.89	2.06	1.09, 3.89	1.65	0.85, 3.19	0.03
	Postmenopausal	1.00	1.16	0.89, 1.53	1.08	0.81, 1.44	1.17	0.88, 1.55	
DPA	Premenopausal	1.00	1.14	0.64, 2.01	0.75	0.41, 1.37	1.27	0.74, 2.17	0.17
	Postmenopausal	1.00	0.94	0.72, 1.22	0.69	0.52, 0.92	0.88	0.67, 1.17	
DHA	Premenopausal	1.00	1.11	0.64, 1.94	0.79	0.45, 1.41	1.09	0.63, 1.88	0.13
	Postmenopausal	1.00	0.90	0.69, 1.18	0.74	0.56, 0.99	0.70	0.53, 0.93	
EPA	Premenopausal	1.00	1.18	0.67, 2.09	0.93	0.54, 1.63	1.14	0.65, 2.01	0.13
	Postmenopausal	1.00	0.89	0.68, 1.16	0.70	0.52, 0.93	0.76	0.58, 1.01	
ω-6	Premenopausal	1.00	1.15	0.58, 2.28	1.51	0.80, 2.85	1.63	0.87, 3.05	0.09
	Postmenopausal	1.00	1.11	0.85, 1.46	1.00	0.75, 1.33	1.26	0.95, 1.67	
LA	Premenopausal	1.00	1.16	0.59, 2.30	1.51	0.80, 2.85	1.62	0.86, 3.02	0.09
	Postmenopausal	1.00	1.16	0.88, 1.51	1.00	0.75, 1.34	1.28	0.97, 1.70	
AA	Premenopausal	1.00	0.86	0.46, 1.62	1.17	0.65, 2.11	1.40	0.80, 2.45	0.11
	Postmenopausal	1.00	1.07	0.81, 1.41	0.87	0.66, 1.17	1.18	0.89, 1.57	
ω-3/ω-6	Premenopausal	1.00	1.06	0.63, 1.78	0.69	0.37, 1.30	1.27	0.74, 2.19	0.01
	Postmenopausal	1.00	0.84	0.62, 1.13	1.00	0.76, 1.31	0.78	0.58, 1.03	

Table A.35 Age-adjusted HRs and 95% CIs for the potential association between PUFA and all-cause mortality stratified by hormone therapy treatment

PUFA	Hormone Therapy	Q1	Q2		Q3		Q4		LRT
		HR	HR	95% CI	HR	95% CI	HR	95% CI	p
PUFA	No	1.00	0.96	0.52, 1.78	1.21	0.68, 2.17	1.23	0.66, 2.29	0.34
	Yes	1.00	1.14	0.76, 1.70	0.99	0.65, 1.50	1.20	0.79, 1.81	
ω-3	No	1.00	0.89	0.48, 1.66	1.12	0.63, 1.99	1.08	0.60, 1.96	0.87
	Yes	1.00	0.94	0.62, 1.42	1.10	0.74, 1.65	1.09	0.72, 1.65	
ALA	No	1.00	0.86	0.47, 1.60	1.12	0.63, 2.02	1.03	0.56, 1.87	0.54
	Yes	1.00	1.03	0.69, 1.55	1.12	0.74, 1.70	1.20	0.80, 1.80	
DPA	No	1.00	1.15	0.65, 2.06	0.71	0.37, 1.35	0.88	0.48, 1.62	0.20
	Yes	1.00	0.85	0.56, 1.29	0.66	0.44, 1.00	0.97	0.65, 1.45	
DHA	No	1.00	0.79	0.43, 1.44	0.76	0.42, 1.37	0.66	0.36, 1.21	0.64
	Yes	1.00	0.88	0.58, 1.32	0.79	0.52, 1.19	0.79	0.53, 1.18	
EPA	No	1.00	0.81	0.44, 1.49	0.75	0.42, 1.34	0.66	0.36, 1.20	0.40
	Yes	1.00	0.83	0.55, 1.25	0.69	0.46, 1.06	0.83	0.56, 1.23	
ω-6	No	1.00	1.12	0.60, 2.10	1.33	0.73, 2.41	1.43	0.76, 2.68	0.69
	Yes	1.00	1.07	0.71, 1.62	1.10	0.74, 1.66	1.23	0.81, 1.86	
LA	No	1.00	1.24	0.66, 2.33	1.38	0.75, 2.53	1.53	0.81, 2.89	0.63
	Yes	1.00	1.12	0.74, 1.68	1.11	0.74, 1.66	1.24	0.82, 1.87	
AA	No	1.00	1.00	0.58, 1.72	0.45	0.24, 0.87	0.93	0.51, 1.70	0.02
	Yes	1.00	1.20	0.78, 1.84	1.07	0.70, 1.65	1.25	0.82, 1.92	
ω-3/ω-6	No	1.00	0.60	0.32, 1.13	0.77	0.44, 1.35	0.54	0.31, 0.95	0.14
	Yes	1.00	0.89	0.58, 1.35	0.92	0.61, 1.38	0.88	0.58, 1.34	

Table A.36 Age-adjusted HRs and 95% CIs for the potential association between PUFA and all-cause mortality stratified by chemotherapy treatment

PUFA	Chemo	Q1	Q2		Q3		Q4		LRT
		HR	HR	95% CI	HR	95% CI	HR	95% CI	p
PUFA	No	1.00	0.88	0.58, 1.34	0.89	0.58, 1.38	0.92	0.58, 1.44	0.02
	Yes	1.00	1.58	0.91, 2.75	1.37	0.80, 2.33	1.64	0.95, 2.84	
ω-3	No	1.00	0.82	0.54, 1.24	0.86	0.56, 1.34	0.81	0.51, 1.28	0.01
	Yes	1.00	1.10	0.62, 1.95	1.66	1.01, 2.74	1.54	0.92, 2.55	
ALA	No	1.00	0.89	0.59, 1.36	0.91	0.58, 1.43	0.82	0.52, 1.30	0.01
	Yes	1.00	1.20	0.69, 2.09	1.71	1.03, 2.83	1.59	0.96, 2.62	
DPA	No	1.00	0.89	0.57, 1.37	0.58	0.37, 0.92	1.02	0.66, 1.56	0.33
	Yes	1.00	0.99	0.60, 1.63	0.82	0.49, 1.36	0.94	0.57, 1.54	
DHA	No	1.00	0.85	0.54, 1.31	0.87	0.56, 1.35	0.71	0.45, 1.11	0.40
	Yes	1.00	0.80	0.48, 1.31	0.67	0.41, 1.11	0.79	0.49, 1.26	
EPA	No	1.00	0.77	0.49, 1.19	0.64	0.41, 1.00	0.75	0.48, 1.17	0.24
	Yes	1.00	0.87	0.52, 1.45	0.86	0.53, 1.41	0.78	0.48, 1.26	
ω-6	No	1.00	0.95	0.62, 1.46	0.95	0.61, 1.47	1.07	0.68, 1.67	0.06
	Yes	1.00	1.40	0.80, 2.45	1.51	0.90, 2.55	1.56	0.90, 2.70	
LA	No	1.00	1.01	0.66, 1.53	0.93	0.59, 1.45	1.09	0.70, 1.70	0.04
	Yes	1.00	1.49	0.85, 2.62	1.61	0.95, 2.73	1.62	0.93, 2.82	
AA	No	1.00	0.92	0.60, 1.43	0.65	0.41, 1.03	1.06	0.68, 1.65	0.15
	Yes	1.00	1.30	0.79, 2.14	1.08	0.63, 1.83	1.23	0.73, 2.08	
ω-3/ω-6	No	1.00	0.84	0.53, 1.34	0.91	0.59, 1.40	0.65	0.42, 1.02	0.05
	Yes	1.00	0.73	0.45, 1.20	0.74	0.45, 1.22	1.04	0.65, 1.68	

Table A.37 Age-adjusted HRs and 95% CIs for the potential association between PUFA and all-cause mortality stratified by radiation treatment

PUFA	Radiation	Q1	Q2		Q3		Q4		LRT
		HR	HR	95% CI	HR	95% CI	HR	95% CI	p
PUFA	No	1.00	0.89	0.52, 1.53	0.81	0.46, 1.43	1.32	0.79, 2.21	0.04
	Yes	1.00	1.28	0.85, 1.93	1.26	0.84, 1.89	1.10	0.70, 1.72	
ω-3	No	1.00	0.68	0.39, 1.20	1.14	0.67, 1.93	1.13	0.68, 1.91	0.08
	Yes	1.00	1.12	0.73, 1.71	1.16	0.77, 1.74	1.04	0.68, 1.61	
ALA	No	1.00	0.80	0.47, 1.39	1.05	0.61, 1.81	1.16	0.69, 1.96	0.19
	Yes	1.00	1.14	0.75, 1.74	1.26	0.83, 1.91	1.08	0.70, 1.66	
DPA	No	1.00	1.14	0.67, 1.93	0.73	0.42, 1.27	0.95	0.56, 1.60	0.26
	Yes	1.00	0.81	0.53, 1.23	0.65	0.42, 1.00	0.94	0.62, 1.43	
DHA	No	1.00	1.01	0.59, 1.70	0.75	0.44, 1.28	0.75	0.44, 1.28	0.24
	Yes	1.00	0.73	0.48, 1.12	0.79	0.52, 1.20	0.74	0.49, 1.12	
EPA	No	1.00	1.06	0.64, 1.78	0.73	0.42, 1.27	0.74	0.43, 1.27	0.09
	Yes	1.00	0.66	0.43, 1.01	0.69	0.46, 1.04	0.76	0.51, 1.14	
ω-6	No	1.00	0.90	0.51, 1.59	0.91	0.52, 1.59	1.41	0.84, 2.36	0.05
	Yes	1.00	1.27	0.84, 1.92	1.37	0.91, 2.06	1.16	0.73, 1.82	
LA	No	1.00	0.98	0.56, 1.71	0.91	0.52, 1.59	1.44	0.86, 2.42	0.05
	Yes	1.00	1.32	0.87, 2.01	1.40	0.93, 2.10	1.19	0.75, 1.88	
AA	No	1.00	1.22	0.70, 2.12	1.06	0.59, 1.91	1.19	0.67, 2.13	0.25
	Yes	1.00	1.04	0.69, 1.56	0.73	0.47, 1.11	1.13	0.75, 1.72	
ω-3/ω-6	No	1.00	0.71	0.41, 1.22	0.80	0.47, 1.36	0.91	0.55, 1.50	0.18
	Yes	1.00	0.82	0.53, 1.26	0.81	0.54, 1.22	0.68	0.45, 1.04	

Table A.38 Models for adjusting for total energy intake

Model	Covariates	Interpretation
Age-adjusted only	age	Adding PUFAs to your current diet
Model 1: Multivariate adjustment	age total energy	Substituting PUFAs for any other source of energy
Model 2: Standard multivariate adjustment	**Set 1**: age total energy protein carbohydrates	Substituting PUFAs for any other fat or alcohol
	Set 2: age total energy protein carbohydrates alcohol	Substituting PUFAs for any other fat
Model 3: Standard multivariate adjustment	**Set 1:** age total energy protein carbohydrates non-saturated fats	Substituting PUFAs for saturated fats or alcohol
	Set 2: age total energy protein carbohydrates non-saturated fats alcohol	Substituting PUFAs for saturated fats
Model 4: Standard multivariate adjustment	**Set 1:** age total energy protein carbohydrates saturated fats	Substituting PUFAs for non-saturated fats or alcohol
	Set 2: age total energy protein carbohydrates saturated fats alcohol	Substituting PUFAs for non-saturated fats

Adapted from [387]

Table A.39 Comparison of different energy adjustment methods (no alcohol adjustment) for breast cancer incidence

PUFA	Model	Q1		Q2		Q3		Q4	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
ω-3	Age-adjusted	1.00		1.20	0.98, 1.47	1.15	0.93, 1.41	1.05	0.85, 1.29
	Model 1	1.00		1.25	1.01, 1.54	1.23	0.98, 1.54	1.20	0.92, 1.58
	Model 2	1.00		1.25	1.01, 1.54	1.21	0.96, 1.53	1.15	0.85, 1.56
	Model 3	1.00		1.25	1.01, 1.54	1.21	0.96, 1.53	1.15	0.85, 1.56
	Model 4	1.00		1.25	1.01, 1.54	1.23	0.97, 1.55	1.19	0.87, 1.62
ω-6	Age-adjusted	1.00		1.11	0.91, 1.37	1.23	1.00, 1.51	1.04	0.84, 1.28
	Model 1	1.00		1.15	0.93, 1.42	1.31	1.05, 1.63	1.18	0.91, 1.55
	Model 2	1.00		1.14	0.92, 1.41	1.29	1.03, 1.62	1.14	0.84, 1.55
	Model 3	1.00		1.14	0.92, 1.41	1.29	1.03, 1.63	1.14	0.83, 1.57
	Model 4	1.00		1.14	0.92, 1.41	1.29	1.02, 1.62	1.13	0.83, 1.53

Interpretation:

Age-adjusted: Adding PUFAs to your current diet
Model 1: Substituting PUFAs for any other source of energy
Model 2: Substituting PUFAs for any other fat or alcohol
Model 3: Substituting PUFAs for saturated fats or alcohol
Model 4: Substituting PUFAs for non-saturated fats or alcohol

Table A.40 Comparison of different energy adjustment methods (alcohol adjustment) for breast cancer incidence

PUFA	Model	Q1		Q2		Q3		Q4	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
ω-3	Age-adjusted	1.00		1.20	0.98, 1.47	1.15	0.93, 1.41	1.05	0.85, 1.29
	Model 1	1.00		1.25	1.01, 1.55	1.24	0.99, 1.55	1.21	0.92, 1.58
	Model 2	1.00		1.25	1.01, 1.54	1.22	0.97, 1.54	1.17	0.86, 1.59
	Model 3	1.00		1.25	1.01, 1.54	1.22	0.97, 1.54	1.17	0.86, 1.59
	Model 4	1.00		1.25	1.01, 1.54	1.23	0.97, 1.55	1.19	0.87, 1.63
ω-6	Age-adjusted	1.00		1.11	0.91, 1.37	1.23	1.00, 1.51	1.03	0.84, 1.28
	Model 1	1.00		1.15	0.94, 1.42	1.32	1.06, 1.64	1.19	0.91, 1.55
	Model 2	1.00		1.15	0.93, 1.42	1.30	1.04, 1.64	1.16	0.85, 1.59
	Model 3	1.00		1.15	0.93, 1.42	1.30	1.03, 1.64	1.15	0.83, 1.58
	Model 4	1.00		1.14	0.93, 1.41	1.29	1.02, 1.63	1.13	0.82, 1.55

Interpretation:

Age-adjusted: Adding PUFAs to your current diet
Model 1: Substituting PUFAs for any other source of energy
Model 2: Substituting PUFAs for any other fat
Model 3: Substituting PUFAs for saturated fats
Model 4: Substituting PUFAs for non-saturated fats

Table A.41 Comparison of different energy adjustment methods (no alcohol adjustment) for breast cancer survival

PUFA	Model	Q1		Q2		Q3		Q4	
		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
ω-3	Age-adjusted	1.00		0.97	0.75, 1.25	1.15	0.90, 1.48	1.14	0.89, 1.46
	Model 1	1.00		0.93	0.71, 1.21	1.07	0.82, 1.41	1.00	0.72, 1.38
	Model 2	1.00		0.90	0.69, 1.17	1.00	0.75, 1.32	0.83	0.58, 1.21
	Model 3	1.00		0.90	0.69, 1.17	0.99	0.75, 1.32	0.83	0.57, 1.20
	Model 4	1.00		0.89	0.69, 1.16	0.95	0.71, 1.26	0.75	0.51, 1.10
ω-6	Age-adjusted	1.00		1.12	0.87, 1.43	1.07	0.83, 1.38	1.34	1.04, 1.72
	Model 1	1.00		1.10	0.85, 1.42	1.04	0.79, 1.38	1.27	0.92, 1.76
	Model 2	1.00		1.07	0.82, 1.38	0.98	0.73, 1.31	1.11	0.77, 1.60
	Model 3	1.00		1.06	0.82, 1.38	0.97	0.72, 1.29	1.06	0.72, 1.56
	Model 4	1.00		1.06	0.81, 1.37	0.98	0.74, 1.31	1.14	0.79, 1.66

Interpretation:

Age-adjusted: Adding PUFAs to your current diet
Model 1: Substituting PUFAs for any other source of energy
Model 2: Substituting PUFAs for any other fat or alcohol
Model 3: Substituting PUFAs for saturated fats or alcohol
Model 4: Substituting PUFAs for non-saturated fats or alcohol

Table A.42 Comparison of different energy adjustment methods (alcohol adjustment) for breast cancer survival

PUFA	Model	Q1		Q2		Q3		Q4	
		HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
ω-3	Age-adjusted	1.00		0.97	0.75, 1.25	1.15	0.90, 1.48	1.14	0.89, 1.47
	Model 1	1.00		0.93	0.71, 1.20	1.07	0.81, 1.40	1.00	0.72, 1.38
	Model 2	1.00		0.89	0.69, 1.17	0.98	0.74, 1.30	0.80	0.55, 1.17
	Model 3	1.00		0.89	0.69, 1.17	0.98	0.74, 1.30	0.81	0.55, 1.17
	Model 4	1.00		0.89	0.69, 1.16	0.95	0.71, 1.26	0.75	0.51, 1.10
ω-6	Age-adjusted	1.00		1.10	0.85, 1.42	1.04	0.79, 1.38	1.27	0.92, 1.75
	Model 1	1.00		1.10	0.85, 1.42	1.04	0.79, 1.38	1.27	0.92, 1.75
	Model 2	1.00		1.05	0.81, 1.37	0.97	0.72, 1.29	1.07	0.73, 1.55
	Model 3	1.00		1.05	0.81, 1.37	0.96	0.72, 1.29	1.06	0.72, 1.56
	Model 4	1.00		1.06	0.82, 1.38	0.99	0.74, 1.32	1.16	0.79, 1.71

Interpretation:

Age-adjusted: Adding PUFAs to your current diet
Model 1: Substituting PUFAs for any other source of energy
Model 2: Substituting PUFAs for any other fat
Model 3: Substituting PUFAs for saturated fats
Model 4: Substituting PUFAs for non-saturated fats

Table A.43 Sensitivity analyses examining PUFA adjustment for breast cancer incidence

PUFA	Covariates included in model:	Q1	Q2		Q3		Q4	
		OR	OR	95% CI	OR	95% CI	OR	95% CI
ω-3	Age	1.00	1.20	0.98, 1.47	1.15	0.93, 1.41	1.05	0.85, 1.29
	Age, energy	1.00	1.25	1.01, 1.54	1.23	0.98, 1.54	1.20	0.92, 1.58
	Age, energy, ω-6	1.00	1.17	0.93, 1.48	1.08	0.82, 1.43	1.07	0.76, 1.50
	Age, energy, LA, AA	1.00	1.17	0.92, 1.48	1.09	0.82, 1.45	1.07	0.75, 1.52
	Age, energy, ω-6, LA, AA	1.00	1.17	0.92, 1.48	1.09	0.82, 1.45	1.07	0.75, 1.52
ω-6	Age	1.00	1.11	0.91, 1.37	1.23	1.00, 1.51	1.04	0.84, 1.28
	Age, energy	1.00	1.15	0.93, 1.42	1.31	1.05, 1.63	1.18	0.91, 1.55
	Age, energy, ω-3	1.00	1.09	0.86, 1.38	1.25	0.96, 1.64	1.16	0.83, 1.63
	Age, energy, ALA, DPA, DHA, EPA	1.00	1.05	0.82, 1.34	1.17	0.88, 1.56	1.07	0.75, 1.53
	Age, energy, ω-3, ALA, DPA, DHA, EPA	1.00	1.05	0.82, 1.35	1.20	0.89, 1.60	1.11	0.78, 1.60

Table A.44 Sensitivity analyses examining PUFA adjustment for breast cancer survival

PUFA	Covariates included in model:	Q1	Q2		Q3		Q4	
		HR	HR	95% CI	HR	95% CI	HR	95% CI
ω-3	Age	1.00	0.97	0.75, 1.25	1.15	0.90, 1.48	1.14	0.89, 1.46
	Age, energy	1.00	0.93	0.71, 1.21	1.07	0.82, 1.41	1.00	0.72, 1.38
	Age, energy, ω-6	1.00	0.90	0.68, 1.20	1.01	0.73, 1.41	0.85	0.57, 1.28
	Age, energy, LA, AA	1.00	0.90	0.67, 1.21	1.00	0.71, 1.41	0.83	0.55, 1.26
	Age, energy, ω-6, LA, AA	1.00	0.92	0.68, 1.23	1.02	0.73, 1.45	0.85	0.56, 1.29
ω-6	Age	1.00	0.97	0.75, 1.25	1.15	0.90, 1.48	1.14	0.89, 1.46
	Age, energy	1.00	0.93	0.71, 1.21	1.07	0.82, 1.41	1.00	0.72, 1.38
	Age, energy, ω-3	1.00	1.12	0.84, 1.49	1.06	0.76, 1.49	1.37	0.92, 2.04
	Age, energy, ALA, DPA, DHA, EPA	1.00	1.02	0.75, 1.38	0.98	0.69, 1.38	1.26	0.83, 1.91
	Age, energy, ω-3, ALA, DPA, DHA, EPA	1.00	1.02	0.75, 1.39	0.97	0.68, 1.39	1.23	0.80, 1.88

Table A.45 Power for PUFA/Fish intake & Incidence (Aim 1a)

Exposure	Detectable OR	Power (%) for three different proportions of exposed controls		
		0.25	**0.33**	**0.50**
Total ω-3, Fish intake, or the PUFA ratio	0.5	>99%	>99%	>99%
	0.6	>99%	>99%	>99%
	0.7	98%	99%	>99%
Total ω-6	1.4	99%	99%	>99%
	1.7	>99%	>99%	>99%
	2.0	>99%	>99%	>99%

Note:
n = 1481 cases and 1518 controls with FFQ data available

Power presented for a range of: (1) exposure levels for PUFAs (based on considering intake categorized as quartiles (0.25), tertiles (0.33) or as a dichotomous variable (0.5); and (2) minimum ORs for ω-3 (0.5-0.7) and for ω-6 (1.4-2.0), which is consistent with previously published effect estimates [422].

