INFLAMMATION AND BREAST CANCER RISK

by

Gretchen L. Gierach

BS, Pennsylvania State University, 1998

MPH, University of Pittsburgh, 2004

Submitted to the Graduate Faculty of

Department of Epidemiology

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2006

UNIVERSITY OF PITTSBURGH

Graduate School of Public Health

This dissertation was presented

by

Gretchen L. Gierach

It was defended on

April 7, 2006

and approved by

Dissertation Advisor:
Francesmary Modugno, PhD, MPH
Assistant Professor of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Jane A. Cauley, DrPH, MPH
Professor of Epidemiology
Associate Professor of Nursing
Graduate School of Public Health
School of Nursing
University of Pittsburgh

Joel L. Weissfeld, MD, MPH
Associate Professor of Epidemiology
Assistant Professor of Medicine
Graduate School of Public Health
School of Medicine
University of Pittsburgh

John W. Wilson, PhD
Assistant Professor of Biostatistics
Graduate School of Public Health
University of Pittsburgh

Victor G. Vogel III, MD, MHS
Professor of Medicine and Epidemiology
Graduate School of Public Health
School of Medicine
University of Pittsburgh

Francesmary Modugno, PhD, MPH

INFLAMMATION AND BREAST CANCER RISK

Gretchen L. Gierach, PhD

University of Pittsburgh, 2006

Mammographic density is one of the strongest risk factors for breast cancer. Exactly how breast density increases breast cancer risk is unknown, although it is believed that dense breast areas may reflect exposure to estrogen. Breast cancer incidence is highest in postmenopause, when most estrogens are produced in non-ovarian tissues. Cyclooxygenase (COX)-2 and the cytokine tumor necrosis factor (TNF)-alpha may play a role in regulating estrogen synthesis in postmenopausal women. The aim of the present study was to explore the association between inflammation and breast cancer risk in two populations of postmenopausal women. Different exposures associated with inflammation (i.e. non-steroidal anti-inflammatory drug (NSAID) use, circulating receptors for TNF-alpha, and a polymorphism in the TNF receptor-II gene) were measured and tested for their association with incident breast cancer or mammographic density. In the first study, the Study of Osteoporotic Fractures (SOF), complete NSAID medication and breast cancer risk factor information was available for 6695 women, mean (SD) age 73 (5) years. During a mean (SD) of 13.2 (3.8) years of follow-up, 372 women were diagnosed with primary breast cancer. There were no differences in incident breast cancer by NSAID use, either before or after adjusting for covariates. In the second study, Mammograms and Masses (MAMS), mean mammographic density was lower among women in the highest quartiles of circulating soluble TNF receptor levels. After adjustment for body mass index, the inverse association disappeared. In evaluating the TNFR2 –196 M/R polymorphism (T>G), the unadjusted mean (SD) mammographic density was higher in women with the TT genotype (32.3% (21.0)) as compared to women with the TG/GG genotypes (26.6% (17.2)), p=0.003. The association remained statistically significant after adjustment for age and BMI (p=0.03); however, inclusion of additional covariates reduced the level of statistical significance (p=0.08). There was no observable difference in circulating sTNFR2 levels between the TNFR2 genotypes. An increased understanding of factors that affect mammographic density and their underlying

mechanisms is needed, and inflammation may be involved. An association between breast cancer risk and inflammation would have important pubic health implications for screening and primary prevention of breast cancer.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the people who have offered me guidance and support throughout graduate school and have helped me, each in their own way, to attain my doctoral degree. As my advisor and mentor throughout my training and research, Dr. Francesmary Modugno has been an invaluable resource. Dr. Modugno's scholarly insights and enthusiasm for scientific research have been a driving force behind the successful completion of this dissertation. I cannot thank her enough for her interest, generosity of time and encouragement. Without the support of Dr. Jane A. Cauley, a principal investigator of the Study of Osteoporotic Fractures, this work would not have been possible. Dr. Joel L. Weissfeld, Dr. John W. Wilson, and Dr. Victor G. Vogel III also each provided critical guidance and direction.

This project would not have been feasible without the assistance of the dedicated Mammograms and Masses Study research team. I am especially indebted to Dr. Jennifer K. Simpson not only for her countless contributions to MAMS, but also for her patience, time, and moral support. I thank Alana Hudson for her collaboration on MAMS and thoughtful feedback on this project, and I am sincerely grateful for her friendship. I would also like to thank Glenn Allen for his meticulous management of the MAMS database and Betty Kotowski for her outstanding work in patient enrollment and mammogram retrieval. Special thanks to Martine Salane for the mammographic density readings, Dr. Marjorie Romkes for the genotyping, and Dr. Russell Tracy for the cytokine assays. I dedicate this work to the numerous participants who volunteered in the hopes of furthering the study of women's health.

Many thanks to Dr. Roberta B. Ness for fostering an environment that encourages critical thinking and sound epidemiologic research and for her support of this project through the Department of Epidemiology's Small Grants Program. I would also like to thank those with whom I worked at the Epidemiology Data Center, along with my other friends and colleagues at the University of Pittsburgh. I could not have wished for a better group of people with whom to work and grow throughout my graduate school experience.

I must also acknowledge the love and support of my dear colleagues and friends Dr. Phyllis Kernoff Mansfield, Dr. Patricia Barthalow Koch, Dr. Susannah Heyer Barsom, and Dr. Ann Voda who taught me early in my academic career to approach research with passion and a critical eye.

Lastly, I would like to thank my family and friends for their patience and tolerance of the many ups and downs that often times accompany graduate school. I thank my parents for their constant support of my goals. I would not be where I am today without their love and encouragement. My sister, Kimberlee, and my extended family in Pittsburgh, helped to make Pittsburgh feel like home, always reminding me what matters most in life. My deepest gratitude I extend to my husband, Andy: my best friend and most unwavering supporter. His creativity and ingenuity are a never-ending source of inspiration to me.

1.0 INTRODUCTION

1.1 BACKGROUND AND SIGNIFICANCE

In the following literature review, a brief description of the epidemiology of breast cancer, cytokines and mammographic density will be followed by a review of the potential relationship among these factors.

1.1.1 Epidemiology of breast cancer

Breast cancer incidence rates in the United States are 20%-40% higher in white women than in non-white women; however, U.S. incidence rates are higher in young (age < 40) black women than in young white women (Figure 1). Worldwide, incidence rates for 1988-1992 were low in Asia, moderate in South America and Eastern Europe, and high in North America and Western Europe (1). Migrant studies of increasing breast cancer rates among first-generation daughters of Japanese American women suggest that environmental and lifestyle factors are of greater significance than genetic factors in explaining international differences in breast cancer risk (1-3). The identification of potentially modifiable risk factors for breast cancer therefore provides opportunities for breast cancer prevention among women both at average and high risk.

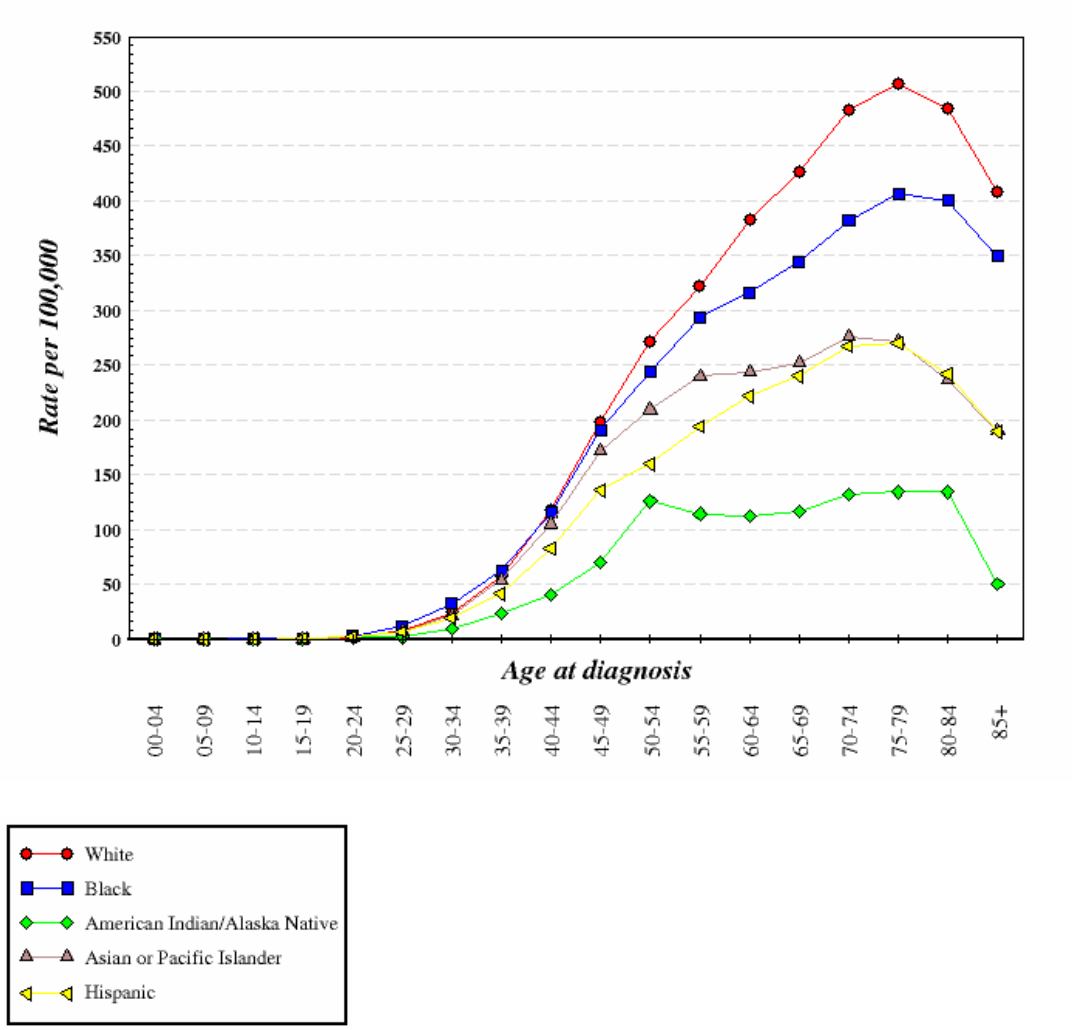


Figure 1. Age-specific incidence of invasive breast cancer among US women, 2001

SEER*Stat Database: 9 SEER Incidence Registries for Public-Use, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2003, based on the November 2002 data (for the years 1973-2000).

1.1.1.1 Risk factors for breast cancer

Few breast cancer risk factors have prevalence in the population of greater than 10% to 15%, although some are associated with very large relative risks (e.g., mutated genes, cellular atypia). Age is one of the most important risk factors for breast cancer (4). While age-adjusted incidence rates continue to rise, breast cancer mortality has fallen in the past decade in the U.S (5). The relationship of age to invasive breast cancer incidence in 2001 in U.S. women is also depicted in Figure 1. Recently investigated epidemiologic risk factors for breast cancer are shown in Table 1, along with the magnitude of their associated risks (6). Traits associated with large relative risks are rare; common risk factors are associated with relative risks less than 2.0 so that the attributable risk for any particular risk factor is small (7).

Table 1. Newer epidemiologic risk factors for breast cancer

Risk factor	Effect	Odds ratio/relative risk (95% confidence interval)
Anthropometry (BMI)		
Premenopausal	Negative	For BMI $\geq$ 31 kg/m^2 vs. BMI < 21 kg/m^2: RR = 0.54 (0.34, 0.85) (8)
Postmenopausal	Positive	For BMI $\geq$ 25 kg/m^2 vs. BMI < 21 kg/m^2: RR = 1.26 (1.09, 1.46) (8) For BMI > 22.6 kg/m^2 vs. BMI $\leq$ 22.6 kg/m^2: RR = 2.52 (1.62, 3.93) (9)
Endogenous hormones	Positive	For increasing quintiles of free estradiol vs. the lowest quintile: (10) RR = 1.38 (0.94, 2.03) RR = 1.84 (1.24, 2.74) RR = 2.24 (1.53, 3.27) RR = 2.58 (1.76, 3.78)
Estrogen metabolism (2:16 OHE1 Ratio [EMR])	Negative?	For EMR in the highest tertile vs. the lowest two-thirds: (11) OR = 0.71 (0.29, 1.75) By menopausal status: (12) OR (pre) = 0.58 (0.25, 1.34) OR (post) = 1.29 (0.53, 3.10)

Table 1 (continued)

Risk factor	Effect	Odds ratio/relative risk (95% confidence interval)
Reproductive factors		For breast cancer diagnosed pre- and post-menopause: (13)
Age at menarche	Negative	OR (pre) = 0.91 (0.89, 0.93) OR (post) = 0.96 (0.95, 0.98)
Age at first live birth	Positive	OR (pre) = 1.05 (1.05, 1.06) OR (post) = 1.03 (1.02, 1.04)
Parity	Negative	OR (pre) = 0.97 (0.94, 0.99) OR (post) = 0.88 (0.86, 0.90)
Breastfeeding	Negative	For every 12 months of breast-feeding: (14) OR = 0.96 (0.94, 0.97)
Preeclampsia	Negative	ORs range from 0.27 (0.08, 0.63) to 0.81 (0.61, 1.1) (15)
Induced abortion	Null	RR = 1.00 (0.94, 1.06) (16)
Bone mineral density	Positive	For the highest quartile of BMD vs. the lowest quartile: (17) RR = 2.7 (1.4, 5.3)
Bone fracture	Negative	For history of fracture vs. no fracture in past 5 years: (18) OR = 0.80 (0.68, 0.94)
Biological growth factors		
TGF-β1	Positive	Protective effect for women lacking the common TGF-β1 genetic polymorphism vs. women with the common variant: (19) Hazard ratio = 0.36 (0.17, 0.75)
IGF-I	Positive	Top vs. bottom tertile of IGF-I: (20) RR = 2.9 (1.21, 6.85)
Exogenous hormones		
Oral contraceptives	Null?	RR = 1.24 (1.15, 1.33) (21) RR = 1.11 (0.94, 1.32) (22)
Hormone therapy	Positive	Hazard ratio = 1.26 (1.00, 1.59) (23)
Exercise/physical activity	Negative	ORs range from 0.3 to 1.6, with an average risk reduction of 30-40% (24)
Alcohol consumption	Positive	For 12 g/day vs. nondrinkers: RR = 1.06 (1.00, 1.11) (25) HT for ≥ 5 years plus ≥ 20 g/day: RR = 1.99 (1.42, 2.79) (26)

Table 1 (continued)

Risk factor	Effect	Odds ratio/relative risk (95% confidence interval)
Smoking		
Premenopausal	Positive	OR (parous) = 1.69 (1.13, 2.51) (27) OR (nulliparous) = 7.08 (1.63, 30.8) (27)
Postmenopausal	Negative	OR = 0.49 (0.27, 0.89) (27)
Breast implants	Null?	RR = 0.72 (0.61, 0.85) (28)
Diet	Null	Fats compared with equivalent energy intake from carbohydrates for an increment of 5% of energy: (29) RR = 1.09 (1.00, 1.19), saturated RR = 0.93 (0.84, 1.03), monounsaturated RR = 1.05 (0.96, 1.16), polyunsaturated
Dietary Micronutrients		
Beta-carotene	Negative	OR = 0.41 (0.22, 0.79) (30)
Lycopene	Negative	OR = 0.55 (0.29, 1.06) (30)
Total carotene	Negative	OR = 0.55 (0.29, 1.03) (30)
Folate	Negative?	For lowest 10^{th} percentile of folate intake vs. $\geq 50^{th}$ percentile: (31) RR = 1.21 (0.91, 1.61) Among drinkers of > 4 gm per day: RR = 1.59 (1.05, 2.41)
Phytoestrogens	Null	For the highest vs. lowest quartile: (32) OR = 1.0, (0.80, 1.3)
Ionizing radiation	Positive	OR = 1.4 (1.2, 1.8) (33) RR = 4.1 (2.5, 5.7) (34)
Environmental toxins		
p,p'-bis(4-chlorophenyl)-1,1-dichloroethene	Null	OR = 1.20 (0.76, 1.90) (35)
chlordane	Null	OR = 0.98 (0.62, 1.55) (35)
dieldrin	Null	OR = 1.37 (0.69, 2.72) (35)
PCB congeners	Null	OR = 0.83 (0.54, 1.29) (35)
Electromagnetic fields	Null	OR = 1.12 (1.09, 1.15), (36) probable bias due to misclassification of exposure
HIV infection	Null?	OR = 0.18 (0.04, 0.76) (37)

1.1.1.2 Estrogens and the risk of breast cancer

Based upon the association of hormonally related risk factors such as age at menarche and age at first live birth with the risk of developing breast cancer, differences in endogenous estrogen levels are theorized to affect the risk of breast cancer (38). Exposure to estrogen over prolonged durations and higher concentrations has been consistently related to an increased risk of postmenopausal breast cancer in many epidemiologic studies (10, 39). In a pooled analysis of nine prospective studies of endogenous hormone concentrations and breast cancer risk, serum estradiol concentrations predicted risk for postmenopausal breast cancer (10): the relative risk (RR) for women with the highest quintile of free estradiol concentration, relative to the lowest quintile, was 2.58 (95% CI: 1.76-3.78). Hence, a single measurement of bioavailable estradiol may be used to estimate a woman's risk for breast cancer. In postmenopausal women, BMI is a critical determinant of estrogen production (40). Results from the Women's Health Initiative (WHI) Observational Study confirmed the effect of increasing BMI on breast cancer risk among postmenopausal women, but only among those women who had never taken HT, with heavier women (baseline BMI >31.1) having an increased risk of postmenopausal breast cancer (RR=2.52; 95% CI: 1.62-3.93), compared to slimmer women (baseline BMI <22.6) (9). In addition, bone mineral density (BMD), perhaps one of the best surrogate measures of lifetime estrogen exposure, is positively associated with breast cancer risk (17, 41). Extensive data also link the use of HT after menopause, a major source of exogenous estrogen exposure in postmenopausal women, to the risk of developing breast cancer (23, 42). *Taken together, these studies all suggest that increased lifetime endogenous and exogenous estrogen exposure appears to increase breast cancer risk. Despite the evidence implicating estrogens in breast cancer, the underlying mechanism by which estrogens exert their effects remains unclear.*

1.1.2 Cytokines and the risk of breast cancer

Although data strongly implicate estrogen in breast cancer risk, increasing evidence suggests that cytokines may play crucial roles in postmenopausal breast cancer etiology (43). In particular, the cytokine TNF-α has emerged as an important regulator of estrogen synthesis in the breast (43). Moreover, inflammatory cytokines induce a range of inflammatory enzymes, including

cyclooxygenase (COX)-2. COX-2 cyclizes and oxygenates arachidonic acid eventually producing prostaglandin E_2 (PGE_2) (44, 45). COX-2 is believed to drive production of estrogen in the breast, in turn facilitating tumorigenesis (46), as evidenced by a positive correlation between 1) the level of COX-2 and expression of cytochrome P19 (CYP19) in human breast cancer (47) and 2) increased aromatase gene (P450) expression, the product of CYP19, in cultured breast cells (45, 48). This paracrine loop may explain why inhibition of COX-2 activity could have a protective effect on breast cancer. Indeed, studies have consistently shown that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX-2 and thereby PGE_2 production (49), hinder breast tumor cell growth *in vitro* and in animal models (47, 50-53). Consistent with this biologic mechanism, several epidemiologic studies have examined the association between use of NSAIDs and breast cancer risk (reviewed in (54) and (55)) with most, but not all (56, 57), of case-control studies finding risk reductions between ~20-40% (58-68). Results from prospective cohort studies have been less consistent, with seven studies finding no association (69-75), one study observing an increased risk (76), and five studies, including the WHI Observational Study, demonstrating a protective effect from use of NSAIDs (77-81). Recently the Women's Health Study, a randomized controlled trial, found that alternate-day use of low-dose aspirin for an average of 10 years of treatment did *not* reduce the risk of breast cancer (82).

It is beyond the scope of this project to discuss all cytokines involved in estrogen synthesis and expressed in the breast. Based upon preliminary data from the Mammograms and Masses Study suggesting a relationship between TNF-alpha soluble receptors and breast density (unpublished), as well as evidence from the literature suggesting a role for TNF-alpha in estrogen biosynthesis in the breast, we focused on the two soluble receptors for TNF-alpha.

1.1.2.1 TNF-alpha

The cytokine tumor necrosis factor (TNF)-alpha, secreted by macrophages of the immune system and also by adipocytes, has a central role in regulating estrogen synthesis within both normal and malignant breast tissue (43, 83, 84). In fact, TNF-α can stimulate the activities of all of the enzymes involved in estrogen synthesis: TNF-α enhances the activities of aromatase (85), 17β-estradiol dehydrogenase (86, 87), and estrone sulfatase (88), the three enzymes involved in the conversion of androstenedione to estrone (E1), the reduction of E1 to estradiol (E2), and the

hydrolysis of estrone sulfate to E1, respectively (84). Furthermore, TNF-α is one of the most potent promoters of aromatase activity in adipose fibroblasts, resulting in the peripheral conversion of androgens to estrogens in the adipose tissue (89). There is evidence that the production of TNF-α is also increased in obese women (90); thus, TNF-α may be one explanation for the positive association observed between obesity and breast cancer among postmenopausal women (9). Plasma concentrations of TNF-α have also been shown to increase with age (91, 92), potentially influencing the development of breast tumors in some postmenopausal women. Several studies have observed the regulation of estrogen production by TNF-α in breast fibroblasts, undifferentiated cells formed around malignant breast epithelial cells (83, 85, 86, 93). Thus, aberrant TNF-α function that ultimately elevates estrogens may promote malignant transformation in the postmenopausal breast (83); however, to date, two prospective epidemiologic studies have shown no association between TNF-α and breast cancer risk (94, 95).

1.1.2.2 TNF-alpha receptor activity: Laboratory and epidemiologic observations

TNF-α exerts its effects by binding to two transmembrane cell surface receptors: the p60 TNF receptor 1 (TNFR1) and the p80 TNF receptor 2 (TNFR2) (96), both of which are expressed in virtually all mammalian cells, including mammary epithelial cells (97). The TNFRs have similar ligand-binding domains, but each differs in cytoplasmic domains, suggesting distinct signal transduction pathways. TNF-α binds to the two receptors with similar affinity; when engaged, the extracellular domains of the soluble TNF receptors may be shed into the circulation, activating downstream pathways, and leading to the stimulation of pro-inflammatory cytokines and immunomodulatory genes (98). In contrast, these shed sTNFRs can also compete for TNF-α with the cell surface receptors and thus block TNF-α activity (99). The soluble TNF receptors (sTNFRs) are believed to play a central role in TNF-alpha-mediated cytotoxic (100), mitogenic (101), anti-proliferative (101), and apoptotic effects (102), but the specific roles of the two receptors are highly debated. The functional role of sTNFRs *in vivo* is yet to be elucidated since these soluble receptors have been shown to inhibit TNF in cytotoxic assays (103, 104), and they have also been reported to enhance TNF-α activity *in vitro* (104). Unlike TNF-α., which has a relatively short half-life in circulation, determination of sTNFR concentrations in healthy individuals at time lapses of 1 year demonstrated that the concentrations of the receptors are stable in each individual (correlation coefficients of 0.84 and 0.90 for sTNFR1 and sTNFR2,

respectively), possibly reflecting genetically determined differences (105); this observation was supported by studies of identical twins, who unlike discordant twins, were more likely to have similar levels of sTNFRs (105). The mRNAs of both TNF receptors are up-regulated in adipose tissue in obese women (106, 107), and circulating levels are positively correlated with BMI and age (108-110).

Excessive signaling through TNF receptors may cause severe inflammatory reactions and tissue damage (98). Solid malignant tumor cell lines shed sTNFRs spontaneously, and levels of the receptors were elevated in the sera of cancer patients (111-115), and in the plasma (116) and serum (117) of breast cancer patients as compared to healthy individuals. Deficient expression of TNFR2 mRNA was found in the endometrium of women at the earliest stages of endometriosis (118); whether low producers of soluble TNFRs may be predisposed to an over-response to TNF in pathological conditions remains to be determined (119). To date, only one nested case-control study has prospectively examined the relationship between serum levels of sTNFRs and breast cancer risk (95). The investigators found no association between serum levels of the soluble receptors TNFR1 and TNFR2 and breast cancer risk; however, this study had limited power to detect an association in postmenopausal women, with only 61 postmenopausal case-control pairs (95).

1.1.2.3 Tumor necrosis factor receptor 2 (TNFR2) gene variations

Polymorphic variations in the TNFR2 gene have been more extensively studied than those in TNFR1. The TNFR2 gene has been localized to chromosome 1p36.2 and spans about 43kb, consisting of 10 exons, only three of which (exons 4, 6, and 9) contain polymorphic sites that lead to a non-conservative amino acid change (120, 121). Whereas the TNFR1 gene does not contain any known functional variants, the TNFR2 gene contains a non-synonymous single nucleotide polymorphism (SNP) with potential functional significance with respect to circulating TNFR2 levels. In particular, the TNFR2 –196 M/R polymorphism (T > G; Exon 6; results in substitution of methionine by arginine; rs1061622) is located in the extracellular region of the receptor, the region responsible for its proteolytic cleavage and solubilization. This polymorphism appears to be functionally significant; in a cohort of patients with rheumatoid arthritis, the TT genotype was associated with a threefold higher chance of responding to anti-TNF-alpha therapy as compared to patients with TG/GG genotypes (122). (Interestingly, anti-

TNF-alpha therapy was successfully tested for toxicity, biological activity and therapeutic efficacy in metastatic breast cancer (123)). Furthermore, the –196 SNP has been found to influence serum TNFR2 levels in previous studies (carriers of the G allele have higher circulating levels) (124, 125), and has been associated with autoimmune diseases (126-130), hypercholesterolemia (124), hyperandrogenism (131), and polycystic ovarian syndrome (131). A recent study of 113 postmenopausal breast cancer cases and 157 pre- and postmenopausal controls in Tunisia demonstrated a significant association between the –196 M/R heterozygous genotype (TG) and breast carcinoma (OR=2.28, 95% CI: 1.36-3.83), yet among cases, the R allele was associated with increased survival after 3 years of follow-up (132); these results are to be interpreted with caution, as they might be specific to Tunisians, and the investigators did not attempt to control for potential confounding factors. No prior studies have examined this SNP in relation to breast density.