Table A.46 Power for PUFA-gene interactions & incidence (Aim 1b)

Prevalence of exposure		θ* = 2 OR(x,y) = 2.1	θ = 3 OR(x,y) = 3.2	θ = 0.5 OR(x,y) = 0.53	θ = 0.3 OR(x,y) = 0.32
Variant Genotype	**ω-3 or PUFA ratio**	**Power**	**Power**	**Power**	**Power**
0.10	0.25	66%	98%	47%	84%
	0.33	73%	99%	56%	91%
	0.50	77%	99%	66%	97%
0.25	0.25	90%	>99%	77%	99%
	0.33	94%	>99%	86%	>99%
	0.50	96%	>99%	93%	>99%
0.40	0.25	93%	>99%	86%	>99%
	0.33	96%	>99%	93%	>99%
	0.50	98%	>99%	97%	>99%

Note:
n = 1067 cases and 1110 controls with genotyping and PUFA estimates

Power presented for a range of: (1) exposure levels for PUFAs (ω-3 fatty acids, or the ratio of ω-3 to ω-6 fatty acids), based on considering PUFAs categorized as quartiles (0.25), tertiles (0.33) or as a dichotomous variable (0.5); (2) genotype prevalence (= minor allele frequencies (MAF from 0.10 to 0.40)); and (3) plausible minimum detectable ORs for the joint effect of PUFAs and genotype (2.1 and 3.2, or 0.53 and 0.32) [344, 422].

*θ (departure from multiplicativity) = OR (x,y)/ [OR(x)*OR(y)], where OR(x)=the odds ratio for genotype relative to low risk exposure to factor y; OR(y)=the odds ratio for factor y relative to the low risk genotype; OR(x,y)=the hypothesized OR for persons with the high risk genotype and exposure.

Table A.47 Power for PUFA/Fish intake & Survival (Aim 2a)

All-cause mortality				
Exposure	**Detectable HR**	**Power for 3 different proportions of exposed breast cancer cases**		
		0.25	**0.33**	**0.50**
Total ω-3, fish or the PUFA ratio	0.5	>99%	>99%	>99%
	0.6	95%	98%	>99%
	0.7	75%	83%	89%
Total ω-6	1.4	88%	92%	94%
	1.7	>99%	>99%	>99%
	2.0	>99%	>99%	>99%
Breast cancer-specific mortality				
Exposure	**Detectable HR**	**Power for 3 different proportions of exposed breast cancer cases**		
		0.25	**0.33**	**0.50**
Total ω-3, fish or the PUFA ratio	0.5	89%	94%	97%
	0.6	70%	79%	86%
	0.7	45%	53%	60%
Total ω-6	1.4	60%	66%	69%
	1.7	96%	98%	98%
	2.0	>99%	>99%	>99%

Note:
n = 1481 cases with LIBCSP FFQ responses

Power presented for a range of: (1) exposure levels for PUFA intake (based on considering intake categorized as quartiles (0.25), tertiles (0.33) or as a dichotomous variable (0.5); and (2) minimum detectable HR for ω-3 (0.5-0.7) and ω-6 (1.4-2.0) fatty acids. We have assumed the outcome includes 436 overall deaths (199 breast cancer-specific deaths) over an average of 12.7 years of follow-up. Power will be better than illustrated here when we consider PUFA intake as a continuous variable.

Table A.48 Power for Genotypes & Survival (Aim 2b)

All-cause mortality			
Detectable	**Power (%) for 3 different proportions of variant genotypes**		
HR	**0.10**	**0.25**	**0.40**
2.0	>99%	>99%	>99%
1.7	89%	>99%	>99%
1.5	64%	90%	95%
Breast cancer-specific mortality			
Detectable	**Power (%) for 3 different proportions of variant genotypes**		
HR	**0.10**	**0.25**	**0.40**
2.0	89%	99%	99%
1.7	63%	88%	93%
1.5	38%	64%	72%

Note:
n= 1067 cases with LIBCSP with available DNA

Power presented for a range of: (1) genotype prevalence (= minor allele frequencies (MAF from 0.10 to 0.40)); and (2) minimum detectable HR ranging from 1.3 - 2.0. We have assumed the outcome to include 314 overall deaths (144 breast cancer-specific deaths) over an average of 12.7 years of follow-up.

Table A.49 Age- and multivariate-adjusted ORs and 95% CI for the association between dietary fish intake (by various cooking methods) and breast cancer incidence

Method	Model	Never			< 1 serving per month				1-3 servings per month				> 1 per week			
		Co	Ca	OR	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI	Co	Ca	OR	95% CI
Grilled/BBQ	Age-adjusted	821	807	1.00	248	213	0.94	0.76, 1.16	241	232	1.06	0.86, 1.30	94	96	1.09	0.80, 1.47
	Multivariate			1.00			0.85	0.68, 1.05			1.00	0.80, 1.24			0.98	0.72, 1.35
Pan Fried	Age-adjusted	774	782	1.00	246	228	0.93	0.76, 1.15	291	260	0.89	0.73, 1.08	78	77	0.96	0.69, 1.33
	Multivariate			1.00			0.88	0.71, 1.09			0.86	0.70, 1.05			0.86	0.61, 1.22
Broiled	Age-adjusted	562	540	1.00	218	205	1.01	0.81, 1.27	441	429	1.02	0.85, 1.22	195	185	0.95	0.75, 1.21
	Multivariate			1.00			0.93	0.73, 1.17			0.91	0.76, 1.10			0.88	0.68, 1.12
Baked	Age-adjusted	534	490	1.00	226	238	1.17	0.94, 1.46	474	460	1.06	0.88, 1.26	176	195	1.17	0.92, 1.48
	Multivariate			1.00			1.09	0.86, 1.37			0.98	0.81, 1.18			1.14	0.89, 1.47
Microwaved	Age-adjusted	1235	1205	1.00	49	38	0.79	0.52, 1.22	52	42	0.82	0.54, 1.24	21	23	1.02	0.56, 1.85
	Multivariate			1.00			0.78	0.50, 1.21			0.75	0.49, 1.16			0.87	0.46, 1.67

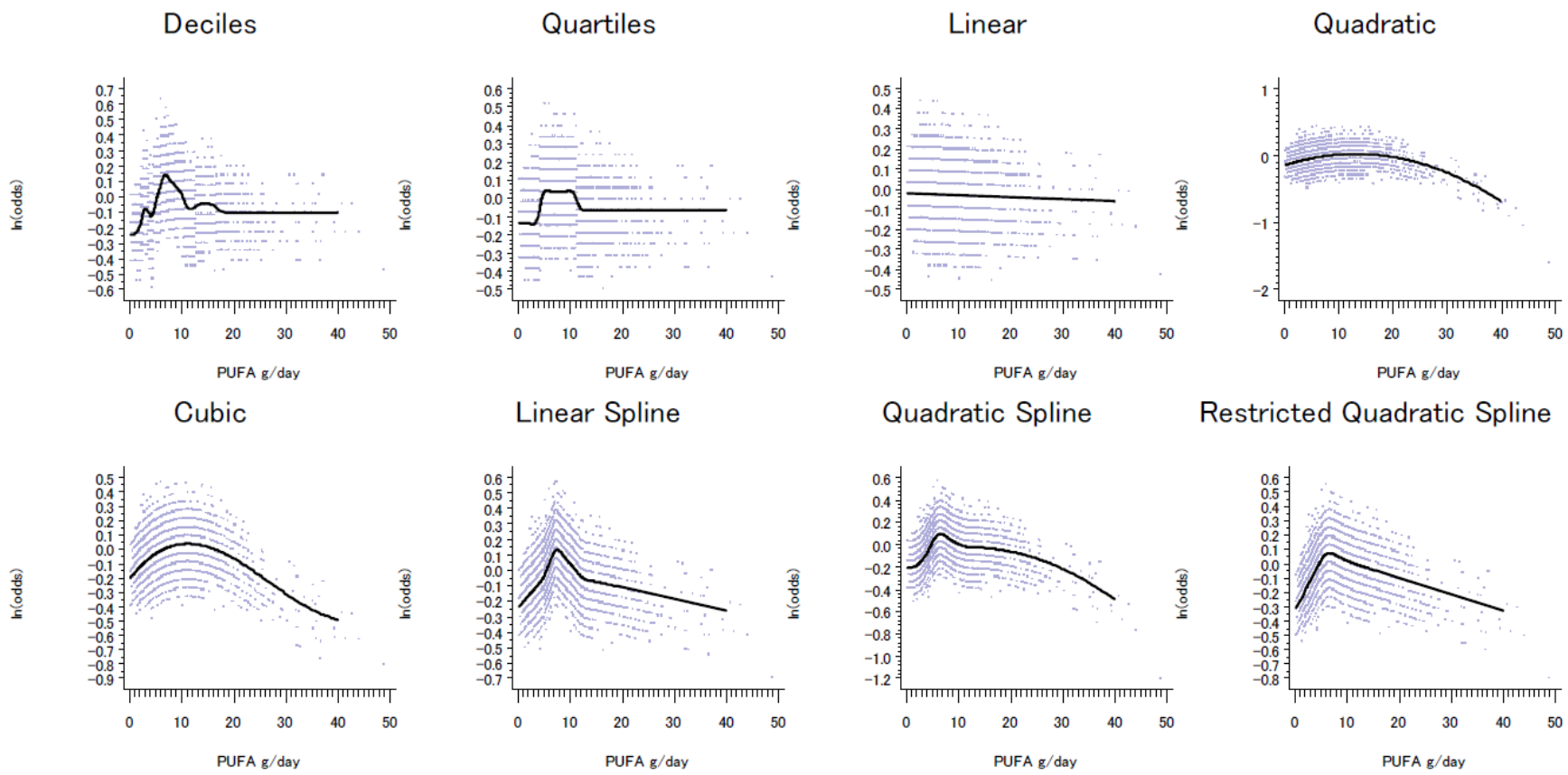
Note:
Multivariate ORs and 95% CI adjusted for matching factor (5-year age group), energy intake (kcal/day), and NSAID use (aspirin, acetaminophen, ibuprofen)
Co=Controls, Ca=Cases

Table A.50 Age- and multivariate-adjusted HRs and 95% CI for the association between dietary fish intake (by various cooking methods) and all-cause mortality

Method	Model	Never			< 1 serving per month				1-3 servings per month				> 1 per week			
		D	Co	HR	D	Co	HR	95% CI	D	Co	HR	95% CI	D	Co	HR	95% CI
Grilled/BBQ	Age-adjusted	300	507	1.00	49	164	0.76	0.56, 1.04	57	175	0.79	0.59, 1.05	26	70	0.82	0.55, 1.22
	Multivariate			1.00			0.76	0.56, 1.04			0.78	0.58, 1.04			0.80	0.53, 1.19
Pan Fried	Age-adjusted	248	534	1.00	65	163	1.01	0.77, 1.33	96	164	1.27	1.00, 1.61	27	50	1.10	0.74, 1.63
	Multivariate			1.00			1.01	0.77, 1.32			1.24	0.98, 1.57			1.07	0.72, 1.59
Broiled	Age-adjusted	191	349	1.00	65	140	1.00	0.75, 1.33	123	306	0.79	0.63, 0.99	59	126	0.77	0.58, 1.03
	Multivariate			1.00			1.00	0.75, 1.32			0.79	0.63, 0.99			0.75	0.56, 1.01
Baked	Age-adjusted	162	328	1.00	71	167	0.95	0.72, 1.25	145	315	0.93	0.75, 1.17	74	121	1.06	0.80, 1.39
	Multivariate			1.00			0.94	0.71, 1.24			0.93	0.74, 1.16			1.04	0.79, 1.37
Microwaved	Age-adjusted	399	806	1.00	10	28	0.84	0.45, 1.56	11	31	0.74	0.41, 1.35	8	15	0.79	0.39, 1.60
	Multivariate			1.00			0.85	0.45, 1.59			0.74	0.41, 1.35			0.78	0.39, 1.57

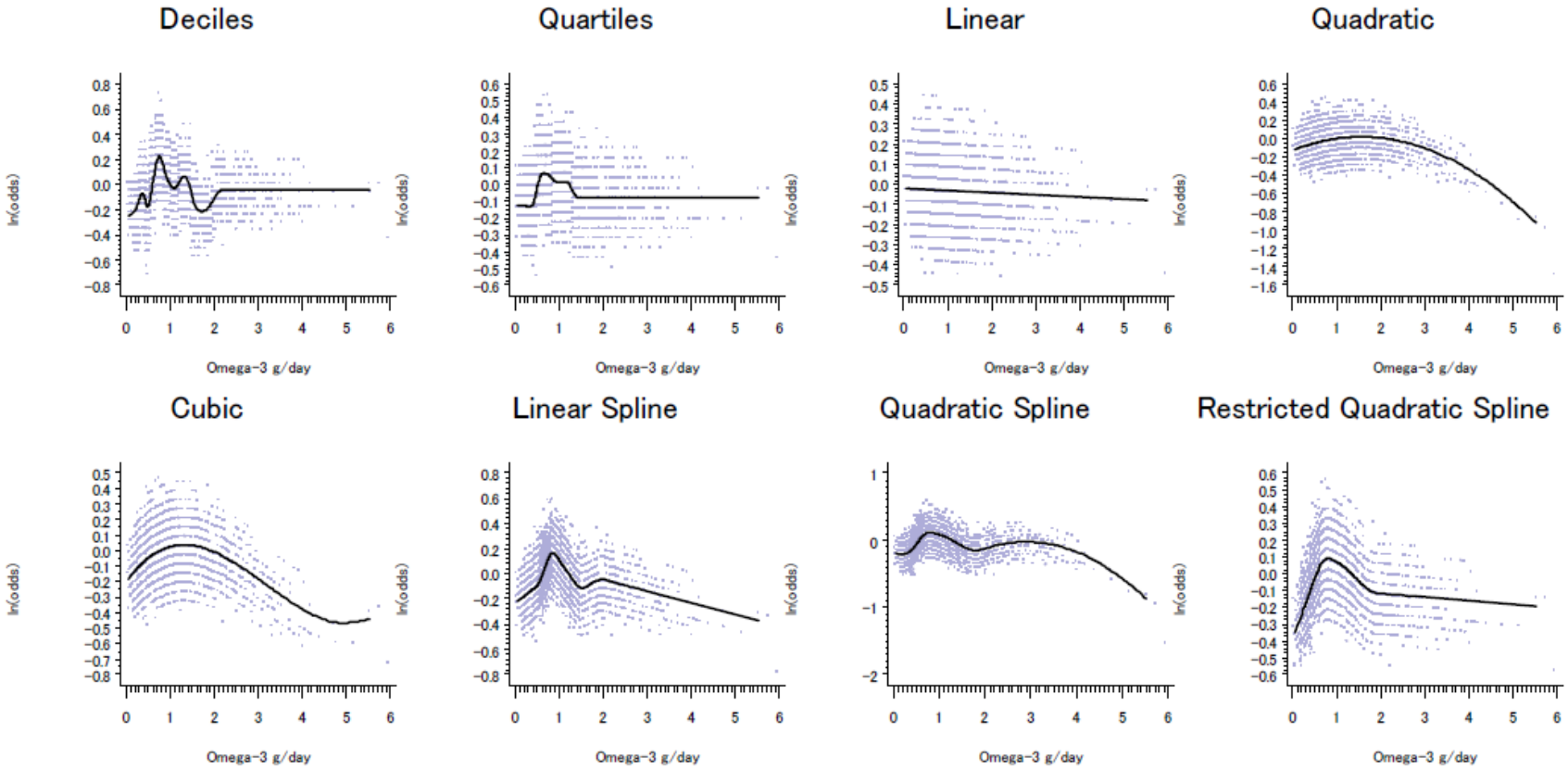
Note:
Multivariate HRs and 95% CI adjusted for age (5-year age group) and energy intake (kcal/day)
Co=Cohort, D=deaths

Figure A.1 Dose-response between total PUFA intake (g/day) and the age-adjusted log-odds of breast cancer in the LIBCSP



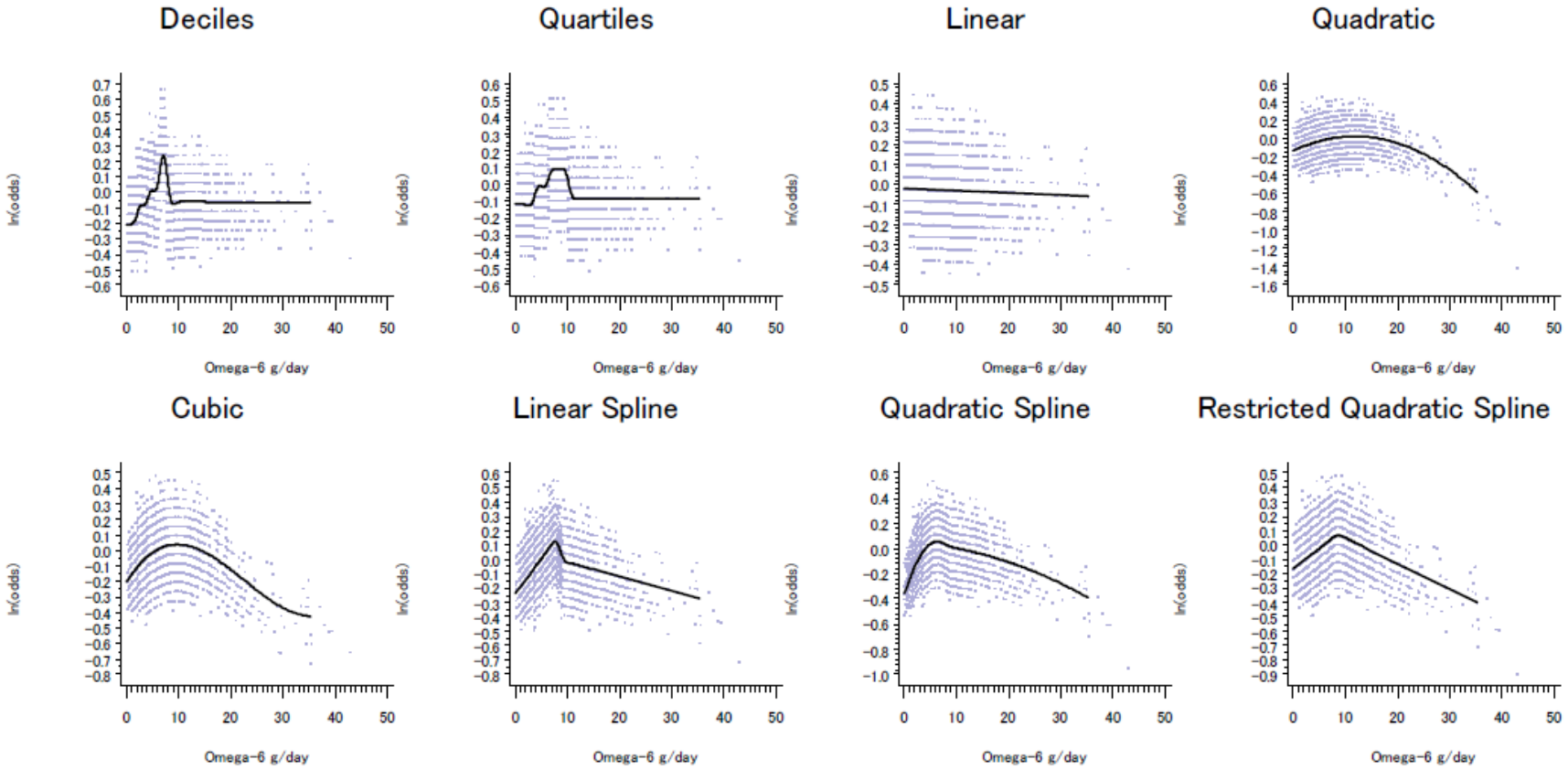
Note: Line represents women 54-59 years old

Figure A.2 Dose-response between total ω-3 intake (g/day) and the age-adjusted log-odds of breast cancer in the LIBCSP



Note: Line represents women 54-59 years old

Figure A.3 Dose-response between total ω-6 intake (g/day) and the age-adjusted log-odds of breast cancer in the LIBCSP



Note: Line represents women 54-59 years old

Figure A.4 Kaplan-Meier Survival curves for total PUFA, total ω-3, total ω-6, and ω-3/ω-6 intake (quartiles) and all-cause mortality

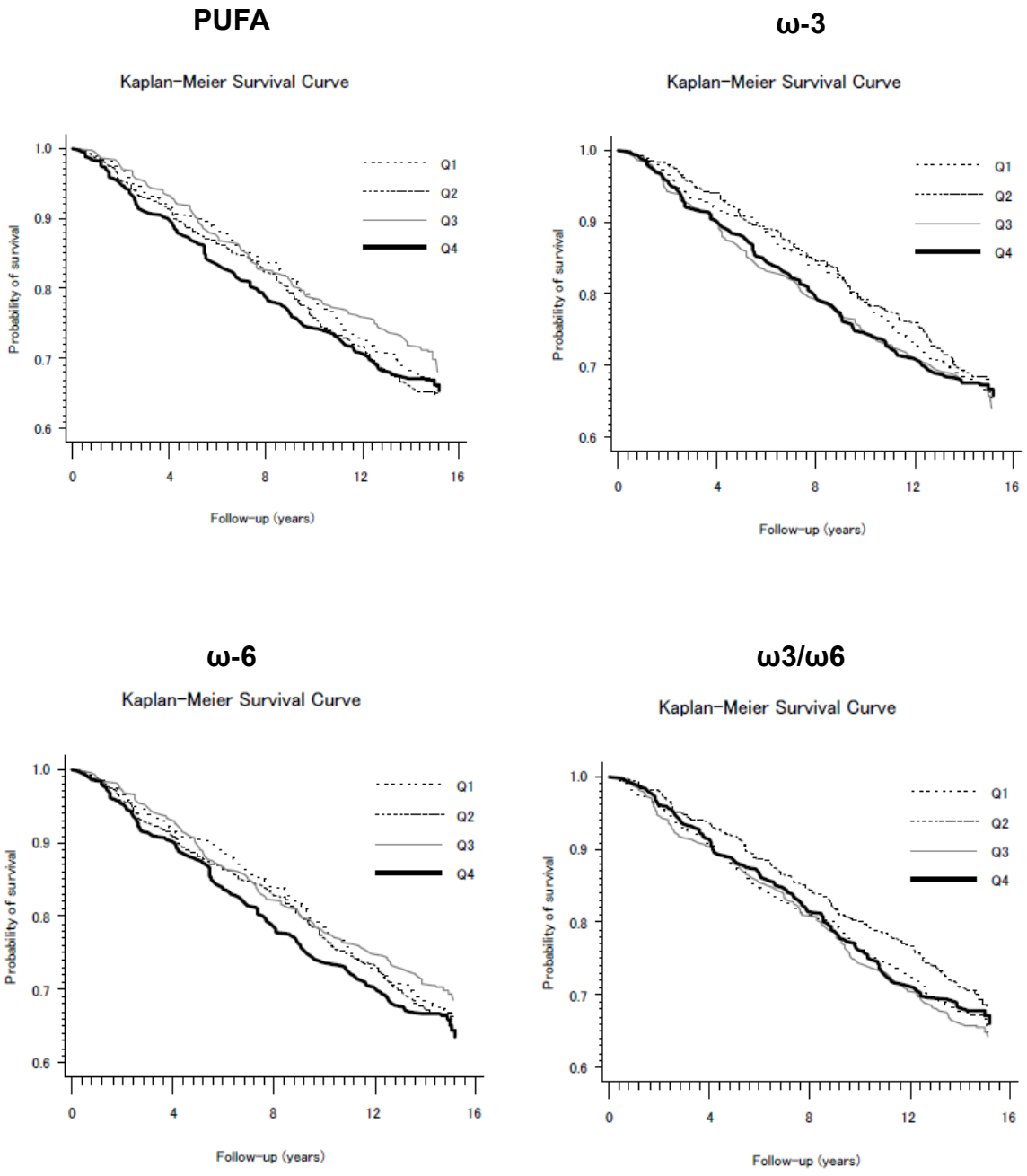


Figure A.5 Kaplan-Meier Survival curves for ALA, DPA, DHA, and EPA intake (quartiles) and all-cause mortality