Hence, TNF-alpha and its soluble receptors may play a role in breast cancer. Evidence also suggests that they are related to estrogen synthesis. Furthermore, prospective studies indicate that circulating levels of sTNFRs may be genetically determined. *However, no adequately powered study has investigated the relationship between these cytokine levels or polymorphisms in the TNFR2 gene with postmenopausal mammographic density.*

1.1.3 Mammographic breast density as a breast cancer biomarker

With the exception of age and carriage of BRCA1/2 mutations, mammographic density is the greatest risk factor for breast cancer (133, 134). The histologic composition of the breast is reflected mammographically by density and parenchymal pattern. The higher the fat content of the breast the lower the radiologic density. Conversely, a high proportion of connective, ductal/epithelial, and glandular tissue increases density (135-138). The first method to associate breast parenchymal patterns and breast cancer risk was proposed by Dr. John Wolfe in 1976 (139). His classification consisted of four patterns: N1-radiolucent breast, low risk; P1-linear radiographic densities or ductal prominence of lesser extent than P2, intermediate risk; P2-ductal prominence to a greater extent, intermediate risk; and DY-radiographically dense, risk highest (139). In an effort to reduce intra- and inter-observer variability, various methods have been developed to quantitatively assess mammographic parenchymal patterns. These methods

encompass visual estimation of dense tissue, digitized images utilizing computer-assisted methods, and planimetry to measure the area of density within the total breast area; the area of dense tissue divided by the total breast area is known as the proportion or percentage of mammographic density (133, 140-145). In both case-control (146-155) and prospective cohort studies (156-158) using Wolfe's method, increased breast density was associated with increased risk for breast cancer with ORs ranging from 1.4 to 6.2 (Table 2). Likewise, case-control (146, 147, 149, 151-155, 159-164) and prospective cohort studies (165) using quantitative methods have found similar associations, with ORs ranging from 1.8 to 6.0, and most studies yielding an OR of 4.0 or greater (Table 2). These associations remain even after adjusting for factors known to influence breast density and breast cancer risk, such as age at menarche, menopausal status, parity, age at first birth, family history, HT use, and BMI (discussed further in "Breast Cancer Risk Factors and their Association with Mammographic Density"). As Wolfe's classification method is subjective and may vary between observers (e.g. radiologists) (166), the quantitative methods have been deemed more effective in identifying women at increased risk for developing breast cancer (167, 168). Indeed, the majority of studies have shown a stronger association with breast cancer risk for the quantitative methods than for those using Wolfe's classification (133).

The best method of utilizing the information obtained from the dense and nondense components of a mammogram is currently under debate. While the dense area itself is related to the risk of breast cancer (169), the percentage of breast density appears to confer a greater risk and is the measure reported in the vast majority of studies (134, 155, 170, 171). Most risk factors for breast cancer that are related to mammographic density have the opposite relationship with the nondense area of the mammogram, largely comprised of fat tissue (172). Since body mass index correlates strongly and positively with both the nondense and total breast areas (173), and thus correlates inversely with percent breast density (163, 174, 175), potential confounding by adiposity is of particular concern when studying factors that are related to both percent breast density and BMI. Under such circumstances, investigators have argued for examination of the absolute area of dense breast tissue, instead of percent breast density (169, 173).

Mammographic density has also been positively associated with breast cancer tumor characteristics, such as tumor size, lymph node status, and lymphatic or vascular invasion, in both case control studies (176, 177) and in recent case-only studies of women diagnosed with screen-detected (178) and interval-detected (179) invasive breast cancer. The positive

association observed between density and tumor size could be due to a delayed diagnosis of tumors in women with dense breasts (a "masking" effect because density impairs mammographic sensitivity (180)); alternatively, dense breasts may be associated with increased cell proliferation (discussed further in "Heritability, Genetic Variations and Breast Density") (177). While many studies have evaluated mammographic density and its association with risk of incident primary breast cancer, the National Surgical Adjuvant Breast and Bowel Project (NSABP) recently evaluated mammographic density among women with a previous diagnosis of DCIS, and who were prospectively followed to measure breast cancer recurrence (181). NSABP investigators found that women with highly dense breasts had 2.8 (95% CI: 1.3 to 6.1) times the risk of breast cancer recurrence (DCIS or invasive) and three times the risk of subsequent invasive breast cancer (95% CI: 1.2 to 7.5) (181). Notably, a recent retrospective study of diagnostic mammograms from consecutive women diagnosed with DCIS at the USC/Norris Comprehensive Cancer Center demonstrated that DCIS lesions occurred overwhelmingly in areas of mammographically dense tissue; further, the majority of lesions occurred in the mammographic quandrant with the highest percentage density (182). All available pre-DCIS films showed that the areas in which DCIS subsequently arose were also dense at the time of the earlier mammogram (182). These results strongly suggest that, indeed, some characteristic of the mammographically dense tissue is directly influencing the carcinogenic process in the local breast glandular tissue (182). *Since mammographic breast density is a non-invasive, reliable and quantitative measure that is strongly associated with breast cancer risk, breast density provides a useful intermediate marker in studies aimed at understanding breast cancer etiology and prevention (183).*

Table 2. Selected studies of breast density and breast cancer risk: Wolfe's method and quantitative methods

First Author	Study Design	Participants	Wolfe Odds Ratio* (95% CI)	Quantitative Odds Ratio** (95% CI)	Quantitative Method	Threshold (%)	Adjustments
Boyd et al. 1982 (146)	Case-control	183 Cases 183 Controls	Range of 3 reading radiologists 1.9-3.7	Range of 3 reading radiologists OR 2.8-6.0 (1.4-5.6 to 2.5-14.1)	Visual Estimation	<10 vs. ≥75	Age at first birth, parity, family history
Brisson et al. 1982 (147)	Case-control	408 Cases 1021 Controls	OR DY vs N1 1.9 (1.1-3.3)	homogeneous density: OR 5.4 (2.5-11.4) nodular density: OR 3.8 (1.6, 8.7)	Visual Estimation	0 vs ≥60	Parity, age at first birth, family history, age at menopause, hormone use
Tabar & Dean 1982 (156)	Prospective	21,157 women 1857 Prevalent Cases 31 Incident Cases	Age 60+ 0.97 RR DY vs N1 Prevalent 2.9 Incident 6.2	NA			
Chaudry et al. 1983 (148)	Case-Control	104 Cases 937 Controls	OR DY vs N1 1.4				
Brisson et al. 1984 (149)	Case-control	362 Cases 686 Controls	OR DY vs N1 2.7 (1.5-4.8)	OR 4.4 (2.5-7.9)	Visual Estimation	0 vs ≥60	Weight, height
Carlile et al. 1985 (150)	Case-Control	706 Cases 1412 Controls	OR DY vs N1 3.1	NA			
Gravelle et al. 1986 (157)	Prospective	4,044 women 31 cancer	RR DY vs N1 4.4 (0.54-36.7)	NA			

Table 2 (continued)

First Author	Study Design	Participants	Wolfe Odds Ratio* (95% CI)	Quantitative Odds Ratio** (95% CI)	Quantitative Method	Threshold (%)	Adjustments
Wolfe et al. 1987 (151)	Case-control	160 Cases 160 Controls	OR P2/DY vs. N1/P1 3.3 (1.9-5.7)	OR 4.3 (1.8-10.4)	Manual Planimetry	<25 vs. ≥70	Parity
Brisson et al. 1989 (152)	Case-control	290 Cases 645 Controls	OR DY vs N1 3.7 (1.8-7.4)	OR 5.5 (2.3-13.2)	Visual Estimation	0 vs ≥60	Age, parity, education, weight, height
de Stavola et al. 1990 (158)	Prospective	4,044 women 69 cancer	RR P2/DY vs P1/N1 1.7 (0.72-4.0)	NA			
Saftlas et al. 1991 (153)	N. Case-control	260 Cases 301 Controls	OR DY vs N1 2.6 (1.3-5.4)	OR 4.3 (2.1-8.8)	Manual Planimetry	<5 vs ≥65	Age, weight, parity
Boyd et al. 1995 (161)	N. Case-control	354 Cases 354 Controls	NA	OR 4.0 (2.1, 7.7) OR 6.0 (2.8-13.0)	Computerized (thresholding) Visual Estimation	0 vs. ≥75	Age, parity, age at first birth, weight, height, age at menarche, family history
Kato et al. 1995 (154)	N. Case-control	73 PRE Cases 281 PRE Controls 124 POST Cases 240 POST Controls	PRE women; OR P2/DY vs P1/N1 OR 6.0 (1.3-27.3) POST women; OR P2/DY vs P1/N1 OR 1.9 (1.2-3.1)	OR 3.6 (1.7-7.9) 2.1 (1.1, 3.8)	Manual Planimetry	<48 vs ≥65 <28 vs ≥44	BMI, parity, menopausal status

Table 2 (continued)

First Author	Study Design	Participants	Wolfe Odds Ratio* (95% CI)	Quantitative Odds Ratio** (95% CI)	Quantitative Method	Threshold (%)	Adjustments
Byrne et al. 1995 (155)	N. Case-control	1880 Cases	OR DY vs N1				Weight, age at first birth, family history, education, alcohol use, prior biopsies, reproductive years
		2152 Controls	2.7 (2.0-3.7)	OR 4.3 (3.1-6.1)	Computerized Planimetry	0 vs. ≥75	
van Gils et al. 1999 (162)	N. Case-control	108 Cases					Menopausal status, BMI
		400 Controls	NA	OR 3.3 (1.5-7.2)	Computerized (automated)	<5 vs >25	
Lam et al. 2000 (163)	N. Case-control	529 Cases					Weight
		2116 Controls	NA	OR 4.5 (1.9-10.6)	BIRADS	Entirely fatty vs extremely dense	
Maskarinec & Meng 2000 (159)	Case-control	647 Cases					Age at menarche, menopausal status, parity, age at first birth, family history, hormone use, previous breast problems
		647 Controls	NA	OR 1.8 (1.1-3.0)	Computerized (thresholding)	<10 vs ≥50	

Table 2 (continued)

First Author	Study Design	Participants	Wolfe Odds Ratio* (95% CI)	Quantitative Odds Ratio** (95% CI)	Quantitative Method	Threshold (%)	Adjustments
Ursin et al. 2003 (171)	Case-control	622 Cases					Age, BMI, age at menarche, family history of breast cancer, # full-term pregnancies, menopausal status, hormone therapy use, age at first birth
		443 Controls	NA	OR 5.2 (1.7-16.1)	Computerized (thresholding)	<1 vs ≥75	
Vacek & Geller 2004 (165)	Prospective	24,238 PRE women					
		337 PRE cancer	NA	RR 4.6 (1.7, 12.6)	BIRADS	Entirely fatty vs extremely dense	these are unadjusted estimates
		37,606 POST women					
		854 POST cancer	NA	RR 3.9 (2.6, 5.8)	BIRADS	Entirely fatty vs extremely dense	these are unadjusted estimates
Kerlikowske et al. 2005 (160)	N. Case-control	200 Cases					
		431 Controls	NA	OR 2.7 (1.4-5.4)	Computerized (thresholding)	<23.9 vs. ≥66.8 (lowest vs. highest sextile)	Age, family history, age at first birth, hip BMD, race, BMI

Table 2 (continued)

First Author	Study Design	Participants	Wolfe Odds Ratio* (95% CI)	Quantitative Odds Ratio** (95% CI)	Quantitative Method	Threshold (%)	Adjustments
Maskarinec et al. 2005 (164)	N. Case-control	607 Cases					Ethnicity, age at mammogram, BMI, age at first live birth, # children, age at menarche and menopause, family history of breast cancer
		667 Controls	NA	OR 3.1 (2.0-4.9)	Computerized (thresholding)	<10 vs ≥50	

*Wolfe's Method: N1-radiolucent breast, low risk; P1-linear radiographic densities or ductal prominence of lesser extent than P2, intermediate risk; P2-ductal prominence to a greater extent, intermediate risk; and DY-radiographically dense, risk highest

**ORs shown for total density unless otherwise specified

BIRADS=Breast Imaging Reporting and Data System; BMD=bone mineral density; BMI=body mass index; CI=confidence interval; N.=nested; NA=not applicable; OR=odds ratio; PRE=premenopausal; POST=postmenopausal

1.1.3.1 Breast cancer risk factors and their association with breast density

Several reproductive and hormonal factors are known to influence both the mammographic appearance of the breast and the risk of breast cancer in a similar fashion. For instance, mammographic density is inversely related to parity, and increased mammographic density is observed in nulliparous women, and in women with a later age at first birth and later age at menopause (133, 158, 184-192); the same negative and positive relationships have been observed for these reproductive factors with respect to breast cancer risk (193). However, while breast cancer risk increases with age (4), breast density decreases with age (194-197). This apparent inconsistency can be explained by comparing breast density to breast cancer incidence rates in the population (167, 198). The rate of increase in breast cancer incidence begins to slow around age 50 (199); around the same time, glandular and ductal tissue decreases and fibrous connective tissue is replaced by fat (194-196). In contrast to its inverse relationship with age, breast density is very hormonally responsive and is positively associated with hormone therapy use (133, 200-203); elevated breast cancer risk has also been found with HT use in the WHI clinical trial (23). In addition, selective estrogen receptor modulators have been associated with both a reduction in breast density and breast cancer risk (143, 204-207). Studies of BMD, a proxy measure of lifetime estrogen exposure, and breast density have been equivocal. One cross-sectional analysis from the Postmenopausal Estrogen/Progestin Interventions Study (PEPI) showed a positive association between BMD and breast density only among women who had not recently used HT (208). Although BMD is positively associated with breast cancer risk (17, 41), additional studies have not observed an association between BMD and breast density (160, 209, 210). Finally, breast density potentially may be influenced by lifestyle and anthropometric factors. Evidence for an association between diet, physical activity, and breast cancer is not entirely consistent (24, 29); these relationships remain unclear for breast density as well (211-218). BMI has been inversely associated with breast density in several studies (163, 174, 175), while breast cancer risk has been shown to be positively associated with BMI in postmenopausal non-HT users (9). Taken together, these observations suggest that variations in exposure to both endogenous and exogenous hormones may be responsible for the variations in breast tissue composition that are reflected in inter-individual differences in the extent of mammographic density. *Hence, the associations between other breast cancer risk factors and breast density, as*

well as the responsiveness of breast density to hormones, further supports breast density as a surrogate marker of breast cancer risk.

1.1.3.2 Heritability, genetic variations and breast density

The amount of breast density may be due in part to genetic heredity (219). A cohort study of families with a history of breast cancer demonstrated evidence for a genetic effect as sister-sister correlations in breast density were significant (r=0.16-0.27) (220), and these results were further clarified in a sib-pair linkage analysis (221). A twin study conducted in Australia and North America estimated that genetic factors likely account for 63% of the unexplained variance in mammographic density in all twins studied (219). In contrast to women at low risk for developing breast cancer, women with known BRCA1 or BRCA2 mutations have been shown to have denser breast tissue (222). A study of 6146 women in the San Francisco Mammography Registry demonstrated an association between increased breast density with a positive family history of breast cancer (223).

Despite the findings which suggest that genetics plays a strong role in breast density, relatively few studies to date have demonstrated strong, consistent relationships between polymorphisms in genes and breast density in postmenopausal women (224-227). In a study of breast cancer patients, longer CAG repeat lengths of the androgen receptor gene were associated with higher mean breast density only among postmenopausal women who were current HT users (228). In a cross-sectional study of 328 healthy women (only 60 of which were postmenopausal), carriers of the catechol-O-methyl transferase (COMT) and CYP1A1 variant alleles had lower mammographic density as compared to women with the common alleles, although this result was in the opposite of what is commonly hypothesized with respect to the enzyme function of these genes involved in estrogen synthesis (229). A study of healthy women in Toronto found a strong relationship between polymorphisms in the IGFBP-3 gene in premenopausal women only (230); while the IGF-1 19 repeat allele was positively associated with breast density among postmenopausal women, this polymorphic locus was not related to serum levels of IGF-1 in this population (230). A study in postmenopausal women, who had participated in one of two clinical trials with hormone therapy, demonstrated that polymorphisms in genes involved in the metabolism of estrogen (cytochrome P450 1B1, CYP1B1) and progesterone (aldo-keto reductase 1C4, AKR1C4) were associated with mammographic density

changes between baseline and 12 months in the group of women using estrogen plus progestin therapy (EPT) (231). These findings suggest that the increase in breast density in women using combined hormone therapy may be greater in those with genetically determined lower activity of enzymes that metabolize estrogen and progesterone; however, these data should be considered preliminary as they are based on small numbers (EPT group: n=33 genotyped for CYP1B1 and n=32 genotyped for AKR1C4) (231). Other genetic associations that have not yet been replicated include polymorphisms in the estrogen receptor alpha (ESR1) and progesterone receptor (PGR) genes, which modified the association between hormone therapy use and mammographic density (232, 233), along with two SNPs in the pituitary growth hormone gene (GH1), one of which was also associated with serum growth hormone levels (234).

In spite of the paucity of data relating genetic polymorphisms to breast density, the factors known to influence density suggest that genes related to sex steroid hormone regulation may be involved, and no prior study has analyzed breast density in relation to polymorphisms in the TNF receptor-II gene. *Genes regulating cytokine production are ideal candidates to assess whether breast density may vary by particular polymorphisms.*

1.1.4 Association between endogenous hormones, cytokines, and breast density

Despite the relationship between breast cancer and breast density, and their associations with estrogen exposure, relatively few studies have examined the association between endogenous hormones and breast density in postmenopausal women. The relationship between insulin-like growth factors (IGFs) and mammographic density is complex; similar to the observed relations between IGFs and breast cancer risk (20, 235), associations with breast density vary by menopausal status and history of hormone therapy use, and may be confounded by body mass index (173). In a cross-sectional analysis of the Nurses' Health Study, mammographic density was significantly associated with insulin-like growth factors in premenopausal but not postmenopausal women (236). In contrast, among postmenopausal women who were former hormone therapy users, mammographic density was inversely associated with the IGF-1/IGFBP-3 ratio (237). In a study of healthy premenopausal women, IGF binding protein-3 was inversely related to breast density, and the IGF-1/IGFBP-3 ratio was positively associated with breast density (238), and these results were subsequently confirmed (239). Breast tissue from healthy

women with little or no density compared to breast tissue from healthy women with dense breasts has increased IGF-1, matched for age at biopsy (240). Lastly, among healthy pre- and postmenopausal women, levels of C-peptide, a marker of insulin secretion, are not associated with breast density after adjustment for adiposity (241).

In postmenopausal women, variations in mammographic density have been associated with blood levels of prolactin in a dose-response fashion (242), although not consistently (243, 244). Bioavailable estradiol has been negatively associated with breast density in several studies of postmenopausal women (237, 242, 244). However, in the Nurses' Health Study negative associations between circulating estrogens and mammographic density were attenuated after adjustment for BMI (244), and in PEPI significant *positive* associations between circulating estrogens and mammographic density were observed despite adjustment for potential confounders, including BMI and prior use of hormone therapy (245).

Sex hormone binding globulin (SHBG) has been positively related to postmenopausal percent density (242), albeit inconsistently. Positive associations between SHBG and breast density disappeared after adjustment for BMI in pre- (246) and postmenopausal women (244). Riza et al (247) evaluated the role of urinary estrogen metabolites and their relationship with mammographic density in 70 postmenopausal women with high-density Wolfe mammographic parenchymal patterns (P2/DY) and 70 women with low-density patterns (N1). The ratio of 2-hydroxyestrone (OHE1):16α-OHE1 was 35% higher (p=0.005) in women with a P2/DY pattern. These data are not consistent with observed associations between low 2-OHE1:16α-OHE1 ratios and increased breast cancer risk (11, 12); additional larger studies are needed.

Thus, several studies have demonstrated associations between levels of breast mitogens and mammographic density, perhaps suggesting a biological basis for the associated risk of breast density with breast cancer. *However, no studies have investigated the association between cytokines and breast density, and studies are needed to understand this relationship.*

1.1.5 Summary of Background and Significance

The association between estrogen and breast cancer risk is well-established. However, few studies have investigated the underlying biological mechanisms mediating the inflammation-breast cancer relationship, despite the evidence that cytokines play a role in estrogen synthesis in the breast. The association between breast cancer risk factors and mammographic breast density suggests that the radiologic features of breast tissue may provide an index of exposure of breast tissue to current and past endocrine events that influence breast cancer susceptibility (183). These radiologic features can be quantitatively measured and are directly related to the risk of breast cancer. Thus, mammographic breast density can be used as a surrogate marker for breast cancer risk. Despite evidence linking inflammation to breast cancer and breast density to breast cancer, to the best of our knowledge this project is the first to investigate the association between TNF receptors and breast density. It is our hope that this study may provide us with a deeper, more comprehensive understanding of the role of cytokines in breast cancer risk.

1.2 LITERATURE CITED

1. Lacey JV, Jr., Devesa SS, and Brinton LA, *Recent trends in breast cancer incidence and mortality.* Environmental & Molecular Mutagenesis, 2002. **39**(2-3): p. 82-8.

2. Johnson JW, *Transactions of the sixty-third annual meeting of the South Atlantic Association of Obstetricians and Gynecologyists. The millennial mark: presidential address.* American Journal of Obstetrics & Gynecology, 2001. **185**(2): p. 261-7.

3. Deapen D, Liu L, Perkins C, Bernstein L, and Ross RK, *Rapidly rising breast cancer incidence rates among Asian-American women.* International Journal of Cancer, 2002. **99**(5): p. 747-50.

4. Alberg AJ and Singh S, *Epidemiology of breast cancer in older women: implications for future healthcare.* Drugs & Aging, 2001. **18**(10): p. 761-72.

5. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, and Thun MJ, *Cancer Statistics, 2006.* CA Cancer J Clin, 2006. **56**(2): p. 106-130.

6. Gierach G and Vogel V, *Epidemiology of Breast Cancer*, in *Advanced Therapy of Breast Disease, 2nd Edition*, Singletary SE, Robb GL, and Hortobagyi GN, Editors. 2004, BC Decker, Inc.: Hamilton, Ontario. p. 58-74.

7. Vogel VG, *Breast cancer risk factors and preventive approaches to breast cancer.*, in *Cancer in Women*, Kavanagh J SS, Einhorn N, DePetrillo AD, Eds., Editor. 1998, Blackwell Scientific Publications, Inc.: Cambridge, MA. p. 58-91.

8. van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, Fraser G, Goldbohm RA, Graham S, Kushi L, Marshall JR, Miller AB, Rohan T, Smith-Warner SA, Speizer FE, Willett WC, Wolk A, and Hunter DJ, *Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk.* American Journal of Epidemiology, 2000. **152**(6): p. 514-27.

9. Morimoto L, White E, Chen Z, Chlebowski R, Hays J, Kuller L, Lopez A, Manson J, Margolis K, Muti P, Stefanick M, and McTiernan A, *Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States).* Cancer Causes Control, 2002. **13(8)**: p. 741-51.

10. The Endogenous Hormones and Breast Cancer Collaborative Group, *Endogenous Sex Hormones and Breast Cancer in Postmenopausal Women: Reanalysis of Nine Prospective Studies.* JNCI Cancer Spectrum, 2002. **94**(8): p. 606-616.

11. Meilahn EN, De Stavola B, Allen DS, Fentiman I, Bradlow HL, Sepkovic DW, and Kuller LH, *Do urinary oestrogen metabolites predict breast cancer? Guernsey III cohort follow-up.* British Journal of Cancer, 1998. **78**(9): p. 1250-5.

12. Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, Schunemann HJ, Stanulla M, Yang J, Sepkovic DW, Trevisan M, and Berrino F, *Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women.* Epidemiology, 2000. **11**(6): p. 635-40.

13. Clavel-Chapelon F and Gerber M, *Reproductive factors and breast cancer risk. Do they differ according to age at diagnosis?* Breast Cancer Research & Treatment, 2002. **72**(2): p. 107-15.

14. Collaborative Group on Hormonal Factors in Breast C, *Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease.[comment].* Lancet, 2002. **360**(9328): p. 187-95.

15. Innes KE and Byers TE, *Preeclampsia and breast cancer risk.* Epidemiology, 1999. **10**(6): p. 722-32.

16. Melbye M, Wohlfahrt J, Olsen JH, Frisch M, Westergaard T, Helweg-Larsen K, and Andersen PK, *Induced abortion and the risk of breast cancer.[comment].* New England Journal of Medicine, 1997. **336**(2): p. 81-5.

17. Zmuda JM, Cauley JA, Ljung BM, Bauer DC, Cummings SR, Kuller LH, and The Study of Osteoporotic Fractures Research G, *Bone mass and breast cancer risk in older women: differences by stage at diagnosis.* Journal of the National Cancer Institute, 2001. **93**(12): p. 930-6.

18. Newcomb PA, Trentham-Dietz A, Egan KM, Titus-Ernstoff L, Baron JA, Storer BE, Willett WC, Stampfer MJ, Wohlfahrt J, Andersen PK, Mouridsen HT, and Melbye M, *Fracture History and Risk of Breast and Endometrial Cancer.* American Journal of Epidemiology, 2001. **153**(11): p. 1071-1078.

19. Ziv E, Cauley J, Morin PA, Saiz R, and Browner WS, *Association between the T29-->C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures.* JAMA, 2001. **285**(22): p. 2859-63.

20. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, and Pollak M, *Circulating concentrations of insulin-like growth factor-I and risk of breast cancer.[comment].* Lancet, 1998. **351**(9113): p. 1393-6.

21. Collaborative Group on Hormonal Factors in Breast C, *Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies.[comment].* Lancet, 1996. **347**(9017): p. 1713-27.

22. Hankinson SE, Colditz GA, Manson JE, Willett WC, Hunter DJ, Stampfer MJ, and Speizer FE, *A prospective study of oral contraceptive use and risk of breast cancer (Nurses' Health Study, United States).* Cancer Causes & Control, 1997. **8**(1): p. 65-72.

23. Writing Group for the Women's Health Initiative I, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* Jama, 2002. **288**(3): p. 321-33.

24. Friedenreich CM and Orenstein MR, *Physical activity and cancer prevention: etiologic evidence and biological mechanisms.* Journal of Nutrition, 2002. **132**(11 Suppl): p. 3456S-3464S.

25. Ellison RC, Zhang Y, McLennan CE, and Rothman KJ, *Exploring the Relation of Alcohol Consumption to Risk of Breast Cancer. .* American Journal of Epidemiology, 2001. **154**(8): p. 740-747.

26. Chen WY, Colditz GA, Rosner B, Hankinson SE, Hunter DJ, Manson JE, Stampfer MJ, Willett WC, and Speizer FE, *Use of postmenopausal hormones, alcohol, and risk for invasive breast cancer.[summary for patients in Ann Intern Med. 2002 Nov 19;137(10):I43; PMID: 12435234].* Annals of Internal Medicine Online, 2002. **137**(10): p. 798-804.

27. Band PR, Le ND, Fang R, and Deschamps M, *Carcinogenic and endocrine disrupting effects of cigarette smoke and risk of breast cancer.[comment].* Lancet, 2002. **360**(9339): p. 1044-9.

28. Hoshaw SJ, Klein PJ, Clark BD, Cook RR, and Perkins LL, *Breast implants and cancer: causation, delayed detection, and survival.* Plastic & Reconstructive Surgery, 2001. **107**(6): p. 1393-407.

29. Smith-Warner SA, Spiegelman D, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, Miller AB, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, and Hunter DJ, *Types of dietary fat and breast cancer: a pooled analysis of cohort studies.* International Journal of Cancer, 2001. **92**(5): p. 767-74.

30. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, and Comstock GW, *Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer.* Cancer Epidemiology Biomarkers & Prevention, 2002. **11**(5): p. 451-7.

31. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, Olson JE, Therneau TM, and Folsom AR, *Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women.* Epidemiology, 2001. **12**(4): p. 420-8.

32. Horn-Ross PL, John EM, Lee M, Stewart SL, Koo J, Sakoda LC, Shiau AC, Goldstein J, Davis P, Perez-Stable EJ, Gilliland FD, Li Y-F, Baumgartner K, Crumley D, and Samet JM, *Phytoestrogen Consumption and Breast Cancer Risk in a Multiethnic Population -*

The Bay Area Breast Cancer Study. American Journal of Epidemiology 2001. **154**(5): p. 434-441.

33. Hill D, Preston-Martin S, Ross R, and L B, *Medical radiation, family history of cancer, and benign breast disease in relation to breast cancer risk in young women, USA.* Cancer Causes Control, 2002. **13(8)**: p. 711-8.

34. Hancock SL, Tucker MA, and Hoppe RT, *Breast cancer after treatment of Hodgkin's disease.* Journal of the National Cancer Institute, 1993. **85**(1): p. 25-31.

35. Gammon MD, Wolff MS, Neugut AI, Eng SM, Teitelbaum SL, Britton JA, Terry MB, Levin B, Stellman SD, Kabat GC, Hatch M, Senie R, Berkowitz G, Bradlow HL, Garbowski G, Maffeo C, Montalvan P, Kemeny M, Citron M, Schnabel F, Schuss A, Hajdu S, Vinceguerra V, Niguidula N, Ireland K, and Santella RM, *Environmental toxins and breast cancer on Long Island. II. Organochlorine compound levels in blood.* Cancer Epidemiology Biomarkers & Prevention, 2002. **11**(8): p. 686-97.

36. Erren TC, *A meta-analysis of epidemiologic studies of electric and magnetic fields and breast cancer in women and men.* Bioelectromagnetics, 2001. **Suppl**(5): p. S105-19.

37. Amir H, Makwaya C, Mhalu F, Mbonde MP, and Schwartz-Albiez R, *Breast cancer during the HIV epidemic in an African population.* Oncology Reports, 2001. **8**(3): p. 659-61.

38. Hilakivi-Clarke L, Cabanes A, Olivo S, Kerr L, Bouker KB, and Clarke R, *Do estrogens always increase breast cancer risk?* Journal of Steroid Biochemistry & Molecular Biology, 2002. **80**(2): p. 163-74.

39. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, and Cummings SR, *Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group.[comment].* Annals of Internal Medicine., 1999. **130**(4 Pt 1): p. 270-7.

40. Lipworth L, Adami HO, Trichopoulos D, Carlstrom K, and Mantzoros C, *Serum steroid hormone levels, sex hormone-binding globulin, and body mass index in the etiology of postmenopausal breast cancer.* Epidemiology, 1996. **7**(1): p. 96-100.

41. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, and Cummings SR, *Bone mineral density and risk of breast cancer in older women: the study of osteoporotic fractures. Study of Osteoporotic Fractures Research Group.* Jama, 1996. **276**(17): p. 1404-8.

42. Collaborative Group on Hormonal Factors in Breast C, *Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. [comment][erratum appears in Lancet 1997 Nov 15;350(9089):1484].* Lancet., 1997. **350**(9084): p. 1047-59.

43. Reed MJ and Purohit A, *Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis.* Endocr Rev, 1997. **18**(5): p. 701-15.

44. Morita I, *Distinct functions of COX-1 and COX-2.* Prostaglandins Other Lipid Mediat, 2002. **68-69**: p. 165-75.

45. DuBois RN, *Aspirin and breast cancer prevention: the estrogen connection.* Jama, 2004. **291**(20): p. 2488-9.

46. Gasparini G, Longo R, Sarmiento R, and Morabito A, *Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?* Lancet Oncol, 2003. **4**(10): p. 605-15.

47. Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, and Robertson FM, *Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens.* Cancer Letters, 1999. **140**(1-2): p. 27-35.

48. Zhao Y, Agarwal VR, Mendelson CR, and Simpson ER, *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, 1996. **137**(12): p. 5739-42.

49. Howe LR, Subbaramaiah K, Brown AM, and Dannenberg AJ, *Cyclooxygenase-2: a target for the prevention and treatment of breast cancer.* Endocrine-Related Cancer, 2001. **8**(2): p. 97-114.

50. Harris R, Beebe-Donk J, and Alshafie G, *Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors.* BMC Cancer, 2006. **6**(1): p. 27.

51. Ip MM, Mazzer C, Watson D, and Ip C, *The effect of eicosanoid synthesis inhibitors on DMBA-induced rat mammary carcinogenesis.* Proc Am Assoc Cancer Res, 1989. **30**: p. 182.

52. Lee PP and Ip MM, *Regulation of proliferation of rat mammary tumor cells by inhibitors of cyclooxygenase and lipoxygenase.* Prostaglandins Leukot Essent Fatty Acids, 1992. **45**: p. 21-31.

53. Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, McCormick DL, Pereira MA, Crowell JA, Bagheri D, and et al., *Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program.* J Cell Biochem Suppl, 1994. **20**: p. 32-54.

54. Khuder SA and Mutgi AB, *Breast cancer and NSAID use: a meta-analysis.* British Journal of Cancer, 2001. **84**(9): p. 1188-92.

55. Harris RE, Beebe-Donk J, Doss H, and Burr Doss D, *Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review).* Oncol Rep, 2005. **13**(4): p. 559-83.

56. Langman MJS, Cheng KK, Gilman EA, and Lancashire RJ, *Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database.* BMJ, 2000. **320**(7250): p. 1642-1646.

57. Neugut A, Rosenberg D, Ahsan H, Jacobson J, Wahid N, Hagan M, Rahman M, Khan Z, Chen L, Pablos-Mendez A, and Shea S, *Association between coronary heart disease and cancers of the breast, prostate, and colon.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(10): p. 869-873.

58. Coogan PF, Rao SR, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD, and Shapiro S, *The Relationship of Nonsteroidal Anti-inflammatory Drug Use to the Risk of Breast Cancer,.* Preventive Medicine, 1999. **29**(2): p. 72-76.

59. Cotterchio M, Kreiger N, Sloan M, and Steingart A, *Nonsteroidal Anti-inflammatory Drug Use and Breast Cancer Risk.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(11): p. 1213-1217.

60. Harris RE, Namboodiri K., Stellman S. D., and Wynder E. L., *Breast Cancer and NSAID Use: Heterogeneity of Effect in a Case-Control Study.* Preventive Medicine, 1995. **24**(2): p. 119-120.

61. Harris RE, Namboodiri KK, and Farrar WB, *Nonsteroidal antiinflammatory drugs and breast cancer.* Epidemiology, 1996. **7**(2): p. 203-5.

62. Meier CR, Schmitz S, and Jick H, *Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer.* Pharmacotherapy, 2002. **22**(3): p. 303-9.

63. Moorman P, Grubber J, Millikan R, and Newman B, *Association between non-steroidal anti-inflammatory drugs (NSAIDs) and invasive breast cancer and carcinoma in situ of the breast.* Cancer Causes and Control, 2003. **14**(10): p. 915-922.

64. Rahme E, Ghosn J, Dasgupta K, Rajan R, and Hudson M, *Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer.* BMC Cancer, 2005. **5**(1): p. 159.

65. Rosenberg L, *Nonsteroidal Anti-inflammatory Drugs and Cancer.* Preventive Medicine, 1995. **24**(2): p. 107-109.

66. Swede H, Mirand AL, Menezes RJ, and Moysich KB, *Association of Regular Aspirin Use and Breast Cancer Risk.* Oncology, 2005. **68**(1): p. 40-47.

67. Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, Subbaramaiah K, Dannenberg AJ, and Neugut AI, *Association of Frequency and Duration of Aspirin Use and Hormone Receptor Status With Breast Cancer Risk.* JAMA, 2004. **291**(20): p. 2433-2440.

68. Zhang Y, Coogan PF, Palmer JR, Strom BL, and Rosenberg L, *Use of Nonsteroidal Antiinflammatory Drugs and Risk of Breast Cancer: The Case-Control Surveillance Study Revisited.* Am J Epidemiol, 2005. **162**(2): p. 165-170.

69. Egan K, Stampfer M, Giovannucci E, Rosner B, and Colditz G, *Prospective study of regular aspirin use and the risk of breast cancer.* J Natl Cancer Inst, 1996. **88**(14): p. 988a-993.

70. Friis S, Sorensen HT, McLaughlin JK, Johnsen SP, Blot WJ, and Olsen JH, *A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin.* Br J Cancer, 2003. **88**(5): p. 684-8.

71. Garcia Rodriguez LA and Gonzalez-Perez A, *Risk of breast cancer among users of aspirin and other anti-inflammatory drugs.* Br J Cancer, 2004. **91**(3): p. 525-9.

72. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, Patel AV, Flanders WD, and Calle EE, *Aspirin and Other Nonsteroidal Anti-inflammatory Drugs and Breast Cancer Incidence in a Large U.S. Cohort.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(1): p. 261-264.

73. Paganini-Hill A, Chao A, Ross RK, and Henderson BE, *Aspirin use and chronic diseases: a cohort study of the elderly.* Bmj, 1989. **299**(6710): p. 1247-50.

74. Sorensen HT, Friis S, Norgard B, Mellemkjaer L, Blot WJ, McLaughlin JK, Ekbom A, and Baron JA, *Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study.* Br J Cancer, 2003. **88**(11): p. 1687-92.

75. Thun M, Namboodiri M, Calle E, Flanders W, and Heath C, Jr, *Aspirin use and risk of fatal cancer.* Cancer Res, 1993. **53**(6): p. 1322-1327.

76. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A, and Ross RK, *Nonsteroidal Anti-Inflammatory Drug Use and Breast Cancer Risk by Stage and Hormone Receptor Status.* J Natl Cancer Inst, 2005. **97**(11): p. 805-812.

77. Harris RE, Kasbari S, and Farrar WB, *Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer.* Oncol Rep, 1999. **6**(1): p. 71-3.

78. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, and McTiernan A, *Breast Cancer and Nonsteroidal Anti-Inflammatory Drugs: Prospective Results from the Women's Health Initiative.* Cancer Res, 2003. **63**(18): p. 6096-6101.

79. Johnson TW, Anderson KE, Lazovich D, and Folsom AR, *Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(12): p. 1586-1591.

80. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, and Hanley JA, *Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage.* Br J Cancer, 2000. **83**(1): p. 112-20.

81. Schreinemachers DM and Everson RB, *Aspirin use and lung, colon, and breast cancer incidence in a prospective study.* Epidemiology, 1994. **5**(2): p. 138-46.

82. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE, *Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial.* JAMA, 2005. **294**(1): p. 47-55.

83. Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, and Bulun SE, *Estrogen Regulates Expression of Tumor Necrosis Factor Receptors in Breast Adipose Fibroblasts.* J Clin Endocrinol Metab, 2004. **89**(8): p. 4018-4024.

84. Purohit A, Newman S, and Reed M, *The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer.* Breast Cancer Res, 2002. **4**(2): p. 65 - 69.

85. Macdiarmid F, Wang D, Duncan LJ, Purohit A, Ghilchick MW, and Reed MJ, *Stimulation of aromatase activity in breast fibroblasts by tumor necrosis factor alpha.* Mol Cell Endocrinol, 1994. **106**(1-2): p. 17-21.

86. Duncan LJ, Coldham NG, and Reed MJ, *The interaction of cytokines in regulating oestradiol 17 beta-hydroxysteroid dehydrogenase activity in MCF-7 cells.* J Steroid Biochem Mol Biol, 1994. **49**(1): p. 63-8.

87. Adams EF, Rafferty B, and White MC, *Interleukin 6 is secreted by breast fibroblasts and stimulates 17 beta-oestradiol oxidoreductase activity of MCF-7 cells: possible paracrine regulation of breast 17 beta-oestradiol levels.* Int J Cancer, 1991. **49**(1): p. 118-21.

88. Purohit A, Duncan LJ, Wang DY, Coldham NG, Ghilchick MW, and Reed MJ, *Paracrine control of oestrogen production in breast cancer.* Endocr Relat Cancer, 1997. **4**: p. 323-330.

89. de Jong PC, Blankenstein MA, van de Ven J, Nortier JWR, Blijham GH, and Thijssen JHH, *Importance of local aromatase activity in hormone-dependent breast cancer: a review.* The Breast, 2001. **10**(2): p. 91-99.

90. Reed MJ and Purohit A, *Aromatase regulation and breast cancer.* Clinical Endocrinology, 2001. **54**(5): p. 563-71.

91. Pfeilschifter J, Koditz R, Pfohl M, and Schatz H, *Changes in Proinflammatory Cytokine Activity after Menopause.* Endocr Rev, 2002. **23**(1): p. 90-119.

92. Sites CK, Toth MJ, Cushman M, L'Hommedieu GD, Tchernof A, Tracy RP, and Poehlman ET, *Menopause-related differences in inflammation markers and their*

relationship to body fat distribution and insulin-stimulated glucose disposal. Fertil Steril, 2002. **77**(1): p. 128-35.

93. Danforth DN, Jr. and Sgagias MK, *Tumour necrosis factor-alpha modulates oestradiol responsiveness of MCF-7 breast cancer cells in vitro.* J Endocrinol, 1993. **138**(3): p. 517-28.

94. Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, and Kritchevsky SB, *Circulating Levels of Inflammatory Markers and Cancer Risk in the Health Aging and Body Composition Cohort.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(10): p. 2413-2418.

95. Krajcik RA, Massardo S, and Orentreich N, *No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(9): p. 945-6.

96. Hohmann H, Remy R, Brockhaus M, and van Loon A, *Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha).* J Biol Chem, 1989. **264**(25): p. 14927-14934.

97. Varela LM, Darcy KM, and Ip MM, *The Epidermal Growth Factor Receptor Is Not Required for Tumor Necrosis Factor-{alpha} Action in Normal Mammary Epithelial Cells.* Endocrinology, 1997. **138**(9): p. 3891-3900.

98. Bazzoni F and Beutler B, *The tumor necrosis factor ligand and receptor families.* N Engl J Med, 1996. **334**(26): p. 1717-25.

99. Aderka D, *The potential biological and clinical significance of the soluble tumor necrosis factor receptors.* Cytokine Growth Factor Rev, 1996. **7**(3): p. 231-40.

100. Heller RA, Song K, Fan N, and Chang DJ, *The p70 tumor necrosis factor receptor mediates cytotoxicity.* Cell, 1992. **70**(1): p. 47-56.

101. Ininns EK, Gatanaga M, Cappuccini F, Dett CA, Yamamoto RS, Granger GA, and Gatanaga T, *Growth of the endometrial adenocarcinoma cell line AN3 CA is modulated by tumor necrosis factor and its receptor is up-regulated by estrogen in vitro.* Endocrinology, 1992. **130**(4): p. 1852-6.

102. Higuchi M and Aggarwal BB, *P80 form of the human tumor necrosis factor receptor is involved in DNA fragmentation.* FEBS Letters, 1993. **331**(3): p. 252-5.

103. Dror Y, Hahn T, and Barash Y, *Soluble tumor necrosis factor receptor levels in familial Mediterranean fever.* Journal of Rheumatology, 1998. **25**(12): p. 2481-2.

104. Medvedev AE, Espevik T, Ranges G, and Sundan A, *Distinct Roles of the Two Tumor Necrosis Factor (TNF) Receptors in Modulating TNF and Lymphotoxin alpha Effects.* J Biol Chem, 1996. **271**(16): p. 9778-9784.

105. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, and Wallach D, *Variation in serum levels of the soluble TNF receptors among healthy individuals.* Lymphokine and Cytokine Research, 1992. **11**(3): p. 157-9.

106. Hotamisligil GS, Arner P, Atkinson RL, and Spiegelman BM, *Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance.* Diabetes, 1997. **46**(3): p. 451-5.

107. Hube F, Birgel M, Lee YM, and Hauner H, *Expression pattern of tumour necrosis factor receptors in subcutaneous and omental human adipose tissue: role of obesity and non-insulin-dependent diabetes mellitus.* European Journal of Clinical Investigation, 1999. **29**(8): p. 672-8.

108. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, and Coppack SW, *Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo.* Am J Physiol, 1999. **277**(6 Pt 1): p. E971-5.

109. Hauner H, Bender M, Haastert B, and Hube F, *Plasma concentrations of soluble TNF-alpha receptors in obese subjects.* Int J Obes Relat Metab Disord, 1998. **22**(12): p. 1239-43.

110. Hasegawa Y, Sawada M, Ozaki N, Inagaki T, and Suzumura A, *Increased soluble tumor necrosis factor receptor levels in the serum of elderly people.* Gerontology, 2000. **46**(4): p. 185-8.

111. Aderka D, Englemann H, Hornik V, Skornick Y, Levo Y, Wallach D, and Kushtai G, *Increased serum levels of soluble receptors for tumor necrosis factor in cancer patients.* Cancer Research, 1991. **51**(20): p. 5602-7.

112. Kaminska J, Nowacki MP, Kowalska M, Rysinska A, Chwalinski M, Fuksiewicz M, Michalski W, and Chechlinska M, *Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I--an independent prognostic factor.* Tumour Biol, 2005. **26**(4): p. 186-94.

113. Grosen EA, Granger GA, Gatanaga M, Ininns EK, Hwang C, DiSaia P, Berman M, Manetta A, Emma D, and Gatanaga T, *Measurement of the soluble membrane receptors for tumor necrosis factor and lymphotoxin in the sera of patients with gynecologic malignancy.* Gynecol Oncol, 1993. **50**(1): p. 68-77.

114. Gadducci A, Ferdeghini M, Castellani C, Annicchiarico C, Gagetti O, Prontera C, Bianchi R, and Facchini V, *Serum Levels of Tumor Necrosis Factor (TNF), Soluble Receptors for TNF (55- and 75-kDa sTNFr), and Soluble CD14 (sCD14) in Epithelial Ovarian Cancer.* Gynecologic Oncology, 1995. **58**(2): p. 184-188.

115. Gadducci A, Ferdeghini M, Fanucchi A, Annicchiarico C, Prato B, Prontera C, Facchini V, and Genazzani AR, *Serum levels of soluble receptors for tumor necrosis factor (p55 and p75 sTNFr) in endometrial cancer.* Anticancer Res, 1996. **16**(5B): p. 3125-8.

116. Tesarova P, Kvasnicka J, Umlaufova A, Homolkova H, Jirsa M, and Tesar V, *Soluble TNF and IL-2 receptors in patients with breast cancer.* Med Sci Monit, 2000. **6**(4): p. 661-7.

117. Jablonska E, Kiluk M, and Markiewicz W, *Soluble TNF receptors release by polymorphonuclear cells and the serum levels in breast cancer patients before and after treatment.* Neoplasma, 1998. **45**(1): p. 17-20.

118. Kharfi A, Labelle Y, Mailloux J, and Akoum A, *Deficient expression of tumor necrosis factor receptor type 2 in the endometrium of women with endometriosis.* Am J Reprod Immunol, 2003. **50**(1): p. 33-40.

119. Aderka D, Engelmann H, Maor Y, Brakebusch C, and Wallach D, *Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors.* J Exp Med, 1992. **175**(2): p. 323-9.

120. Beltinger CP, White PS, Maris JM, Sulman EP, Jensen SJ, LePaslier D, Stallard BJ, Goeddel DV, de Sauvage FJ, and Brodeur GM, *Physical mapping and genomic structure of the human TNFR2 gene.* Genomics, 1996. **35**(1): p. 94-100.

121. Santee SM and Owen-Schaub LB, *Human tumor necrosis factor receptor p75/80 (CD120b) gene structure and promoter characterization.* Journal of Biological Chemistry, 1996. **271**(35): p. 21151-9.

122. Fabris M, Tolusso B, Di Poi E, Assaloni R, Sinigaglia L, and Ferraccioli G, *Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis.* J Rheumatol, 2002. **29**(9): p. 1847-50.

123. Madhusudan S, Foster M, Muthuramalingam SR, Braybrooke JP, Wilner S, Kaur K, Han C, Hoare S, Balkwill F, Talbot DC, Ganesan TS, and Harris AL, *A Phase II Study of Etanercept (Enbrel), a Tumor Necrosis Factor {alpha} Inhibitor in Patients with Metastatic Breast Cancer.* Clin Cancer Res, 2004. **10**(19): p. 6528-6534.

124. Glenn CL, Wang WY, Benjafield AV, and Morris BJ, *Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor.* Human Molecular Genetics, 2000. **9**(13): p. 1943-9.

125. Tolusso B, Sacco S, Gremese E, La Torre G, Tomietto P, and Ferraccioli GF, *Relationship between the tumor necrosis factor receptor II (TNF-RII) gene polymorphism and sTNF-RII plasma levels in healthy controls and in rheumatoid arthritis.* Human Immunology, 2004. **65**(12): p. 1420-1426.

126. Komata T, Tsuchiya N, Matsushita M, Hagiwara K, and Tokunaga K, *Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus.* Tissue Antigens, 1999. **53**(6): p. 527-33.

127. Barton A, John S, Ollier WE, Silman A, and Worthington J, *Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not*

tumor necrosis factor receptor I, in Caucasians. Arthritis and Rheumatism, 2001. **44**(1): p. 61-5.

128. Constantin A, Dieude P, Lauwers-Cances V, Jamard B, Mazieres B, Cambon-Thomsen A, Cornelis F, and Cantagrel A, *Tumor necrosis factor receptor II gene polymorphism and severity of rheumatoid arthritis.* Arthritis Rheum, 2004. **50**(3): p. 742-7.

129. Morita C, Horiuchi T, Tsukamoto H, Hatta N, Kikuchi Y, Arinobu Y, Otsuka T, Sawabe T, Harashima S, Nagasawa K, and Niho Y, *Association of tumor necrosis factor receptor type II polymorphism 196R with Systemic lupus erythematosus in the Japanese: molecular and functional analysis.* Arthritis Rheum, 2001. **44**(12): p. 2819-27.

130. Dieudé P, Petit E, Cailleau-Moindrault S, Osorio J, Pierlot C, Martinez M, Fauré S, Alibert O, Lasbleiz S, De Toma C, Bardin T, Prum B, and Cornélis F, *Association between tumor necrosis factor receptor II and familial, but not sporadic, rheumatoid arthritis: Evidence for genetic heterogeneity.* Arthritis & Rheumatism, 2002. **46**(8): p. 2039-2044.

131. Peral B, San Millan JL, Castello R, Moghetti P, and Escobar-Morreale HF, *Comment: the methionine 196 arginine polymorphism in exon 6 of the TNF receptor 2 gene (TNFRSF1B) is associated with the polycystic ovary syndrome and hyperandrogenism.* Journal of Clinical Endocrinology and Metabolism, 2002. **87**(8): p. 3977-83.

132. Mestiri S, Bouaouina N, Ben Ahmed S, and Chouchane L, *A functional polymorphism of the tumor necrosis factor receptor-II gene associated with the survival and relapse prediction of breast carcinoma.* Cytokine, 2005. **30**(4): p. 182-187.

133. Harvey JA and Bovbjerg VE, *Quantitative Assessment of Mammographic Breast Density: Relationship with Breast Cancer Risk.* Radiology, 2004. **230**(1): p. 29-41.

134. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, and Paterson AD, *Mammographic breast density as an intermediate phenotype for breast cancer.* The Lancet Oncology, 2005. **6**(10): p. 798-808.

135. Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, and Meyer JE, *Relationship between mammographic and histologic features of breast tissue in women with benign biopsies.* Cancer, 1988. **61**(2): p. 266-71.

136. Fisher ER, Palekar A, Kim WS, and Redmond C, *The histopathology of mammographic patterns.* American Journal of Clinical Pathology, 1978. **69**(4): p. 421-6.

137. Page DL and Winfield AC, *The dense mammogram.* AJR. American Journal of Roentgenology, 1986. **147**(3): p. 487-9.

138. Wellings SR and Wolfe JN, *Correlative studies of the histological and radiographic appearance of the breast parenchyma.* Radiology, 1978. **129**(2): p. 299-306.

139. Wolfe JN, *Risk for breast cancer development determined by mammographic parenchymal pattern.* Cancer, 1976. **37**(5): p. 2486-92.

140. Byng JW, Yaffe MJ, Jong RA, Shumak RS, Lockwood GA, Tritchler DL, and Boyd NF, *Analysis of mammographic density and breast cancer risk from digitized mammograms.* Radiographics, 1998. **18**(6): p. 1587-98.

141. Byng JW, Boyd NF, Little L, Lockwood G, Fishell E, Jong RA, and Yaffe MJ, *Symmetry of projection in the quantitative analysis of mammographic images.* Eur J Cancer Prev, 1996. **5**(5): p. 319-27.

142. Byng JW, Boyd NF, Fishell E, Jong RA, and Yaffe MJ, *Automated analysis of mammographic densities.* Phys Med Biol, 1996. **41**(5): p. 909-23.

143. Chow CK, Venzon D, Jones EC, Premkumar A, O'Shaughnessy J, and Zujewski J, *Effect of tamoxifen on mammographic density.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(9): p. 917-21.

144. Pawluczyk O, Augustine BJ, Yaffe MJ, Rico D, Yang J, Mawdsley GE, and Boyd NF, *A volumetric method for estimation of breast density on digitized screen-film mammograms.* Med Phys, 2003. **30**(3): p. 352-64.

145. Boyd NF, Greenberg C, Lockwood G, Little L, Martin L, Byng J, Yaffe M, and Tritchler D, *Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. Canadian Diet and Breast Cancer Prevention Study Group.* J Natl Cancer Inst, 1997. **89**(7): p. 488-96.

146. Boyd NF, O'Sullivan B, Campbell JE, Fishell E, Simor I, Cooke G, and Germanson T, *Mammographic signs as risk factors for breast cancer.* Br J Cancer, 1982. **45**(2): p. 185-93.

147. Brisson J, Merletti F, Sadowsky NL, Twaddle JA, Morrison AS, and Cole P, *Mammographic features of the breast and breast cancer risk.* Am J Epidemiol, 1982. **115**(3): p. 428-37.

148. Chaudary MA, Gravelle IH, Bulstrode JC, Wang DY, Bulbrook RD, Millis RR, and Hayward JL, *Breast parenchymal patterns in women with bilateral primary breast cancer.* Br J Radiol, 1983. **56**(670): p. 703-6.

149. Brisson J, Morrison AS, Kopans DB, Sadowsky NL, Kalisher L, Twaddle JA, Meyer JE, Henschke CI, and Cole P, *Height and weight, mammographic features of breast tissue, and breast cancer risk.* Am J Epidemiol, 1984. **119**(3): p. 371-81.

150. Carlile T, Kopecky KJ, Thompson DJ, Whitehead JR, Gilbert FI, Jr., Present AJ, Threatt BA, Krook P, and Hadaway E, *Breast cancer prediction and the Wolfe classification of mammograms.* Jama, 1985. **254**(8): p. 1050-3.

151. Wolfe JN, Saftlas AF, and Salane M, *Mammographic parenchymal patterns and quantitative evaluation of mammographic densities: a case-control study.* AJR. American Journal of Roentgenology, 1987. **148**(6): p. 1087-92.

152. Brisson J, Verreault R, Morrison AS, Tennina S, and Meyer F, *Diet, mammographic features of breast tissue, and breast cancer risk.* Am J Epidemiol, 1989. **130**(1): p. 14-24.

153. Saftlas AF, Hoover RN, Brinton LA, Szklo M, Olson DR, Salane M, and Wolfe JN, *Mammographic densities and risk of breast cancer.* Cancer, 1991. **67**(11): p. 2833-8.

154. Kato I, Beinart C, Bleich A, Su S, Kim M, and Toniolo PG, *A nested case-control study of mammographic patterns, breast volume, and breast cancer (New York City, NY, United States).* Cancer Causes Control, 1995. **6**(5): p. 431-8.

155. Byrne C, Schairer C, Wolfe J, Parekh N, Salane M, Brinton LA, Hoover R, and Haile R, *Mammographic features and breast cancer risk: effects with time, age, and menopause status.* Journal of the National Cancer Institute, 1995. **87**(21): p. 1622-9.

156. Tabar L and Dean PB, *Mammographic parenchymal patterns. Risk indicator for breast cancer?* Jama, 1982. **247**(2): p. 185-9.

157. Gravelle IH, Bulstrode JC, Bulbrook RD, Wang DY, Allen D, and Hayward JL, *A prospective study of mammographic parenchymal patterns and risk of breast cancer.* Br J Radiol, 1986. **59**(701): p. 487-91.

158. de Stavola BL, Gravelle IH, Wang DY, Allen DS, Bulbrook RD, Fentiman IS, Hayward JL, and Chaudary MC, *Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study.* International Journal of Epidemiology, 1990. **19**(2): p. 247-54.

159. Maskarinec G and Meng L, *A case-control study of mammographic densities in Hawaii.* Breast Cancer Res Treat, 2000. **63**(2): p. 153-61.

160. Kerlikowske K, Shepherd J, Creasman J, Tice JA, Ziv E, and Cummings SR, *Are Breast Density and Bone Mineral Density Independent Risk Factors for Breast Cancer?* J Natl Cancer Inst, 2005. **97**(5): p. 368-374.

161. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, and Yaffe MJ, *Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study.* J Natl Cancer Inst, 1995. **87**(9): p. 670-5.

162. van Gils CH, Hendriks JH, Holland R, Karssemeijer N, Otten JD, Straatman H, and Verbeek AL, *Changes in mammographic breast density and concomitant changes in breast cancer risk.* European Journal of Cancer Prevention, 1999. **8**(6): p. 509-15.

163. Lam PB, Vacek PM, Geller BM, and Muss HB, *The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont.* Cancer, 2000. **89**(2): p. 369-75.

164. Maskarinec G, Pagano I, Lurie G, Wilkens LR, and Kolonel LN, *Mammographic Density and Breast Cancer Risk.* Am. J. Epidemiol., 2005: p. kwi270.

165. Vacek PM and Geller BM, *A Prospective Study of Breast Cancer Risk Using Routine Mammographic Breast Density Measurements.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(5): p. 715-722.

166. Boyd NF, O'Sullivan B, Fishell E, Simor I, and Cooke G, *Mammographic patterns and breast cancer risk: methodologic standards and contradictory results.* J Natl Cancer Inst, 1984. **72**(6): p. 1253-9.

167. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, and Yaffe MJ, *Mammographic densities and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(12): p. 1133-44.

168. Gram I, Bremnes Y, Ursin G, Maskarinec G, Bjurstam N, and Lund E, *Percentage density, Wolfe's and Tabar's mammographic patterns: agreement and association with risk factors for breast cancer.* Breast Cancer Research, 2005. **7**(5): p. R854 - R861.

169. Haars G, van Noord PAH, van Gils CH, Grobbee DE, and Peeters PHM, *Measurements of Breast Density: No Ratio for a Ratio.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2634-2640.

170. Torres-Mejia G, De Stavola B, Allen DS, Perez-Gavilan JJ, Ferreira JM, Fentiman IS, and dos Santos Silva I, *Mammographic Features and Subsequent Risk of Breast Cancer: A Comparison of Qualitative and Quantitative Evaluations in the Guernsey Prospective Studies.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(5): p. 1052-1059.

171. Ursin G, Ma H, Wu AH, Bernstein L, Salane M, Parisky YR, Astrahan M, Siozon CC, and Pike MC, *Mammographic density and breast cancer in three ethnic groups.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(4): p. 332-8.

172. Heng D, Gao F, Jong R, Fishell E, Yaffe M, Martin L, Li T, Stone J, Sun L, Hopper J, and Boyd NF, *Risk Factors for Breast Cancer Associated with Mammographic Features in Singaporean Chinese Women.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(11): p. 1751-1758.

173. Kaaks R, Diorio C, and Brisson J, *Insulin-Like Growth Factor-I and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(12): p. 3019-.

174. McCormack VA, dos Santos Silva I, De Stavola BL, Perry N, Vinnicombe S, Swerdlow AJ, Hardy R, and Kuh D, *Life-course body size and perimenopausal mammographic parenchymal patterns in the MRC 1946 British birth cohort.* Br J Cancer, 2003. **89**(5): p. 852-9.

175. Modugno F, Ngo D, Allen G, Kuller L, Ness R, Vogel V, Costantino J, and Cauley J, *Breast cancer risk factors and mammographic breast density in women over age 70.* Breast Cancer Research and Treatment, 2005: p. 1-10.

176. Sala E, Warren R, McCann J, Duffy S, Day N, and Luben R, *Mammographic parenchymal patterns and mode of detection: implications for the breast screening programme.* J Med Screen, 1998. **5**(4): p. 207-12.

177. Harrison DA, Duffy SW, Sala E, Warren RML, Couto E, and Day NE, *Deterministic models for breast cancer progression:: Application to the association between mammographic parenchymal pattern and histologic grade of breast cancers.* Journal of Clinical Epidemiology, 2002. **55**(11): p. 1113-1118.

178. Aiello EJ, Buist DSM, White E, and Porter PL, *Association between Mammographic Breast Density and Breast Cancer Tumor Characteristics.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(3): p. 662-668.

179. Roubidoux MA, Bailey JE, Wray LA, and Helvie MA, *Invasive Cancers Detected after Breast Cancer Screening Yielded a Negative Result: Relationship of Mammographic Density to Tumor Prognostic Factors.* Radiology, 2004. **230**(1): p. 42-48.

180. Egan RL and Mosteller RC, *Breast cancer mammography patterns.* Cancer, 1977. **40**(5): p. 2087-90.

181. Habel LA, Dignam JJ, Land SR, Salane M, Capra AM, and Julian TB, *Mammographic Density and Breast Cancer After Ductal Carcinoma In Situ.* J Natl Cancer Inst, 2004. **96**(19): p. 1467-1472.

182. Ursin G, Hovanessian-Larsen L, Parisky Y, Pike M, and Wu A, *Greatly increased occurrence of breast cancers in areas of mammographically dense tissue.* Breast Cancer Research, 2005. **7**(5): p. R605 - R608.

183. Boyd NF, Lockwood GA, Martin LJ, Byng JW, Yaffe MJ, and Tritchler DL, *Mammographic density as a marker of susceptibility to breast cancer: a hypothesis.* IARC Scientific Publications, 2001. **154**: p. 163-9.

184. de Waard F, Rombach JJ, Collette HJ, and Slotboom B, *Breast cancer risk associated with reproductive factors and breast parenchymal patterns.* J Natl Cancer Inst, 1984. **72**(6): p. 1277-82.

185. Vachon CM, Kuni CC, Anderson K, Anderson VE, and Sellers TA, *Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States).* Cancer Causes and Control, 2000. **11**(7): p. 653-62.

186. Ernster VL, Sacks ST, Peterson CA, and Schweitzer RJ, *Mammographic parenchymal patterns and risk factors for breast cancer.* Radiology, 1980. **134**(3): p. 617-20.

187. Bergkvist L, Tabar L, Bergstrom R, and Adami HO, *Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program.* American Journal of Epidemiology, 1987. **126**(6): p. 1075-81.

188. Grove JS, Goodman MJ, Gilbert FI, Jr., and Mi MP, *Factors associated with mammographic pattern.* British Journal of Radiology, 1985. **58**(685): p. 21-5.

189. Kaufman Z, Garstin WI, Hayes R, Michell MJ, and Baum M, *The mammographic parenchymal patterns of nulliparous women and women with a family history of breast cancer.* Clinical Radiology, 1991. **43**(6): p. 385-8.

190. Gram IT, Funkhouser E, and Tabar L, *Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women.* British Journal of Cancer, 1995. **71**(3): p. 647-50.

191. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao M-S, Khokha R, Martin L, and Boyd N, *The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 343-349.

192. Jeffreys M, Warren R, Gunnell D, McCarron P, and Smith GD, *Life course breast cancer risk factors and adult breast density (United Kingdom).* Cancer Causes and Control, 2004. **15**(9): p. 947-955.

193. Kelsey JL, Gammon MD, and John EM, *Reproductive factors and breast cancer.* Epidemiologic Reviews, 1993. **15**(1): p. 36-47.

194. Whitehouse GH and Leinster SJ, *The variation of breast parenchymal patterns with age.* British Journal of Radiology, 1985. **58**(688): p. 315-8.

195. Whitehead J, Carlile T, Kopecky KJ, Thompson DJ, Gilbert FI, Jr., Present AJ, Threatt BA, Krook P, and Hadaway E, *Wolfe mammographic parenchymal patterns. A study of the masking hypothesis of Egan and Mosteller.* Cancer, 1985. **56**(6): p. 1280-6.

196. van Gils CH, Otten JD, Verbeek AL, and Hendriks JH, *Short communication: breast parenchymal patterns and their changes with age.* British Journal of Radiology, 1995. **68**(814): p. 1133-5.

197. Riza E, dos Santos Silva I, De Stavola B, Perry N, Karadedou-Zafiriadou E, Linos D, Remoundos D-D, and Linos A, *Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece.* European Journal of Cancer, 2005. **41**(4): p. 590-600.

198. Pike MC, Krailo MD, Henderson BE, Casagrande JT, and Hoel DG, *'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer.* Nature, 1983. **303**(5920): p. 767-70.

199. *SEER*Stat Database: 9 SEER Incidence Registries for Public-Use, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, Released April 2003, based on the November 2002 data (for the years 1973-2000).* [cited November 11, 2004].

200. *Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial.* Jama, 1995. **273**(3): p. 199-208.

201. Christodoulakos GE, Lambrinoudaki IV, Panoulis KP, Vourtsi AD, Vlachos L, Georgiou E, and Creatsas GC, *The effect of various regimens of hormone replacement therapy on mammographic breast density.* Maturitas, 2003. **45**(2): p. 109-18.

202. Christodoulakos GE, Lambrinoudaki IV, Vourtsi AD, Vlachou S, Creatsa M, Panoulis KP, and Botsis D, *The effect of low dose hormone therapy on mammographic breast density.* Maturitas, 2006. **54**: p. 78-85.

203. Harvey J, Scheurer C, Kawakami FT, Quebe-Fehling E, de Palacios PI, and Ragavan VV, *Hormone replacement therapy and breast density changes.* Climacteric, 2005. **8**(2): p. 185-92.

204. Atkinson C, Warren R, Bingham SA, and Day NE, *Mammographic patterns as a predictive biomarker of breast cancer risk: effect of tamoxifen.* Cancer Epidemiol Biomarkers Prev, 1999. **8**(10): p. 863-6.

205. Brisson J, Brisson B, Cote G, Maunsell E, Berube S, and Robert J, *Tamoxifen and mammographic breast densities.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(9): p. 911-5.

206. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, and Wolmark N, *Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study.* Journal of the National Cancer Institute, 1998. **90**(18): p. 1371-88.

207. Cuzick J, Warwick J, Pinney E, Warren RML, and Duffy SW, *Tamoxifen and Breast Density in Women at Increased Risk of Breast Cancer.* J Natl Cancer Inst, 2004. **96**(8): p. 621-628.

208. Crandall C, Palla S, Reboussin B, Ursin G, and Greendale G, *Positive association between mammographic breast density and bone mineral density in the Postmenopausal Estrogen/Progestin Interventions Study.* Breast Cancer Research, 2005. **7**(6): p. R922 - R928.

209. Dite G, Wark J, Giles G, English D, McCredie M, and Hopper J, *Is there a positive association between mammographic density and bone mineral density?* Breast Cancer Research, 2006. **8**(1): p. 401.

210. Dite GS, Wark JD, Giles GG, English DR, McCredie MR, and Hopper JL, *Is there overlap between the genetic determinants of mammographic density and bone mineral density?* Cancer Epidemiol Biomarkers Prev, 2005. **14**(9): p. 2266-8.

211. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, and Palli D, *Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population.* International Journal of Cancer, 2006. **118**(7): p. 1782-1789.

212. Vachon CM, Kushi LH, Cerhan JR, Kuni CC, and Sellers TA, *Association of diet and mammographic breast density in the Minnesota breast cancer family cohort.* Cancer Epidemiol Biomarkers Prev, 2000. **9**(2): p. 151-60.

213. Maskarinec G, Williams AE, and Carlin L, *Mammographic densities in a one-year isoflavone intervention.* European Journal of Cancer Prevention, 2003. **12**(2): p. 165-9.

214. Berube S, Diorio C, Verhoek-Oftedahl W, and Brisson J, *Vitamin D, calcium, and mammographic breast densities.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(9): p. 1466-72.

215. Lopez P, Van Horn L, Colangelo LA, Wolfman JA, Hendrick RE, and Gapstur SM, *Physical inactivity and percent breast density among Hispanic women.* International Journal of Cancer, 2003. **107**(6): p. 1012-6.

216. Irwin M, Aiello E, McTiernan A, Baumgartner R, Baumgartner K, Bernstein L, Gilliland F, and Ballard-Barbash R, *Pre-diagnosis physical activity and mammographic density in breast cancer survivors.* Breast Cancer Research and Treatment, 2005: p. 1-8.

217. Suijkerbuijk KPM, Van Duijnhoven FJB, Van Gils CH, Van Noord PAH, Peeters PHM, Friedenreich CM, and Monninkhof EM, *Physical Activity in Relation to Mammographic Density in the Dutch Prospect-European Prospective Investigation into Cancer and Nutrition Cohort.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(3): p. 456-460.

218. Maskarinec G, Takata Y, Pagano I, Lurie G, Wilkens LR, and Kolonel LN, *Alcohol consumption and mammographic density in a multiethnic population.* Int J Cancer, 2006. **118**(10): p. 2579-83.

219. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, and Hopper JL, *Heritability of mammographic density, a risk factor for breast cancer.* New England Journal of Medicine, 2002. **347**(12): p. 886-94.

220. Pankow JS, Vachon CM, Kuni CC, King RA, Arnett DK, Grabrick DM, Rich SS, Anderson VE, and Sellers TA, *Genetic analysis of mammographic breast density in adult women: evidence of a gene effect.* Journal of the National Cancer Institute, 1997. **89**(8): p. 549-56.

221. Vachon CM, King RA, Atwood LD, Kuni CC, and Sellers TA, *Preliminary sibpair linkage analysis of percent mammographic density.* Journal of the National Cancer Institute, 1999. **91**(20): p. 1778-9.

222. Huo Z, Giger ML, Olopade OI, Wolverton DE, Weber BL, Metz CE, Zhong W, and Cummings SA, *Computerized analysis of digitized mammograms of BRCA1 and BRCA2 gene mutation carriers.* Radiology, 2002. **225**(2): p. 519-26.

223. Ziv E, Shepherd J, Smith-Bindman R, and Kerlikowske K, *Mammographic breast density and family history of breast cancer.* J Natl Cancer Inst, 2003. **95**(7): p. 556-8.

224. Haiman CA, Bernstein L, Berg D, Ingles SA, Salane M, and Ursin G, *Genetic determinants of mammographic density.* Breast Cancer Research, 2002. **4**(3): p. R5.

225. Haiman CA, Hankinson SE, De Vivo I, Guillemette C, Ishibe N, Hunter DJ, and Byrne C, *Polymorphisms in steroid hormone pathway genes and mammographic density.* Breast Cancer Research and Treatment, 2003. **77**(1): p. 27-36.

226. Hong CC, Thompson HJ, Jiang C, Hammond GL, Tritchler D, Yaffe M, and Boyd NF, *Val158Met Polymorphism in catechol-O-methyltransferase gene associated with risk factors for breast cancer.* Cancer Epidemiology, Biomarkers and Prevention, 2003. **12**(9): p. 838-47.

227. Hong CC, Thompson HJ, Jiang C, Hammond GL, Tritchler D, Yaffe M, and Boyd NF, *Association between the T27C polymorphism in the cytochrome P450 c17alpha (CYP17) gene and risk factors for breast cancer.* Breast Cancer Res Treat, 2004. **88**(3): p. 217-30.

228. Lillie EO, Bernstein L, Ingles SA, Gauderman WJ, Rivas GE, Gagalang V, Krontiris T, and Ursin G, *Polymorphism in the Androgen Receptor and Mammographic Density in Women Taking and Not Taking Estrogen and Progestin Therapy.* Cancer Res, 2004. **64**(4): p. 1237-1241.

229. Maskarinec G, Lurie G, Williams AE, and Le Marchand L, *An investigation of mammographic density and gene variants in healthy women.* Int J Cancer, 2004. **112**(4): p. 683-8.

230. Lai JH, Vesprini D, Zhang W, Yaffe MJ, Pollak M, and Narod SA, *A Polymorphic Locus in the Promoter Region of the IGFBP3 Gene Is Related to Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(4): p. 573-582.

231. Lord S, Mack W, Van Den Berg D, Pike M, Ingles S, Haiman C, Wang W, Parisky Y, Hodis H, and Ursin G, *Polymorphisms in genes involved in estrogen and progesterone metabolism and mammographic density changes in women randomized to postmenopausal hormone therapy: results from a pilot study.* Breast Cancer Research, 2005. **7**(3): p. R336 - R344.

232. van Duijnhoven FJB, Bezemer ID, Peeters PHM, Roest M, Uitterlinden AG, Grobbee DE, and van Gils CH, *Polymorphisms in the Estrogen Receptor {alpha} Gene and*

Mammographic Density. Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2655-2660.

233. van Duijnhoven FJB, Peeters PHM, Warren RML, Bingham SA, Uitterlinden AG, van Noord PAH, Monninkhof EM, Grobbee DE, and van Gils CH, *Influence of Estrogen Receptor {alpha} and Progesterone Receptor Polymorphisms on the Effects of Hormone Therapy on Mammographic Density.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(3): p. 462-467.

234. Mulhall C, Hegele RA, Cao H, Tritchler D, Yaffe M, and Boyd NF, *Pituitary Growth Hormone and Growth Hormone-Releasing Hormone Receptor Genes and Associations with Mammographic Measures and Serum Growth Hormone.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2648-2654.

235. Kaaks R, Lundin E, Rinaldi S, Manjer J, Biessy C, Soderberg S, Lenner P, Janzon L, Riboli E, Berglund G, and Hallmans G, *Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden.* Cancer Causes & Control, 2002. **13**(4): p. 307-16.

236. Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, and Hankinson SE, *Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density.* Cancer Research, 2000. **60**(14): p. 3744-8.

237. Aiello EJ, Tworoger SS, Yasui Y, Stanczyk FZ, Potter J, Ulrich CM, Irwin M, and McTiernan A, *Associations among Circulating Sex Hormones, Insulin-Like Growth Factor, Lipids, and Mammographic Density in Postmenopausal Women.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(6): p. 1411-1417.

238. Maskarinec G, Williams AE, and Kaaks R, *A cross-sectional investigation of breast density and insulin-like growth factor I.* International Journal of Cancer, 2003. **107**(6): p. 991-6.

239. Diorio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, and Brisson J, *Insulin-Like Growth Factor-I, IGF-Binding Protein-3, and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(5): p. 1065-1073.

240. Guo YP, Martin LJ, Hanna W, Banerjee D, Miller N, Fishell E, Khokha R, and Boyd NF, *Growth factors and stromal matrix proteins associated with mammographic densities.* Cancer Epidemiology, Biomarkers and Prevention, 2001. **10**(3): p. 243-8.

241. Diorio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, and Brisson J, *Levels of C-Peptide and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2661-2664.

242. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, and Minkin S, *The association of breast mitogens with mammographic densities.* British Journal of Cancer, 2002. **87**(8): p. 876-82.

243. Soysal S, Soysal ME, Karabulut N, Gul N, and Gezgin T, *The basal and TRH stimulated levels of prolactin in low risk climacteric patients with increased breast density: A matched pair case control trial.* Maturitas, 2006. **In Press**.

244. Tamimi RM, Hankinson SE, Colditz GA, and Byrne C, *Endogenous Sex Hormone Levels and Mammographic Density among Postmenopausal Women.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2641-2647.

245. Greendale GA, Palla SL, Ursin G, Laughlin GA, Crandall C, Pike MC, and Reboussin BA, *The Association of Endogenous Sex Steroids and Sex Steroid Binding Proteins with Mammographic Density: Results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study.* Am J Epidemiol, 2005. **162**(9): p. 826-34.

246. Noh JJ, Maskarinec G, Pagano I, Cheung LW-K, and Stanczyk FZ, *Mammographic densities and circulating hormones: A cross-sectional study in premenopausal women.* The Breast, 2006. **15**(1): p. 20-28.

247. Riza E, dos Santos Silva I, De Stavola B, Bradlow HL, Sepkovic DW, Linos D, and Linos A, *Urinary estrogen metabolites and mammographic parenchymal patterns in postmenopausal women.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(6): p. 627-34.

2.0 NONSTEROIDAL ANTI-INFLAMMATORY DRUG USE AND BREAST CANCER IN OLDER WOMEN: THE STUDY OF OSTEOPOROTIC FRACTURES

To be submitted for publication

Gretchen L. Gierach, PhD, MPH,[1] Francesmary Modugno, PhD, MPH,[1, 2] Joel L. Weissfeld, MD, MPH,[1, 2] John W. Wilson, PhD,[3, 4] Victor G. Vogel, MD, MHS,[5] Steven R. Cummings, MD,[6, 7] Jeffrey Tice, MD, MPH,[8] Elad Ziv, MD,[8] and Jane A. Cauley, DrPH, MPH[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]National Surgical Adjuvant Breast and Bowel Project Center, University of Pittsburgh, Pittsburgh, PA

[5]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

[6]Department of Epidemiology and Biostatistics, University of California (San Francisco), San Francisco, California

[7]Research Institute, California Pacific Medical Center, San Francisco, California

[8]Department of Medicine, University of California (San Francisco), San Francisco, California

Running Title: NSAID use and breast cancer

This work was supported by contracts from: National Cancer Institute; Grant Numbers: K07-CA80668, R21-CA95113, and R25-CA57703-07; National Institute of Aging; Grant Numbers: AG05407, AR35582, AG05394, AR35584, and AR35583.

2.1 ABSTRACT

Purpose: To test reported use of aspirin and non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs) for their effect on incident breast cancer among older women. We further investigated whether the relationship between NSAIDs and breast cancer incidence differed by hormone receptor status and tumor type at diagnosis.

Methods: The Study of Osteoporotic Fractures is a multi-center, prospective cohort study of white women recruited from four U.S. centers, 1986-1988. Complete NSAID medication and breast cancer risk factor information was available for 6695 women, mean (SD) age 73 (5) years.

Results: During a mean (SD) of 13.2 (3.8) years of follow-up, 372 women were diagnosed with primary breast cancer: *in situ* (14%), invasive (81%), or unknown stage (5%). Weekly use of any NSAID during the past 12 months was reported by 3646 (54%) women. Daily use of any NSAID for at least one year was reported by 2097 (31%) women. There were no differences in the risk of incident breast cancer by use of aspirin, non-aspirin NSAID, or any NSAID, before and after adjusting for age, current use of estrogen therapy, body mass index, surgical menopause, total hip bone mineral density, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension. Further, we observed no difference in breast cancer risk by frequency and duration of NSAID use. Results were similar irrespective of hormone receptor status and tumor type.

Conclusions: Our results do not support a protective effect of nonprescription NSAIDs among older postmenopausal women.

2.2 INTRODUCTION

Breast cancer is the most common cancer in women around the world; in the United States this year, it is expected that breast cancer alone will account for 31% (212,920) of all new cancer cases among women and 40,970 women are expected to die from the disease (1). Estrogen is believed to be a key contributor to breast cancer development (2). Most breast tumors are initially dependent on estrogen for survival; paradoxically, the highest incidence of breast cancer occurs in postmenopausal women when ovarian production of estrogens is minimal (3). In postmenopausal women, estrogens continue to be produced in non-ovarian sites, such as adipose tissue, as well as in normal and cancerous breast tissues (4). In fact, the more biologically active form of estrogen, estradiol, has been detected in breast tumors at 50 to 100 times the concentration of that found in sera of postmenopausal women (5). Further, postmenopausal breast cancer is largely estrogen receptor (ER) or progesterone receptor (PR) positive, and is more responsive to anti-estrogen therapy and aromatase inhibitors, even after controlling for stage and other prognostic factors (6). Increased understanding of the mechanism by which estrogens are synthesized in the postmenopausal breast may inform preventative strategies.

In breast tissue, estrogen biosynthesis increases with over-expression of the cyclooxygenase-2 (COX-2) gene and subsequent deregulation of prostaglandin E_2 (PGE_2) production (7), which may facilitate tumorigenesis (8). COX-2-driven production of estrogen in the breast is evidenced by a positive correlation between 1) the level of COX-2 and expression of cytochrome P19 (CYP19) in human breast cancer (9) and 2) increased aromatase gene (P450) expression, the product of CYP19, in cultured breast cells (10). This paracrine loop may explain why inhibition of COX-2 activity could have a protective effect on breast cancer. Indeed, studies have consistently shown that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit COX-2 and thereby PGE_2 production (7), hinder breast tumor cell growth *in vitro* and in animal models (9, 11-14). Epidemiologic studies have examined the association between use of NSAIDs and breast cancer risk (reviewed in (15) and (16)) with most, but not all (17, 18), of case-control studies finding risk reductions between ~20-40% (19-29). Results from prospective cohort studies have been less consistent, with seven studies finding no association

(30-36), one study observing an increased risk (37), and five studies demonstrating a protective effect from use of NSAIDs (38-42). Recently the Women's Health Study, a randomized controlled trial, found that alternate-day use of low-dose aspirin for an average of 10 years of treatment did not reduce the risk of breast cancer (43).