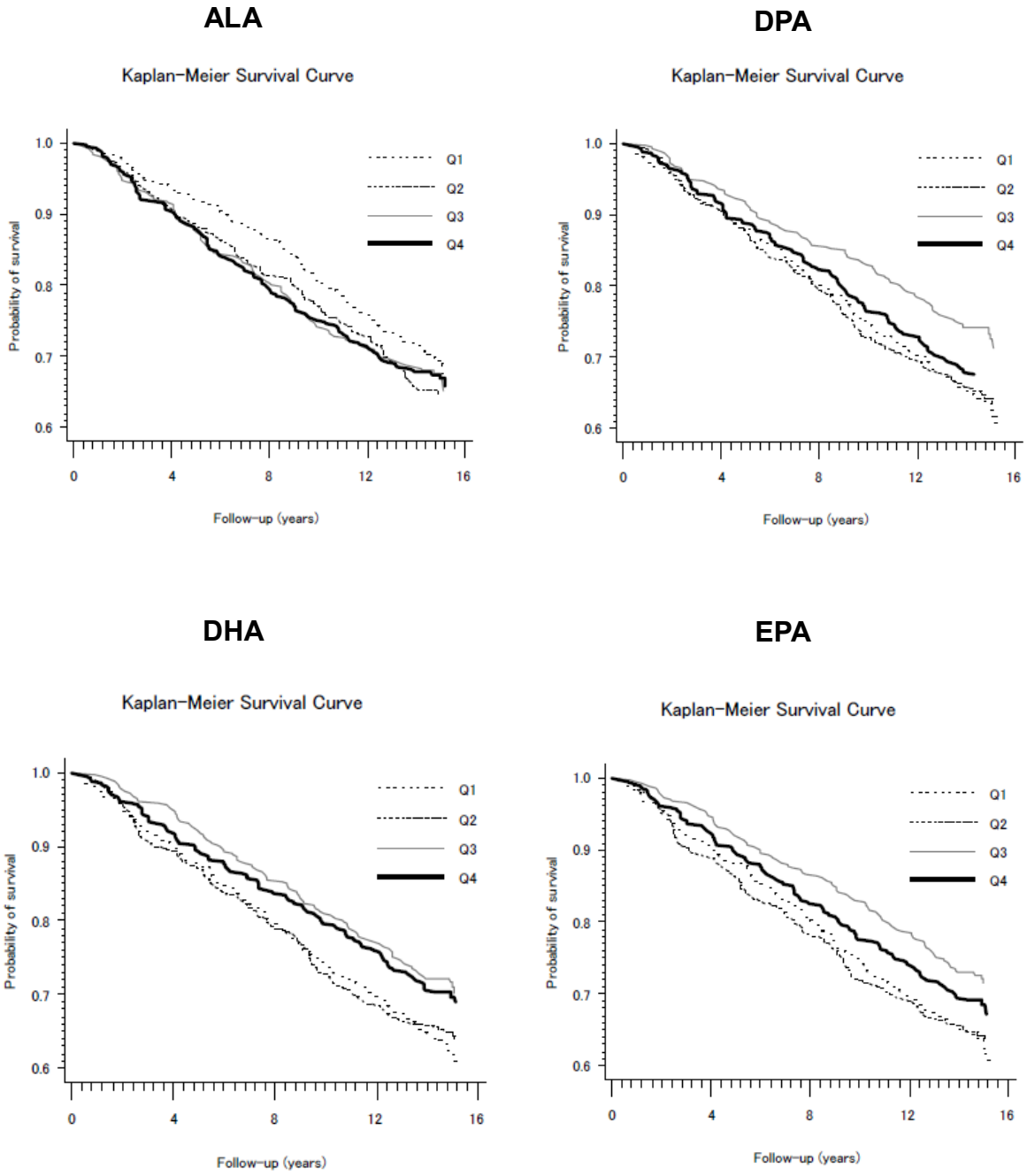
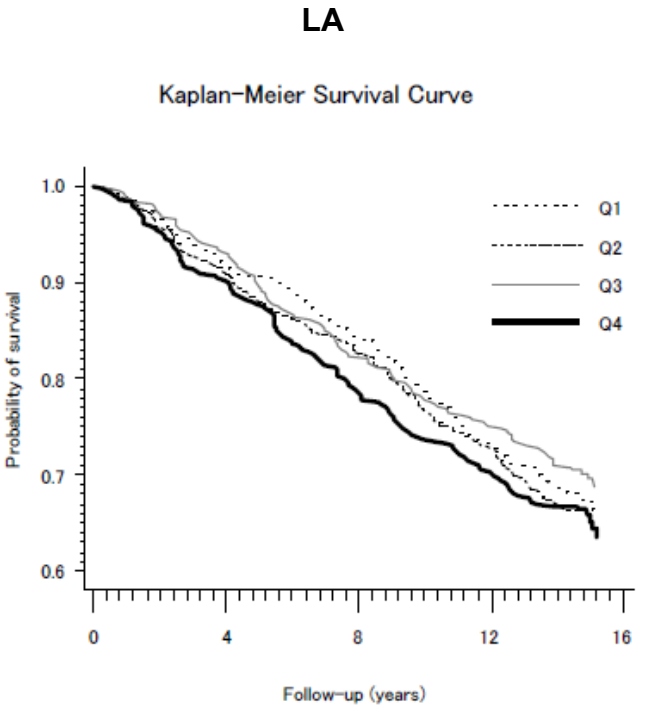


Figure A.6 Kaplan-Meier Survival curves for LA and AA intake (quartiles) and all-cause mortality



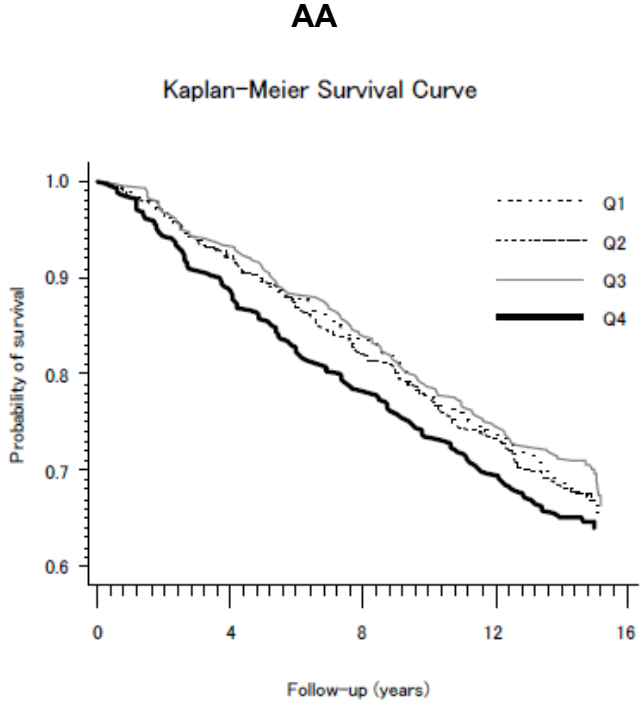
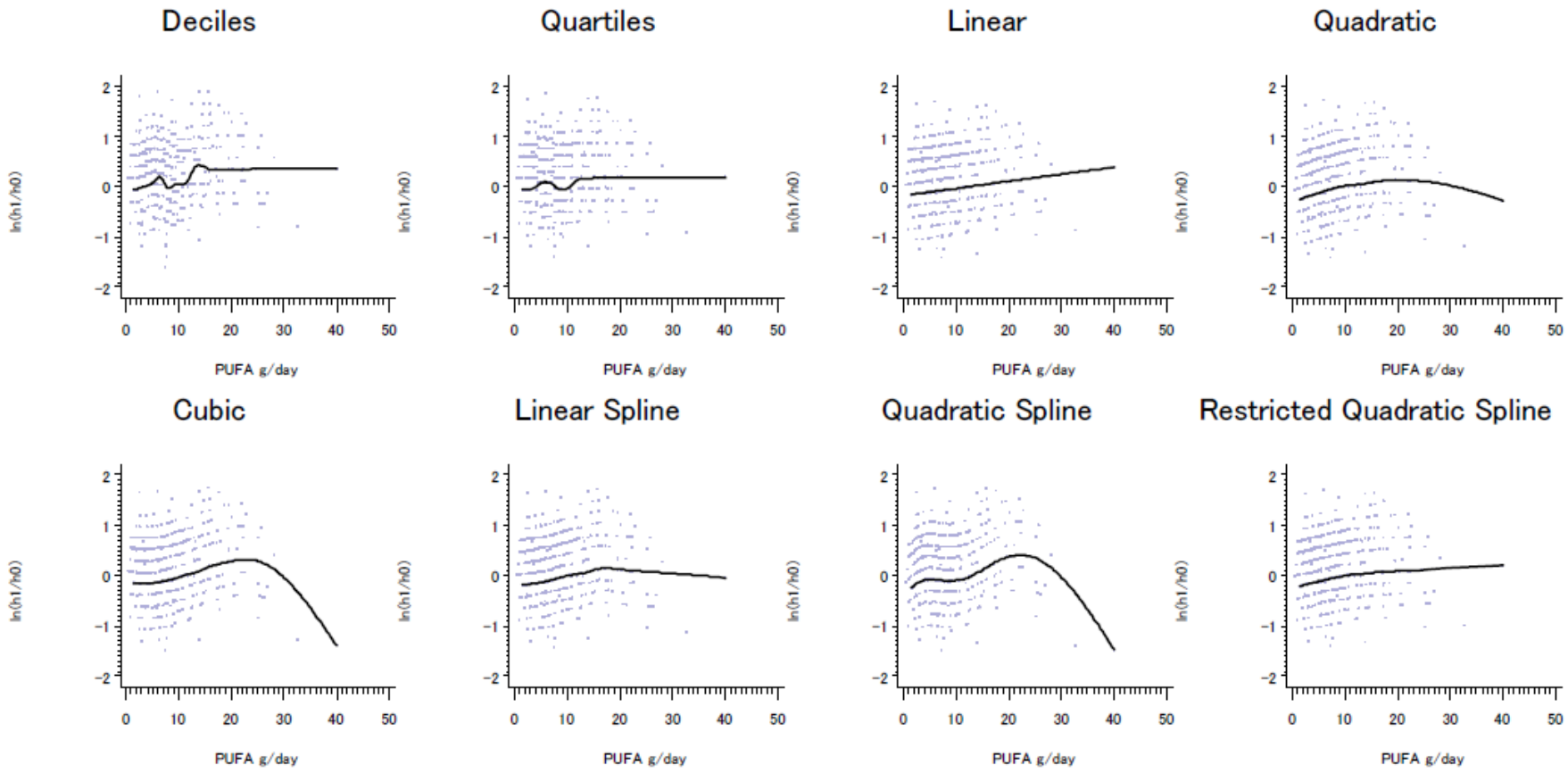
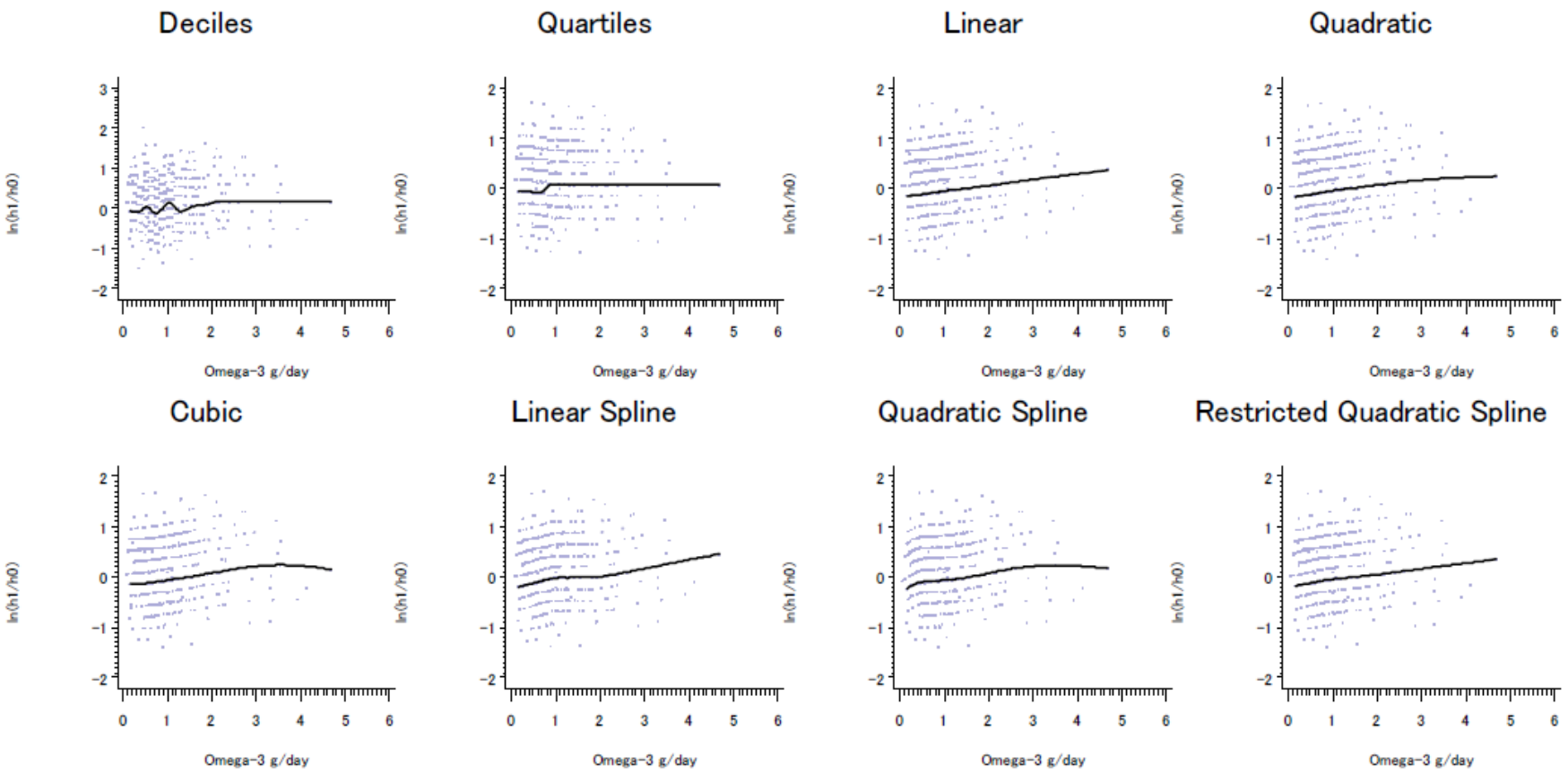


Figure A.7 Dose-response between total PUFA intake (g/day) and the age-adjusted difference in log-hazards of all-cause mortality among women with breast cancer in the LIBCSP



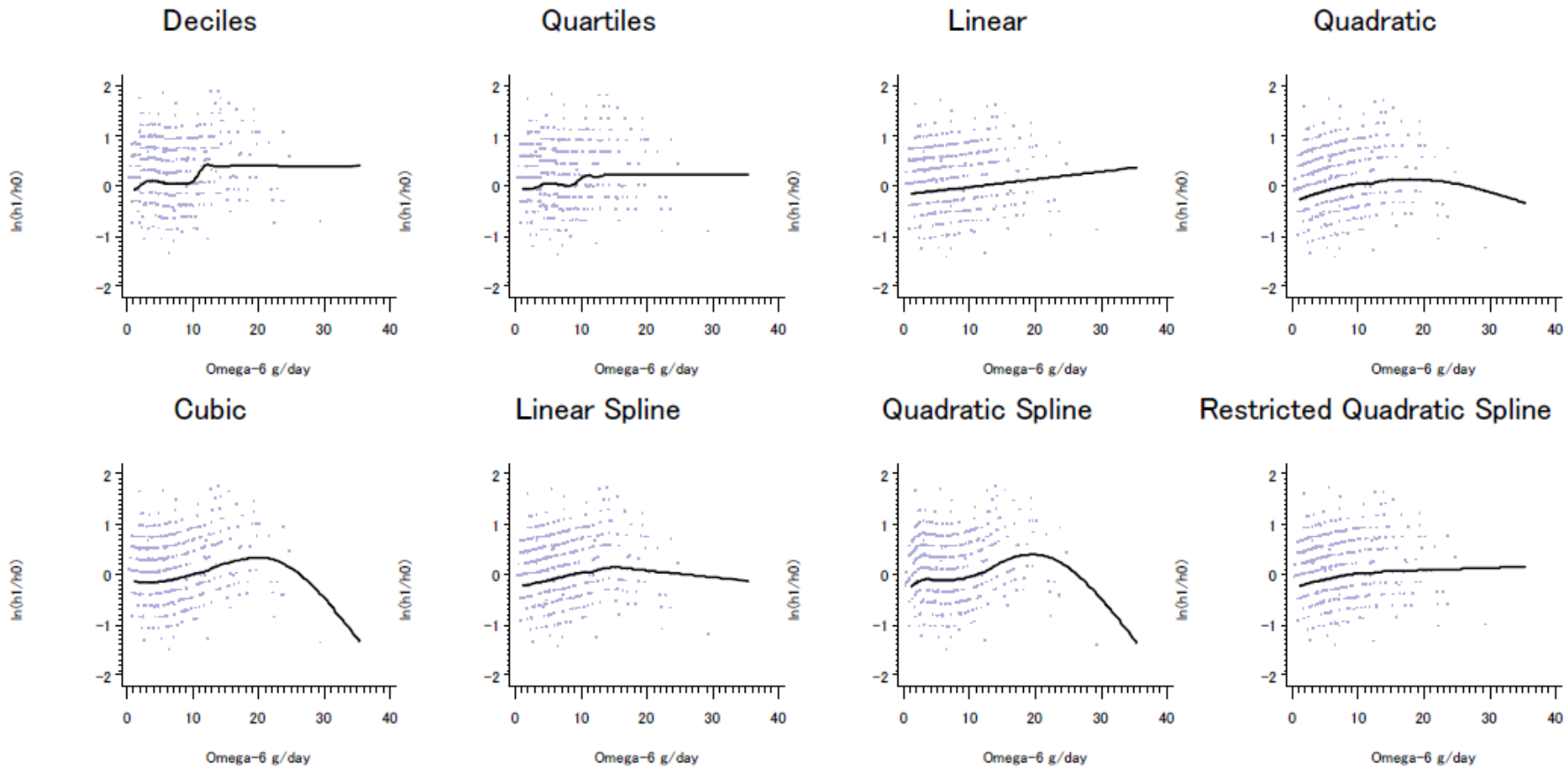
Note: Line represents women 54-59 years old

Figure A.8 Dose-response between total ω-3 intake (g/day) and the age-adjusted difference in log-hazards of all-cause mortality among women with breast cancer in the LIBCSP



Note: Line represents women 54-59 years old

Figure A.9 Dose-response between total ω-6 intake (g/day) and the age-adjusted difference in log-hazards of all-cause mortality among women with breast cancer in the LIBCSP



Note: Line represents women 54-59 years old

REFERENCES

1. Desantis, C., Ma, J., Bryan, L. and Jemal, A. (2014) Breast cancer statistics, 2013. *CA Cancer. J. Clin.*, **64**, 52-62

2. American Cancer Society. (2014) Cancer Facts & Figures 2014

3. Adami, H., Hunter, D.J., Trichopoulos, D. and Sadie Jenkins Harmon Collection. (2002) *Textbook of cancer epidemiology*. Oxford University Press, Oxford ; New York, N.Y.

4. Chie, W.C., Chen, C.F., Chen, C.J., Chang, C.L., Liaw, Y.P. and Lin, R.S. (1995) Geographic variation of breast cancer in Taiwan: international and migrant comparison. *Anticancer Res.*, **15**, 2745-2749

5. Stanford, J.L., Herrinton, L.J., Schwartz, S.M. and Weiss, N.S. (1995) Breast cancer incidence in Asian migrants to the United States and their descendants. *Epidemiology*, **6**, 181-183

6. Wu, A.H. and Bernstein, L. (1998) Breast Cancer among Asian Americans and Pacific Islanders. *Asian Am. Pac. Isl. J. Health.*, **6**, 327-343

7. Althuis, M.D., Dozier, J.M., Anderson, W.F., Devesa, S.S. and Brinton, L.A. (2005) Global trends in breast cancer incidence and mortality 1973-1997. *Int. J. Epidemiol.*, **34**, 405-412

8. Hankinson, S.E., Colditz, G.A. and Willett, W.C. (2004) Towards an integrated model for breast cancer etiology: the lifelong interplay of genes, lifestyle, and hormones. *Breast Cancer Res.*, **6**, 213-218

9. Soerjomataram, I., Louwman, M.W., Ribot, J.G., Roukema, J.A. and Coebergh, J.W. (2008) An overview of prognostic factors for long-term survivors of breast cancer. *Breast Cancer Res. Treat.*, **107**, 309-330

10. Dickson, R.B., Thompson, E.W. and Lippman, M.E. (1990) Regulation of proliferation, invasion and growth factor synthesis in breast cancer by steroids. *J. Steroid Biochem. Mol. Biol.*, **37**, 305-316

11. Clemons, M. and Goss, P. (2001) Estrogen and the risk of breast cancer. *N. Engl. J. Med.*, **344**, 276-285

12. Dao, T.L. and Chan, P.C. (1983) Hormones and dietary fat as promoters in mammary carcinogenesis. *Environ. Health Perspect.*, **50**, 219-225

13. Henderson, B.E. and Bernstein, L. (1991) The international variation in breast cancer rates: an epidemiological assessment. *Breast Cancer Res. Treat.*, **18 Suppl 1**, S11-7

14. Kelsey, J.L., Gammon, M.D. and John, E.M. (1993) Reproductive factors and breast cancer. *Epidemiol. Rev.*, **15**, 36-47

15. Li, C.I., Malone, K.E., Porter, P.L., Weiss, N.S., Tang, M.T. and Daling, J.R. (2003) Reproductive and anthropometric factors in relation to the risk of lobular and ductal breast carcinoma among women 65-79 years of age. *Int. J. Cancer*, **107**, 647-651

16. Liu, Y.T., Gao, C.M., Ding, J.H., Li, S.P., Cao, H.X., Wu, J.Z., Tang, J.H., Qian, Y. and Tajima, K. (2011) Physiological, reproductive factors and breast cancer risk in Jiangsu province of China. *Asian Pac. J. Cancer. Prev.*, **12**, 787-790

17. Opdahl, S., Alsaker, M.D., Janszky, I., Romundstad, P.R. and Vatten, L.J. (2011) Joint effects of nulliparity and other breast cancer risk factors. *Br. J. Cancer*, **105**, 731-736

18. Iwasaki, M. and Tsugane, S. (2011) Risk factors for breast cancer: epidemiological evidence from Japanese studies. *Cancer. Sci.*, **102**, 1607-1614

19. Russo, J., Tay, L.K. and Russo, I.H. (1982) Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res. Treat.*, **2**, 5-73

20. Newcomb, P.A., Egan, K.M., Titus-Ernstoff, L., Trentham-Dietz, A., Greenberg, E.R., Baron, J.A., Willett, W.C. and Stampfer, M.J. (1999) Lactation in relation to postmenopausal breast cancer. *Am. J. Epidemiol.*, **150**, 174-182

21. Shantakumar, S., Terry, M.B., Teitelbaum, S.L., Britton, J.A., Millikan, R.C., Moorman, P.G., Neugut, A.I. and Gammon, M.D. (2007) Reproductive factors and breast cancer risk among older women. *Breast Cancer Res. Treat.*, **102**, 365-374

22. Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J. and Writing Group for the Women's Health Initiative Investigators. (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*, **288**, 321-333

23. Beral, V. and Million Women Study Collaborators. (2003) Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*, **362**, 419-427

24. Greene, M.H. (1997) Genetics of breast cancer. *Mayo Clin. Proc.*, **72**, 54-65

25. Pharoah, P.D., Day, N.E., Duffy, S., Easton, D.F. and Ponder, B.A. (1997) Family history and the risk of breast cancer: a systematic review and meta-analysis. *Int. J. Cancer*, **71**, 800-809

26. Ellisen, L.W. and Haber, D.A. (1998) Hereditary breast cancer. *Annu. Rev. Med.*, **49**, 425-436

27. Peto, J., Collins, N., Barfoot, R., Seal, S., Warren, W., Rahman, N., Easton, D.F., Evans, C., Deacon, J. and Stratton, M.R. (1999) Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J. Natl. Cancer Inst.,* **91**, 943-949

28. Matsuzawa, Y., Shimomura, I., Nakamura, T., Keno, Y., Kotani, K. and Tokunaga, K. (1995) Pathophysiology and pathogenesis of visceral fat obesity. *Obes. Res.,* **3 Suppl 2**, 187S-194S

29. Judd, H.L., Shamonki, I.M., Frumar, A.M. and Lagasse, L.D. (1982) Origin of serum estradiol in postmenopausal women. *Obstet. Gynecol.,* **59**, 680-686

30. van den Brandt, P.A., Spiegelman, D., Yaun, S.S., Adami, H.O., Beeson, L., Folsom, A.R., Fraser, G., Goldbohm, R.A., Graham, S., Kushi, L., Marshall, J.R., Miller, A.B., Rohan, T., Smith-Warner, S.A., Speizer, F.E., Willett, W.C., Wolk, A. and Hunter, D.J. (2000) Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. *Am. J. Epidemiol.,* **152**, 514-527

31. Eng, S.M., Gammon, M.D., Terry, M.B., Kushi, L.H., Teitelbaum, S.L., Britton, J.A. and Neugut, A.I. (2005) Body size changes in relation to postmenopausal breast cancer among women on Long Island, New York. *Am. J. Epidemiol.,* **162**, 229-237

32. Trentham-Dietz, A., Newcomb, P.A., Egan, K.M., Titus-Ernstoff, L., Baron, J.A., Storer, B.E., Stampfer, M. and Willett, W.C. (2000) Weight change and risk of postmenopausal breast cancer (United States). *Cancer Causes Control,* **11**, 533-542

33. Wenten, M., Gilliland, F.D., Baumgartner, K. and Samet, J.M. (2002) Associations of weight, weight change, and body mass with breast cancer risk in Hispanic and non-Hispanic white women. *Ann. Epidemiol.,* **12**, 435-434

34. Lahmann, P.H., Lissner, L., Gullberg, B., Olsson, H. and Berglund, G. (2003) A prospective study of adiposity and postmenopausal breast cancer risk: the Malmo Diet and Cancer Study. *Int. J. Cancer,* **103**, 246-252

35. Yoo, K., Tajima, K., Park, S., Kang, D., Kim, S., Hirose, K., Takeuchi, T. and Miura, S. (2001) Postmenopausal obesity as a breast cancer risk factor according to estrogen and progesterone receptor status (Japan). *Cancer Lett.*, **167**, 57-63

36. Suzuki, R., Iwasaki, M., Inoue, M., Sasazuki, S., Sawada, N., Yamaji, T., Shimazu, T., Tsugane, S. and Japan Public Health Center-based Prospective Study Group. (2011) Body weight at age 20 years, subsequent weight change and breast cancer risk defined by estrogen and progesterone receptor status--the Japan public health center-based prospective study. *Int. J. Cancer,* **129**, 1214-1224

37. Chow, L.W., Lui, K.L., Chan, J.C., Chan, T.C., Ho, P.K., Lee, W.Y., Leung, L.H., Sy, W.M., Yeung, C.C. and Yung, A.K. (2005) Association between body mass index and risk of

formation of breast cancer in Chinese women. *Asian J. Surg.*, **28**, 179-184

38. Coates, R.J., Uhler, R.J., Hall, H.I., Potischman, N., Brinton, L.A., Ballard-Barbash, R., Gammon, M.D., Brogan, D.R., Daling, J.R., Malone, K.E., Schoenberg, J.B. and Swanson, C.A. (1999) Risk of breast cancer in young women in relation to body size and weight gain in adolescence and early adulthood. *Br. J. Cancer*, **81**, 167-174

39. Peacock, S.L., White, E., Daling, J.R., Voigt, L.F. and Malone, K.E. (1999) Relation between obesity and breast cancer in young women. *Am. J. Epidemiol.*, **149**, 339-346

40. Friedenreich, C.M., Courneya, K.S. and Bryant, H.E. (2002) Case-control study of anthropometric measures and breast cancer risk. *Int. J. Cancer*, **99**, 445-452

41. Key, T.J., Allen, N.E., Verkasalo, P.K. and Banks, E. (2001) Energy balance and cancer: the role of sex hormones. *Proc. Nutr. Soc.*, **60**, 81-89

42. Potischman, N., Swanson, C.A., Siiteri, P. and Hoover, R.N. (1996) Reversal of relation between body mass and endogenous estrogen concentrations with menopausal status. *J. Natl. Cancer Inst.*, **88**, 756-758

43. Kossman, D.A., Williams, N.I., Domchek, S.M., Kurzer, M.S., Stopfer, J.E. and Schmitz, K.H. (2011) Exercise lowers estrogen and progesterone levels in premenopausal women at high risk of breast cancer. *J. Appl. Physiol.*, **111**, 1687-1693

44. McTiernan, A., Ulrich, C., Slate, S. and Potter, J. (1998) Physical activity and cancer etiology: associations and mechanisms. *Cancer Causes Control*, **9**, 487-509

45. Rundle, A. (2005) Molecular epidemiology of physical activity and cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 227-236

46. Shephard, R.J., Rhind, S. and Shek, P.N. (1995) The impact of exercise on the immune system: NK cells, interleukins 1 and 2, and related responses. *Exerc. Sport Sci. Rev.*, **23**, 215-241

47. Hamer, M., Sabia, S., Batty, G.D., Shipley, M.J., Tabak, A.G., Singh-Manoux, A. and Kivimaki, M. (2012) Physical Activity and Inflammatory Markers Over 10 Years: Follow-Up in Men and Women From the Whitehall II Cohort Study. *Circulation*, **126**, 928-933

48. Monninkhof, E.M., Elias, S.G., Vlems, F.A., van der Tweel, I., Schuit, A.J., Voskuil, D.W., van Leeuwen, F.E. and TFPAC. (2007) Physical activity and breast cancer: a systematic review. *Epidemiology*, **18**, 137-157

49. Sprague, B.L., Trentham-Dietz, A., Newcomb, P.A., Titus-Ernstoff, L., Hampton, J.M. and Egan, K.M. (2007) Lifetime recreational and occupational physical activity and risk of in situ and invasive breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 236-243

50. Eliassen, A.H., Hankinson, S.E., Rosner, B., Holmes, M.D. and Willett, W.C. (2010) Physical activity and risk of breast cancer among postmenopausal women. *Arch. Intern. Med.*, **170**, 1758-1764

51. Gao, C.M., Tajima, K., Ding, J.H., Tang, J.H., Wu, J.Z., Li, S.P., Cao, H.X., Liu, Y.T., Su, P., Qian, Y., Chang, J. and Takezaki, T. (2009) Body size, physical activity and risk of breast cancer - a case control study in Jangsu Province of China. *Asian Pac. J. Cancer. Prev.*, **10**, 877-881

52. McCullough, L.E., Eng, S.M., Bradshaw, P.T., Cleveland, R.J., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2012) Fat or fit: The joint effects of physical activity, weight gain, and body size on breast cancer risk. *Cancer*, **118**, 4860-4868

53. Hyde, C.A. and Missailidis, S. (2009) Inhibition of arachidonic acid metabolism and its implication on cell proliferation and tumour-angiogenesis. *Int. Immunopharmacol.*, **9**, 701-715

54. Neugut, A.I., Rosenberg, D.J., Ahsan, H., Jacobson, J.S., Wahid, N., Hagan, M., Rahman, M.I., Khan, Z.R., Chen, L., Pablos-Mendez, A. and Shea, S. (1998) Association between coronary heart disease and cancers of the breast, prostate, and colon. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 869-873

55. Thompson, H.J., Briggs, S., Paranka, N.S., Piazza, G.A., Brendel, K., Gross, P.H., Sperl, G.J., Pamukcu, R. and Ahnen, D.J. (1995) Inhibition of mammary carcinogenesis in rats by sulfone metabolite of sulindac. *J. Natl. Cancer Inst.*, **87**, 1259-1260

56. Khuder, S.A. and Mutgi, A.B. (2001) Breast cancer and NSAID use: a meta-analysis. *Br. J. Cancer*, **84**, 1188-1192

57. Agrawal, A. and Fentiman, I.S. (2008) NSAIDs and breast cancer: a possible prevention and treatment strategy. *Int. J. Clin. Pract.*, **62**, 444-449

58. Bardia, A., Olson, J.E., Vachon, C.M., Lazovich, D., Vierkant, R.A., Wang, A.H., Limburg, P.J., Anderson, K.E. and Cerhan, J.R. (2011) Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: results from a prospective cohort study. *Breast Cancer Res. Treat.*, **126**, 149-155

59. Cronin-Fenton, D.P., Pedersen, L., Lash, T.L., Friis, S., Baron, J.A. and Sorensen, H.T. (2010) Prescriptions for selective cyclooxygenase-2 inhibitors, non-selective non-steroidal anti-inflammatory drugs, and risk of breast cancer in a population-based case-control study. *Breast Cancer Res.*, **12**, R15

60. Vinogradova, Y., Coupland, C. and Hippisley-Cox, J. (2011) Exposure to cyclooxygenase-2 inhibitors and risk of cancer: nested case-control studies. *Br. J. Cancer*, **105**, 452-459

61. Eliassen, A.H., Chen, W.Y., Spiegelman, D., Willett, W.C., Hunter, D.J. and Hankinson, S.E. (2009) Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. *Arch. Intern. Med.,* **169**, 115-21; discussion 121

62. Ashok, V., Dash, C., Rohan, T.E., Sprafka, J.M. and Terry, P.D. (2011) Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. *Breast,* **20**, 66-70

63. Brasky, T.M., Bonner, M.R., Moysich, K.B., Ochs-Balcom, H.M., Marian, C., Ambrosone, C.B., Nie, J., Tao, M.H., Edge, S.B., Trevisan, M., Shields, P.G. and Freudenheim, J.L. (2011) Genetic variants in COX-2, non-steroidal anti-inflammatory drugs, and breast cancer risk: the Western New York Exposures and Breast Cancer (WEB) Study. *Breast Cancer Res. Treat.,* **126**, 157-165

64. Terry, M.B., Gammon, M.D., Zhang, F.F., Tawfik, H., Teitelbaum, S.L., Britton, J.A., Subbaramaiah, K., Dannenberg, A.J. and Neugut, A.I. (2004) Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. *JAMA,* **291**, 2433-2440

65. Shen, J., Gammon, M.D., Terry, M.B., Teitelbaum, S.L., Neugut, A.I. and Santella, R.M. (2006) Genetic polymorphisms in the cyclooxygenase-2 gene, use of nonsteroidal anti-inflammatory drugs, and breast cancer risk. *Breast Cancer Res.,* **8**, R71

66. Walter, R.B., Milano, F., Brasky, T.M. and White, E. (2011) Long-term use of acetaminophen, aspirin, and other nonsteroidal anti-inflammatory drugs and risk of hematologic malignancies: results from the prospective Vitamins and Lifestyle (VITAL) study. *J. Clin. Oncol.,* **29**, 2424-2431

67. Huang, E.S., Strate, L.L., Ho, W.W., Lee, S.S. and Chan, A.T. (2011) Long-term use of aspirin and the risk of gastrointestinal bleeding. *Am. J. Med.,* **124**, 426-433
68. Rose, D.P. and Connolly, J.M. (1999) Antiangiogenicity of docosahexaenoic acid and its role in the suppression of breast cancer cell growth in nude mice. *Int. J. Oncol.,* **15**, 1011-1015

69. Coronado, G.D., Beasley, J. and Livaudais, J. (2011) Alcohol consumption and the risk of breast cancer. *Salud Publica Mex.,* **53**, 440-447

70. Longnecker, M.P. (1994) Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. *Cancer Causes Control,* **5**, 73-82

71. Smith-Warner, S.A., Spiegelman, D., Yaun, S.S., van den Brandt, P.A., Folsom, A.R., Goldbohm, R.A., Graham, S., Holmberg, L., Howe, G.R., Marshall, J.R., Miller, A.B., Potter, J.D., Speizer, F.E., Willett, W.C., Wolk, A. and Hunter, D.J. (1998) Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA,* **279**, 535-540

72. Allen, N.E., Beral, V., Casabonne, D., Kan, S.W., Reeves, G.K., Brown, A., Green, J.

and Million Women Study Collaborators. (2009) Moderate alcohol intake and cancer incidence in women. *J. Natl. Cancer Inst.,* **101**, 296-305

73. Chen, W.Y., Rosner, B., Hankinson, S.E., Colditz, G.A. and Willett, W.C. (2011) Moderate alcohol consumption during adult life, drinking patterns, and breast cancer risk. *JAMA*, **306**, 1884-1890

74. Feigelson, H.S., Jonas, C.R., Robertson, A.S., McCullough, M.L., Thun, M.J. and Calle, E.E. (2003) Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol. Biomarkers Prev.,* **12**, 161-164

75. Tjonneland, A., Christensen, J., Olsen, A., Stripp, C., Thomsen, B.L., Overvad, K., Peeters, P.H., van Gils, C.H., Bueno-de-Mesquita, H.B., Ocke, M.C., Thiebaut, A., Fournier, A., Clavel-Chapelon, F., Berrino, F., Palli, D., Tumino, R., Panico, S., Vineis, P., Agudo, A., Ardanaz, E., Martinez-Garcia, C., Amiano, P., Navarro, C., Quiros, J.R., Key, T.J., Reeves, G., Khaw, K.T., Bingham, S., Trichopoulou, A., Trichopoulos, D., Naska, A., Nagel, G., Chang-Claude, J., Boeing, H., Lahmann, P.H., Manjer, J., Wirfalt, E., Hallmans, G., Johansson, I., Lund, E., Skeie, G., Hjartaker, A., Ferrari, P., Slimani, N., Kaaks, R. and Riboli, E. (2007) Alcohol intake and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Causes Control*, **18**, 361-373

76. Wu, A.H., Pike, M.C. and Stram, D.O. (1999) Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. *J. Natl. Cancer Inst.,* **91**, 529-534

77. Bruning, P.F. and Bonfrer, J.M. (1986) Free fatty acid concentrations correlated with the available fraction of estradiol in human plasma. *Cancer Res.,* **46**, 2606-2609

78. World Cancer Research Fund / American Institute for Cancer Research. (2007) Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective

79. Kushi, L.H., Sellers, T.A., Potter, J.D., Nelson, C.L., Munger, R.G., Kaye, S.A. and Folsom, A.R. (1992) Dietary fat and postmenopausal breast cancer. *J. Natl. Cancer Inst.,* **84**, 1092-1099

80. Byrne, C., Rockett, H. and Holmes, M.D. (2002) Dietary fat, fat subtypes, and breast cancer risk: lack of an association among postmenopausal women with no history of benign breast disease. *Cancer Epidemiol. Biomarkers Prev.,* **11**, 261-265

81. Howe, G.R., Friedenreich, C.M., Jain, M. and Miller, A.B. (1991) A cohort study of fat intake and risk of breast cancer. *J. Natl. Cancer Inst.,* **83**, 336-340

82. Kushi, L.H., Potter, J.D., Bostick, R.M., Drinkard, C.R., Sellers, T.A., Gapstur, S.M., Cerhan, J.R. and Folsom, A.R. (1995) Dietary fat and risk of breast cancer according to hormone receptor status. *Cancer Epidemiol. Biomarkers Prev.,* **4**, 11-19

83. Mattisson, I., Wirfalt, E., Johansson, U., Gullberg, B., Olsson, H. and Berglund, G. (2004) Intakes of plant foods, fibre and fat and risk of breast cancer--a prospective study in the Malmo Diet and Cancer cohort. *Br. J. Cancer,* **90**, 122-127

84. Sieri, S., Krogh, V., Muti, P., Micheli, A., Pala, V., Crosignani, P. and Berrino, F. (2002) Fat and protein intake and subsequent breast cancer risk in postmenopausal women. *Nutr. Cancer*, **42**, 10-17

85. Wirfalt, E., Mattisson, I., Gullberg, B., Johansson, U., Olsson, H. and Berglund, G. (2002) Postmenopausal breast cancer is associated with high intakes of omega6 fatty acids (Sweden). *Cancer Causes Control,* **13**, 883-893

86. Graham, S., Zielezny, M., Marshall, J., Priore, R., Freudenheim, J., Brasure, J., Haughey, B., Nasca, P. and Zdeb, M. (1992) Diet in the epidemiology of postmenopausal breast cancer in the New York State Cohort. *Am. J. Epidemiol.*, **136**, 1327-1337

87. Kim, E.H., Willett, W.C., Colditz, G.A., Hankinson, S.E., Stampfer, M.J., Hunter, D.J., Rosner, B. and Holmes, M.D. (2006) Dietary fat and risk of postmenopausal breast cancer in a 20-year follow-up. *Am. J. Epidemiol.,* **164**, 990-997

88. Aro, A., Mannisto, S., Salminen, I., Ovaskainen, M.L., Kataja, V. and Uusitupa, M. (2000) Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women. *Nutr. Cancer,* **38**, 151-157

89. Braga, C., La Vecchia, C., Negri, E., Franceschi, S. and Parpinel, M. (1997) Intake of selected foods and nutrients and breast cancer risk: an age- and menopause-specific analysis. *Nutr. Cancer,* **28**, 258-263

90. Challier, B., Perarnau, J.M. and Viel, J.F. (1998) Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. *Eur. J. Epidemiol.,* **14**, 737-747

91. Gerber, M., Richardson, S., Crastes de Paulet, P., Pujol, H. and Crastes de Paulet, A. (1989) Relationship between vitamin E and polyunsaturated fatty acids in breast cancer. Nutritional and metabolic aspects. *Cancer,* **64**, 2347-2353

92. Hirohata, T., Shigematsu, T., Nomura, A.M., Nomura, Y., Horie, A. and Hirohata, I. (1985) Occurrence of breast cancer in relation to diet and reproductive history: a case-control study in Fukuoka, Japan. *Natl. Cancer Inst. Monogr.,* **69**, 187-190

93. Holmberg, L., Ohlander, E.M., Byers, T., Zack, M., Wolk, A., Bergstrom, R., Bergkvist, L., Thurfjell, E., Bruce, A. and Adami, H.O. (1994) Diet and breast cancer risk. Results from a population-based, case-control study in Sweden. *Arch. Intern. Med.,* **154**, 1805-1811