Relatively few studies have examined the effect of NSAIDs on breast cancer risk by hormone receptor status (28, 29, 37) or carcinoma type (*in situ* (stage 0) vs. stages 1-4) at diagnosis (30, 37, 40, 41), and these also present conflicting results. In particular, results from the Long Island Breast Cancer Prevention Project (LIBCP), a population-based case control study, demonstrated that the protective effect of NSAIDs was greater for hormone receptor-positive than for hormone receptor negative breast cancer (28). Furthermore, the protective effect of NSAIDs was significant for postmenopausal but not for premenopausal women; these results persisted after considering potential confounding by numerous variables, including medical conditions not considered in previous studies (e.g. hypertension and myocardial infarction) (28). In contrast, in a hospital-based case-control study the protective effect of NSAIDs was *not* modified by hormone receptor status (29), and one cohort study found that long-term daily aspirin use was associated with an *increased* risk of hormone receptor-negative breast cancer (37). Of the four studies assessing the association between NSAID use and stage of breast cancer, three cohort studies have found no relationship (30, 37, 40), and one nested case-control study reported a decreased risk of distant, but not regional, lymph node metastasis (41). Finally, interpretation of prior research is complicated by a paucity of attention to the effect of individual NSAIDs, with few studies providing separate risk estimates for ibuprofen and aspirin (16). As these two types of NSAIDs may be associated with different biologic effects, separate evaluation is needed (39, 44). Hence, we undertook a study to test reported use of aspirin and non-aspirin NSAIDs (ibuprofen and naproxen) for their effect on incident breast cancer among older women participating in the Study of Osteoporotic Fractures (SOF) cohort. We further investigated whether the relationship between NSAIDs and breast cancer incidence differed by hormone receptor status and tumor type at diagnosis.

2.3 METHODS

The complete SOF study design and methodology have been described in detail (45-47). Briefly, SOF is a multi-center, prospective cohort study of risk factors for osteoporotic fractures. As part of the study, SOF has also tracked cases of incident breast cancer. Women were recruited between 1986 and 1988 from four centers in the United States (Baltimore, Pittsburgh, Minneapolis and Portland), resulting in a cohort of 9704 community-dwelling, primarily white (99.7%) women age ≥65 years. Women were excluded from the study if they reported a bilateral hip replacement or were unable to walk unassisted. The procedures followed were in accordance with each clinic's institutional guidelines, and all participants gave informed consent for participation.

In this report, we used the information on aspirin and non-aspirin NSAID (ibuprofen or naproxen) use that was collected at the second clinical examination (visit 2: 1989-1990) and incident breast cancers that occurred after this examination until June, 2004, representing an average follow-up period of 13.2 years. For the analyses presented here, only women with complete NSAID medication and breast cancer risk factor information were considered. Of the 9339 women who attended visit two, 522 were excluded because of prevalent breast cancer, and 114 women were excluded due to unavailable information on their breast cancer status during follow-up. Reported aspirin or non-aspirin NSAID use was missing for 438 women, and 1570 women had missing breast cancer risk factor data; these women were also excluded. Thus, 6695 women were included in the present analyses.

2.3.1 Ascertainment of breast cancer

Breast cancer incidence was captured by self-report on annual follow-up questionnaires and by review of death records obtained from state health departments. All women who reported that they had been diagnosed with breast cancer (or for decedents, the next-of-kin) were asked for permission to obtain relevant hospital records and histopathology reports. Date of breast cancer diagnosis, stage at diagnosis, and estrogen and progesterone-receptor status were confirmed by medical record and pathology report review by a study physician. Cases of breast cancer were staged according to the American Joint Committee on Cancer (AJCC) methodology using

standard tumor-node-metastasis (TNM) staging criteria. An expert breast pathologist reviewed a random sample of 10% of the cases.

2.3.2 Non-steroidal anti-inflammatory drug exposure

At the second clinical examination, trained clinic staff asked participants to look at three lists of medications for pain, arthritis, headaches and other discomforts, and then to answer the question, "In the past 12 months, have you taken any of these at least once a week?" Separate lists were shown for aspirin (Aspirin, Aspirin Plus Codeine, Anacin, Ascriptin, Bufferin, Another Aspirin Product), acetaminophen (Tylenol, Tylenol Plus Codeine, Anacin III, No Aspirin, Acetaminophen, Another Aspirin Substitute), and non-aspirin NSAIDs (e.g. Advil, Nuprin, Ibuprofen, Motrin, Naproxen, etc.). If the answer was yes, participants were asked to indicate for how many days per week (1-4 or 5-7), on average, they took each type of medication. Participants were also asked, "Have you taken any of the medications on this list every day or almost every day for a year or longer?" If the answer was yes, participants were asked to indicate for how many years they took each type of medication; this question was not specific to recent use, but rather captured the number of total years during the lifespan that the particular medication was taken on an almost daily basis for a year or longer. We created categories for duration of use (no regular use, <5 years, and ≥5 years) based on previous epidemiologic investigations which suggest that continuing daily intake of aspirin or ibuprofen for at least five years reduces breast cancer risk by ~25-30% or 50-60%, respectively (16). In this report, "any NSAID" combines the use of aspirin and non-aspirin NSAIDs. Acetaminophen does not inhibit COX-2 gene expression (48); however, since lifestyle factors and response patterns may be similar between NSAIDs and acetaminophen use, we analyzed both NSAIDs and acetaminophen to assess whether any observed associations were specific to NSAIDs.

2.3.3 Demographic and risk factor data

All measures were collected by trained clinic staff at visit 2 unless noted otherwise (Tables 3-6). Weight was measured in light clothes with a balance beam scale, and height was measured on a Harpenden Stadiometer (Dyved, U.K.). Weight and height were used to calculate body mass

index (BMI, weight in kilograms divided by height in meters squared), and BMI was categorized according to national guidelines: underweight or normal weight (<25 kg/m^2) vs. overweight or obese (≥25 kg/m^2) (49). Lifestyle and reproductive history were obtained by questionnaire and interview, including education (<high school vs. high school graduate), parity, age at menopause, type of menopause (surgical vs. natural), estrogen therapy use (current, former, never), number of alcoholic drinks per week, cigarette smoking (current, former, never), takes walks for exercise, self-reported diagnosis by a physician of stroke or myocardial infarction, and family history of breast cancer. A family history of breast cancer was defined as a report of breast cancer in a participant's mother or sister. Cut points for age at menarche (<12, 12-13, ≥14 years) and age at first live birth (≤20 vs. >20 years) were determined based on those used in the Gail Model for 5-year risk of breast cancer (50). Blood pressure was measured during the baseline clinic visit (1986-1988), and hypertension was defined as systolic blood pressure >160 mmHg, or diastolic blood pressure >90 mmHg, or use of thiazide at baseline. At visit 3 (1990-1992) participants were asked if they had ever had a mammogram. Total hip bone mineral density (BMD) was measured by dual energy x-ray absorptiometry (DEXA, QDR 1000; Hologic Inc., Waltham, Massachusetts).

2.3.4 Statistical analysis

Characteristics of women reporting use of the medication of interest (any NSAID, aspirin, non-aspirin NSAID, acetaminophen) were compared to women not regularly using that medication by the Wilcoxon rank-sum test for continuous measures and the chi-square test for discrete measures. Fisher's exact test for discrete measures was used when expected cell counts were less than five. Cox proportional hazards models were used to estimate hazard ratios and 95% confidence intervals for the associations between medication type (any NSAID, aspirin, non-aspirin NSAID, acetaminophen) and breast cancer. To test the hypotheses that the relationship between NSAIDs and breast cancer incidence differs by hormone receptor status and tumor type, we conducted subgroup analyses, modeling the incidence of breast cancer separately for hormone receptor positive breast cancer (ER+PR+, ER+PR-, or ER-PR+) and after excluding *in situ* cases. For most medication types, cell sizes were too sparse to model the incidence of hormone receptor negative (ER-PR-) breast cancer and *in situ* breast cancer separately.

Cox proportional hazards models were used to estimate hazard ratios and 95% confidence intervals for the associations between NSAID type (any NSAID, aspirin, non-aspirin NSAIDs, acetaminophen) and breast cancer, initially adjusting for age. Due to anonymized high extreme values for age in the public release SOF database, age was dichotomized at the median (≤72 vs. >73 years) in order to avoid having missing covariate information for these women (n=26). Subsequent models controlled for covariates that were shown to differ between users and non-users of NSAIDs in univariate analyses, in addition to those that are known to be associated with breast cancer risk. These were included in multivariate modeling as follows: current use of estrogen therapy, BMI, surgical menopause, total hip BMD, cigarette smoking, family history of breast cancer, study center, takes walks for exercise, nulliparity, and hypertension. As hypertension was highly collinear with self-reported myocardial infarction and stroke, only hypertension was included in multivariate analyses. In subsequent models, we also adjusted individually for several risk factors for breast cancer, including age at menarche, first birth, and menopause, and mammogram at visit 3; results were essentially the same and are not shown here.

Several approaches were used to check the proportional hazards assumption. We regressed Schoenfeld residuals for each medication variable on follow-up time; probability values ranged from 0.33 to 0.99, suggesting no departure from proportionality. To test the proportional hazards assumption for our final multivariate model, we generated time dependent covariates by including interactions of each predictor with the natural log of follow-up time in the model; probability values for all time dependent covariates were >0.05, consistent with hazards that are proportional. Probability values of ≤ 0.05 were considered statistically significant. All tests of statistical significance were two-tailed. Analyses were performed using SAS software release 8.02 (SAS Institute Inc., Cary, NC).

2.4 RESULTS

Among the 6695 mostly white (99.8%) SOF participants in this report, weekly use of any NSAID during the past 12 months was reported by 3646 (54%) women. Among weekly users of any NSAID, 752 (21%) reported using both aspirin and a non-aspirin NSAID, 1876 (51%)

reported using aspirin, and 1018 (28%) reported using a non-aspirin NSAID; 1047 (29%) women reported using both an NSAID and acetaminophen on a weekly basis. Among weekly users of any NSAID, 1445 (40%) took an NSAID 5-7 days/week. Daily use of any NSAID for at least one year during the lifespan was reported by 2097 (31%) women, including 279 (13%) users of both aspirin and a non-aspirin NSAID, 1206 (58%) users of aspirin, and 612 (29%) users of a non-aspirin NSAID; 300 women reported using both an NSAID and acetaminophen on a daily basis for at least one year (14%). Among daily users of any NSAID, 884 (42%) used an NSAID for 5 or more years. Reported mean (SD) duration of daily use was 6 (8) years for any NSAID, 7 (9) years for aspirin, 4 (4) years for a non-aspirin NSAID, and 5 (7) years for acetaminophen. The shorter duration for daily non-aspirin NSAID use is consistent with a more limited time of availability, as ibuprofen was licensed for over-the-counter use in 1984 (four years prior to visit 2) in the United States (51).

Table 3 shows the characteristics of the population by reported NSAID use. Women who used any NSAID on a regular basis (≥1/week) in the past 12 months were slightly older, had a higher BMI, and were more likely to be hypertensive, have a history of heart attack or stroke, to be former smokers, and to have been enrolled at clinic A than were nonusers. In addition, regular users of NSAIDs were more likely to have a younger age at first birth, currently use estrogen therapy, and have a greater total hip BMD; and were less likely to be nulliparous and walk for exercise. Similar differences were observed between daily users and nonusers of any NSAID for ≥1 year with the exception of the following variables: daily users of any NSAID for ≥1 year tended to have an earlier age at menarche and menopause and were no different with respect to parity.

In contrast to women who used any NSAID on a regular basis in the past 12 months, regular users and nonusers of acetaminophen were no different with respect to age and were more likely to have a mammogram at visit three (Table 4). Regular acetaminophen users also had an earlier age at menopause; were more likely to have had a surgical menopause; were less likely to be high school graduates; and were no different from non-users with respect to walking for exercise and total hip BMD. For the most part, daily users of acetaminophen for ≥1 year were similar to women who used acetaminophen on a regular basis in the past 12 months, except that daily users tended to be older; were less likely to take walks for exercise; and were no

different with respect to cigarette smoking, nulliparity, and having a mammogram as compared to nonusers (Table 4).

In general, aspirin and non-aspirin NSAID users shared similar characteristics, although daily aspirin users were more likely to be high school graduates, while weekly and daily non-aspirin NSAID users were less likely to have graduated from high school (Tables 5 and 6). In addition, weekly and daily users of non-aspirin NSAID were more likely to have had a surgical menopause, while no such difference was observed for aspirin use.

During a mean (SD) of 13.2 (3.8) years of follow-up, 372 women were diagnosed with primary *in situ* (14%) or invasive (81%) breast cancer (Tables 7 and 8). Because axillary dissection was not performed in all cases of invasive cancer, staging could not be determined in 17 (4.6%) women and was missing in 2 (0.05%) cases. Clinical characteristics of cases did not differ by NSAID (Table 7) or acetaminophen use (Table 8).

2.4.1 Non-steroidal anti-inflammatory drug use and breast cancer

There were no differences in the risk of incident breast cancer by weekly use of aspirin, non-aspirin NSAID, any NSAID, or acetaminophen in SOF (Table 9). In multivariable analyses, the hazard ratios for incident breast cancer associated with reported use of aspirin, non-aspirin NSAID, or any NSAID, respectively, for ≥1/week in the past 12 months were as follows: 0.96 (95% CI: 0.78, 1.18); 0.96 (95% CI: 0.76, 1.21); and 0.89 (95% CI: 0.73, 1.10). Further evaluation of weekly aspirin, non-aspirin NSAID, NSAID, and acetaminophen use by frequency (no regular use, 1-4 days/week, 5-7 days/week) did not reveal any differences in breast cancer risk. Likewise, there were no differences in the risk of incident breast cancer by daily use of aspirin, non-aspirin NSAID, or any NSAID for ≥1 year, with multivariable hazard ratios of 0.90 (0.70, 1.16), 0.95 (0.70, 1.29), and 0.89 (0.71, 1.12) for aspirin, non-aspirin NSAID, and any NSAID, respectively. Women who reported using acetaminophen daily for ≥1 year had a decreased age-adjusted risk of breast cancer of borderline significance (HR=0.58, 95% CI: 0.34, 0.99); however, only 14 women with breast cancer reported using acetaminophen daily for ≥1 year and contributed to this analysis. Again, there were no apparent differences in breast cancer risk when evaluating daily use of aspirin, non-aspirin NSAID, and any NSAID by duration (no regular use, <5 years, ≥5 years). Cell sizes were too small to evaluate daily acetaminophen use

by duration. Results were similar when we modeled the incidence of breast cancer separately for hormone receptor positive breast cancer (Table 10) and after excluding *in situ* cases (Table 11).

2.5 DISCUSSION

In this prospective study of postmenopausal, mostly white, women we found no association between NSAID use and incident breast cancer. Further, we observed no difference in breast cancer risk by frequency and duration of NSAID use. Results were similar irrespective of hormone receptor status and tumor type.

Previously, thirteen prospective studies and one randomized controlled trial have evaluated the influence of NSAIDs on breast cancer risk. Our results are consistent with seven large prospective studies finding no association (30-36), and with null results from the randomized controlled trial of alternate-day low-dose aspirin (43). However, our results are not consistent with five prospective studies demonstrating a protective effect from use of NSAIDs (38-42) and one suggesting an increased risk. (37).

Although the reason for these differing results is unclear, one explanation may be due to differences in exposure assessment across studies. Among the seven prior prospective studies with null results, four studies assessed exposure of aspirin or non-aspirin NSAIDs using self-reported questionnaire data (30, 33, 34, 36), with none verifying use against pill bottles; one used a general practitioners' database containing information on aspirin use (32); and two used databases of prescribed low-dose aspirin (31) or non-aspirin NSAIDs (35). In contrast, the Women's Health Initiative Observational Study, which observed a 21% reduction in breast cancer incidence with regular NSAID use (≥2 tablets/week) for 5-9 years, validated self-reported use with pill bottle labels and prescription records (39). In the present study, a medication inventory was not conducted until visit 4, at which time patients were asked to bring all medications to the clinic for verification of use; however, the questions regarding frequency and duration of aspirin and non-aspirin NSAID use were only asked at visit two. Further, at visit two only non-prescription NSAID use was captured. This is an important limitation, especially given that a recent case-control study demonstrated a 71% reduction in breast cancer risk associated with use of selective COX-2 inhibitors (11). If in the present analysis users of prescription

NSAIDs were included in the referent group (i.e. non-NSAID users), results would be biased toward the null.

Nevertheless, this study has several strengths. Its prospective design and assessment of NSAID use before cancer diagnosis eliminate recall bias. We controlled for many factors that differed among NSAID users and nonusers. In addition, we examined the effect of individual NSAIDs on breast cancer risk, and the prevalence of aspirin and non-aspirin NSAID use in SOF is consistent with that observed in other studies (28, 30, 40). While acetaminophen does not inhibit COX-2 gene expression (48), we analyzed both NSAIDs and acetaminophen to assess whether any observed associations were specific to NSAIDs. Weekly acetaminophen use was not associated with incident breast cancer; however, daily use of acetaminophen for 1 year or more at an unknown period during the lifespan appeared to be associated with a decrease in breast cancer risk. This finding is to be interpreted with caution as it is likely due to small cell sizes; only 14 cases reported daily use of acetaminophen. In addition, when we excluded *in situ* breast cancer cases, the age-adjusted relative hazard associated with acetaminophen use was no longer significant (HR=0.56, 95% CI: 0.31, 1.03). Lastly, we attempted to separately examine the effect of NSAIDs on hormone receptor positive and invasive breast cancer. To date, only one other prospective study has evaluated the association by hormone receptor status, finding that long-term daily aspirin use was associated with an *increased* risk of hormone receptor-negative breast cancer (37). Only 31 cases were ER/PR negative in SOF, a subgroup of too small a size to evaluate separately. Our non-significant findings for invasive breast cancer are consistent with three out of four prospective studies evaluating the association between NSAID use and stage of breast cancer (30, 37, 40).

In conclusion, our results do not support a protective effect of nonprescription NSAIDs among older postmenopausal women. Given the potential public health impact should NSAIDs be successful chemopreventive agents for breast cancer, these findings warrant further investigation in larger populations with carefully defined exposure assessment.

Table 3. Characteristics of women by NSAID use in the Study of Osteoporotic Fractures, 1989-90

	Overall n=6695		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year				
			No (n=3049)		Yes (n=3646)			No (n=4598)		Yes (n=2097)		
Variable	**n**	**%**	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Clinic*							<0.0001					0.003
A	1781	27	739	24	1042	29		1174	26	607	29	
B	1635	24	729	24	906	25		1104	24	531	25	
C	1489	22	768	25	721	20		1051	23	438	21	
D	1790	27	813	27	977	27		1269	28	521	25	
Age (±SD), years	73 (5)		73 (5)		73 (5)		0.2	73 (5)		74 (5)		<0.0001
Age (years)							0.05					0.0015
≤72	3528	53	1647	54	1881	52		2483	54	1045	50	
73+	3167	47	1402	46	1765	48		2115	46	1052	50	
Education*							0.38					0.94
<High school	1431	21	637	21	794	22		984	21	447	21	
High school graduate	5264	79	2412	79	2852	78		3614	79	1650	79	
Family history of breast cancer*							0.54					0.23
No	5809	87	2637	86	3172	87		3974	86	1835	88	
Yes	886	13	412	14	474	13		624	14	262	12	
Age at first Menses							0.38					0.02
<12	794	12	350	12	444	12		511	11	283	14	
12-13	3541	54	1636	55	1905	53		2452	55	1089	53	
14+	2192	34	979	33	1213	34		1520	34	672	33	
Missing	168		84		84			115		53		
Parity*							0.04					0.51
Nulliparous	1246	19	612	20	634	17		845	18	401	19	
1	922	14	431	14	491	13		632	14	290	14	
2	1828	27	830	27	998	27		1252	27	576	27	
3	1363	20	590	19	773	21		929	20	434	21	
4	697	10	310	10	387	11		502	11	195	9	
5+	639	9	276	9	363	10		438	10	201	10	

Table 3 (continued)

Variable	Overall n=6695		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year				
			No (n=3049)		Yes (n=3646)			No (n=4598)		Yes (n=2097)		
	n	%	n	%	n	%	p-value	n	%	n	%	p-value
Nulliparous*							0.005					0.47
No	5449	81	2437	80	3012	83		3753	82	1696	81	
Yes	1246	19	612	20	634	17		845	18	401	19	
Age at first live birth							0.004					0.002
≤20	836	15	336	14	500	17		538	14	298	18	
>20	4603	85	2098	86	2505	83		3209	86	1394	82	
Missing	1256		615		641			851		405		
Age (years) at menopause*							0.36					0.03
≤40	580	10	250	10	330	11		376	10	204	12	
41-45	1082	19	511	20	571	19		769	20	313	18	
46-50	2144	38	975	38	1169	38		1467	37	677	39	
≥51	1834	32	856	33	978	32		1294	33	540	31	
Missing	1055		457		598			692		363		
Surgical menopause*							0.13					0.12
No	5873	88	2695	88	3178	87		4053	88	1820	87	
Yes	822	12	354	12	468	13		545	12	277	13	
Estrogen only therapy (Oral)*							<0.0001					<0.0001
Never use	3835	57	1864	61	1971	54		2766	60	1069	51	
Past use	1883	28	805	26	1078	30		1258	27	625	30	
Current use	977	15	380	12	597	16		574	12	403	19	
Estrogen only therapy (Any Current)*							<0.0001					<0.0001
No	5718	85	2669	87	3049	84		4024	87	1694	81	
Yes	977	15	380	12	597	16		574	12	403	19	
Average no. of alcoholic drinks/week (±SD)*	2 (4)		2 (4)		2 (4)		0.82	2 (4)		2 (4)		0.26

Table 3 (continued)

Variable	Overall n=6695		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year				
			No (n=3049)		Yes (n=3646)			No (n=4598)		Yes (n=2097)		
	n	%	n	%	n	%	p-value	n	%	n	%	p-value
Average no. of alcoholic drinks/week*							0.77					0.53
None	1932	29	887	29	1045	29		1341	29	591	28	
<1	2265	34	1043	34	1222	33		1571	34	694	33	
1-2	830	12	361	12	469	13		559	12	271	13	
2-7	1106	16	500	16	606	17		754	16	352	17	
>7	562	8	258	8	304	8		373	8	189	9	
Cigarette Smoking							0.05					0.12
Never	4047	60	1874	61	2173	60		2810	61	1237	59	
Former	2128	32	925	30	1203	33		1425	31	703	33	
Current	520	8	250	8	270	7		363	8	157	7	
Walks for exercise*							0.02					0.001
No	3174	47	1398	46	1776	49		2118	46	1056	50	
Yes	3521	53	1651	54	1870	51		2480	54	1041	50	
Body mass index §							<0.0001					<0.0001
<25	2999	45	1528	50	1471	40		2161	47	838	40	
25+	3696	55	1521	50	2175	60		2437	53	1259	60	
Hypertension*							<0.0001					<0.0001
Never	4186	62	2057	67	2129	58		3033	66	1153	55	
Ever	2509	37	992	32	1517	42		1565	34	944	45	
Hip bone mineral density (±SD), g/cm²	0.76 (0.13)		0.75 (0.13)		0.76 (0.13)		<0.0001	0.75 (0.13)		0.76 (0.14)		0.002
Stroke							<0.0001					<0.0001
Never	6378	96	2947	97	3431	95		4440	97	1938	93	
Ever	276	4	89	3	187	5		133	3	143	7	
Missing	41		13		28			25		16		

Table 3 (continued)

	Overall n=6695		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year				
			No (n=3049)		Yes (n=3646)			No (n=4598)		Yes (n=2097)		
Variable	**n**	**%**	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Heart Attack							<0.0001					<0.0001
Never	5375	93	2548	95	2827	91		3755	94	1620	90	
Ever	396	7	133	5	263	9		222	6	174	10	
Missing	924		368		556			621		303		
Mammogram at visit 3							0.91					0.91
No	875	20	410	20	465	20		606	20	269	20	
Yes	3484	80	1640	80	1844	80		2406	80	1078	80	
Missing	2336		999		1337			1586		750		

***Information collected at baseline clinical visit; † History of breast cancer in a mother or sister; § Weight (kg)/height² (m²).**

Table 4. Characteristics of women by acetaminophen use in the Study of Osteoporotic Fractures, 1989-90

	Regular use of acetaminophen in past 12 months					Daily use of acetaminophen for at least 1 year				
	No (n=5173)		Yes (n=1513)			No (n=6234)		Yes (n=446)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Clinic*					<0.0001					0.003
A	1369	26	409	27		1642	26	135	30	
B	1241	24	394	26		1505	24	129	29	
C	1218	24	269	18		1410	23	76	17	
D	1345	26	441	29		1677	27	106	24	
Age (±SD), years	73 (5)		73 (5)		0.46	73 (5)		74 (5)		0.0009
Age (years)					0.50					0.008
≤72	2715	52	809	53		3312	53	208	47	
73+	2458	47	704	47		2922	47	238	53	
Education*					0.0002					0.004
<High school	1053	20	376	25		1306	21	119	27	
High school grad	4120	80	1137	75		4928	79	327	73	
Family history of breast cancer*					0.86					0.06
No	4487	87	1315	87		5398	87	400	90	
Yes	686	13	198	13		836	13	46	10	
Age at first Menses					0.39					0.08
<12	621	12	171	12		726	12	67	15	
12-13	2742	54	796	54		3312	55	221	50	
14+	1669	33	519	35		2036	33	150	34	
Missing	141		27			160		8		
Parity*					0.31					0.17
Nulliparous	990	19	255	17		1159	19	82	18	
1	722	14	200	13		876	14	46	10	
2	1395	27	426	28		1700	27	125	28	
3	1052	20	311	20		1265	20	94	21	
4	530	10	166	11		651	10	45	10	
5+	484	9	155	10		583	9	54	12	
Nulliparous*					0.04					0.91
No	4183	81	1258	83		5075	81	364	82	
Yes	990	19	255	17		1159	19	82	18	
Age at first live birth					0.002					<0.0001
≤20	607	14	227	18		749	15	83	23	
>20	3569	85	1028	82		4316	85	281	77	
Missing	997		258			1169		82		
Age (years) at menopause*					0.007					0.01
≤40	420	10	156	13		524	10	55	15	
41-45	839	19	241	20		1010	19	72	20	
46-50	1690	38	453	37		2009	38	129	36	
≥51	1458	33	375	31		1725	33	105	29	
Missing	766		288			966		85		

Table 4 (continued)