94. London, S.J., Sacks, F.M., Stampfer, M.J., Henderson, I.C., Maclure, M., Tomita, A., Wood, W.C., Remine, S., Robert, N.J. and Dmochowski, J.R. (1993) Fatty acid composition

of the subcutaneous adipose tissue and risk of proliferative benign breast disease and breast cancer. *J. Natl. Cancer Inst.*, **85**, 785-793

95. McCann, S.E., Ip, C., Ip, M.M., McGuire, M.K., Muti, P., Edge, S.B., Trevisan, M. and Freudenheim, J.L. (2004) Dietary intake of conjugated linoleic acids and risk of premenopausal and postmenopausal breast cancer, Western New York Exposures and Breast Cancer Study (WEB Study). *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1480-1484

96. Miller, A.B., Kelly, A., Choi, N.W., Matthews, V., Morgan, R.W., Munan, L., Burch, J.D., Feather, J., Howe, G.R. and Jain, M. (1978) A study of diet and breast cancer. *Am. J. Epidemiol.*, **107**, 499-509

97. Richardson, S., Gerber, M. and Cenee, S. (1991) The role of fat, animal protein and some vitamin consumption in breast cancer: a case control study in southern France. *Int. J. Cancer*, **48**, 1-9

98. van't Veer, P., Kok, F.J., Hermus, R.J. and Sturmans, F. (1989) Alcohol dose, frequency and age at first exposure in relation to the risk of breast cancer. *Int. J. Epidemiol.*, **18**, 511-517

99. Van 't Veer, P., van Leer, E.M., Rietdijk, A., Kok, F.J., Schouten, E.G., Hermus, R.J. and Sturmans, F. (1991) Combination of dietary factors in relation to breast-cancer occurrence. *Int. J. Cancer*, **47**, 649-653

100. Wakai, K., Tamakoshi, K., Date, C., Fukui, M., Suzuki, S., Lin, Y., Niwa, Y., Nishio, K., Yatsuya, H., Kondo, T., Tokudome, S., Yamamoto, A., Toyoshima, H., Tamakoshi, A. and JACC Study Group. (2005) Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. *Cancer. Sci.*, **96**, 590-599

101. Bonilla-Fernandez, P., Lopez-Cervantes, M., Torres-Sanchez, L.E., Tortolero-Luna, G. and Lopez-Carrillo, L. (2003) Nutritional factors and breast cancer in Mexico. *Nutr. Cancer*, **45**, 148-155

102. Graham, S., Hellmann, R., Marshall, J., Freudenheim, J., Vena, J., Swanson, M., Zielezny, M., Nemoto, T., Stubbe, N. and Raimondo, T. (1991) Nutritional epidemiology of postmenopausal breast cancer in western New York. *Am. J. Epidemiol.*, **134**, 552-566

103. Pryor, M., Slattery, M.L., Robison, L.M. and Egger, M. (1989) Adolescent diet and breast cancer in Utah. *Cancer Res.*, **49**, 2161-2167

104. Zaridze, D., Lifanova, Y., Maximovitch, D., Day, N.E. and Duffy, S.W. (1991) Diet, alcohol consumption and reproductive factors in a case-control study of breast cancer in Moscow. *Int. J. Cancer*, **48**, 493-501

105. Smith-Warner, S.A., Spiegelman, D., Adami, H.O., Beeson, W.L., van den Brandt, P.A., Folsom, A.R., Fraser, G.E., Freudenheim, J.L., Goldbohm, R.A., Graham, S., Kushi, L.H.,

Miller, A.B., Rohan, T.E., Speizer, F.E., Toniolo, P., Willett, W.C., Wolk, A., Zeleniuch-Jacquotte, A. and Hunter, D.J. (2001) Types of dietary fat and breast cancer: a pooled analysis of cohort studies. *Int. J. Cancer,* **92**, 767-774

106. Yildirim, E., Dalgic, T. and Berberoglu, U. (2000) Prognostic significance of young age in breast cancer. *J. Surg. Oncol.,* **74**, 267-272

107. Schairer, C., Mink, P.J., Carroll, L. and Devesa, S.S. (2004) Probabilities of death from breast cancer and other causes among female breast cancer patients. *J. Natl. Cancer Inst.,* **96**, 1311-1321

108. Swanson, G.M. and Lin, C.S. (1994) Survival patterns among younger women with breast cancer: the effects of age, race, stage, and treatment. *J. Natl. Cancer. Inst. Monogr.,* **(16)**, 69-77

109. Carey, L.A., Perou, C.M., Livasy, C.A., Dressler, L.G., Cowan, D., Conway, K., Karaca, G., Troester, M.A., Tse, C.K., Edmiston, S., Deming, S.L., Geradts, J., Cheang, M.C., Nielsen, T.O., Moorman, P.G., Earp, H.S. and Millikan, R.C. (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA,* **295**, 2492-2502

110. O'Brien, K.M., Cole, S.R., Tse, C.K., Perou, C.M., Carey, L.A., Foulkes, W.D., Dressler, L.G., Geradts, J. and Millikan, R.C. (2010) Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina Breast Cancer Study. *Clin. Cancer Res.*, **16**, 6100-6110

111. Gordon, N.H. (2003) Socioeconomic factors and breast cancer in black and white Americans. *Cancer Metastasis Rev.,* **22**, 55-65

112. Page, D.L., Jensen, R.A. and Simpson, J.F. (1998) Routinely available indicators of prognosis in breast cancer. *Breast Cancer Res. Treat.,* **51**, 195-208

113. Michaelson, J.S., Silverstein, M., Wyatt, J., Weber, G., Moore, R., Halpern, E., Kopans, D.B. and Hughes, K. (2002) Predicting the survival of patients with breast carcinoma using tumor size. *Cancer,* **95**, 713-723

114. Reeves, G.K., Patterson, J., Vessey, M.P., Yeates, D. and Jones, L. (2000) Hormonal and other factors in relation to survival among breast cancer patients. *Int. J. Cancer,* **89**, 293-299

115. Garne, J.P., Aspegren, K., Linell, F., Rank, F. and Ranstam, J. (1994) Primary prognostic factors in invasive breast cancer with special reference to ductal carcinoma and histologic malignancy grade. *Cancer*, **73**, 1438-1448

116. Putti, T.C., El-Rehim, D.M., Rakha, E.A., Paish, C.E., Lee, A.H., Pinder, S.E. and Ellis, I.O. (2005) Estrogen receptor-negative breast carcinomas: a review of morphology and immunophenotypical analysis. *Mod. Pathol.,* **18**, 26-35

117. Maynard, P.V., Blamey, R.W., Elston, C.W., Haybittle, J.L. and Griffiths, K. (1978) Estrogen receptor assay in primary breast cancer and early recurrence of the disease. *Cancer Res.*, **38**, 4292-4295

118. Parl, F.F., Schmidt, B.P., Dupont, W.D. and Wagner, R.K. (1984) Prognostic significance of estrogen receptor status in breast cancer in relation to tumor stage, axillary node metastasis, and histopathologic grading. *Cancer,* **54**, 2237-2242

119. Potter, J.D., Cerhan, J.R., Sellers, T.A., McGovern, P.G., Drinkard, C., Kushi, L.R. and Folsom, A.R. (1995) Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there?. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 319-326

120. Allemani, C., Sant, M., Berrino, F., Aareleid, T., Chaplain, G., Coebergh, J.W., Colonna, M., Contiero, P., Danzon, A., Federico, M., Gafa, L., Grosclaude, P., Hedelin, G., Mace-Lesech, J., Garcia, C.M., Paci, E., Raverdy, N., Tretarre, B. and Williams, E.M. (2004) Prognostic value of morphology and hormone receptor status in breast cancer - a population-based study. *Br. J. Cancer,* **91**, 1263-1268

121. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet,* **365**, 1687-1717

122. Bardou, V.J., Arpino, G., Elledge, R.M., Osborne, C.K. and Clark, G.M. (2003) Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J. Clin. Oncol.*, **21**, 1973-1979

123. Goss, P.E., Ingle, J.N., Martino, S., Robert, N.J., Muss, H.B., Piccart, M.J., Castiglione, M., Tu, D., Shepherd, L.E., Pritchard, K.I., Livingston, R.B., Davidson, N.E., Norton, L., Perez, E.A., Abrams, J.S., Therasse, P., Palmer, M.J. and Pater, J.L. (2003) A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N. Engl. J. Med.*, **349**, 1793-1802

124. Anonymous (1998) Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet,* **351**, 1451-1467

125. Ragaz, J., Olivotto, I.A., Spinelli, J.J., Phillips, N., Jackson, S.M., Wilson, K.S., Knowling, M.A., Coppin, C.M., Weir, L., Gelmon, K., Le, N., Durand, R., Coldman, A.J. and Manji, M. (2005) Locoregional radiation therapy in patients with high-risk breast cancer receiving adjuvant chemotherapy: 20-year results of the British Columbia randomized trial. *J. Natl. Cancer Inst.*, **97**, 116-126

126. Kwan, M.L., Chen, W.Y., Kroenke, C.H., Weltzien, E.K., Beasley, J.M., Nechuta, S.J., Poole, E.M., Lu, W., Holmes, M.D., Quesenberry, C.P.,Jr, Pierce, J.P., Shu, X.O. and Caan,

B.J. (2012) Pre-diagnosis body mass index and survival after breast cancer in the After Breast Cancer Pooling Project. *Breast Cancer Res. Treat.,* **132**, 729-739

127. Cleveland, R.J., Eng, S.M., Abrahamson, P.E., Britton, J.A., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2007) Weight gain prior to diagnosis and survival from breast cancer. *Cancer Epidemiol. Biomarkers Prev.,* **16**, 1803-1811

128. Bradshaw, P.T., Ibrahim, J.G., Stevens, J., Cleveland, R., Abrahamson, P.E., Satia, J.A., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2012) Postdiagnosis change in bodyweight and survival after breast cancer diagnosis. *Epidemiology,* **23**, 320-327

129. Cleveland, R.J., Eng, S.M., Stevens, J., Bradshaw, P.T., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2012) Influence of prediagnostic recreational physical activity on survival from breast cancer. *Eur. J. Cancer Prev.,* **21**, 46-54

130. West-Wright, C.N., Henderson, K.D., Sullivan-Halley, J., Ursin, G., Deapen, D., Neuhausen, S., Reynolds, P., Chang, E., Ma, H. and Bernstein, L. (2009) Long-term and recent recreational physical activity and survival after breast cancer: the California Teachers Study. *Cancer Epidemiol. Biomarkers Prev.,* **18**, 2851-2859

131. Chen, X., Lu, W., Zheng, W., Gu, K., Matthews, C.E., Chen, Z., Zheng, Y. and Shu, X.O. (2011) Exercise after diagnosis of breast cancer in association with survival. *Cancer. Prev. Res. (Phila),* **4**, 1409-1418

132. Holick, C.N., Newcomb, P.A., Trentham-Dietz, A., Titus-Ernstoff, L., Bersch, A.J., Stampfer, M.J., Baron, J.A., Egan, K.M. and Willett, W.C. (2008) Physical activity and survival after diagnosis of invasive breast cancer. *Cancer Epidemiol. Biomarkers Prev.,* **17**, 379-386

133. Bradshaw, P.T., Ibrahim, J.G., Khankari, N., Cleveland, R.J., Abrahamson, P.E., Stevens, J., Satia, J.A., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2014) Post-diagnosis physical activity and survival after breast cancer diagnosis: the Long Island Breast Cancer Study. *Breast Cancer Res. Treat.,*

134. Borugian, M.J., Sheps, S.B., Kim-Sing, C., Van Patten, C., Potter, J.D., Dunn, B., Gallagher, R.P. and Hislop, T.G. (2004) Insulin, macronutrient intake, and physical activity: are potential indicators of insulin resistance associated with mortality from breast cancer?. *Cancer Epidemiol. Biomarkers Prev.,* **13**, 1163-1172

135. Jain, M., Miller, A.B. and To, T. (1994) Premorbid diet and the prognosis of women with breast cancer. *J. Natl. Cancer Inst.,* **86**, 1390-1397

136. McEligot, A.J., Largent, J., Ziogas, A., Peel, D. and Anton-Culver, H. (2006) Dietary fat, fiber, vegetable, and micronutrients are associated with overall survival in postmenopausal women diagnosed with breast cancer. *Nutr. Cancer,* **55**, 132-140

137. Zhang, S., Folsom, A.R., Sellers, T.A., Kushi, L.H. and Potter, J.D. (1995) Better breast cancer survival for postmenopausal women who are less overweight and eat less fat. The Iowa Women's Health Study. *Cancer,* **76**, 275-283

138. Dal Maso, L., Zucchetto, A., Talamini, R., Serraino, D., Stocco, C.F., Vercelli, M., Falcini, F., Franceschi, S. and Prospective Analysis of Case-control studies on Environmental factors and health (PACE) study group. (2008) Effect of obesity and other lifestyle factors on mortality in women with breast cancer. *Int. J. Cancer,* **123**, 2188-2194

139. Nomura, A.M., Marchand, L.L., Kolonel, L.N. and Hankin, J.H. (1991) The effect of dietary fat on breast cancer survival among Caucasian and japanese women in Hawaii. *Breast Cancer Res. Treat.,* **18 Suppl 1**, S135-41

140. Chlebowski, R.T., Blackburn, G.L., Thomson, C.A., Nixon, D.W., Shapiro, A., Hoy, M.K., Goodman, M.T., Giuliano, A.E., Karanja, N., McAndrew, P., Hudis, C., Butler, J., Merkel, D., Kristal, A., Caan, B., Michaelson, R., Vinciguerra, V., Del Prete, S., Winkler, M., Hall, R., Simon, M., Winters, B.L. and Elashoff, R.M. (2006) Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J. Natl. Cancer Inst.,* **98**, 1767-1776

141. Pierce, J.P., Faerber, S., Wright, F.A., Rock, C.L., Newman, V., Flatt, S.W., Kealey, S., Jones, V.E., Caan, B.J., Gold, E.B., Haan, M., Hollenbach, K.A., Jones, L., Marshall, J.R., Ritenbaugh, C., Stefanick, M.L., Thomson, C., Wasserman, L., Natarajan, L., Thomas, R.G., Gilpin, E.A. and Women's Healthy Eating and Living (WHEL) study group. (2002) A randomized trial of the effect of a plant-based dietary pattern on additional breast cancer events and survival: the Women's Healthy Eating and Living (WHEL) Study. *Control. Clin. Trials,* **23**, 728-756

142. Pierce, J.P., Natarajan, L., Caan, B.J., Parker, B.A., Greenberg, E.R., Flatt, S.W., Rock, C.L., Kealey, S., Al-Delaimy, W.K., Bardwell, W.A., Carlson, R.W., Emond, J.A., Faerber, S., Gold, E.B., Hajek, R.A., Hollenbach, K., Jones, L.A., Karanja, N., Madlensky, L., Marshall, J., Newman, V.A., Ritenbaugh, C., Thomson, C.A., Wasserman, L. and Stefanick, M.L. (2007) Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. *JAMA,* **298**, 289-298

143. Holmes, M.D., Chen, W.Y., Li, L., Hertzmark, E., Spiegelman, D. and Hankinson, S.E. (2010) Aspirin intake and survival after breast cancer. *J. Clin. Oncol.,* **28**, 1467-1472

144. Li, Y., Brasky, T.M., Nie, J., Ambrosone, C.B., McCann, S.E., Shields, P.G., Trevisan, M., Edge, S.B. and Freudenheim, J.L. (2012) Use of nonsteroidal anti-inflammatory drugs and survival following breast cancer diagnosis. *Cancer Epidemiol. Biomarkers Prev.,* **21**, 239-242

145. Benatti, P., Peluso, G., Nicolai, R. and Calvani, M. (2004) Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. *J. Am. Coll. Nutr.,* **23**, 281-302

146. Kris-Etherton, P.M., Taylor, D.S., Yu-Poth, S., Huth, P., Moriarty, K., Fishell, V.,

Hargrove, R.L., Zhao, G. and Etherton, T.D. (2000) Polyunsaturated fatty acids in the food chain in the United States. *Am. J. Clin. Nutr.,* **71**, 179S-88S

147. Echarte, M., Zulet, M.A. and Astiasaran, I. (2001) Oxidation process affecting fatty acids and cholesterol in fried and roasted salmon. *J. Agric. Food Chem.,* **49**, 5662-5667

148. Russo, G.L. (2009) Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem. Pharmacol.,* **77**, 937-946

149. Zhou, D., Ghebremeskel, K., Crawford, M.A. and Reifen, R. (2006) Vitamin A deficiency enhances docosahexaenoic and osbond acids in liver of rats fed an alpha linolenic acid-adequate diet. *Lipids,* **41**, 213-219

150. Gerster, H. (1998) Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)?. *Int. J. Vitam. Nutr. Res.,* **68**, 159-173

151. Simopoulos, A.P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.,* **56**, 365-379

152. Blasbalg, T.L., Hibbeln, J.R., Ramsden, C.E., Majchrzak, S.F. and Rawlings, R.R. (2011) Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am. J. Clin. Nutr.,* **93**, 950-962

153. Simopoulos, A.P. (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp. Biol. Med. (Maywood),* **233**, 674-688

154. Sekikawa, A., Curb, J.D., Ueshima, H., El-Saed, A., Kadowaki, T., Abbott, R.D., Evans, R.W., Rodriguez, B.L., Okamura, T., Sutton-Tyrrell, K., Nakamura, Y., Masaki, K., Edmundowicz, D., Kashiwagi, A., Willcox, B.J., Takamiya, T., Mitsunami, K., Seto, T.B., Murata, K., White, R.L., Kuller, L.H. and ERA JUMP (Electron-Beam Tomography, Risk Factor Assessment Among Japanese and U.S. Men in the Post-World War II Birth Cohort) Study Group. (2008) Marine-derived n-3 fatty acids and atherosclerosis in Japanese, Japanese-American, and white men: a cross-sectional study. *J. Am. Coll. Cardiol.,* **52**, 417-424

155. Chenais, B. and Blanckaert, V. (2012) The janus face of lipids in human breast cancer: how polyunsaturated Fatty acids affect tumor cell hallmarks. *Int. J. Breast Cancer.,* **2012**, 712536

156. Half, E., Tang, X.M., Gwyn, K., Sahin, A., Wathen, K. and Sinicrope, F.A. (2002) Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. *Cancer Res.,* **62**, 1676-1681

157. Kundu, N., Yang, Q., Dorsey, R. and Fulton, A.M. (2001) Increased cyclooxygenase-2 (cox-2) expression and activity in a murine model of metastatic breast cancer. *Int. J. Cancer,*

93, 681-686

158. Ristimaki, A., Sivula, A., Lundin, J., Lundin, M., Salminen, T., Haglund, C., Joensuu, H. and Isola, J. (2002) Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res.,* **62**, 632-635

159. Hwang, D., Scollard, D., Byrne, J. and Levine, E. (1998) Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. *J. Natl. Cancer Inst.,* **90**, 455-460

160. Soslow, R.A., Dannenberg, A.J., Rush, D., Woerner, B.M., Khan, K.N., Masferrer, J. and Koki, A.T. (2000) COX-2 is expressed in human pulmonary, colonic, and mammary tumors. *Cancer,* **89**, 2637-2645

161. Shim, J.Y., An, H.J., Lee, Y.H., Kim, S.K., Lee, K.P. and Lee, K.S. (2003) Overexpression of cyclooxygenase-2 is associated with breast carcinoma and its poor prognostic factors. *Mod. Pathol.,* **16**, 1199-1204

162. Howe, L.R. and Dannenberg, A.J. (2003) COX-2 inhibitors for the prevention of breast cancer. *J. Mammary Gland Biol. Neoplasia,* **8**, 31-43

163. Jiang, W.G., Douglas-Jones, A. and Mansel, R.E. (2003) Levels of expression of lipoxygenases and cyclooxygenase-2 in human breast cancer. *Prostaglandins Leukot. Essent. Fatty Acids,* **69**, 275-281

164. Singh, B. and Lucci, A. (2002) Role of cyclooxygenase-2 in breast cancer. *J. Surg. Res.,* **108**, 173-179

165. Singh, B., Berry, J.A., Shoher, A., Ramakrishnan, V. and Lucci, A. (2005) COX-2 overexpression increases motility and invasion of breast cancer cells. *Int. J. Oncol.,* **26**, 1393-1399

166. Singh, B., Berry, J.A., Shoher, A., Ayers, G.D., Wei, C. and Lucci, A. (2007) COX-2 involvement in breast cancer metastasis to bone. *Oncogene,* **26**, 3789-3796

167. Liu, C.H., Chang, S.H., Narko, K., Trifan, O.C., Wu, M.T., Smith, E., Haudenschild, C., Lane, T.F. and Hla, T. (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. *J. Biol. Chem.,* **276**, 18563-18569

168. Pan, M.R., Hou, M.F., Chang, H.C. and Hung, W.C. (2008) Cyclooxygenase-2 up-regulates CCR7 via EP2/EP4 receptor signaling pathways to enhance lymphatic invasion of breast cancer cells. *J. Biol. Chem.,* **283**, 11155-11163

169. Li, Z., Schem, C., Shi, Y.H., Medina, D. and Zhang, M. (2008) Increased COX2 expression enhances tumor-induced osteoclastic lesions in breast cancer bone metastasis. *Clin. Exp. Metastasis,* **25**, 389-400

170. Lu, S., Yu, G., Zhu, Y. and Archer, M.C. (2005) Cyclooxygenase-2 overexpression in MCF-10F human breast epithelial cells inhibits proliferation, apoptosis and differentiation, and causes partial transformation. *Int. J. Cancer,* **116**, 847-852

171. Salhab, M., Singh-Ranger, G., Mokbel, R., Jouhra, F., Jiang, W.G. and Mokbel, K. (2007) Cyclooxygenase-2 mRNA expression correlates with aromatase expression in human breast cancer. *J. Surg. Oncol.,* **96**, 424-428

172. Farooqui, M., Li, Y., Rogers, T., Poonawala, T., Griffin, R.J., Song, C.W. and Gupta, K. (2007) COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. *Br. J. Cancer*, **97**, 1523-1531

173. Stasinopoulos, I., O'Brien, D.R., Wildes, F., Glunde, K. and Bhujwalla, Z.M. (2007) Silencing of cyclooxygenase-2 inhibits metastasis and delays tumor onset of poorly differentiated metastatic breast cancer cells. *Mol. Cancer. Res.,* **5**, 435-442

174. Larkins, T.L., Nowell, M., Singh, S. and Sanford, G.L. (2006) Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. *BMC Cancer*, **6**, 181

175. Basu, G.D., Pathangey, L.B., Tinder, T.L., Lagioia, M., Gendler, S.J. and Mukherjee, P. (2004) Cyclooxygenase-2 inhibitor induces apoptosis in breast cancer cells in an in vivo model of spontaneous metastatic breast cancer. *Mol. Cancer. Res.,* **2**, 632-642

176. Masferrer, J. (2001) Approach to angiogenesis inhibition based on cyclooxygenase-2. *Cancer J.,* **7 Suppl 3**, S144-50

177. Wang, D. and Dubois, R.N. (2010) Eicosanoids and cancer. *Nat. Rev. Cancer.,* **10**, 181-193

178. Chen, E.P. and Smyth, E.M. (2011) COX-2 and PGE2-dependent immunomodulation in breast cancer. *Prostaglandins Other Lipid Mediat.,* **96**, 14-20

179. Hanahan, D. and Weinberg, R.A. (2000) The hallmarks of cancer. *Cell,* **100**, 57-70

180. Chang, S.H., Liu, C.H., Conway, R., Han, D.K., Nithipatikom, K., Trifan, O.C., Lane, T.F. and Hla, T. (2004) Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced breast cancer progression. *Proc. Natl. Acad. Sci. U. S. A.*, **101**, 591-596

181. Timoshenko, A.V., Xu, G., Chakrabarti, S., Lala, P.K. and Chakraborty, C. (2003) Role of prostaglandin E2 receptors in migration of murine and human breast cancer cells. *Exp. Cell Res.,* **289**, 265-274

182. Holt, D., Ma, X., Kundu, N. and Fulton, A. (2011) Prostaglandin E(2) (PGE (2))

suppresses natural killer cell function primarily through the PGE(2) receptor EP4. *Cancer Immunol. Immunother.,* **60**, 1577-1586

183. Subbaramaiah, K., Benezra, R., Hudis, C. and Dannenberg, A.J. (2008) Cyclooxygenase-2-derived prostaglandin E2 stimulates Id-1 transcription. *J. Biol. Chem.,* **283**, 33955-33968

184. Natarajan, R., Esworthy, R., Bai, W., Gu, J.L., Wilczynski, S. and Nadler, J. (1997) Increased 12-lipoxygenase expression in breast cancer tissues and cells. Regulation by epidermal growth factor. *J. Clin. Endocrinol. Metab.,* **82**, 1790-1798

185. Natarajan, R. and Nadler, J. (1998) Role of lipoxygenases in breast cancer. *Front. Biosci.,* **3**, E81-8

186. Reddy, N., Everhart, A., Eling, T. and Glasgow, W. (1997) Characterization of a 15-lipoxygenase in human breast carcinoma BT-20 cells: stimulation of 13-HODE formation by TGF alpha/EGF. *Biochem. Biophys. Res. Commun.,* **231**, 111-116

187. Pasqualini, M.E., Heyd, V.L., Manzo, P. and Eynard, A.R. (2003) Association between E-cadherin expression by human colon, bladder and breast cancer cells and the 13-HODE:15-HETE ratio. A possible role of their metastatic potential. *Prostaglandins Leukot. Essent. Fatty Acids,* **68**, 9-16

188. Honn, K.V., Timar, J., Rozhin, J., Bazaz, R., Sameni, M., Ziegler, G. and Sloane, B.F. (1994) A lipoxygenase metabolite, 12-(S)-HETE, stimulates protein kinase C-mediated release of cathepsin B from malignant cells. *Exp. Cell Res.*, **214**, 120-130

189. Nony, P.A., Kennett, S.B., Glasgow, W.C., Olden, K. and Roberts, J.D. (2005) 15S-Lipoxygenase-2 mediates arachidonic acid-stimulated adhesion of human breast carcinoma cells through the activation of TAK1, MKK6, and p38 MAPK. *J. Biol. Chem.,* **280**, 31413-31419

190. Steele, V.E., Holmes, C.A., Hawk, E.T., Kopelovich, L., Lubet, R.A., Crowell, J.A., Sigman, C.C. and Kelloff, G.J. (2000) Potential use of lipoxygenase inhibitors for cancer chemoprevention. *Expert Opin. Investig. Drugs,* **9**, 2121-2138

191. Panigrahy, D., Kaipainen, A., Greene, E.R. and Huang, S. (2010) Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. *Cancer Metastasis Rev.,* **29**, 723-735

192. Roman, R.J. (2002) P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.,* **82**, 131-185

193. M. H. M. Yousif, I. F. Benter, K. M. J. Dunn, A. J. Dahly-Vernon, S. Akhtar, and R. J. Roman. (2009) Role of 20-hydroxyeicosatetraenoic acid in altering vascular reactivity in diabetes. *Auton Autacoid Pharmacol,* **29**, 1

194. Williams, J.M., Murphy, S., Burke, M. and Roman, R.J. (2010) 20-Hydroxyeicosatetraeonic Acid: a New Target for the Treatment of Hypertension. *J. Cardiovasc. Pharmacol.,* **56**, 336-344

195. Alexanian, A., Rufanova, V.A., Miller, B., Flasch, A., Roman, R.J. and Sorokin, A. (2009) Down-regulation of 20-HETE synthesis and signaling inhibits renal adenocarcinoma cell proliferation and tumor growth. *Anticancer Res.,* **29**, 3819-3824

196. Goodman, A.I., Choudhury, M., da Silva, J.L., Schwartzman, M.L. and Abraham, N.G. (1997) Overexpression of the heme oxygenase gene in renal cell carcinoma. *Proc. Soc. Exp. Biol. Med.,* **214**, 54-61

197. Richards, J.A. and Brueggemeier, R.W. (2003) Prostaglandin E2 regulates aromatase activity and expression in human adipose stromal cells via two distinct receptor subtypes. *J. Clin. Endocrinol. Metab.,* **88**, 2810-2816

198. Zhou, J., Suzuki, T., Kovacic, A., Saito, R., Miki, Y., Ishida, T., Moriya, T., Simpson, E.R., Sasano, H. and Clyne, C.D. (2005) Interactions between prostaglandin E(2), liver receptor homologue-1, and aromatase in breast cancer. *Cancer Res.,* **65**, 657-663

199. Attar, E., Tokunaga, H., Imir, G., Yilmaz, M.B., Redwine, D., Putman, M., Gurates, B., Attar, R., Yaegashi, N., Hales, D.B. and Bulun, S.E. (2009) Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. *J. Clin. Endocrinol. Metab.,* **94**, 623-631

200. Zhao, Y., Agarwal, V.R., Mendelson, C.R. and Simpson, E.R. (1996) Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology*, **137**, 5739-5742

201. Singh, A., Purohit, A., Ghilchik, M.W. and Reed, M.J. (1999) The regulation of aromatase activity in breast fibroblasts: the role of interleukin-6 and prostaglandin E2. *Endocr. Relat. Cancer,* **6**, 139-147

202. Brueggemeier, R.W., Richards, J.A. and Petrel, T.A. (2003) Aromatase and cyclooxygenases: enzymes in breast cancer. *J. Steroid Biochem. Mol. Biol.,* **86**, 501-507

203. Brodie, A.M., Lu, Q., Long, B.J., Fulton, A., Chen, T., Macpherson, N., DeJong, P.C., Blankenstein, M.A., Nortier, J.W., Slee, P.H., van de Ven, J., van Gorp, J.M., Elbers, J.R., Schipper, M.E., Blijham, G.H. and Thijssen, J.H. (2001) Aromatase and COX-2 expression in human breast cancers. *J. Steroid Biochem. Mol. Biol.,* **79**, 41-47

204. Diaz-Cruz, E.S., Shapiro, C.L. and Brueggemeier, R.W. (2005) Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *J. Clin. Endocrinol. Metab.,* **90**, 2563-2570

205. Foghsgaard, L., Lademann, U., Wissing, D., Poulsen, B. and Jaattela, M. (2002)

Cathepsin B mediates tumor necrosis factor-induced arachidonic acid release in tumor cells. *J. Biol. Chem.,* **277**, 39499-39506

206. Wissing, D., Mouritzen, H., Egeblad, M., Poirier, G.G. and Jaattela, M. (1997) Involvement of caspase-dependent activation of cytosolic phospholipase A2 in tumor necrosis factor-induced apoptosis. *Proc. Natl. Acad. Sci. U. S. A.,* **94**, 5073-5077

207. Karuppu, D., Kalus, A., Simpson, E.R. and Clyne, C. (2002) Aromatase and prostaglandin inter-relationships in breast adipose tissue: significance for breast cancer development. *Breast Cancer Res. Treat.,* **76**, 103-109

208. Zhang, B., Sun, T., Xue, L., Han, X., Zhang, B., Lu, N., Shi, Y., Tan, W., Zhou, Y., Zhao, D., Zhang, X., Guo, Y. and Lin, D. (2007) Functional polymorphisms in FAS and FASL contribute to increased apoptosis of tumor infiltration lymphocytes and risk of breast cancer. *Carcinogenesis,* **28**, 1067-1073

209. Ioachim, H.L., Decuseara, R., Giancotti, F. and Dorsett, B.H. (2005) FAS and FAS-L expression by tumor cells and lymphocytes in breast carcinomas and their lymph node metastases. *Pathol. Res. Pract.,* **200**, 743-751

210. Herrnring, C., Reimer, T., Jeschke, U., Makovitzky, J., Kruger, K., Gerber, B., Kabelitz, D. and Friese, K. (2000) Expression of the apoptosis-inducing ligands FasL and TRAIL in malignant and benign human breast tumors. *Histochem. Cell Biol.,* **113**, 189-194

211. Mullauer, L., Mosberger, I., Grusch, M., Rudas, M. and Chott, A. (2000) Fas ligand is expressed in normal breast epithelial cells and is frequently up-regulated in breast cancer. *J. Pathol.,* **190**, 20-30

212. O'Callaghan, G., Kelly, J., Shanahan, F. and Houston, A. (2008) Prostaglandin E2 stimulates Fas ligand expression via the EP1 receptor in colon cancer cells. *Br. J. Cancer,* **99**, 502-512

213. Liu, H., Zang, C., Fenner, M.H., Possinger, K. and Elstner, E. (2003) PPARgamma ligands and ATRA inhibit the invasion of human breast cancer cells in vitro. *Breast Cancer Res. Treat.,* **79**, 63-74

214. Paumi, C.M., Smitherman, P.K., Townsend, A.J. and Morrow, C.S. (2004) Glutathione S-transferases (GSTs) inhibit transcriptional activation by the peroxisomal proliferator-activated receptor gamma (PPAR gamma) ligand, 15-deoxy-delta 12,14prostaglandin J2 (15-d-PGJ2). *Biochemistry,* **43**, 2345-2352

215. Qin, C., Burghardt, R., Smith, R., Wormke, M., Stewart, J. and Safe, S. (2003) Peroxisome proliferator-activated receptor gamma agonists induce proteasome-dependent degradation of cyclin D1 and estrogen receptor alpha in MCF-7 breast cancer cells. *Cancer Res.,* **63**, 958-964

216. Sun, H., Hu, Y., Gu, Z., Owens, R.T., Chen, Y.Q. and Edwards, I.J. (2011) Omega-3 fatty acids induce apoptosis in human breast cancer cells and mouse mammary tissue through syndecan-1 inhibition of the MEK-Erk pathway. *Carcinogenesis,* **32**, 1518-1524

217. Bocca, C., Bozzo, F., Martinasso, G., Canuto, R.A. and Miglietta, A. (2008) Involvement of PPARalpha in the growth inhibitory effect of arachidonic acid on breast cancer cells. *Br. J. Nutr.,* **100**, 739-750

218. Fruchart, J.C., Duriez, P. and Staels, B. (1999) Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. *Curr. Opin. Lipidol.,* **10**, 245-257

219. Flavell, D.M., Pineda Torra, I., Jamshidi, Y., Evans, D., Diamond, J.R., Elkeles, R.S., Bujac, S.R., Miller, G., Talmud, P.J., Staels, B. and Humphries, S.E. (2000) Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. *Diabetologia,* **43**, 673-680

220. Rozic, J.G., Chakraborty, C. and Lala, P.K. (2001) Cyclooxygenase inhibitors retard murine mammary tumor progression by reducing tumor cell migration, invasiveness and angiogenesis. *Int. J. Cancer,* **93**, 497-506

221. Connolly, J.M. and Rose, D.P. (1998) Enhanced angiogenesis and growth of 12-lipoxygenase gene-transfected MCF-7 human breast cancer cells in athymic nude mice. *Cancer Lett.,* **132**, 107-112

222. Noguchi, M., Earashi, M., Minami, M., Kinoshita, K. and Miyazaki, I. (1995) Effects of eicosapentaenoic and docosahexaenoic acid on cell growth and prostaglandin E and leukotriene B production by a human breast cancer cell line (MDA-MB-231). *Oncology,* **52**, 458-464

223. Noguchi, M., Minami, M., Yagasaki, R., Kinoshita, K., Earashi, M., Kitagawa, H., Taniya, T. and Miyazaki, I. (1997) Chemoprevention of DMBA-induced mammary carcinogenesis in rats by low-dose EPA and DHA. *Br. J. Cancer,* **75**, 348-353

224. Rose, D.P., Connolly, J.M. and Coleman, M. (1996) Effect of omega-3 fatty acids on the progression of metastases after the surgical excision of human breast cancer cell solid tumors growing in nude mice. *Clin. Cancer Res.,* **2**, 1751-1756

225. Sauer, L.A., Dauchy, R.T., Blask, D.E., Krause, J.A., Davidson, L.K. and Dauchy, E.M. (2005) Eicosapentaenoic acid suppresses cell proliferation in MCF-7 human breast cancer xenografts in nude rats via a pertussis toxin-sensitive signal transduction pathway. *J. Nutr.,* **135**, 2124-2129

226. Eling, T.E. and Glasgow, W.C. (1994) Cellular proliferation and lipid metabolism: importance of lipoxygenases in modulating epidermal growth factor-dependent mitogenesis. *Cancer Metastasis Rev.,* **13**, 397-410

227. Sauer, L.A., Dauchy, R.T. and Blask, D.E. (2000) Mechanism for the antitumor and anticachectic effects of n-3 fatty acids. *Cancer Res.,* **60**, 5289-5295

228. Przylipiak, A., Hafner, J., Przylipiak, J., Runnebaum, B., Rabe, T. and Kohn, F.M. (1998) Influence of leukotrienes on in vitro growth of human mammary carcinoma cell line MCF-7. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **77**, 61-65

229. Tong, W.G., Ding, X.Z. and Adrian, T.E. (2002) The mechanisms of lipoxygenase inhibitor-induced apoptosis in human breast cancer cells. *Biochem. Biophys. Res. Commun.*, **296**, 942-948

230. Avis, I., Hong, S.H., Martinez, A., Moody, T., Choi, Y.H., Trepel, J., Das, R., Jett, M. and Mulshine, J.L. (2001) Five-lipoxygenase inhibitors can mediate apoptosis in human breast cancer cell lines through complex eicosanoid interactions. *FASEB J.*, **15**, 2007-2009

231. Gonzalez, M.J., Schemmel, R.A., Dugan, L.,Jr, Gray, J.I. and Welsch, C.W. (1993) Dietary fish oil inhibits human breast carcinoma growth: a function of increased lipid peroxidation. *Lipids,* **28**, 827-832

232. Hubbard, N.E., Lim, D. and Erickson, K.L. (1998) Alteration of murine mammary tumorigenesis by dietary enrichment with n-3 fatty acids in fish oil. *Cancer Lett.*, **124**, 1-7

233. Welsch, C.W., Oakley, C.S., Chang, C.C. and Welsch, M.A. (1993) Suppression of growth by dietary fish oil of human breast carcinomas maintained in three different strains of immune-deficient mice. *Nutr. Cancer,* **20**, 119-127

234. Kramer, M.S., Kahn, S.R., Platt, R.W., Genest, J., Rozen, R., Chen, M.F., Goulet, L., Seguin, L., Dassa, C., Lydon, J., McNamara, H., Dahhou, M., Lamoureux, J. and Evans, R.W. (2009) Antioxidant vitamins, long-chain fatty acids, and spontaneous preterm birth. *Epidemiology*, **20**, 707-713

235. Ghosh-Choudhury, T., Mandal, C.C., Woodruff, K., St Clair, P., Fernandes, G., Choudhury, G.G. and Ghosh-Choudhury, N. (2009) Fish oil targets PTEN to regulate NFkappaB for downregulation of anti-apoptotic genes in breast tumor growth. *Breast Cancer Res. Treat.,* **118**, 213-228

236. Mandal, C.C., Ghosh-Choudhury, T., Yoneda, T., Choudhury, G.G. and Ghosh-Choudhury, N. (2010) Fish oil prevents breast cancer cell metastasis to bone. *Biochem. Biophys. Res. Commun.,* **402**, 602-607

237. Rose, D.P., Rayburn, J., Hatala, M.A. and Connolly, J.M. (1994) Effects of dietary fish oil on fatty acids and eicosanoids in metastasizing human breast cancer cells. *Nutr. Cancer,* **22**, 131-141

238. Truan, J.S., Chen, J.M. and Thompson, L.U. (2010) Flaxseed oil reduces the growth of

human breast tumors (MCF-7) at high levels of circulating estrogen. *Mol. Nutr. Food Res.*, **54**, 1414-1421

239. Hardman, W.E., Ion, G., Akinsete, J.A. and Witte, T.R. (2011) Dietary walnut suppressed mammary gland tumorigenesis in the C(3)1 TAg mouse. *Nutr. Cancer*, **63**, 960-970

240. Chen, J., Stavro, P.M. and Thompson, L.U. (2002) Dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor. *Nutr. Cancer*, **43**, 187-192

241. Wang, Z., Butt, K., Wang, L. and Liu, H. (2007) The effect of seal oil on paclitaxel induced cytotoxicity and apoptosis in breast carcinoma MCF-7 and MDA-MB-231 cell lines. *Nutr. Cancer*, **58**, 230-238

242. Barascu, A., Besson, P., Le Floch, O., Bougnoux, P. and Jourdan, M.L. (2006) CDK1-cyclin B1 mediates the inhibition of proliferation induced by omega-3 fatty acids in MDA-MB-231 breast cancer cells. *Int. J. Biochem. Cell Biol.*, **38**, 196-208

243. Schley, P.D., Jijon, H.B., Robinson, L.E. and Field, C.J. (2005) Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells. *Breast Cancer Res. Treat.*, **92**, 187-195

244. Hardman, W.E., Sun, L., Short, N. and Cameron, I.L. (2005) Dietary omega-3 fatty acids and ionizing irradiation on human breast cancer xenograft growth and angiogenesis. *Cancer. Cell. Int.*, **5**, 12

245. Chamras, H., Ardashian, A., Heber, D. and Glaspy, J.A. (2002) Fatty acid modulation of MCF-7 human breast cancer cell proliferation, apoptosis and differentiation. *J. Nutr. Biochem.*, **13**, 711-716

246. Lu, I.F., Hasio, A.C., Hu, M.C., Yang, F.M. and Su, H.M. (2010) Docosahexaenoic acid induces proteasome-dependent degradation of estrogen receptor alpha and inhibits the downstream signaling target in MCF-7 breast cancer cells. *J. Nutr. Biochem.*, **21**, 512-517

247. Yamamoto, D., Kiyozuka, Y., Adachi, Y., Takada, H., Hioki, K. and Tsubura, A. (1999) Synergistic action of apoptosis induced by eicosapentaenoic acid and TNP-470 on human breast cancer cells. *Breast Cancer Res. Treat.*, **55**, 149-160

248. Abdi-Dezfuli, F., Froyland, L., Thorsen, T., Aakvaag, A. and Berge, R.K. (1997) Eicosapentaenoic acid and sulphur substituted fatty acid analogues inhibit the proliferation of human breast cancer cells in culture. *Breast Cancer Res. Treat.*, **45**, 229-239

249. Senzaki, H., Tsubura, A. and Takada, H. (2001) Effect of eicosapentaenoic acid on the suppression of growth and metastasis of human breast cancer cells in vivo and in vitro. *World Rev. Nutr. Diet.*, **88**, 117-125

250. Altenburg, J.D. and Siddiqui, R.A. (2009) Omega-3 polyunsaturated fatty acids down-modulate CXCR4 expression and function in MDA-MB-231 breast cancer cells. *Mol. Cancer. Res.*, **7**, 1013-1020

251. Thoennes, S.R., Tate, P.L., Price, T.M. and Kilgore, M.W. (2000) Differential transcriptional activation of peroxisome proliferator-activated receptor gamma by omega-3 and omega-6 fatty acids in MCF-7 cells. *Mol. Cell. Endocrinol.*, **160**, 67-73

252. Moore, M.R. and King, R.A. (2012) Effects of Omega-3 Fatty Acids on Progestin Stimulation of Invasive Properties in Breast Cancer. *Horm. Cancer.*,

253. Weber, P.C. and Leaf, A. (1991) Cardiovascular effects of omega 3 fatty acids. Atherosclerosis risk factor modification by omega 3 fatty acids. *World Rev. Nutr. Diet.*, **66**, 218-232

254. Stoll, B.A. (2002) N-3 fatty acids and lipid peroxidation in breast cancer inhibition. *Br. J. Nutr.*, **87**, 193-198

255. Germain, E., Chajes, V., Cognault, S., Lhuillery, C. and Bougnoux, P. (1998) Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MDA-MB-231: relationship to lipid peroxidation. *Int. J. Cancer*, **75**, 578-583

256. Bougnoux, P., Hajjaji, N., Maheo, K., Couet, C. and Chevalier, S. (2010) Fatty acids and breast cancer: sensitization to treatments and prevention of metastatic re-growth. *Prog. Lipid Res.*, **49**, 76-86

257. Welsch, C.W. (1995) Review of the effects of dietary fat on experimental mammary gland tumorigenesis: role of lipid peroxidation. *Free Radic. Biol. Med.*, **18**, 757-773

258. Najid, A., Beneytout, J.L. and Tixier, M. (1989) Cytotoxicity of arachidonic acid and of its lipoxygenase metabolite 15-hydroperoxyeicosatetraenoic acid on human breast cancer MCF-7 cells in culture. *Cancer Lett.*, **46**, 137-141