	Regular use of acetaminophen in past 12 months					Daily use of acetaminophen for at least 1 year				
	No (n=5173)		Yes (n=1513)			No (n=6234)		Yes (n=446)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Surgical menopause*					<0.0001					<0.0001
No	4592	89	1276	84		5494	88	365	82	
Yes	581	11	237	16		740	12	81	18	
Estrogen only therapy (Oral)*					0.0006					0.006
Never use	3022	58	808	53		3597	58	233	52	
Past use	1433	28	446	29		1749	28	126	28	
Current use	718	14	259	17		888	14	87	20	
Estrogen only therapy (Any Current)*					0.002					0.002
No	4455	86	1254	83		5346	86	359	80	
Yes	718	14	259	17		888	14	87	20	
Avg. no. of alcoholic drinks/week (±SD)*	2 (4)		2 (5)		0.12	2 (4)		2 (6)		0.99
Avg. no. of alcoholic drinks/week*					0.50					0.43
None	1483	29	444	29		1798	29	125	28	
<1	1730	33	532	35		2101	34	161	36	
1-2	652	13	177	12		785	13	45	10	
2-7	872	17	234	15		1032	17	72	16	
>7	436	8	126	8		518	8	43	10	
Cigarette Smoking					0.06					0.41
Never	3124	60	917	61		3757	60	281	63	
Former	1626	31	499	33		1993	32	129	29	
Current	423	8	97	6		484	8	36	8	
Walks for exercise*					0.16					0.003
No	2428	47	741	49		2925	47	242	54	
Yes	2745	53	772	51		3309	53	204	46	
Body mass index §					<0.0001					0.008
<25	2412	47	583	39		2821	45	173	39	
25+	2761	53	930	61		3413	55	273	61	
Hypertension*					0.0008					0.0002
Never	3289	64	890	59		3938	63	242	54	
Ever	1884	36	623	41		2296	37	204	46	
Hip bone mineral density (±SD), g/cm²	0.76 (0.13)		0.76 (0.13)		0.055	0.76 (0.13)		0.74 (0.14)		0.07
Stroke					0.001					<0.0001
Never	4948	96	1421	94		5960	96	406	92	
Ever	191	4	85	6		236	4	37	8	
Missing	34		7			38		3		

Table 4 (continued)

	Regular use of acetaminophen in past 12 months					Daily use of acetaminophen for at least 1 year				
	No (n=5173)		Yes (n=1513)			No (n=6234)		Yes (n=446)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Heart Attack					0.0003					0.02
Never	4197	94	1173	91		5020	93	345	90	
Ever	277	6	117	9		357	7	37	10	
Missing	699		223			857		64		
Mammogram at visit 3					0.02					0.29
No	703	21	169	17		822	20	52	18	
Yes	2681	79	803	83		3234	80	242	82	
Missing	1789		541			2178		152		

***Information collected at baseline clinical visit.**

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 5. Characteristics of women by aspirin use in the Study of Osteoporotic Fractures, 1989-90

	Regular use of asiprin in past 12 months					Daily use of aspirin for at least 1 year				
	No (n=4067)		Yes (n=2628)			No (n=5210)		Yes (n=1485)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Clinic*					<0.0001					0.0003
A	1001	25	780	30		1335	26	446	30	
B	1013	25	622	24		1264	24	371	25	
C	967	24	522	20		1163	22	326	22	
D	1086	27	704	27		1448	28	342	23	
Age (±SD), years	73 (5)		73 (5)		0.45	73 (5)		74 (5)		0.004
Age (years)					0.12					0.003
≤72	2174	53	1354	52		2796	54	732	49	
73+	1893	47	1274	48		2414	46	753	51	
Education*					0.28					0.02
<High school	887	22	544	21		1147	22	284	19	
High school graduate	3180	78	2084	79		4063	78	1201	81	
Family history of breast cancer*					0.07					0.11
No	3504	86	2305	88		4502	86	1307	88	
Yes	563	14	323	12		708	14	178	12	
Age at first Menses					0.09					0.44
<12	472	12	322	13		605	12	189	13	
12-13	2190	56	1351	53		2771	54	770	53	
14+	1296	32	896	35		1703	34	489	34	
Missing	109		59			131		37		
Parity*					0.59					0.2
Nulliparous	775	19	471	18		954	18	292	20	
1	564	14	358	14		712	14	210	14	
2	1113	27	715	27		1426	27	402	27	
3	800	20	563	21		1051	20	312	21	
4	428	10	269	10		568	11	129	9	
5+	387	9	252	10		499	10	140	9	
Nulliparous*					0.24					0.24
No	3292	81	2157	82		4256	82	1193	80	
Yes	775	19	471	18		954	18	292	20	
Age at first live birth					0.39					0.17
≤20	494	15	342	16		638	15	198	17	
>20	2792	85	1811	84		3611	85	992	83	
Missing	781		475			961		295		
Age (years) at menopause*					0.37					0.21
≤40	351	10	229	10		445	10	135	11	
41-45	656	19	426	19		858	19	224	18	
46-50	1283	37	861	39		1647	37	497	40	
≥51	1147	33	687	31		1450	33	384	31	
Missing	630		425			810		245		

Table 5 (continued)

	Regular use of asiprin in past 12 months					Daily use of aspirin for at least 1 year				
	No (n=4067)		Yes (n=2628)			No (n=5210)		Yes (n=1485)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Surgical menopause*					0.52					0.29
No	3576	88	2297	87		4582	88	1291	87	
Yes	491	12	331	13		628	12	194	13	
Estrogen only therapy (Any Current)*					0.05					<0.0001
No	3501	86	2217	84		4498	86	1220	82	
Yes	566	14	411	16		712	14	265	18	
Average no. of alcoholic drinks/week (±SD)*	2 (4)		2 (4)		0.09	2 (4)		2 (4)		0.04
Average no. of alcoholic drinks/week*					0.37					0.07
None	1197	29	735	28		1517	29	415	28	
<1	1388	34	877	33		1790	34	475	32	
1-2	500	12	330	12		646	12	184	12	
2-7	646	16	460	17		837	16	269	18	
>7	336	8	226	9		420	8	142	9	
Cigarette Smoking					0.59					0.5
Never	2478	61	1569	60		3169	61	878	59	
Former	1275	31	853	32		1640	31	488	33	
Current	314	8	206	8		401	8	119	8	
Walks for exercise*					0.58					0.1
No	1917	47	1257	48		2442	47	732	49	
Yes	2150	53	1371	52		2768	53	753	51	
Body mass index §					<0.0001					0.04
<25	1900	47	1099	42		2369	45	630	42	
25+	2167	53	1529	58		2841	55	855	58	
Hypertension*					<0.0001					<0.0001
Never	2627	65	1559	59		3362	65	824	55	
Ever	1440	35	1069	41		1848	35	661	45	
Hip bone mineral density (±SD), g/cm²	0.75 (0.13)		0.76 (0.13)		0.005	0.76 (0.13)		0.76 (0.14)		0.13
Stroke					<0.0001					<0.0001
Never	3927	97	2451	94		5025	97	1353	92	
Ever	121	3	155	6		158	3	118	8	
Missing	19		22			27		14		
Heart Attack					<0.0001					<0.0001
Never	3384	95	1991	90		4255	94	1120	89	
Ever	181	5	215	10		255	6	141	11	
Missing	502		422			700		224		

Table 5 (continued)

	Regular use of asiprin in past 12 months					Daily use of aspirin for at least 1 year				
	No (n=4067)		Yes (n=2628)			No (n=5210)		Yes (n=1485)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Mammogram at visit 3					0.13					0.77
No	521	19	354	21		676	20	199	20	
Yes	2172	81	1312	79		2708	80	776	80	
Missing	1374		962			1826		510		

***Information collected at baseline clinical visit.**

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 6. Characteristics of women by non-aspirin NSAID use in the Study of Osteoporotic Fractures, 1989-90

	Regular use of a non-aspirin NSAID in past 12 months					Daily use of a non-aspirin NSAID for at least 1 year				
	No (n=4925)		Yes (n=1770)			No (n=5804)		Yes (n=891)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Clinic*					<0.0001					0.0002
A	1290	26	491	28		1528	26	253	28	
B	1163	24	472	27		1391	24	244	27	
C	1171	24	318	18		1340	23	149	17	
D	1301	26	489	28		1545	27	245	27	
Age (±SD), years	73 (5)		73 (5)		0.87	73 (5)		73 (5)		0.36
Age (years)					0.79					0.91
≤72	2600	53	928	52		3060	53	468	53	
73+	2325	47	842	48		2744	47	423	47	
Education*					<0.0001					0.01
<High school	992	20	439	25		1213	21	218	24	
High school graduate	3933	80	1331	75		4591	79	673	76	
Family history of breast cancer*					0.26					0.66
No	4287	87	1522	86		5040	87	769	86	
Yes	638	13	248	14		764	13	122	14	
Age at first Menses					0.85					0.007
<12	577	12	217	12		663	12	131	15	
12-13	2603	54	938	54		3069	54	472	54	
14+	1616	34	576	33		1927	34	265	31	
Missing	129		39			145		23		
Parity*					0.01					0.27
Nulliparous	952	19	294	17		1098	19	148	17	
1	691	14	231	13		809	14	113	13	
2	1353	27	475	27		1582	27	246	28	
3	991	20	372	21		1179	20	184	21	
4	488	10	209	12		592	10	105	12	
5+	450	9	189	11		544	9	95	11	
Nulliparous*					0.01					0.10
No	3973	81	1476	83		4706	81	743	83	
Yes	952	19	294	17		1098	19	148	17	
Age at first live birth					0.0002					0.03
≤20	565	14	271	18		702	15	134	18	
>20	3402	86	1201	82		3996	85	607	82	
Missing	958		298			1106		150		
Age (years) at menopause*					0.64					0.06
≤40	419	10	161	11		486	10	94	13	
41-45	808	19	274	19		948	19	134	19	
46-50	1598	38	546	37		1872	38	272	38	
≥51	1349	32	485	33		1615	33	219	30	
Missing	751		304			883		172		

Table 6 (continued)

	Regular use of a non-aspirin NSAID in past 12 months					Daily use of a non-aspirin NSAID for at least 1 year				
	No (n=4925)		Yes (n=1770)			No (n=5804)		Yes (n=891)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Surgical menopause*					0.05					0.04
No	4343	88	1530	86		5110	88	763	86	
Yes	582	12	240	14		694	12	128	14	
Estrogen only therapy (Any Current)*					<0.0001					<0.0001
No	4266	87	1452	82		5027	87	691	78	
Yes	659	13	318	18		777	13	200	22	
Average no. of alcoholic drinks/week (±SD)*	2 (4)		2 (4)		0.20	2 (4)		2 (4)		0.38
Average no. of alcoholic drinks/week*					0.56					0.67
None	1404	29	528	30		1677	29	255	29	
<1	1673	34	592	33		1953	34	312	35	
1-2	600	12	230	13		712	12	118	13	
2-7	826	17	280	16		969	17	137	15	
>7	422	9	140	8		493	8	69	8	
Cigarette Smoking					0.05					0.33
Never	2992	61	1055	60		3520	61	527	59	
Former	1533	31	595	34		1827	31	301	34	
Current	400	8	120	7		457	8	63	7	
Walks for exercise*					0.006					0.0004
No	2285	46	889	50		2702	47	472	53	
Yes	2640	54	881	50		3102	53	419	47	
Body mass index §					<0.0001					<0.0001
<25	2364	48	635	36		2700	46	299	33	
25+	2561	52	1135	64		3104	53	592	66	
Hypertension*					<0.0001					<0.0001
Never	3190	65	996	56		3713	64	473	53	
Ever	1735	35	774	44		2091	36	418	47	
Hip bone mineral density (±SD), g/cm²	0.75 (0.13)		0.77 (0.13)		<0.0001	0.75 (0.13)		0.77 (0.14)		<0.0001
Stroke					0.49					0.006
Never	4696	96	1682	96		5544	96	834	94	
Ever	198	4	78	4		224	4	52	6	
Missing	31		10			36		5		
Heart Attack					0.57					0.18
Never	3966	93	1409	93		4662	93	713	92	
Ever	287	7	109	7		334	7	62	8	
Missing	672		252			808		116		

Table 6 (continued)

	Regular use of a non-aspirin NSAID in past 12 months					Daily use of a non-aspirin NSAID for at least 1 year				
	No (n=4925)		Yes (n=1770)			No (n=5804)		Yes (n=891)		
Variable	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Mammogram at visit 3					0.17					0.86
No	670	21	205	19		764	20	111	20	
Yes	2590	79	894	81		3034	80	450	80	
Missing	1665		671			2006		330		

***Information collected at baseline clinical visit.**

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 7. Clinical characteristics of breast cancer cases by regular NSAID use, the Study of Osteoporotic Fractures, 1986-1993

	Overall n=372		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year				
			No (n=177)		Yes (n=195)			No (n=265)		Yes (n=107)		
Characteristics	**n**	**%**	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Age at diagnosis, mean (SD), y			78 (5)		79 (5)		0.16	78 (5)		78 (6)		0.34
Estrogen receptor status, No. (%)†							§					§
Positive	269	72	129	73	140	72		188	71	81	76	
Negative	37	10	16	9	21	11		27	10	10	9	
Borderline	1	0.3	0	0	1	1		1	0	0	0	
Unknown	65	17	32	18	33	17		49	18	16	15	
Estrogen receptor status, No. (%)†							0.6					0.71
Positive ‖	270	88	129	89	140	86		189	87	81	89	
Negative	37	12	16	11	21	13		27	12	10	11	
missing	65		0	0	1	1		49		16		
Progesterone receptor status, No. (%)†							§					§
Positive	209	56	99	56	110	56		152	57	57	53	
Negative	88	24	40	23	48	25		55	21	33	31	
Borderline	7	2	3	2	4	2		6	2	1	1	
Unknown	68	18	35	20	33	17		52	20	16	15	
Progesterone receptor status, No. (%)†							0.78					0.07
Positive ‖	216	71	102	72	114	70		158	74	58	64	
Negative	88	29	40	28	48	30		55	26	33	36	
missing	68		35		33			52		16		

Table 7 (continued)

Characteristics	Overall n=372		Regular use of any NSAID in past 12 months					Daily use of any NSAID for at least 1 year					
			No (n=177)		Yes (n=195)			No (n=265)		Yes (n=107)			
	n	%	n	%	n	%	p-value	n	%	n	%	p-value	
Cancer stage at diagnosis, No. (%)‡								0.84				0.82	
O (in situ)			52	14	27	15	25	13		38	14	14	13
I			206	55	98	55	108	55		150	57	56	52
II (no nodes)			41	11	20	11	21	11		29	11	12	11
II (+ nodes)			36	10	20	11	16	8		24	9	12	11
III			14	4	4	2	10	5		10	4	4	4
IV			4	1	1	1	3	1		3	1	1	1
Unknown			19	5	7	4	12	6		11	4	8	8

†Women with unknown estrogen receptor status were excluded in statistical tests.

‡The p value compares women with stage II cancer or greater at diagnosis with other cases. Women with unknown cancer stage at diagnosis were excluded from this analysis.

‖ Borderline recoded to positive

§ Cell sizes too sparse to use chi square test to compare clinical characteristic by medication use.

Table 8. Clinical characteristics of breast cancer cases by regular acetaminophen use, the Study of Osteoporotic Fractures, 1986-1993

	Regular use of acetaminophen in past 12 months					Daily use of acetaminophen for at least 1 year				
	No (n=295)		Yes (n=76)			No (n=358)		Yes (n=14)		
Characteristics	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Age at diagnosis, mean (SD), y	78 (5)		78 (6)		0.29	78 (5)		77 (6)		0.24
Estrogen receptor status, No. (%)†					§					§
Positive	210	71	58	76		260	73	9	64	
Negative	29	10	8	11		36	10	1	7	
Borderline	1	0	0	0		1	0	0	0	
Unknown	55	19	10	13		61	17	4	29	
Estrogen receptor status, No. (%)†					0.99					1.00
Positive ‖	211	88	58	88		261	88	9	90	
Negative	29	12	8	12		36	12	1	10	
missing	55		10			61		4		
Progesterone receptor status, No. (%)†					§					§
Positive	160	54	48	63		204	57	5	36	
Negative	71	24	17	22		83	23	5	36	
Borderline	7	2	0	0		7	2	0	0	
Unknown	57	19	11	14		64	18	4	29	
Progesterone receptor status, No. (%)†					0.56					0.16
Positive ‖	167	70	48	74		211	72	5	50	
Negative	71	30	17	26		83	28	5	50	
missing	57		11			64		4		

Table 8 (continued)

	Regular use of acetaminophen in past 12 months					Daily use of acetaminophen for at least 1 year				
	No (n=295)		Yes (n=76)			No (n=358)		Yes (n=14)		
Characteristics	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Cancer stage at diagnosis, No. (%)‡					0.25					§
O (in situ)	45	15	7	9		50	14	2	14	
I	158	53	47	62		200	56	6	43	
II (no nodes)	35	12	6	8		40	11	1	7	
II (+ nodes)	30	10	6	8		33	9	3	21	
III	10	3	4	5		13	4	1	7	
IV	3	1	1	1		4	1	0	0	
Unknown	14	5	5	7		18	5	1	7	

†Women with unknown estrogen receptor status were excluded in statistical tests.

‡The p value compares women with stage II cancer or greater at diagnosis with other cases. Women with unknown cancer stage at diagnosis were excluded from this analysis.

‖ Borderline recoded to positive

§ Cell sizes too sparse to use chi square test to compare clinical characteristic by medication use.

Table 9. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use in the Study of Osteoporotic Fractures, 1986-1993

Risk Factor	Controls (n=6323)	Cases (n=372)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Aspirin ≥ 1/week in past 12 months								
No	3838	229	1.00		1.00		1.00	
Yes	2485	143	0.98	0.80, 1.21	0.98	0.80, 1.21	0.96	0.78, 1.18
Aspirin frequency:								
No regular use	3838	229	1.00		1.00		1.00	
1-4 days/week	995	62	1.02	0.77, 1.35	1.02	0.77, 1.35	1.00	0.76, 1.33
5-7 days/week	1418	72	0.89	0.68, 1.16	0.90	0.69, 1.17	0.87	0.66, 1.13
Missing	72	9						
Aspirin ~daily for ≥ 1 year								
No	4913	297	1.00		1.00		1.00	
Yes	1410	75	0.93	0.72, 1.19	0.93	0.72, 1.20	0.90	0.70, 1.16
Aspirin duration:								
No regular use	4913	297	1.00		1.00		1.00	
< 5 years	771	40	0.90	0.65, 1.25	0.90	0.65, 1.26	0.89	0.64, 1.23
5+ years	632	35	0.97	0.68, 1.37	0.97	0.69, 1.38	0.92	0.65, 1.31
Missing	7	0						
Non-aspirin NSAID ≥ 1/week in past 12 months								
No	4652	273	1.00		1.00		1.00	
Yes	1671	99	1.01	0.80, 1.27	1.01	0.80, 1.27	0.96	0.76, 1.21
Non-aspirin NSAID frequency:								
No regular use	4652	273	1.00		1.00		1.00	
1-4 days/week	540	35	1.08	0.76, 1.54	1.08	0.76, 1.54	1.05	0.74, 1.50
5-7 days/week	1080	60	0.96	0.72, 1.26	0.96	0.72, 1.27	0.90	0.68, 1.19
Missing	51	4						
Non-aspirin NSAID ~daily for ≥ 1 year								
No	5481	323	1.00		1.00		1.00	
Yes	842	49	1.02	0.75, 1.38	1.02	0.76, 1.38	0.95	0.70, 1.29
Non-aspirin NSAID duration:								
No regular use	5481	323	1.00		1.00		1.00	
< 5 years	565	34	1.06	0.75, 1.51	1.07	0.75, 1.52	1.02	0.71, 1.46
5+ years	271	15	0.95	0.57, 1.60	0.95	0.57, 1.59	0.85	0.50, 1.43
Missing	6	0						
Any NSAIDs ≥ 1/week in past 12 months								
No	2872	177	1.00		1.00		1.00	
Yes	3451	195	0.93	0.76, 1.14	0.94	0.76, 1.15	0.89	0.73, 1.10

Table 9 (continued)

Risk Factor	Controls (n=6323)	Cases (n=372)	Unadjusted* HR‡	95% CI	Adjusted** HR‡	95% CI	Adjusted*** HR‡	95% CI
Any NSAIDs frequency:								
No regular use	2872	177	1.00		1.00		1.00	
1-4 days/week	2014	105	0.88	0.69, 1.12	0.89	0.70, 1.13	0.83	0.65, 1.07
5-7 days/week	1364	81	0.95	0.73, 1.24	0.95	0.73, 1.24	0.92	0.71, 1.20
Missing	73	9						
Any NSAIDs ~daily for ≥ 1 year								
No	4333	265	1.00		1.00		1.00	
Yes	1990	107	0.93	0.74, 1.16	0.93	0.74, 1.17	0.89	0.71, 1.12
NSAIDs duration:								
No regular use	4333	265	1.00		1.00		1.00	
< 5 years	1143	62	0.93	0.71, 1.23	0.94	0.71, 1.24	0.91	0.69, 1.20
5+ years	839	45	0.92	0.67, 1.26	0.93	0.68, 1.27	0.86	0.63, 1.19
Missing	8	0						
Acetaminophen ≥ 1/week in past 12 months								
No	4878	295	1.00		1.00		1.00	
Yes	1437	76	0.89	0.69, 1.15	0.87	0.70, 1.07	0.87	0.67, 1.12
Missing	8	1						
Acetaminophen frequency:								
No regular use	4878	295	1.00		1.00		1.00	
1-4 days/week	842	47	0.92	0.68, 1.26	0.92	0.68, 1.25	0.90	0.66, 1.22
5-7 days/week	495	19	0.68	0.43, 1.08	0.68	0.43, 1.09	0.67	0.42, 1.07
Missing	108	11						
Acetaminophen ~daily for ≥ 1 year								
No	5876	358	1.00		1.00		1.00	
Yes	432	14	0.58	0.34, 0.99	0.58	0.34, 0.99	0.57	0.33, 0.97
Missing	15	0						
Acetaminophen duration: §								
No regular use	5876	358	1.00		1.00		1.00	
< 5 years	276	6						
5+ years	152	8						
Missing	19	0						

*Proportional hazards regression models.

**Age-adjusted hazard ratio

***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.

‡HR, hazard ratio; CI, confidence interval.

§ Too few breast cancer cases to estimate relative hazard associated with medication use.

Table 10. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use by hormone receptor status in the SOF, 1986-1993

Risk Factor	Controls (n=6323)	Cases: ≥ 1 Positive Hormone Receptor (n=275)	Unadjusted*		Adjusted**		Adjusted***	
			HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Aspirin ≥ 1/week in past 12 months								
No	3838	170	1.00		1.00		1.00	
Yes	2485	105	0.97	0.76, 1.24	0.97	0.76, 1.24	0.94	0.74, 1.21
Aspirin frequency:								
No regular use	3838	170	1.00		1.00		1.00	
1-4 days/week	995	46	1.02	0.74, 1.41	1.02	0.73, 1.41	1.00	0.72, 1.39
5-7 days/week	1418	52	0.87	0.64, 1.18	0.87	0.64, 1.19	0.84	0.61, 1.15
Missing	72	7						
Aspirin ~daily for ≥ 1 year								
No	4913	216	1.00		1.00		1.00	
Yes	1410	59	1.00	0.75, 1.33	1.01	0.75, 1.34	0.96	0.72, 1.29
Aspirin duration:								
No regular use	4913	216	1.00		1.00		1.00	
< 5 years	771	30	0.93	0.63, 1.36	0.93	0.64, 1.36	0.90	0.62, 1.33
5+ years	632	29	1.10	0.74, 1.62	1.11	0.75, 1.63	1.04	0.71, 1.54
Missing	7	0						
Non-aspirin NSAID ≥ 1/week in past 12 mos								
No	4652	200	1.00		1.00		1.00	
Yes	1671	75	1.04	0.80, 1.36	1.04	0.80, 1.36	1.00	0.76, 1.30
Non-aspirin NSAID frequency:								
No regular use	4652	200	1.00		1.00		1.00	
1-4 days/week	540	28	1.18	0.79, 1.75	1.18	0.79, 1.75	1.15	0.78, 1.72
5-7 days/week	1080	45	0.98	0.71, 1.35	0.98	0.71, 1.36	0.92	0.66, 1.28
Missing	51	2						
Non-aspirin NSAID ~daily for ≥ 1 year								
No	5481	237	1.00		1.00		1.00	
Yes	842	38	1.08	0.76, 1.51	1.08	0.76, 1.52	1.01	0.71, 1.42
Non-aspirin NSAID duration:								
No regular use	5481	237	1.00		1.00		1.00	
< 5 years	565	26	1.11	0.74, 1.66	1.11	0.74, 1.67	1.06	0.71, 1.60
5+ years	271	12	1.03	0.58, 1.85	1.03	0.58, 1.84	0.92	0.51, 1.65
Missing	6	0						

Table 10 (continued)

Risk Factor	Controls (n=6323)	Cases: ≥ 1 Positive Hormone Receptor (n=275)	Unadjusted*		Adjusted**		Adjusted***	
			HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Any NSAIDs ≥ 1/week in past 12 months								
No	2872	131	1.00		1.00		1.00	
Yes	3451	144	0.93	0.73, 1.18	0.93	0.74, 1.18	0.89	0.70, 1.13
Any NSAIDs frequency:								
No regular use	2872	131	1.00		1.00		1.00	
1-4 days/week	2014	76	0.86	0.65, 1.14	0.87	0.65, 1.15	0.81	0.61, 1.08
5-7 days/week	1364	62	0.98	0.73, 1.33	0.98	0.72, 1.33	0.95	0.70, 1.29
Missing	73	6						
Any NSAIDs ~daily for ≥ 1 year								
No	4333	193	1.00		1.00		1.00	
Yes	1990	82	0.97	0.75, 1.26	0.98	0.76, 1.27	0.93	0.71, 1.20
NSAIDs duration:								
No regular use	4333	193	1.00		1.00		1.00	
< 5 years	1143	46	0.95	0.69, 1.31	0.96	0.69, 1.32	0.92	0.67, 1.27
5+ years	839	36	1.01	0.71, 1.44	1.01	0.71, 1.45	0.94	0.66, 1.35
Missing	8	0						
Acetaminophen ≥ 1/week in past 12 months								
No	4878	215	1.00		1.00		1.00	
Yes	1437	59	0.94	0.71, 1.26	0.94	0.71, 1.26	0.92	0.69, 1.23
Missing	8	1						
Acetaminophen frequency:								
No regular use	4878	215	1.00		1.00		1.00	
1-4 days/week	842	37	0.99	0.70, 1.41	0.99	0.70, 1.40	0.96	0.68, 1.37
5-7 days/week	495	14	0.68	0.40, 1.17	0.69	0.40, 1.18	0.67	0.39, 1.15
Missing	108	9						
Acetaminophen ~daily for ≥ 1 year †								
No	5876	266	1.00		1.00		1.00	
Yes	432	9	0.50	0.26, 0.97	0.50	0.26, 0.98	0.48	0.25, 0.93
Missing	15	0						

Table 10 (continued)

Risk Factor	Controls (n=6323)	Cases: ≥ 1 Positive Hormone Receptor (n=275)	Unadjusted*		Adjusted**		Adjusted***	
			HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Acetaminophen duration: †								
No regular use	5876	266	1.00		1.00		1.00	
< 5 years	276	4						
5+ years	152	5						
Missing	19	0						

*Proportional hazards regression models; **Age-adjusted hazard ratio

***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.

‡HR, hazard ratio; CI, confidence interval.