259. Kang, K.S., Wang, P., Yamabe, N., Fukui, M., Jay, T. and Zhu, B.T. (2010) Docosahexaenoic acid induces apoptosis in MCF-7 cells in vitro and in vivo via reactive oxygen species formation and caspase 8 activation. *PLoS One*, **5**, e10296

260. Bianchi, A., Becuwe, P., Franck, P. and Dauca, M. (2002) Induction of MnSOD gene by arachidonic acid is mediated by reactive oxygen species and p38 MAPK signaling pathway in human HepG2 hepatoma cells. *Free Radic. Biol. Med.*, **32**, 1132-1142

261. Das, U.N., Begin, M.E., Ells, G., Huang, Y.S. and Horrobin, D.F. (1987) Polyunsaturated fatty acids augment free radical generation in tumor cells in vitro. *Biochem. Biophys. Res. Commun.*, **145**, 15-24

262. Cognault, S., Jourdan, M.L., Germain, E., Pitavy, R., Morel, E., Durand, G., Bougnoux, P. and Lhuillery, C. (2000) Effect of an alpha-linolenic acid-rich diet on rat mammary tumor growth depends on the dietary oxidative status. *Nutr. Cancer,* **36**, 33-41

263. Hardman, W.E., Munoz, J.,Jr and Cameron, I.L. (2002) Role of lipid peroxidation and antioxidant enzymes in omega 3 fatty acids induced suppression of breast cancer xenograft growth in mice. *Cancer. Cell. Int.,* **2**, 10

264. Colas, S., Maheo, K., Denis, F., Goupille, C., Hoinard, C., Champeroux, P., Tranquart, F. and Bougnoux, P. (2006) Sensitization by dietary docosahexaenoic acid of rat mammary carcinoma to anthracycline: a role for tumor vascularization. *Clin. Cancer Res.,* **12**, 5879-5886

265. Colas, S., Paon, L., Denis, F., Prat, M., Louisot, P., Hoinard, C., Le Floch, O., Ogilvie, G. and Bougnoux, P. (2004) Enhanced radiosensitivity of rat autochthonous mammary tumors by dietary docosahexaenoic acid. *Int. J. Cancer,* **109**, 449-454

266. DeGraffenried, L.A., Friedrichs, W.E., Fulcher, L., Fernandes, G., Silva, J.M., Peralba, J.M. and Hidalgo, M. (2003) Eicosapentaenoic acid restores tamoxifen sensitivity in breast cancer cells with high Akt activity. *Ann. Oncol.*, **14**, 1051-1056

267. Bougnoux, P., Germain, E., Chajes, V., Hubert, B., Lhuillery, C., Le Floch, O., Body, G. and Calais, G. (1999) Cytotoxic drugs efficacy correlates with adipose tissue docosahexaenoic acid level in locally advanced breast carcinoma. *Br. J. Cancer,* **79**, 1765-1769

268. Bougnoux, P., Hajjaji, N., Ferrasson, M.N., Giraudeau, B., Couet, C. and Le Floch, O. (2009) Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: a phase II trial. *Br. J. Cancer,* **101**, 1978-1985

269. Nkondjock, A., Shatenstein, B. and Ghadirian, P. (2003) A case-control study of breast cancer and dietary intake of individual fatty acids and antioxidants in Montreal, Canada. *Breast,* **12**, 128-135

270. Sczaniecka, A.K., Brasky, T.M., Lampe, J.W., Patterson, R.E. and White, E. (2012) Dietary Intake of Specific Fatty Acids and Breast Cancer Risk Among Postmenopausal Women in the VITAL Cohort. *Nutr. Cancer,* **64**, 1131-1142

271. Willett, W. (1998) *Nutritional epidemiology*. Oxford University Press, New York

272. Arab, L. and Akbar, J. (2002) Biomarkers and the measurement of fatty acids. *Public Health Nutr.,* **5**, 865-871

273. Arab, L. (2003) Biomarkers of fat and fatty acid intake. *J. Nutr.,* **133 Suppl 3**, 925S-932S

274. Rothman, K.J. (1981) Induction and latent periods. *Am. J. Epidemiol.,* **114**, 253-259

275. Yu, S.Z., Lu, R.F., Xu, D.D. and Howe, G.R. (1990) A case-control study of dietary and nondietary risk factors for breast cancer in Shanghai. *Cancer Res.,* **50**, 5017-5021

276. Lee, H.P., Gourley, L., Duffy, S.W., Esteve, J., Lee, J. and Day, N.E. (1992) Risk factors for breast cancer by age and menopausal status: a case-control study in Singapore. *Cancer Causes Control,* **3**, 313-322

277. Kim, J., Lim, S.Y., Shin, A., Sung, M.K., Ro, J., Kang, H.S., Lee, K.S., Kim, S.W. and Lee, E.S. (2009) Fatty fish and fish omega-3 fatty acid intakes decrease the breast cancer risk: a case-control study. *BMC Cancer,* **9**, 216

278. Katsouyanni, K., Trichopoulou, A., Stuver, S., Garas, Y., Kritselis, A., Kyriakou, G., Stoikidou, M., Boyle, P. and Trichopoulos, D. (1994) The association of fat and other macronutrients with breast cancer: a case-control study from Greece. *Br. J. Cancer,* **70**, 537-541

279. Landa, M.C., Frago, N. and Tres, A. (1994) Diet and the risk of breast cancer in Spain. *Eur. J. Cancer Prev.,* **3**, 313-320

280. Martin-Moreno, J.M., Willett, W.C., Gorgojo, L., Banegas, J.R., Rodriguez-Artalejo, F., Fernandez-Rodriguez, J.C., Maisonneuve, P. and Boyle, P. (1994) Dietary fat, olive oil intake and breast cancer risk. *Int. J. Cancer,* **58**, 774-780

281. Tavani, A., Pelucchi, C., Parpinel, M., Negri, E., Franceschi, S., Levi, F. and La Vecchia, C. (2003) n-3 polyunsaturated fatty acid intake and cancer risk in Italy and Switzerland. *Int. J. Cancer,* **105**, 113-116

282. Alothaimeen, A., Ezzat, A., Mohamed, G., Muammar, T. and Al-Madouj, A. (2004) Dietary fat and breast cancer in Saudi Arabia: a case-control study. *East. Mediterr. Health J.,* **10**, 879-886

283. De Stefani, E., Deneo-Pellegrini, H., Mendilaharsu, M. and Ronco, A. (1998) Essential fatty acids and breast cancer: a case-control study in Uruguay. *Int. J. Cancer,* **76**, 491-494

284. Chajes, V., Torres-Mejia, G., Biessy, C., Ortega-Olvera, C., Angeles-Llerenas, A., Ferrari, P., Lazcano-Ponce, E. and Romieu, I. (2012) omega-3 and omega-6 Polyunsaturated fatty acid intakes and the risk of breast cancer in Mexican women: impact of obesity status. *Cancer Epidemiol. Biomarkers Prev.,* **21**, 319-326

285. Witte, J.S., Ursin, G., Siemiatycki, J., Thompson, W.D., Paganini-Hill, A. and Haile, R.W. (1997) Diet and premenopausal bilateral breast cancer: a case-control study. *Breast Cancer Res. Treat.,* **42**, 243-251

286. Goodstine, S.L., Zheng, T., Holford, T.R., Ward, B.A., Carter, D., Owens, P.H. and

Mayne, S.T. (2003) Dietary (n-3)/(n-6) fatty acid ratio: possible relationship to premenopausal but not postmenopausal breast cancer risk in U.S. women. *J. Nutr.,* **133**, 1409-1414

287. Wang, J., John, E.M. and Ingles, S.A. (2008) 5-Lipoxygenase and 5-Lipoxygenase-Activating Protein Gene Polymorphisms, Dietary Linoleic Acid, and Risk for Breast Cancer. *Cancer Epidemiol. Biomarkers Prev.,* **17**, 2748-2754

288. Toniolo, P., Riboli, E., Shore, R.E. and Pasternack, B.S. (1994) Consumption of meat, animal products, protein, and fat and risk of breast cancer: a prospective cohort study in New York. *Epidemiology,* **5**, 391-397

289. Gago-Dominguez, M., Yuan, J.M., Sun, C.L., Lee, H.P. and Yu, M.C. (2003) Opposing effects of dietary n-3 and n-6 fatty acids on mammary carcinogenesis: The Singapore Chinese Health Study. *Br. J. Cancer,* **89**, 1686-1692

290. Murff, H.J., Shu, X.O., Li, H., Yang, G., Wu, X., Cai, H., Wen, W., Gao, Y.T. and Zheng, W. (2010) Dietary polyunsaturated fatty acids and breast cancer risk in Chinese women: A prospective cohort study. *Int. J. Cancer,*

291. Knekt, P., Albanes, D., Seppanen, R., Aromaa, A., Jarvinen, R., Hyvonen, L., Teppo, L. and Pukkala, E. (1990) Dietary fat and risk of breast cancer. *Am. J. Clin. Nutr.*, **52**, 903-908

292. Voorrips, L.E., Brants, H.A., Kardinaal, A.F., Hiddink, G.J., van den Brandt, P.A. and Goldbohm, R.A. (2002) Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. *Am. J. Clin. Nutr.,* **76**, 873-882

293. Lof, M., Sandin, S., Lagiou, P., Hilakivi-Clarke, L., Trichopoulos, D., Adami, H.O. and Weiderpass, E. (2007) Dietary fat and breast cancer risk in the Swedish women's lifestyle and health cohort. *Br. J. Cancer,* **97**, 1570-1576

294. Thiebaut, A.C., Chajes, V., Gerber, M., Boutron-Ruault, M.C., Joulin, V., Lenoir, G., Berrino, F., Riboli, E., Benichou, J. and Clavel-Chapelon, F. (2009) Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. *Int. J. Cancer*, **124**, 924-931

295. Barrett-Connor, E. and Friedlander, N.J. (1993) Dietary fat, calories, and the risk of breast cancer in postmenopausal women: a prospective population-based study. *J. Am. Coll. Nutr.,* **12**, 390-399

296. Holmes, M.D., Hunter, D.J., Colditz, G.A., Stampfer, M.J., Hankinson, S.E., Speizer, F.E., Rosner, B. and Willett, W.C. (1999) Association of dietary intake of fat and fatty acids with risk of breast cancer. *JAMA,* **281**, 914-920

297. Velie, E., Kulldorff, M., Schairer, C., Block, G., Albanes, D. and Schatzkin, A. (2000)

Dietary fat, fat subtypes, and breast cancer in postmenopausal women: a prospective cohort study. *J. Natl. Cancer Inst.*, **92**, 833-839

298. Folsom, A.R. and Demissie, Z. (2004) Fish intake, marine omega-3 fatty acids, and mortality in a cohort of postmenopausal women. *Am. J. Epidemiol.*, **160**, 1005-1010

299. Thiebaut, A.C., Rotival, M., Gauthier, E., Lenoir, G.M., Boutron-Ruault, M.C., Joulin, V., Clavel-Chapelon, F. and Chajes, V. (2009) Correlation between serum phospholipid fatty acids and dietary intakes assessed a few years earlier. *Nutr. Cancer*, **61**, 500-509

300. Sasaki, S., Horacsek, M. and Kesteloot, H. (1993) An ecological study of the relationship between dietary fat intake and breast cancer mortality. *Prev. Med.*, **22**, 187-202

301. Ishimoto, H., Nakamura, H. and Miyoshi, T. (1994) Epidemiological study on relationship between breast cancer mortality and dietary factors. *Tokushima J. Exp. Med.*, **41**, 103-114

302. Witte, J.S., Greenland, S., Haile, R.W. and Bird, C.L. (1994) Hierarchical regression analysis applied to a study of multiple dietary exposures and breast cancer. *Epidemiology*, **5**, 612-21

303. Maillard, V., Bougnoux, P., Ferrari, P., Jourdan, M.L., Pinault, M., Lavillonniere, F., Body, G., Le Floch, O. and Chajes, V. (2002) N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. *Int. J. Cancer*, **98**, 78-83

304. Simonsen, N., van't Veer, P., Strain, J.J., Martin-Moreno, J.M., Huttunen, J.K., Navajas, J.F., Martin, B.C., Thamm, M., Kardinaal, A.F., Kok, F.J. and Kohlmeier, L. (1998) Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer. *Am. J. Epidemiol.*, **147**, 342-352

305. Witt, P.M., Christensen, J.H., Schmidt, E.B., Dethlefsen, C., Tjonneland, A., Overvad, K. and Ewertz, M. (2009) Marine n-3 polyunsaturated fatty acids in adipose tissue and breast cancer risk: a case-cohort study from Denmark. *Cancer Causes Control*, **20**, 1715-1721

306. Kuriki, K., Hirose, K., Wakai, K., Matsuo, K., Ito, H., Suzuki, T., Hiraki, A., Saito, T., Iwata, H., Tatematsu, M. and Tajima, K. (2007) Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids in Japanese. *Int. J. Cancer*, **121**, 377-385

307. Pala, V., Krogh, V., Muti, P., Chajes, V., Riboli, E., Micheli, A., Saadatian, M., Sieri, S. and Berrino, F. (2001) Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. *J. Natl. Cancer Inst.*, **93**, 1088-1095

308. Shannon, J., King, I.B., Moshofsky, R., Lampe, J.W., Gao, D.L., Ray, R.M. and Thomas, D.B. (2007) Erythrocyte fatty acids and breast cancer risk: a case-control study in

Shanghai, China. *Am. J. Clin. Nutr.*, **85**, 1090-1097

309. Saadatian-Elahi, M., Toniolo, P., Ferrari, P., Goudable, J., Akhmedkhanov, A., Zeleniuch-Jacquotte, A. and Riboli, E. (2002) Serum fatty acids and risk of breast cancer in a nested case-control study of the New York University Women's Health Study. *IARC Sci. Publ.*, **156**, 227-230

310. Takata, Y., King, I.B., Neuhouser, M.L., Schaffer, S., Barnett, M., Thornquist, M., Peters, U. and Goodman, G.E. (2009) Association of serum phospholipid fatty acids with breast cancer risk among postmenopausal cigarette smokers. *Cancer Causes Control*, **20**, 497-504

311. Wirfalt, E., Vessby, B., Mattisson, I., Gullberg, B., Olsson, H. and Berglund, G. (2004) No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmo Diet Cancer cohort (Sweden). *Eur. J. Clin. Nutr.*, **58**, 761-770

312. Zaridze, D.G., Chevchenko, V.E., Levtshuk, A.A., Lifanova, Y.E. and Maximovitch, D.M. (1990) Fatty acid composition of phospholipids in erythrocyte membranes and risk of breast cancer. *Int. J. Cancer*, **45**, 807-810

313. Klein, V., Chajes, V., Germain, E., Schulgen, G., Pinault, M., Malvy, D., Lefrancq, T., Fignon, A., Le Floch, O., Lhuillery, C. and Bougnoux, P. (2000) Low alpha-linolenic acid content of adipose breast tissue is associated with an increased risk of breast cancer. *Eur. J. Cancer*, **36**, 335-340

314. Eid, A. and Berry, E.M. (1988) The relationship between dietary fat, adipose tissue composition, and neoplasms of the breast. *Nutr. Cancer*, **11**, 173-177

315. Chajes, V., Hulten, K., Van Kappel, A.L., Winkvist, A., Kaaks, R., Hallmans, G., Lenner, P. and Riboli, E. (1999) Fatty-acid composition in serum phospholipids and risk of breast cancer: an incident case-control study in Sweden. *Int. J. Cancer*, **83**, 585-590

316. Rissanen, H., Knekt, P., Jarvinen, R., Salminen, I. and Hakulinen, T. (2003) Serum fatty acids and breast cancer incidence. *Nutr. Cancer*, **45**, 168-175

317. Bagga, D., Anders, K.H., Wang, H.J. and Glaspy, J.A. (2002) Long-chain n-3-to-n-6 polyunsaturated fatty acid ratios in breast adipose tissue from women with and without breast cancer. *Nutr. Cancer*, **42**, 180-185

318. Petrek, J.A., Hudgins, L.C., Ho, M., Bajorunas, D.R. and Hirsch, J. (1997) Fatty acid composition of adipose tissue, an indication of dietary fatty acids, and breast cancer prognosis. *J. Clin. Oncol.*, **15**, 1377-1384

319. Terry, P., Rohan, T.E., Wolk, A., Maehle-Schmidt, M. and Magnusson, C. (2002) Fish consumption and breast cancer risk. *Nutr. Cancer*, **44**, 1-6

320. Vatten, L.J., Solvoll, K. and Loken, E.B. (1990) Frequency of meat and fish intake and risk of breast cancer in a prospective study of 14,500 Norwegian women. *Int. J. Cancer,* **46**, 12-15

321. Franceschi, S., Favero, A., La Vecchia, C., Negri, E., Dal Maso, L., Salvini, S., Decarli, A. and Giacosa, A. (1995) Influence of food groups and food diversity on breast cancer risk in Italy. *Int. J. Cancer,* **63**, 785-789

322. Favero, A., Parpinel, M. and Franceschi, S. (1998) Diet and risk of breast cancer: major findings from an Italian case-control study. *Biomed. Pharmacother.,* **52**, 109-115

323. Bessaoud, F., Daures, J.P. and Gerber, M. (2008) Dietary factors and breast cancer risk: a case control study among a population in Southern France. *Nutr. Cancer,* **60**, 177-187

324. Stripp, C., Overvad, K., Christensen, J., Thomsen, B.L., Olsen, A., Moller, S. and Tjonneland, A. (2003) Fish intake is positively associated with breast cancer incidence rate. *J. Nutr.,* **133**, 3664-3669

325. Engeset, D., Alsaker, E., Lund, E., Welch, A., Khaw, K.T., Clavel-Chapelon, F., Thiebaut, A., Chajes, V., Key, T.J., Allen, N.E., Amiano, P., Dorronsoro, M., Tjonneland, A., Stripp, C., Peeters, P.H., van Gils, C.H., Chirlaque, M.D., Nagel, G., Linseisen, J., Ocke, M.C., Bueno-de-Mesquita, H.B., Sacerdote, C., Tumino, R., Ardanaz, E., Sanchez, M.J., Panico, S., Palli, D., Trichopoulou, A., Kalapothaki, V., Benetou, V., Quiros, J.R., Agudo, A., Overvad, K., Bjerregaard, L., Wirfalt, E., Schulz, M., Boeing, H., Slimani, N. and Riboli, E. (2006) Fish consumption and breast cancer risk. The European Prospective Investigation into Cancer and Nutrition (EPIC). *Int. J. Cancer,* **119**, 175-182

326. Hirose, K., Tajima, K., Hamajima, N., Inoue, M., Takezaki, T., Kuroishi, T., Yoshida, M. and Tokudome, S. (1995) A large-scale, hospital-based case-control study of risk factors of breast cancer according to menopausal status. *Jpn. J. Cancer Res.,* **86**, 146-154

327. Dai, Q., Shu, X.O., Jin, F., Gao, Y.T., Ruan, Z.X. and Zheng, W. (2002) Consumption of animal foods, cooking methods, and risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.,* **11**, 801-808

328. Hirose, K., Takezaki, T., Hamajima, N., Miura, S. and Tajima, K. (2003) Dietary factors protective against breast cancer in Japanese premenopausal and postmenopausal women. *Int. J. Cancer,* **107**, 276-282

329. Hirose, K., Imaeda, N., Tokudome, Y., Goto, C., Wakai, K., Matsuo, K., Ito, H., Toyama, T., Iwata, H., Tokudome, S. and Tajima, K. (2005) Soybean products and reduction of breast cancer risk: a case-control study in Japan. *Br. J. Cancer,* **93**, 15-22

330. Zhang, C.X., Ho, S.C., Chen, Y.M., Lin, F.Y., Fu, J.H. and Cheng, S.Z. (2009) Meat and egg consumption and risk of breast cancer among Chinese women. *Cancer Causes*

Control, **20**, 1845-1853

331. Bao, P.P., Shu, X.O., Zheng, Y., Cai, H., Ruan, Z.X., Gu, K., Su, Y., Gao, Y.T., Zheng, W. and Lu, W. (2012) Fruit, vegetable, and animal food intake and breast cancer risk by hormone receptor status. *Nutr. Cancer,* **64**, 806-819

332. Holmes, M.D., Colditz, G.A., Hunter, D.J., Hankinson, S.E., Rosner, B., Speizer, F.E. and Willett, W.C. (2003) Meat, fish and egg intake and risk of breast cancer. *Int. J. Cancer,* **104**, 221-227

333. Brasky, T.M., Lampe, J.W., Potter, J.D., Patterson, R.E. and White, E. (2010) Specialty supplements and breast cancer risk in the VITamins And Lifestyle (VITAL) Cohort. *Cancer Epidemiol. Biomarkers Prev.,* **19**, 1696-1708

334. McElroy, J.A., Kanarek, M.S., Trentham-Dietz, A., Robert, S.A., Hampton, J.M., Newcomb, P.A., Anderson, H.A. and Remington, P.L. (2004) Potential exposure to PCBs, DDT, and PBDEs from sport-caught fish consumption in relation to breast cancer risk in Wisconsin. *Environ. Health Perspect.,* **112**, 156-162

335. Torres-Sanchez, L., Lopez-Carrillo, L., Lopez-Cervantes, M., Rueda-Neria, C. and Wolff, M.S. (2000) Food sources of phytoestrogens and breast cancer risk in Mexican women. *Nutr. Cancer,* **37**, 134-139

336. Xu, W.H., Dai, Q., Xiang, Y.B., Zhao, G.M., Zheng, W., Gao, Y.T., Ruan, Z.X., Cheng, J.R. and Shu, X.O. (2006) Animal food intake and cooking methods in relation to endometrial cancer risk in Shanghai. *Br. J. Cancer,* **95**, 1586-1592

337. Chiu, B.C., Ji, B.T., Dai, Q., Gridley, G., McLaughlin, J.K., Gao, Y.T., Fraumeni, J.F.,Jr and Chow, W.H. (2003) Dietary factors and risk of colon cancer in Shanghai, China. *Cancer Epidemiol. Biomarkers Prev.,* **12**, 201-208

338. He, K., Xun, P., Brasky, T.M., Gammon, M.D., Stevens, J. and White, E. (2013) Types of fish consumed and fish preparation methods in relation to pancreatic cancer incidence: the VITAL Cohort Study. *Am. J. Epidemiol.,* **177**, 152-160

339. Patterson, R.E., Flatt, S.W., Newman, V.A., Natarajan, L., Rock, C.L., Thomson, C.A., Caan, B.J., Parker, B.A. and Pierce, J.P. (2011) Marine fatty acid intake is associated with breast cancer prognosis. *J. Nutr.,* **141**, 201-206

340. Kyogoku, S., Hirohata, T., Nomura, Y., Shigematsu, T., Takeshita, S. and Hirohata, I. (1992) Diet and prognosis of breast cancer. *Nutr. Cancer,* **17**, 271-277

341. Lund, E. and Bonaa, K.H. (1993) Reduced breast cancer mortality among fishermen's wives in Norway. *Cancer Causes Control,* **4**, 283-287

342. Gago-Dominguez, M., Castelao, J.E., Sun, C.L., Van Den Berg, D., Koh, W.P., Lee,

H.P. and Yu, M.C. (2004) Marine n-3 fatty acid intake, glutathione S-transferase polymorphisms and breast cancer risk in post-menopausal Chinese women in Singapore. *Carcinogenesis,* **25**, 2143-2147

343. Ferlay, J., Parkin, D., Curado, M., Bray, F., Edwards, B., Shin, H. and Forman, D. Cancer Incidence in Five Continents, Volumes I to IX: IARC CancerBase No.9 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://ci5.iarc.fr

344. Abraham, J.E., Harrington, P., Driver, K.E., Tyrer, J., Easton, D.F., Dunning, A.M. and Pharoah, P.D. (2009) Common polymorphisms in the prostaglandin pathway genes and their association with breast cancer susceptibility and survival. *Clin. Cancer Res.,* **15**, 2181-2191

345. Simopoulos, A.P. (2010) Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. *Exp. Biol. Med. (Maywood),* **235**, 785-795

346. Simopoulos, A.P. (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.,* **21**, 495-505

347. de Lima-Salgado, T.M., Alba-Loureiro, T.C., do Nascimento, C.S., Nunes, M.T. and Curi, R. (2010) Molecular Mechanisms by Which Saturated Fatty Acids Modulate TNF-alpha Expression in Mouse Macrophage Lineage. *Cell Biochem. Biophys.,*

348. Liu, W.H. and Chang, L.S. (2009) Arachidonic acid induces Fas and FasL upregulation in human leukemia U937 cells via Ca2+/ROS-mediated suppression of ERK/c-Fos pathway and activation of p38 MAPK/ATF-2 pathway. *Toxicol. Lett.,* **191**, 140-148

349. Fradet, V., Cheng, I., Casey, G. and Witte, J.S. (2009) Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. *Clin. Cancer Res.,* **15**, 2559-2566

350. Hedelin, M., Chang, E.T., Wiklund, F., Bellocco, R., Klint, A., Adolfsson, J., Shahedi, K., Xu, J., Adami, H.O., Gronberg, H. and Balter, K.A. (2006) Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. *Int J Cancer,* **120**, 398-405

351. Poole, E.M., Hsu, L., Xiao, L., Kulmacz, R.J., Carlson, C.S., Rabinovitch, P.S., Makar, K.W., Potter, J.D. and Ulrich, C.M. (2010) Genetic variation in prostaglandin E2 synthesis and signaling, prostaglandin dehydrogenase, and the risk of colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev.,* **19**, 547-557

352. Poole, E.M., Bigler, J., Whitton, J., Sibert, J.G., Kulmacz, R.J., Potter, J.D. and Ulrich, C.M. (2007) Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. *Carcinogenesis,* **28**, 1259-1263

353. Siezen, C.L., van Leeuwen, A.I., Kram, N.R., Luken, M.E., van Kranen, H.J. and Kampman, E. (2005) Colorectal adenoma risk is modified by the interplay between polymorphisms in arachidonic acid pathway genes and fish consumption. *Carcinogenesis*, **26**, 449-457

354. Koh, W.P., Yuan, J.M., van den Berg, D., Lee, H.P. and Yu, M.C. (2004) Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. *Br. J. Cancer,* **90**, 1760-1764

355. Gammon, M.D., Neugut, A.I., Santella, R.M., Teitelbaum, S.L., Britton, J.A., Terry, M.B., Eng, S.M., Wolff, M.S., Stellman, S.D., Kabat, G.C., Levin, B., Bradlow, H.L., Hatch, M., Beyea, J., Camann, D., Trent, M., Senie, R.T., Garbowski, G.C., Maffeo, C., Montalvan, P., Berkowitz, G.S., Kemeny, M., Citron, M., Schnabe, F., Schuss, A., Hajdu, S., Vincguerra, V., Collman, G.W. and Obrams, G.I. (2002) The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. *Breast Cancer Res. Treat.,* **74**, 235-254

356. Fink, B.N., Gaudet, M.M., Britton, J.A., Abrahamson, P.E., Teitelbaum, S.L., Jacobson, J., Bell, P., Thomas, J.A., Kabat, G.C., Neugut, A.I. and Gammon, M.D. (2006) Fruits, vegetables, and micronutrient intake in relation to breast cancer survival. *Breast Cancer Res. Treat.*, **98**, 199-208

357. Anonymous (2009) About the National Death Index **2011**, 1

358. Cowper, D.C., Kubal, J.D., Maynard, C. and Hynes, D.M. (2002) A primer and comparative review of major US mortality databases. *Ann. Epidemiol.,* **12**, 462-468

359. Block, G., Woods, M., Potosky, A. and Clifford, C. (1990) Validation of a self-administered diet history questionnaire using multiple diet records. *J. Clin. Epidemiol.,* **43**, 1327-1335

360. Schatzkin, A., Subar, A.F., Moore, S., Park, Y., Potischman, N., Thompson, F.E., Leitzmann, M., Hollenbeck, A., Morrissey, K.G. and Kipnis, V. (2009) Observational epidemiologic studies of nutrition and cancer: the next generation (with better observation). *Cancer Epidemiol. Biomarkers Prev.,* **18**, 1026-1032

361. Xu, Z. and Taylor, J.A. (2009) SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res.,* **37**, W600-5

362. Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Neugut, A.I., Josephy, P.D. and Ambrosone, C.B. (2004) Myeloperoxidase genotype, fruit and vegetable consumption, and breast cancer risk. *Cancer Res,* **64**, 7634-9

363. Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Nowell, S., Davis, W., Garza, C., Neugut, A.I. and Ambrosone, C.B. (2005)

Associations between breast cancer risk and the catalase genotype, fruit and vegetable consumption, and supplement use. *Am J Epidemiol*, **162**, 943-52

364. Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Neugut, A.I. and Ambrosone, C.B. (2005) No association between glutathione peroxidase Pro198Leu polymorphism and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2459-2461

365. Gaudet, M.M., Gammon, M.D., Santella, R.M., Britton, J.A., Teitelbaum, S.L., Eng, S.M., Terry, M.B., Bensen, J.T., Schroeder, J., Olshan, A.F., Neugut, A.I. and Ambrosone, C.B. (2005) MnSOD Val-9Ala genotype, pro- and anti-oxidant environmental modifiers, and breast cancer among women on Long Island, New York. *Cancer Causes Control*, **16**, 1225-1234

366. Steck, S.E., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L., Terry, M.B., Neugut, A.I., Santella, R.M. and Gammon, M.D. (2007) Interactions among GSTM1, GSTT1 and GSTP1 polymorphisms, cruciferous vegetable intake and breast cancer risk. *Carcinogenesis*, **28**, 1954-1959

367. Crew, K.D., Gammon, M.D., Terry, M.B., Zhang, F.F., Agrawal, M., Eng, S.M., Sagiv, S.K., Teitelbaum, S.L., Neugut, A.I. and Santella, R.M. (2007) Genetic polymorphisms in the apoptosis-associated genes FAS and FASL and breast cancer risk. *Carcinogenesis*, **28**, 2548-2551

368. Golembesky, A.K., Gammon, M.D., North, K.E., Bensen, J.T., Schroeder, J.C., Teitelbaum, S.L., Neugut, A.I. and Santella, R.M. (2008) Peroxisome proliferator-activated receptor-alpha (PPARA) genetic polymorphisms and breast cancer risk: a Long Island ancillary study. *Carcinogenesis*, **29**, 1944-1949

369. Chen, Y., Gammon, M.D., Teitelbaum, S.L., Britton, J.A., Terry, M.B., Shantakumar, S., Eng, S.M., Wang, Q., Gurvich, I., Neugut, A.I., Santella, R.M. and Ahsan, H. (2008) Estrogen-biosynthesis gene CYP17 and its interactions with reproductive, hormonal and lifestyle factors in breast cancer risk: results from the Long Island Breast Cancer Study Project. *Carcinogenesis*, **29**, 766-771

370. Talbott, K.E., Gammon, M.D., Kibriya, M.G., Chen, Y., Teitelbaum, S.L., Long, C.M., Gurvich, I., Santella, R.M. and Ahsan, H. (2008) A CYP19 (aromatase) polymorphism is associated with increased premenopausal breast cancer risk. *Breast Cancer Res. Treat.*, **111**, 481-487

371. Gaudet, M.M., Bensen, J.T., Schroeder, J., Olshan, A.F., Terry, M.B., Eng, S.M., Teitelbaum, S.L., Britton, J.A., Lehman, T.A., Neugut, A.I., Ambrosone, C.B., Santella, R.M. and Gammon, M.D. (2006) Catechol-O-methyltransferase haplotypes and breast cancer among women on Long Island, New York. *Breast Cancer Res. Treat.*, **99**, 235-240

372. Ahn, J., Gammon, M.D., Santella, R.M., Gaudet, M.M., Britton, J.A., Teitelbaum, S.L.,

Terry, M.B., Neugut, A.I., Eng, S.M., Zhang, Y., Garza, C. and Ambrosone, C.B. (2006) Effects of glutathione S-transferase A1 (GSTA1) genotype and potential modifiers on breast cancer risk. *Carcinogenesis,* **27**, 1876-1882

373. Rothman, K.J., Greenland, S. and Lash, T.L. (2008) *Modern epidemiology*. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia

374. Hosmer, D.W. and Lemeshow, S. (1992) Confidence interval estimation of interaction. *Epidemiology*, **3**, 452-456

375. Allison, P.D. and SAS Institute. (2010) *Survival analysis using SAS: a practical guide*. SAS Institute, Cary, NC

376. Poole, C. (2001) Low P-values or narrow confidence intervals: which are more durable?. *Epidemiology*, **12**, 291-294

377. Ziegler, A. and König, I.R. (2010) *A statistical approach to genetic epidemiology*. Wiley-VCH, Weinheim

378. Wacholder, S., Chanock, S., Garcia-Closas, M., El Ghormli, L. and Rothman, N. (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J. Natl. Cancer Inst.,* **96**, 434-442

379. Wakefield, J. (2007) A Bayesian measure of the probability of false discovery in genetic epidemiology studies. *Am. J. Hum. Genet.,* **81**, 208-227

380. Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N. and Golani, I. (2001) Controlling the false discovery rate in behavior genetics research. *Behav. Brain Res.,* **125**, 279-284

381. Benjamini, Y. and Hochberg, Y. (1995) Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. *Royal Statistical Society,* **57**, 289

382. Lucke, J.F. (2009) A critique of the false-positive report probability. *Genet. Epidemiol.,* **33**, 145-150

383. Fink, B.N., Steck, S.E., Wolff, M.S., Britton, J.A., Kabat, G.C., Schroeder, J.C., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2007) Dietary flavonoid intake and breast cancer risk among women on Long Island. *Am J Epidemiol,* **165**, 514-23

384. Willett, W.C., Howe, G.R. and Kushi, L.H. (1997) Adjustment for total energy intake in epidemiologic studies. *Am. J. Clin. Nutr.,* **65**, 1220S-1228S; discussion 1229S-1231S

385. Dirx, M.J., Zeegers, M.P., Dagnelie, P.C., van den Bogaard, T. and van den Brandt, P.A. (2003) Energy restriction and the risk of spontaneous mammary tumors in mice: a meta-analysis. *Int. J. Cancer,* **106**, 766-770

386. Zhang, F.F., John, E.M., Knight, J.A., Kaur, M., Daly, M., Buys, S., Andrulis, I.L., Stearman, B., West, D. and Terry, M.B. (2013) Total energy intake and breast cancer risk in sisters: the Breast Cancer Family Registry. *Breast Cancer Res. Treat.*, **137**, 541-551

387. Hu, F.B., Stampfer, M.J., Rimm, E., Ascherio, A., Rosner, B.A., Spiegelman, D. and Willett, W.C. (1999) Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. *Am. J. Epidemiol.*, **149**, 531-540

388. Lubin, J.H. and Gail, M.H. (1990) On power and sample size for studying features of the relative odds of disease. *Am. J. Epidemiol.*, **131**, 552-566

389. Garcia-Closas, M. and Lubin, J.H. (1999) Power and sample size calculations in case-control studies of gene-environment interactions: comments on different approaches. *Am. J. Epidemiol.*, **149**, 689-692

390. Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C. and Parkin, D.M. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer*, **127**, 2893-2917

391. Mahaffey, K.R., Clickner, R.P. and Jeffries, R.A. (2009) Adult women's blood mercury concentrations vary regionally in the United States: association with patterns of fish consumption (NHANES 1999-2004). *Environ. Health Perspect.*, **117**, 47-53

392. Tran, N.L., Barraj, L.M., Bi, X., Schuda, L.C. and Moya, J. (2013) Estimated long-term fish and shellfish intake--national health and nutrition examination survey. *J. Expo. Sci. Environ. Epidemiol.*, **23**, 128-136

393. Waksberg, J. (1978) Sampling methods for random digit dialing. *J Amer Statistic Assoc*, 40

394. Terry, M.B., Zhang, F.F., Kabat, G., Britton, J.A., Teitelbaum, S.L., Neugut, A.I. and Gammon, M.D. (2006) Lifetime alcohol intake and breast cancer risk. *Ann. Epidemiol.*, **16**, 230-240

395. Block, G., Hartman, A.M., Dresser, C.M., Carroll, M.D., Gannon, J. and Gardner, L. (1986) A data-based approach to diet questionnaire design and testing. *Am. J. Epidemiol.*, **124**, 453-469

396. Potischman, N., Swanson, C.A., Coates, R.J., Weiss, H.A., Brogan, D.R., Stanford, J.L., Schoenberg, J.B., Gammon, M.D. and Brinton, L.A. (1997) Dietary relationships with early onset (under age 45) breast cancer in a case-control study in the United States: influence of chemotherapy treatment. *Cancer Causes Control*, **8**, 713-721

397. Anonymous (2012) U.S. Department of Agriculture, Agricultural Research Service, USDA National Nutrient Database for Standard Reference, Release 25. Nutrient Data

398. Gammon, M.D., Santella, R.M., Neugut, A.I., Eng, S.M., Teitelbaum, S.L., Paykin, A., Levin, B., Terry, M.B., Young, T.L., Wang, L.W., Wang, Q., Britton, J.A., Wolff, M.S., Stellman, S.D., Hatch, M., Kabat, G.C., Senie, R., Garbowski, G., Maffeo, C., Montalvan, P., Berkowitz, G., Kemeny, M., Citron, M., Schnabel, F., Schuss, A., Hajdu, S. and Vinceguerra, V. (2002) Environmental toxins and breast cancer on Long Island. I. Polycyclic aromatic hydrocarbon DNA adducts. *Cancer Epidemiol. Biomarkers Prev.*, **11**, 677-685

399. Lee, Y.S., Kim, H., Wu, T.X., Wang, X.M. and Dionne, R.A. (2006) Genetically mediated interindividual variation in analgesic responses to cyclooxygenase inhibitory drugs. *Clin. Pharmacol. Ther.*, **79**, 407-418

400. Huang, Q.R., Morris, D. and Manolios, N. (1997) Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. *Mol. Immunol.*, **34**, 577-582

401. Wu, J., Metz, C., Xu, X., Abe, R., Gibson, A.W., Edberg, J.C., Cooke, J., Xie, F., Cooper, G.S. and Kimberly, R.P. (2003) A novel polymorphic CAAT/enhancer-binding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. *J. Immunol.*, **170**, 132-138

402. Elahi, M.M., Asotra, K., Matata, B.M. and Mastana, S.S. (2009) Tumor necrosis factor alpha -308 gene locus promoter polymorphism: an analysis of association with health and disease. *Biochim. Biophys. Acta*, **1792**, 163-172

403. Bastaki, M., Huen, K., Manzanillo, P., Chande, N., Chen, C., Balmes, J.R., Tager, I.B. and Holland, N. (2006) Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. *Pharmacogenet Genomics*, **16**, 279-286

404. Hansson, M., Olsson, I. and Nauseef, W.M. (2006) Biosynthesis, processing, and sorting of human myeloperoxidase. *Arch. Biochem. Biophys.*, **445**, 214-224

405. Forsberg, L., Lyrenas, L., de Faire, U. and Morgenstern, R. (2001) A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radic. Biol. Med.*, **30**, 500-505

406. Hu, Y.J. and Diamond, A.M. (2003) Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. *Cancer Res.*, **63**, 3347-3351

407. Garte, S., Gaspari, L., Alexandrie, A.K., Ambrosone, C., Autrup, H., Autrup, J.L., Baranova, H., Bathum, L., Benhamou, S., Boffetta, P., Bouchardy, C., Breskvar, K., Brockmoller, J., Cascorbi, I., Clapper, M.L., Coutelle, C., Daly, A., Dell'Omo, M., Dolzan,

V., Dresler, C.M., Fryer, A., Haugen, A., Hein, D.W., Hildesheim, A., Hirvonen, A., Hsieh, L.L., Ingelman-Sundberg, M., Kalina, I., Kang, D., Kihara, M., Kiyohara, C., Kremers, P., Lazarus, P., Le Marchand, L., Lechner, M.C., van Lieshout, E.M., London, S., Manni, J.J., Maugard, C.M., Morita, S., Nazar-Stewart, V., Noda, K., Oda, Y., Parl, F.F., Pastorelli, R., Persson, I., Peters, W.H., Rannug, A., Rebbeck, T., Risch, A., Roelandt, L., Romkes, M., Ryberg, D., Salagovic, J., Schoket, B., Seidegard, J., Shields, P.G., Sim, E., Sinnet, D., Strange, R.C., Stucker, I., Sugimura, H., To-Figueras, J., Vineis, P., Yu, M.C. and Taioli, E. (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 1239-1248

408. Lin, H.J., Johansson, A.S., Stenberg, G., Materi, A.M., Park, J.M., Dai, A., Zhou, H., Gim, J.S., Kau, I.H., Hardy, S.I., Parker, M.W. and Mannervik, B. (2003) Naturally occurring Phe151Leu substitution near a conserved folding module lowers stability of glutathione transferase P1-1. *Biochim. Biophys. Acta*, **1649**, 16-23

409. Morel, F., Rauch, C., Coles, B., Le Ferrec, E. and Guillouzo, A. (2002) The human glutathione transferase alpha locus: genomic organization of the gene cluster and functional characterization of the genetic polymorphism in the hGSTA1 promoter. *Pharmacogenetics*, **12**, 277-286

410. Dempster, E.L., Mill, J., Craig, I.W. and Collier, D.A. (2006) The quantification of COMT mRNA in post mortem cerebellum tissue: diagnosis, genotype, methylation and expression. *BMC Med. Genet.*, **7**, 10

411. Feigelson, H.S., Shames, L.S., Pike, M.C., Coetzee, G.A., Stanczyk, F.Z. and Henderson, B.E. (1998) Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Res.*, **58**, 585-587

412. Lachman, H.M., Papolos, D.F., Saito, T., Yu, Y.M., Szumlanski, C.L. and Weinshilboum, R.M. (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics*, **6**, 243-250

413. Sostres, C., Gargallo, C.J. and Lanas, A. (2013) Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. *Arthritis Res. Ther.*, **15 Suppl 3**, S3

414. McKelvey, W., Gwynn, R.C., Jeffery, N., Kass, D., Thorpe, L.E., Garg, R.K., Palmer, C.D. and Parsons, P.J. (2007) A biomonitoring study of lead, cadmium, and mercury in the blood of New York city adults. *Environ. Health Perspect.*, **115**, 1435-1441

415. Mahaffey, K.R., Clickner, R.P. and Bodurow, C.C. (2004) Blood organic mercury and dietary mercury intake: National Health and Nutrition Examination Survey, 1999 and 2000. *Environ. Health Perspect.*, **112**, 562-570

416. Taha, A.Y., Cheon, Y., Faurot, K.F., Macintosh, B., Majchrzak-Hong, S.F., Mann, J.D., Hibbeln, J.R., Ringel, A. and Ramsden, C.E. (2014) Dietary omega-6 fatty acid lowering

increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. *Prostaglandins Leukot. Essent. Fatty Acids,* **90**, 151-157

417. Li, H., Fan, Y.W., Li, J., Tang, L., Hu, J.N. and Deng, Z.Y. (2013) Evaluating and predicting the oxidative stability of vegetable oils with different fatty acid compositions. *J. Food Sci.,* **78**, H633-41

418. Beveridge, M.C., Thilsted, S.H., Phillips, M.J., Metian, M., Troell, M. and Hall, S.J. (2013) Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *J. Fish Biol.,* **83**, 1067-1084

419. Mahaffey, K.R. (2004) Fish and shellfish as dietary sources of methylmercury and the omega-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: risks and benefits. *Environ. Res.,* **95**, 414-428

420. Velentzis, L.S., Keshtgar, M.R., Woodside, J.V., Leathem, A.J., Titcomb, A., Perkins, K.A., Mazurowska, M., Anderson, V., Wardell, K. and Cantwell, M.M. (2011) Significant changes in dietary intake and supplement use after breast cancer diagnosis in a UK multicentre study. *Breast Cancer Res. Treat.,* **128**, 473-482

421. Mairesse, G., Thomas, M., Gardeur, J.N. and Brun-Bellut, J. (2006) Effects of geographic source, rearing system, and season on the nutritional quality of wild and farmed Perca fluviatilis. *Lipids,* **41**, 221-229

422. Boyd, N.F., Stone, J., Vogt, K.N., Connelly, B.S., Martin, L.J. and Minkin, S. (2003) Dietary fat and breast cancer risk revisited: a meta-analysis of the published literature. *Br. J. Cancer,* **89**, 1672-1685