† Too few hormone receptor-positive breast cancer cases to estimate HR associated with medication use.

Table 11. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use by tumor type at diagnosis in the SOF (1986-1993)

Risk Factor	Cases Stages I to IV (n=301)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Aspirin ≥ 1/week in past 12 months							
No	184	1.00		1.00		1.00	
Yes	117	1.00	0.79, 1.26	1.00	0.76, 1.26	0.97	0.77, 1.22
Aspirin frequency:							
No regular use	184	1.00		1.00		1.00	
1-4 days/week	52	1.07	0.78, 1.45	1.06	0.78, 1.44	1.04	0.76, 1.41
5-7 days/week	59	0.91	0.68, 1.22	0.91	0.68, 1.23	0.88	0.65, 1.18
Missing	6						
Aspirin ~daily for ≥ 1 year							
No	242	1.00		1.00		1.00	
Yes	59	0.89	0.67, 1.19	0.90	0.68, 1.19	0.86	0.65, 1.15
Aspirin duration:							
No regular use	242	1.00		1.00		1.00	
< 5 years	30	0.83	0.57, 1.21	0.83	0.57, 1.22	0.81	0.55, 1.19
5+ years	29	0.98	0.67, 1.44	0.99	0.67, 1.45	0.93	0.63, 1.37
Missing	0						
Non-aspirin NSAID ≥ 1/week in past 12 months							
No	221	1.00		1.00		1.00	
Yes	80	1.01	0.78, 1.30	1.01	0.78, 1.30	0.96	0.74, 1.24
Non-aspirin NSAID frequency:							
No regular use	221	1.00		1.00		1.00	
1-4 days/week	29	1.11	0.75, 1.63	1.11	0.75, 1.63	1.08	0.73, 1.59
5-7 days/week	49	0.96	0.71, 1.31	0.97	0.71, 1.32	0.90	0.66, 1.23
Missing	2						
Non-aspirin NSAID ~daily for ≥ 1 year							
No	261	1.00		1.00		1.00	
Yes	40	1.03	0.74, 1.44	1.03	0.74, 1.44	0.96	0.68, 1.34
Non-aspirin NSAID duration:							
No regular use	261	1.00		1.00		1.00	
< 5 years	28	1.08	0.73, 1.60	1.09	0.74, 1.61	1.03	0.70, 1.53
5+ years	12	0.94	0.53, 1.68	0.94	0.53, 1.68	0.83	0.45, 1.49
Missing	0						
Any NSAIDs ≥ 1/week in past 12 months							
No	143	1.00		1.00		1.00	
Yes	158	0.93	0.75, 1.17	0.94	0.75, 1.18	0.89	0.71, 1.12
Any NSAIDs frequency:							
No regular use	143	1.00		1.00		1.00	
1-4 days/week	86	0.89	0.68, 1.16	0.90	0.69, 1.17	0.84	0.64, 1.10
5-7 days/week	67	0.97	0.73, 1.30	0.97	0.73, 1.30	0.94	0.70, 1.26
Missing	5						

Table 11 (continued)

Risk Factor	Cases Stages I to IV (n=301)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Any NSAIDs ~daily for ≥ 1 year							
No	216	1.00		1.00		1.00	
Yes	85	0.90	0.70, 1.16	0.91	0.71, 1.17	0.86	0.66, 1.10
NSAIDs duration:							
No regular use	216	1.00		1.00		1.00	
< 5 years	48	0.89	0.65, 1.21	0.89	0.65, 1.22	0.86	0.63, 1.18
5+ years	37	0.93	0.65, 1.31	0.93	0.66, 1.32	0.86	0.60, 1.22
Missing	0						
Acetaminophen ≥ 1/week in past 12 months							
No	236	1.00		1.00		1.00	
Yes	64	0.94	0.71, 1.23	0.93	0.71, 1.23	0.90	0.68, 1.19
Missing	1						
Acetaminophen frequency:							
No regular use	236	1.00		1.00		1.00	
1-4 days/week	40	0.98	0.70, 1.37	0.98	0.70, 1.37	0.95	0.68, 1.33
5-7 days/week	16	0.71	0.43, 1.18	0.72	0.43, 1.19	0.69	0.41, 1.15
Missing	9						
Acetaminophen ~daily for ≥ 1 year							
No	290	1.00		1.00		1.00	
Yes	11	0.56	0.31, 1.02	0.56	0.31, 1.03	0.53	0.29, 0.98
Missing	0						
Acetaminophen duration: †							
No regular use	290	1.00		1.00		1.00	
< 5 years	4						
5+ years	7						
Missing	0						

*Proportional hazards regression models.

**Age-adjusted hazard ratio

***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.

‡HR, hazard ratio; CI, confidence interval.

† Too few invasive breast cancer cases to estimate relative hazard associated with medication use.

2.6 LITERATURE CITED

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, and Thun MJ, *Cancer Statistics, 2006.* CA Cancer J Clin, 2006. **56**(2): p. 106-130.

2. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, and Hopper JL, *Heritability of mammographic density, a risk factor for breast cancer.* New England Journal of Medicine, 2002. **347**(12): p. 886-94.

3. Alberg AJ and Singh S, *Epidemiology of breast cancer in older women: implications for future healthcare.* Drugs & Aging, 2001. **18**(10): p. 761-72.

4. Miller WR and Forrest AP, *Oestradiol synthesis by a human breast carcinoma.* Lancet, 1974. **2**(7885): p. 866-8.

5. Purohit A, Newman S, and Reed M, *The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer.* Breast Cancer Res, 2002. **4**(2): p. 65 - 69.

6. Abeloff MD, Lichter AS, Niederhuber JE, Pierce LJ, and Love RR, *Breast*, in *Clinical Oncology*, Abeloff MD, et al., Editors. 2000, Churchill Livingstone: Philadelphia.

7. Howe LR, Subbaramaiah K, Brown AM, and Dannenberg AJ, *Cyclooxygenase-2: a target for the prevention and treatment of breast cancer.* Endocrine-Related Cancer, 2001. **8**(2): p. 97-114.

8. Gasparini G, Longo R, Sarmiento R, and Morabito A, *Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?* Lancet Oncol, 2003. **4**(10): p. 605-15.

9. Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, and Robertson FM, *Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens.* Cancer Letters, 1999. **140**(1-2): p. 27-35.

10. Zhao Y, Agarwal VR, Mendelson CR, and Simpson ER, *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, 1996. **137**(12): p. 5739-42.

11. Harris R, Beebe-Donk J, and Alshafie G, *Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors.* BMC Cancer, 2006. **6**(1): p. 27.

12. Ip MM, Mazzer C, Watson D, and Ip C, *The effect of eicosanoid synthesis inhibitors on DMBA-induced rat mammary carcinogenesis.* Proc Am Assoc Cancer Res, 1989. **30**: p. 182.

13. Lee PP and Ip MM, *Regulation of proliferation of rat mammary tumor cells by inhibitors of cyclooxygenase and lipoxygenase.* Prostaglandins Leukot Essent Fatty Acids, 1992. **45**: p. 21-31.

14. Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, McCormick DL, Pereira MA, Crowell JA, Bagheri D, and et al., *Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program.* J Cell Biochem Suppl, 1994. **20**: p. 32-54.

15. Khuder SA and Mutgi AB, *Breast cancer and NSAID use: a meta-analysis.* British Journal of Cancer, 2001. **84**(9): p. 1188-92.

16. Harris RE, Beebe-Donk J, Doss H, and Burr Doss D, *Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review).* Oncol Rep, 2005. **13**(4): p. 559-83.

17. Langman MJS, Cheng KK, Gilman EA, and Lancashire RJ, *Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database.* BMJ, 2000. **320**(7250): p. 1642-1646.

18. Neugut A, Rosenberg D, Ahsan H, Jacobson J, Wahid N, Hagan M, Rahman M, Khan Z, Chen L, Pablos-Mendez A, and Shea S, *Association between coronary heart disease and cancers of the breast, prostate, and colon.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(10): p. 869-873.

19. Coogan PF, Rao SR, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD, and Shapiro S, *The Relationship of Nonsteroidal Anti-inflammatory Drug Use to the Risk of Breast Cancer,*. Preventive Medicine, 1999. **29**(2): p. 72-76.

20. Cotterchio M, Kreiger N, Sloan M, and Steingart A, *Nonsteroidal Anti-inflammatory Drug Use and Breast Cancer Risk.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(11): p. 1213-1217.

21. Harris RE, Namboodiri K., Stellman S. D., and Wynder E. L., *Breast Cancer and NSAID Use: Heterogeneity of Effect in a Case-Control Study.* Preventive Medicine, 1995. **24**(2): p. 119-120.

22. Harris RE, Namboodiri KK, and Farrar WB, *Nonsteroidal antiinflammatory drugs and breast cancer.* Epidemiology, 1996. **7**(2): p. 203-5.

23. Meier CR, Schmitz S, and Jick H, *Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer.* Pharmacotherapy, 2002. **22**(3): p. 303-9.

24. Moorman P, Grubber J, Millikan R, and Newman B, *Association between non-steroidal anti-inflammatory drugs (NSAIDs) and invasive breast cancer and carcinoma in situ of the breast.* Cancer Causes and Control, 2003. **14**(10): p. 915-922.

25. Rahme E, Ghosn J, Dasgupta K, Rajan R, and Hudson M, *Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer.* BMC Cancer, 2005. **5**(1): p. 159.

26. Rosenberg L, *Nonsteroidal Anti-inflammatory Drugs and Cancer.* Preventive Medicine, 1995. **24**(2): p. 107-109.

27. Swede H, Mirand AL, Menezes RJ, and Moysich KB, *Association of Regular Aspirin Use and Breast Cancer Risk.* Oncology, 2005. **68**(1): p. 40-47.

28. Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, Subbaramaiah K, Dannenberg AJ, and Neugut AI, *Association of Frequency and Duration of Aspirin Use and Hormone Receptor Status With Breast Cancer Risk.* JAMA, 2004. **291**(20): p. 2433-2440.

29. Zhang Y, Coogan PF, Palmer JR, Strom BL, and Rosenberg L, *Use of Nonsteroidal Antiinflammatory Drugs and Risk of Breast Cancer: The Case-Control Surveillance Study Revisited.* Am. J. Epidemiol., 2005. **162**(2): p. 165-170.

30. Egan K, Stampfer M, Giovannucci E, Rosner B, and Colditz G, *Prospective study of regular aspirin use and the risk of breast cancer.* J Natl Cancer Inst, 1996. **88**(14): p. 988a-993.

31. Friis S, Sorensen HT, McLaughlin JK, Johnsen SP, Blot WJ, and Olsen JH, *A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin.* Br J Cancer, 2003. **88**(5): p. 684-8.

32. Garcia Rodriguez LA and Gonzalez-Perez A, *Risk of breast cancer among users of aspirin and other anti-inflammatory drugs.* Br J Cancer, 2004. **91**(3): p. 525-9.

33. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, Patel AV, Flanders WD, and Calle EE, *Aspirin and Other Nonsteroidal Anti-inflammatory Drugs and Breast Cancer Incidence in a Large U.S. Cohort.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(1): p. 261-264.

34. Paganini-Hill A, Chao A, Ross RK, and Henderson BE, *Aspirin use and chronic diseases: a cohort study of the elderly.* Bmj, 1989. **299**(6710): p. 1247-50.

35. Sorensen HT, Friis S, Norgard B, Mellemkjaer L, Blot WJ, McLaughlin JK, Ekbom A, and Baron JA, *Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study.* Br J Cancer, 2003. **88**(11): p. 1687-92.

36. Thun M, Namboodiri M, Calle E, Flanders W, and Heath C, Jr, *Aspirin use and risk of fatal cancer.* Cancer Res, 1993. **53**(6): p. 1322-1327.

37. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A, and Ross RK, *Nonsteroidal Anti-Inflammatory Drug Use and Breast Cancer Risk by Stage and Hormone Receptor Status.* J Natl Cancer Inst, 2005. **97**(11): p. 805-812.

38. Harris RE, Kasbari S, and Farrar WB, *Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer.* Oncol Rep, 1999. **6**(1): p. 71-3.

39. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, and McTiernan A, *Breast Cancer and Nonsteroidal Anti-Inflammatory Drugs: Prospective Results from the Women's Health Initiative.* Cancer Res, 2003. **63**(18): p. 6096-6101.

40. Johnson TW, Anderson KE, Lazovich D, and Folsom AR, *Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(12): p. 1586-1591.

41. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, and Hanley JA, *Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage.* Br J Cancer, 2000. **83**(1): p. 112-20.

42. Schreinemachers DM and Everson RB, *Aspirin use and lung, colon, and breast cancer incidence in a prospective study.* Epidemiology, 1994. **5**(2): p. 138-46.

43. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE, *Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial.* JAMA, 2005. **294**(1): p. 47-55.

44. Kadar D, *Anti-inflammatory analgesics*, in *Principles of Medical Pharmacology*, Kalant H and Roschlau WHE, Editors. 1998, Oxford University Press: New York. p. 410-30.

45. Ziv E, Cauley J, Morin PA, Saiz R, and Browner WS, *Association between the T29-->C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures.* JAMA, 2001. **285**(22): p. 2859-63.

46. Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK, Mascioli SR, Scott JC, Seeley DG, Steiger P, and et al., *Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group.* JAMA, 1990. **263**(5): p. 665-8.

47. Cauley JA, Cummings SR, Black DM, Mascioli SR, and Seeley DG, *Prevalence and determinants of estrogen replacement therapy in elderly women.* Am J Obstet Gynecol, 1990. **163**(5 Pt 1): p. 1438-44.

48. Chandrasekharan NV, Dai H, Roos KLT, Evanson NK, Tomsik J, Elton TS, and Simmons DL, *From the Cover: COX-3, a cyclooxygenase-1 variant inhibited by*

acetaminophen and other analgesic/antipyretic drugs: Cloning, structure, and expression. PNAS, 2002. **99**(21): p. 13926-13931.

49. *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report,* 1998. NIH Publication No. 98-4083.

50. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, and Mulvihill JJ, *Projecting individualized probabilities of developing breast cancer for white females who are being examined annually.[comment].* Journal of the National Cancer Institute, 1989. **81**(24): p. 1879-86.

51. Rainsford KD, *Discovery, mechanisms of action and safety of ibuprofen.* Int J Clin Pract Suppl, 2003(135): p. 3-8.

3.0 CIRULATING LEVELS OF SOLUBLE TUMOR NECROSIS FACTOR-ALPHA RECEPTORS I AND II AND MAMMOGRAPHIC DENSITY AMONG POSTMENOPAUSAL WOMEN: THE MAMMOGRAMS AND MASSES STUDY (MAMS)

To be submitted for publication

Gretchen L. Gierach, PhD, MPH,[1] Francesmary Modugno, PhD, MPH,[1, 2] Jane A. Cauley, DrPH, MPH,[1] Joel L. Weissfeld, MD, MPH,[1, 2] John W. Wilson, PhD,[3, 4] Jennifer K. Simpson, PhD,[1] Glenn O. Allen, MPH,[1, 2] and Victor G. Vogel, MD, MHS[5]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]National Surgical Adjuvant Breast and Bowel Project Center, University of Pittsburgh, Pittsburgh, PA

[5]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

Running Title: sTNFR-I, sTNFR-II and mammographic density

This work was supported in part by National Institutes of Health grants R25-CA57703, K07-CA80668, R21-CA95113, and P20 CA103730-02; by Department of Defense grants DAMD17-02-1-0553 and by PA Department of Health grant P2777693. Additional support was provided by funds received from the NIH/NCRR/GCRC Grant MO1-RR000056.

3.1 ABSTRACT

Background: Mammographic density is one of the strongest risk factors for breast cancer. Exactly how mammographic density increases breast cancer risk is unknown, although it has been posited that dense breast areas may reflect exposure to estrogen. The cytokine tumor necrosis factor (TNF)-alpha has a central role in regulating estrogen synthesis in the breast. Circulating TNF receptors may block TNF-α activity, thereby preventing induction of COX-2 gene expression, thus resulting in decreased aromatase activity, and ultimately suppression of estrogen biosynthesis in the breast.

Methods: The association between mammographic density and plasma levels of soluble TNF receptors (sTNFR1 and sTNFR2) was examined among 376 cancer-free, mostly white, postmenopausal women, participating in a cross-sectional study of mammographic density (Pittsburgh, PA, 2001-2005). Women were not taking hormone therapy at the time of blood collection. Percent breast density was calculated using a quantitative method (planimetry).

Results: The mean percent mammographic density was lower among women in the highest quartiles of circulating levels of sTNFR1 and sTNFR2. After adjustment for BMI, the inverse association initially observed between the circulating sTNFRs and percent mammographic density disappeared. While not the primary aim of the study, recent NSAID use reported at blood collection was associated with lower percent mammographic density.

Conclusion: Our findings suggest that mammographic density is independent of circulating sTNFRs in postmenopausal women.

3.2 INTRODUCTION

Breast cancer is the most common cancer in women around the world; in the United States this year, it is expected that breast cancer alone will account for 31% (212,920) of all new cancer cases among women and 40,970 women are expected to die from the disease (1). Mammographic density is a known risk factor for breast cancer (2, 3). The histologic composition of the breast is reflected mammographically by density and parenchymal pattern. The higher the fat content of the breast the lower the radiologic density. Conversely, a high proportion of connective, ductal/epithelial, and glandular tissue increases density (4-7). Notably, the risk associated with mammographic density is greater than that associated with almost all other risk factors for breast cancer (3, 8). Therefore, understanding factors that affect breast density and their underlying mechanism is an important research question.

Many reproductive and hormonal factors associated with breast cancer (9-12) are also associated with breast density. For instance, increased mammographic density is observed in nulliparous women, and in women with a later age at first birth and later age at menopause (3, 9, 10, 13-20); similar relationships have been observed for these reproductive factors with respect to breast cancer risk (21). A positive association between hormone therapy (HT) and density further supports a hormonal influence on breast density (3, 11, 22). Together, these observations suggest that the radiologic features of breast tissue may provide an index of breast tissue exposure to current and past endocrine events that influence breast cancer susceptibility (23).

The cytokine tumor necrosis factor (TNF)-alpha, secreted by macrophages of the immune system and also by adipocytes, has a central role in regulating estrogen synthesis within both normal and malignant breast tissue (24-26). Further, TNF-α is one of the most potent promoters of the aromatase gene, CYP19, resulting in the peripheral conversion of androgens to estrogens in adipose tissue (27). TNF-α induces a range of inflammatory enzymes, including cyclooxygenase (COX)-2. COX-2 cyclizes and oxygenates arachidonic acid eventually producing prostaglandin E_2 (PGE_2) (28, 29). COX-2 is believed to drive production of estrogen in the breast, in turn facilitating tumorigenesis (30), as evidenced by a positive correlation between 1) the level of COX-2 and expression of CYP19 in human breast cancer (31) and 2)

increased aromatase gene (P450) expression, the product of CYP19, in cultured breast cells (29, 32). This paracrine loop may explain why inhibition of COX-2 activity could have a protective effect on breast cancer (33) and quite possibly, mammographic density. TNF-α exerts its effects by binding to two transmembrane cell surface receptors: TNF receptor 1 (TNFR1, also referred to as p55, p60, TNFRSF1A) and the TNF receptor 2 (TNFR2, p75, p80, TNFRSF1B) (34, 35), both of which are expressed in virtually all mammalian cells, including mammary epithelial cells (36). The TNFRs have similar ligand-binding domains, but each differs in cytoplasmic domains, suggesting distinct signal transduction pathways. TNF-α binds to the two receptors with similar affinity; when engaged, the extracellular domains of the soluble TNF receptors (sTNFRs) may be shed into the circulation (35). These shed sTNFRs can compete for TNF-α with cell surface receptors and thus block TNF-α activity (37).

To date, only one nested case-control study has prospectively examined the relationship between serum levels of sTNFRs and breast cancer risk (38). The investigators found no association between serum levels of the soluble receptors TNFR1 and TNFR2 and breast cancer risk; however, this study had limited power to detect an association in postmenopausal women, with only 61 postmenopausal case-control pairs (38). In light of the evidence linking TNF-α to estrogen synthesis and cellular proliferation in the breast, and the potential for sTNFRs to inhibit TNF-α, we sought to examine the association between circulating sTNFR1 and sTNFR2 levels with mammographic density among cancer-free postmenopausal women.

3.3 METHODS

The Mammograms and Masses Study (MAMS) is a cross-sectional study of correlates of mammographic density. Pre- and postmenopausal women were recruited between 2001 and 2005 through the Magee Womens Hospital Mammographic Screening and Diagnostic Imaging Program in the greater Pittsburgh area (Pennsylvania, USA). Women were excluded from the study if they reported a previous personal history of any cancer (except nonmelanoma skin cancer). Volunteers arose from two sources: 1) women undergoing outpatient needle breast biopsy through the Breast Biopsy Service at Magee-Womens Hospital (Pittsburgh, PA) and 2) women receiving screening mammography through Magee-Womens Hospital or through a

suburban Pittsburgh Magee Womancare Center. To identify and recruit eligible participants, a trained research assistant personally solicited patients visiting the Breast Biopsy Service between September 2001 and May 2005 and women visiting Magee-Womencare Center North (Wexford, PA) and East (Monroeville, PA) between July 2002 and September 2003. To boost recruitment, Magee-Womens Hospital attached study flyers to screening result reports mailed to Magee-Womancare Center patients with negative mammography between November 2003 and April 2005. The procedures followed were in accordance with both the Magee-Womens Hospital and the University of Pittsburgh's Institutional Review Boards, and all participants gave informed consent.

For this ancillary study, only cancer-free postmenopausal women were considered because both cytokine levels (39) and mammographic density (40) vary with the menstrual cycle. In addition, we excluded women who were taking hormone therapy at study enrollment, the time of blood draw, as HT use is related to elevations in mammographic density (3, 11, 22), and the relationship between HT and circulating sTNFR levels has not been well-established (41). We further restricted the population to women with completed study questionnaires and available sTNFR and mammographic density results. Of 856 Breast Biopsy Service patients approached, 573 (67%) women lacked a personal cancer history, provided informed consent, completed a personal interview, and provided a blood sample. A subsequent review of breast biopsy pathology reports verified non-breast cancer outcomes in 311 (54% of 573) women and confirmed primary breast cancer in 262 (46% of 573) women; breast cancer cases were excluded from this report. Of the 311 cancer-free women, 109 (35% of 311) were postmenopausal and not taking HT at study enrollment. Of these, 69 (63% of 109) had available questionnaire, sTNFR and mammographic density results. Of approximately 100 Magee-Womancare Center North and East patients approached directly, 86 women lacked a personal cancer history, provided informed consent, completed a personal interview, and provided a blood sample. Subsequent follow-up verified non-breast cancer outcomes in 85 (99%) women and a primary breast cancer in one remaining woman; this breast cancer case was excluded. Of the 85 cancer-free women, 43 (51%) were postmenopausal and not taking HT at study enrollment; of these, 30 (70%) had available questionnaire, sTNFR and mammographic density results. Finally, mailing study flyers to 21,606 women with negative mammography produced 1,025 responses (5%), including 857 (84% of 1,025) responses from women without a personal cancer history. Of 451 (53% of 857)

women providing informed consent and a blood sample, 297 (66% of 451) were postmenopausal and not taking HT at study enrollment. Of the 297 women, 277 (93%) had available questionnaire, sTNFR and mammographic density results. Thus, 376 women, consisting of 69 (18%) from the Breast Biopsy Service, 30 (8%) from Magee-Womancare Center North/East, and 277 (74%) from the mass mailings, were included in the present analysis. The 376 women were similar to the 73 women who were excluded due to missing questionnaire and/or mammographic density results with respect to age and body mass index; however, women excluded from the present study were less likely to attend post-secondary education, to walk for exercise, and to be nulliparous, and they were more likely to have been enrolled in the Breast Biopsy Service. Women excluded from the study due to missing questionnaire data were very similar to the 376 women with respect to mammographic density.

3.3.1 Data collection

At study enrollment, trained clinical staff conducted a personal interview and recorded information on standardized study forms including age, race, menopausal status, history of hormone therapy use, aspirin or other anti-inflammatory drug use in the last 48 hours, weight without shoes or heavy clothing (measured in kilograms with a standard balance beam scale), and height without shoes at full inspiration (measured in centimeters with a stadiometer). Weight and height were used to calculate body mass index (BMI, weight in kilograms divided by height in meters squared).

Lifestyle and reproductive history were obtained through a standardized self-administered take-home questionnaire, including education (high school graduate vs. any post-secondary education), cigarette smoking (current, former, never), number of alcohol drinks per week (among those who reported consuming alcohol ≥1/week for ≥6 months), takes walks for exercise ≥10 minutes without stopping (rarely/1-3 times per month vs. at least 2-3 times per week), parity, breastfeeding duration (never or ≤1 month, 1-12 months, 13+ months), type of menopause (surgical vs. natural), and number of breast biopsies prior to study enrollment (0, 1, 2+). Current alcohol use was defined as reported consumption of beer, wine, or spirits for ≥1/week for ≥6 months during the year prior to study enrollment. Ethanol exposure in grams/day was calculated and standardized across the different types of alcoholic beverages as

previously reported (42). Current alcohol consumption was defined as: no current use, <12 g/day (the equivalent of ~1 alcoholic beverage/day), ≥12 g/day. A family history of breast cancer was defined as a report of breast cancer in a participant's mother or sister. Age at menarche (<12, 12-13, ≥14) and age at first live birth (<30 vs. ≥30 or nulliparous) were categorized according to the Gail Model for 5-year risk of breast cancer (43). Age at menopause was defined according to methods outlined by the Women's Health Initiative (44), where age at menopause was the minimum age at which the participant last had any natural menstrual bleeding, had a bilateral oophorectomy, or began using HT. For a hysterectomized woman without a bilateral oophorectomy, age at menopause was the earliest age at which she began using HT or first had menopausal symptoms. If neither occurred and her age at hysterectomy was 50 years or older, then age at menopause was her age at hysterectomy (44). In this report, one participant has a missing value for her age at menopause because she: 1) did not have a hysterectomy, and 2) has a missing value for age last had any menstrual bleeding, and 3) has a missing value for age of bilateral oophorectomy, and 4) has a missing value for age beginning HT. An additional nine participants have a missing value for their age at menopause because they: 1) had a hysterectomy, but not a bilateral oophorectomy, and 2) had their hysterectomy when <50 years of age, and 3) had a missing value for age beginning HT, and 4) had a missing value for age at which she first experienced menopausal symptoms. All ten women missing age at menopause were age 51 or greater at study enrollment. Years since menopause was calculated by subtracting age at menopause from age at study enrollment.

3.3.2 Non-steroidal anti-inflammatory drug exposure

Non-steroidal anti-inflammatory drug use among MAMS participants was recorded in three different ways. First, at study enrollment, trained clinical staff asked participants if they had used aspirin or another anti-inflammatory agent within the last 48 hours (yes/no). Second, medication use was self-reported in the study questionnaire, with an open-ended question asking participants to list all medications they were currently taking. Lastly, in February 2005, IRB-approval was obtained to send a follow-up questionnaire to capture medications participants may have taken for pain or inflammation prior to study enrollment. The follow-up survey was sent to all MAMS participants who indicated on their study consent form that they agreed to be

contacted by researchers at a future time to answer additional questions. All 376 women included in the present study had agreed to follow-up. To date, 304 (81%) of the follow-up surveys for participants included in this report have been returned, reviewed, and entered into the MAMS database. Each mini-survey was accompanied with a personalized cover letter explaining that investigators were interested in learning about medications taken for pain or inflammation before study enrollment, and each woman's enrollment date was clearly specified in the cover letter and throughout the mini-survey in bold face type. Participants were asked to look at three lists of medications and then to answer the question, "During the year before you joined our study, did you ever take any of these products on a regular basis—that is, for at least once a week, every week, for 6 months or more?" Separate lists were shown for aspirin (e.g. Aspirin, Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Emprin, Another Aspirin Product), acetaminophen (e.g. Tylenol, Anacin III, Acetaminophen, APAP), and non-aspirin NSAIDs (e.g. Advil, Nuprin, Ibuprofen, Motrin, Naproxen). If the answer was yes, participants were asked to indicate for how many days per week (1, 2-4, or 5-7), on average, they took each type of medication. Participants were also asked for duration of use (in months) for each type of medication. We used information from both questions to create a new variable indicating daily use of each medication for one year or more (yes/no). In this report, "any NSAID" combines the use of aspirin and non-aspirin NSAIDs. After editing study questionnaires for completeness and consistency, a trained research assistant telephoned subjects, when necessary, to retrieve missing information and to resolve inconsistencies.

3.3.3 Mammographic density assessment

Copies of screen-film mammograms completed within ~4.5 months of study enrollment (95% completed within 3 months; mean (SD) =33 (24) days), were assessed by a consultant expert reader (M Salane), initially trained by Wolfe in both Wolfe's method and planimetry (7). With respect to women enrolled through the Breast Biopsy Service, the unaffected side was sent for evaluation, with the exception of five women, for whom only the affected side was available and assessed. The cranio-caudal view of one breast chosen at random was evaluated for each participant. For two women, only the medio-lateral view was available and assessed. Density measurements from both sides and views have shown a high degree of symmetry (45).

Both a qualitative method, Wolfe's classification, and a quantitative method were used to assess breast density. As Wolfe's classification method is subjective and may vary between observers (46), the quantitative measurements have been deemed more effective in identifying women at increased risk for developing breast cancer (47, 48). Indeed, the majority of studies have shown a stronger association with breast cancer risk for the quantitative methods than for those using Wolfe's classification (3). For this analysis, we therefore chose to examine the quantitative measures only. Using the mammogram image and excluding biopsy scars, Cooper's ligaments, and breast masses, the reader used a wax pencil to outline the entire breast and the portions of breast containing radio-densities. The reader used a compensating polar planimeter (LASICO, Los Angeles, CA) and traced the outline of the entire breast and outlines of dense breast to compute total breast area and dense breast area, respectively. Percentage breast density was calculated by dividing the dense breast area by the total area. Nondense area was calculated by subtracting dense breast area from total breast area. All films were relabeled with a study ID so that the reader remained masked to the participant's identity. We assessed the internal reliability of the reader's readings by randomly sending a masked set of 28 mammograms (8 from the lowest tertile of percent breast density, and 10 each from the remaining two tertiles of percent breast density) for re-review. The intraclass correlation coefficient (ICC) for intra-observer agreement was ρ=0.86 for the continuous measurement of area of dense tissue, ρ=0.99 for total area of the breast, and ρ=0.89 for the measurement of percent breast density. Our ICC estimate for percent breast density is consistent with estimates reported by Boyd et al. (49), who observed an ICC of ρ=0.897 for 150 sets of films in the Canadian National Breast Screening Study, and Byrne et al. (50), who reported an ICC of ρ=0.93 for computer-assisted breast density measurements in the Nurses' Health Study. In addition, the reader's reproducibility in our study is comparable to her reliability as evaluated in the Breast Cancer Detection Demonstration Project (BCDDP), with an ICC of ρ=0.915 (adjusted for case status, age, weight, and film type) for measurement of percent breast density in 193 sets of films (51).

3.3.4 Biological specimen collection

At study enrollment, trained clinical staff drew 40 mL of peripheral blood using standardized phlebotomy procedures; 20 mL was collected with EDTA anticoagulant, which provided 8 mL of plasma. The blood was processed immediately according to standardized protocols at the Magee Womens Hospital Satellite Clinical Research Center. Plasma was separated and placed into individually-labeled 1 mL cryovials and stored at –70°C until analyzed.

3.3.5 Soluble TNFR1 and TNFR2 assays

We used the Laboratory for Clinical Biochemistry Research at the University of Vermont (RP Tracy) and commercially available sTNFR1 and sTNFR2 antibody bead kits for human plasma (BioSource International, Camarillo, CA, USA) to measure sTNFR1 and sTNFR2 plasma levels. Multiplex immunoassays, combining the principle of a sandwich immunoassay with fluorescent-bead-based technology, were conducted according to the manufacturer's specifications. To minimize inter-assay variability, all assays were performed with a single lot of sTNFR kits for human plasma. The plates were read on a Bio-Rad Bio-Plex instrument (Hercules, CA) using Bio-Plex Manager Software Version 3.0, with the instrument calibrated using a low RP1 setting, and gates adjusted to 3000 and 10000. The standard curve ranges were 23,400 to 30 pg/mL and 11,400 to 20 pg/mL for sTNFR1 and sTNFR2, respectively. All samples read well within the standard curve range, with the exception of one potential outlier point for sTNFR1 (23,442.3 pg/mL). Assays were run in duplicate and were preformed by two different technicians, who were masked to the mammographic density results. Using control plasma, the laboratory reported within-assay coefficients of variation of 17.5% and 17.4% for sTNFR1 and sTNFR2 concentrations, respectively. The inter-assay coefficients of variation calculated from the analytic results for 40 masked duplicate plasma samples were 30.0% and 22.4% for sTNFR1 and sTNFR2 concentrations, respectively.

3.3.6 Statistical analysis

Descriptive statistics for all baseline measures were calculated (means for continuous measures and frequencies for nominal variables) to assess the distribution of demographic variables and potential confounding variables. Baseline characteristics were compared across quartiles of percent mammographic density, sTNFR1, and sTNFR2 using generalized linear models (analysis of variance) for continuous measures and the chi-squared test for discrete measures. Fisher's exact test for discrete measures was used when expected cell counts were less than five. Pearson's correlation was used to examine the magnitude of the relationship between sTNFR1, sTNFR2 and percent mammographic density. Fisher's z transformation was used to estimate 95% confidence limits for the correlation coefficients. Although percent mammographic density was the primary focus of this study, the best method of utilizing the information obtained from the dense and nondense components of a mammogram is currently under debate (52). We therefore report the associations between the sTNFRs and dense breast area, total breast area, and nondense breast area for descriptive purposes only, as we did not account for these multiple comparisons *a priori.*

Age at enrollment, BMI (kg/m^2), history of breast biopsy prior to study enrollment (yes/no), former hormone therapy use (yes/no), current alcohol use (yes/no), age at first birth (<30 vs. ≥30 or nulliparous), education (high school graduate vs. any post-secondary education), aspirin or other anti-inflammatory drug use with 48 hrs of blood draw (yes/no), age at menopause (continuous), years since menopause (continuous), site of enrollment (Breast Biopsy Service vs. screening mammography), number of live births (0, 1, 2, 3, 4, or 5+; continuous), nulliparity (yes/no), current bisphosphonate use (e.g. Risedronate, Alendronate; yes/no), and laboratory technician (tech #1 vs. #2) were all evaluated as potential confounding variables due to their known associations with breast density or with sTNFRs, evidence for a difference in the covariate across quartiles of percent breast density or sTNFRs ($p<0.10$), or concerns about possible bias due to use of two different laboratory technicians. Since age and BMI have been previously shown to be positively associated with circulating sTNFR levels (53-55) and negatively associated with percent mammographic density (56-62), the multivariate linear regression models assessing the relation between the sTNFRs and percent mammographic

density were first adjusted for age, and then for age and BMI. We then used forward, stepwise multivariable linear regression to develop a model describing the factors associated with percent breast density (excluding sTNFR1 and sTNFR2). The model building process proceeded as follows. First, we separately regressed on the outcome variable (percent mammographic density) each potential confounding variable. The variable that explained the largest proportion of the variation in percent breast density (R^2) was then selected as the first variable to be entered into the regression equation. Each remaining explanatory variable was then regressed on percent breast density jointly with the first variable, and partial F statistics were determined. The variable with the largest partial F statistic (providing the largest gain in explanatory power) was then added as the second variable in the multiple regression equation (p-value to enter model=0.10), and this process was repeated for the remaining variables until the final model was reached (e.g. the test for the partial F statistic was not significant for the variables not yet in the model). Finally, multiple linear regression was used to assess the relation of the sTNFRs with percent breast density controlling for: 1) age, 2) age and BMI, 3) covariates determined to explain the largest proportion of variation in percent breast density in the stepwise linear regression process described above, and 4) any other covariate shown to be significantly associated ($p<0.05$) with percent breast density in separate linear regression models. Nine women reported using hormone therapy within 3 months, but not within the 2 weeks, prior to study enrollment (blood draw); analyses were run with and without these women, and results remained essentially the same. Likewise, analyses were run with and without the potential outlier for sTNFR1; again, results remained essentially the same, and the participant was not excluded from analyses. sTNFR1 levels were natural logarithm transformed to meet correlation and regression assumptions. Both mammographic density and sTNFR2 levels were normally distributed and analyses were conducted with these variables in the natural scale. Bonferroni correction was used to control for Type I error across the 2 cytokine comparisons; a p-value of less than 0.025 (0.05/2) was required for statistical significance. All tests of statistical significance were two-tailed. Analyses were performed using SAS software release 8.02 (SAS Institute Inc., Cary, NC).

3.4 RESULTS

Among the 376 MAMS participants in this report, the mean (SD) age was 62 (8) years, ranging from 42-85 (Table 12). The majority of the population was Caucasian (94%) and attended post-secondary education (75%). The mean (SD) years since menopause was 14 (10), ranging from 1-43. The mean (SD) percent breast density was 29.7% (19.5), ranging from 0-94.9%. sTNFR1 and sTNFR2 levels were positively correlated with one another ($r=0.49$, $p<0.0001$). One hundred sixty three (44%) of this population reported taking aspirin or another anti-inflammatory agent within 48 hours of blood draw, a proportion similar to those who reported current use of aspirin and non-aspirin NSAIDs medications in their original study questionnaire (40%), and slightly less than those who reported any NSAID use at least once a week in the 12 months prior to study enrollment in their follow-up questionnaire (51%) (Table 13).

Table 14 shows the characteristics of the study population by quartiles of percent mammographic density. Women with higher percent mammographic density were younger, and thus had fewer years since menopause; had a lower BMI; were more likely to have attended post-secondary education, to report current consumption of alcohol, to be nulliparous and/or have a later age at first birth, to be former hormone therapy users, and to report a history of breast biopsy; and were less likely to have taken aspirin or another anti-inflammatory agent at blood draw than women with lower percent mammographic density. No other self-reported medication use was related to percent mammographic density.

In contrast, women with higher sTNFR1 and sTNFR2 levels had a higher BMI than women with lower sTNFR levels (Tables 15 and 16). Women with higher sTNFR1 were more likely to be enrolled in the Breast Biopsy Service, and tended to be less likely to have a family history of breast cancer than women with lower sTNFR1 levels, although this difference was not statistically significant. Women with higher sTNFR2 levels were older, had a greater number of years since menopause and were less educated than women with lower sTNFR2 levels. Self-reported current use of bisphosphonates in the study questionnaire differed across quartiles of sTNFR2, but no clear pattern emerged.

Before adjusting for covariates, both sTNFR1 and sTNFR2 were inversely correlated with percent mammographic density to a similar degree (r = -0.14, p=0.007, and r = -0.13, p=0.01, respectively) (Table 17). As expected, the opposite relationship was observed for total breast area and nondense breast area, which were both positively correlated with sTNFR1 and sTNFR2 levels (Tables 19 and 20). However, sTNFR levels did not appear to be associated with the dense area of the breast (r= -0.05, p=0.31 and r = -0.02, p=0.65) (Table 18).

Results of the multivariable linear regression analyses are shown in Tables 21-24. The inverse associations observed between the sTNFRs and percent mammographic density remained after adjustment for age (Table 21); however, age and each sTNFR explained only 3% of the variation in percent mammographic density (R^2=0.03). After adjustment for BMI, 24% of the variation in percent mammographic density was explained, but the inverse association between the sTNFRs and percent mammographic density diminished and was no longer statistically significant. The covariates associated with percent mammographic density in the stepwise model were age, BMI, ever had breast biopsy prior to enrollment, nulliparity and current alcohol consumption. Addition of these covariates, along with others individually associated with percent breast density, did not further influence the relationship between the sTNFRs and percent mammographic density, and increased the R^2 to 28% and 30%, respectively. No association was observed between the sTNFRs and dense breast area (Table 22), while positive age-adjusted associations resulted between the sTNFRs and both total and nondense breast areas (Tables 23 and 24). Again, these associations were no longer apparent after adjustment for BMI.

3.5 DISCUSSION

In this cross-sectional study of cancer-free, postmenopausal women, we investigated the association between circulating soluble TNF-alpha receptors-I and II with percent mammographic density. Although previous studies have suggested distinct signal transduction pathways for sTNFR1 and sTNFR2 (35), both receptors had similar relationships with mammographic density. The mean percent mammographic density was lower among women in the highest quartiles of circulating levels of sTNFR1 and sTNFR2. After adjustment for BMI, the inverse association initially observed between the circulating sTNFRs and percent

mammographic density disappeared. Thus, these data do not support an independent association between sTNFR1, sTNFR2, and percent mammographic density.

To our knowledge, this is the first study to examine the association between circulating sTNFRs and percent mammographic density. We chose to evaluate percent mammographic density, as the percentage appears to confer a greater risk and is the measure reported in the vast majority of studies (8, 63-65). Our initial findings of an inverse correlation between the sTNFRs and percent breast density were consistent with the idea that circulating sTNFRs may block TNF-α activity (37), thereby preventing induction of COX-2 gene expression (33), resulting in decreased biosynthesis of estrogen and ultimately reducing mammographic density, a marker of breast cancer risk. Furthermore, we observed positive correlations between the sTNFRs and the nondense area of the breast; most risk factors for breast cancer that are related to mammographic density have the opposite relationship with the nondense area of the mammogram, largely comprised of fat tissue (66). However, since body mass index correlates strongly and positively with both the nondense and total breast areas (52), and thus correlates inversely with percent breast density (60-62), potential confounding by adiposity is of particular concern when studying factors, such as circulating sTNFRs, which are positively correlated with BMI. Under such circumstances, investigators have recently argued for examination of the absolute area of dense breast tissue, instead of percent breast density (52, 67). Indeed, circulating sTNFRs were not associated with dense breast area, both before and after adjustment for potential confounding factors in our population.

In spite of the lack of a BMI-adjusted association between sTNFRs and mammographic density in this report, we cannot rule out the hypothesis that inflammation plays a role in mammographic density. Notably, MAMS participants who reported use of aspirin or another anti-inflammatory agent within 48 hours of blood collection were significantly more likely to have lower percent mammographic density. Consistent with this biologic mechanism, several epidemiologic studies have examined the association between use of NSAIDs and breast cancer risk (reviewed in (68) and (69)) with most, but not all (70, 71), of case-control studies finding risk reductions between ~20-40% (72-82). Results from prospective cohort studies have been less consistent, with seven studies finding no association (83-89), one study observing an increased risk (90), and five studies, including the WHI Observational Study, demonstrating a protective effect from use of NSAIDs (91-95). Recently the Women's Health Study, a

randomized controlled trial, found that alternate-day use of low-dose aspirin for an average of 10 years of treatment did *not* reduce the risk of breast cancer (96). Hence, while our finding may be biologically plausible, it is equally likely that this single association may have been a spurious finding due to multiple statistical comparisons. In addition, we observed no association between percent mammographic density and any of our other measures of NSAID exposure. Although previous studies have not evaluated the effect of aspirin and non-aspirin NSAIDs on mammographic density, several clinical trials are underway.

Some limitations of this study deserve consideration. The main limitation of this study is its cross sectional design. We only obtained one mammogram, and used a single measure of circulating sTNFRs, measured with fair reproducibility. Unlike TNF-α, however, which has a relatively short half-life in circulation, determination of sTNFR concentrations in healthy individuals at time lapses of one year demonstrated that the concentrations of the receptors are stable in each individual (correlation coefficients of 0.84 and 0.90 for sTNFR1 and sTNFR2, respectively), possibly reflecting genetically determined differences (97); this observation is supported by studies of identical twins, who unlike discordant twins, are more likely to have similar levels of sTNFRs (97). In addition, medication use was self-reported, and with the exception of a general question about use of "aspirin or anti-inflammatory agents in the last 48 hours" elicited by trained clinical staff, detailed use and dosage of specific medications were not recorded at the time of blood collection nor were they verified against pill bottles or prescription records. Self-reported information on duration and past use of NSAIDs in the MAMS follow-up survey is also subject to recall bias. For example, participants at a higher risk of breast cancer (and thus with greater breast density) may have been more likely to report NSAID use prior to study enrollment with greater frequency and duration; however, we did not observe any difference in mammographic density with self-reported use of any of the medications listed in follow-up survey. Finally, the lack of ethnic diversity within MAMS, in combination with the postmenopausal study population, reduces the generalizability of our findings to other ethnic groups and to younger women. Strengths of the study include the use of a quantitative, highly reproducible measure of mammographic density. Further, we assessed several reproductive and anthropometric variables potentially related to mammographic density and/or sTNFRs, and the relationships between these factors were consistent with previous studies of breast density (3, 9-11, 13-20, 22, 56-62, 98, 99) and sTNFR levels (53-55).

While the mechanism by which mammographic density increases breast cancer risk is unknown, it is thought that dense areas may be associated with increased cell proliferation (100). A recent retrospective study of diagnostic mammograms from women diagnosed with DCIS demonstrated that DCIS lesions occurred overwhelmingly in areas of mammographically dense tissue, suggesting that some characteristic of the dense tissue is directly influencing the carcinogenic process in the breast (101). While our findings suggest that mammographic density is independent of circulating sTNFRs in postmenopausal women, these findings should be replicated with improved measurement of sTNFRs in larger populations. We hope this report will encourage other investigators to examine potential mechanisms by which inflammation may be related to mammographic density and breast cancer risk.

Table 12. Characteristics of the study population, Mammograms and Masses Study (2001-2005)

Variable	n=376	% or (range)
Clinic, %		
Biopsy	69	18
Screening clinic	307	82
Age (years), *mean (SD)*	62 (8)	(42-85)
Race (%)		
White	353	94
Other	23	6
Education level (%)		
High school	93	25
> High school	274	75
Missing	9	
Body mass index §, *mean (SD)*	28.4 (6.1)	(16.8-46.6)
Cigarette Smoking (%)		
Never	219	58
Former	133	35
Current	23	6
Missing	1	
Cigarette Smoking (%)		
Never	219	58
Ever	156	42
Missing	1	
Current alcohol use ≥ 1/week for ≥ 6 months (%)		
No	263	72
Yes	104	28
Missing	9	
Current alcohol consumption ≥1/week for ≥6 months (%)		
No	263	72
Yes		
<12g/day	68	19
≥12g/day	35	9
Missing	10	
Walks for exercise (%)		
Rarely/1-3 times/month	148	40
At least 2-3 times/week	219	60
Missing	9	

Table 12 (continued)

Variable	n=376	% or (range)
Age at menarche (years), %		
<12	72	19
12-13	223	59
≥14	80	21
Missing	1	
Nulliparous (%)		
No	295	78
Yes	81	21
Parity (%)		
Nulliparous	81	21
1	43	11
2	113	30
3	79	21
4	32	9
5+	28	7
Age at first birth (years), %		
<30	249	66
≥30 or nulliparous	127	34
Breastfeeding (%)		
Never or ≤1 month	226	60
Ever	149	40
Missing	1	
Breastfeeding (%)		
Never or ≤1 month	226	60
Ever		
1-12 months	97	26
≥13 months	52	14
Missing	1	
Age at menopause (years), *mean (SD)*	48 (5)	(26-60)
Missing	10	
Years since menopause, *mean (SD)*	14 (10)	(1-43)
Missing	10	
Surgical menopause (%)		
No	340	93
Yes	26	7
Missing	10	

Table 12 (continued)

Variable	n=376	% or (range)
Hormone therapy (%)		
Never use	151	40
Past use	225	60
Family history of breast cancer † (%)		
No	318	85
Yes	54	14
Missing	4	
Breast biopsy prior to enrollment (%)		
Never	305	81
Ever	70	19
Missing	1	
Breast biopsy prior to enrollment (%)		
0	305	81
1	48	13
2+	22	6
Missing	1	
Dense breast area, *mean (SD)*	40.9 (26.2)	(0-188.1)
Total breast area, *mean (SD)*	162.5 (76.4)	(31.6-442.0)
Nondense breast area, *mean (SD)*	121.5 (78.0)	(3.6-389.0)
% Breast density, *mean (SD)*	29.7 (19.5)	(0-94.9)
TNFR1 pg/mL, *mean (SD)*	2794.7 (2314.9)	(107.6-23442.29)
TNFR2 pg/mL, *mean (SD)*	2662.6 (1202.8)	(84.3-8517.8)

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 13. Self-reported medication use in the Mammograms and Masses Study (MAMS), 2001-2005

Variable	n=376	%
Aspirin or other anti-inflammatory agent within 48 hours of blood draw		
% yes	163	44
Missing	2	
Self-reported current medication use in study questionnaire:		
Aspirin, % yes	111	29
Non-aspirin NSAID, % yes	53	14
Acetaminophen, % yes	22	6
Any NSAID, % yes	150	40
Cox-2 inhibitors, % yes	14	4
Selective Estrogen Receptor Modulator (Raloxifene or Tamoxifen), % yes	31	8
Bisphosphonates (Risedronate or Alendronate), % yes	51	14
Self-reported medication use in NSAIDs follow-up questionnaire:		
Aspirin ≥ 1/week in past 12 months, % yes	122	40
Missing	72	
Aspirin ~daily for 1 year or more, % yes	74	24
Missing	72	
Non-aspirin NSAID ≥ 1/week in past 12 months, % yes	69	23
Missing	72	
Non-aspirin NSAID ~daily for 1 year or more, % yes	19	6
Missing	72	
Acetaminophen ≥ 1/week in past 12 months, % yes	79	26
Missing	73	
Acetaminophen ~daily for 1 year or more, % yes	15	5
Missing	73	
Any NSAID ≥ 1/week in past 12 months, % yes	155	51
Missing	72	
Any NSAID ~daily for 1 year or more, % yes	88	29
Missing	72	

Table 14. Characteristics of the study population by percentage of mammographic density quartiles (Q), Mammograms and Masses Study (2001-2005), n=376

	Mammographic density (%)				
Variable	**Q1 (0-14.0)**	**Q2 (14.1-27.4)**	**Q3 (27.5-42.0)**	**Q4 (42.2-94.9)**	**p-value**
Median % mammographic density	8.6	20.4	34.1	56.6	
Median sTNFR1 pg/mL	2612.9	2522.7	2188.9	1961.5	
Median sTNFR2 pg/mL	2541.0	2680.6	2497.5	2433.9	
Mean (SD)					
Age (years)	62 (8)	63 (8)	63 (8)	60 (8)	0.03
Body mass index §	32.0 (6.1)	29.7 (5.9)	27.2 (5.1)	24.7 (4.6)	<0.0001
Age at menopause (years)	48 (5)	48 (5)	48 (6)	48 (5)	0.83
Missing	4	5	1	0	
Years since menopause	14 (9)	15 (10)	15 (10)	11 (9)	0.05
Missing	4	5	1	0	
Dense breast area	15.8 (10.9)	36.4 (15.8)	45.9 (17.8)	65.6 (28.1)	<0.0001
Total breast area	213.3 (79.9)	183.6 (78.4)	134.3 (52.4)	118.7 (49.3)	<0.0001
Nondense breast area	197.6 (76.3)	147.2 (64.8)	88.4 (36.0)	53.1 (28.8)	<0.0001
Frequency, n (%)					
Enrolled in biopsy clinic	12 (13)	14 (15)	19 (20)	24 (26)	0.10
White	88 (94)	86 (91)	90 (96)	89 (95)	0.65
> High school	62 (67)	63 (68)	72 (78)	77 (85)	0.02
Missing	2	2	2	3	
Ever smoker	40 (43)	39 (41)	43 (46)	34 (37)	0.64
Missing	0	0	0	1	
Current alcohol consumption ≥1/week for ≥6 months	15 (16)	24 (26)	31 (34)	34 (37)	0.01
Missing	2	3	2	2	
Walks for exercise at least 2-3 times/week	53 (58)	47 (51)	58 (63)	61 (67)	0.14
Missing	2	2	2	3	
Age at menarche (years)					0.68
<12	21 (22)	19 (20)	19 (20)	13 (14)	
12-13	53 (56)	52 (56)	59 (63)	59 (63)	
≥14	20 (21)	22 (24)	16 (17)	22 (23)	
Missing	0	1	0	0	
Nulliparous	15 (16)	15 (16)	20 (21)	31 (33)	0.01

Table 14 (continued)

Variable	Mammographic density (%) Q1 (0-14.0)	Q2 (14.1-27.4)	Q3 (27.5-42.0)	Q4 (42.2-94.9)	p-value
Age at first birth ≥30 years or nulliparous	27 (29)	26 (28)	32 (34)	42 (45)	0.05
Ever breastfed	37 (39)	36 (38)	38 (40)	38 (41)	0.98
Missing	0	0	0	1	
Surgical menopause	8 (9)	10 (11)	4 (4)	4 (4)	0.17
Missing	4	5	1	0	
Former hormone therapy use	44 (47)	56 (60)	69 (73)	56 (60)	0.003
Family history of breast cancer †	17 (18)	13 (14)	8 (9)	16 (17)	0.25
Missing	1	1	2	0	
Breast biopsy prior to enrollment	7 (7)	14 (15)	22 (23)	27 (29)	0.001
Missing	0	1	0	0	
Aspirin or other anti-inflammatory agent within 48 hours of blood draw	53 (56)	39 (41)	39 (42)	32 (34)	0.02
Missing	0	0	1	1	
Self-reported current medication use in study questionnaire:					
Any NSAID	39 (41)	39 (41)	37 (39)	35 (37)	0.92
Selective Estrogen Receptor Modulator	8 (9)	8 (9)	8 (9)	7 (7)	0.99
Bisphosphonates	9 (10)	11 (12)	18 (19)	13 (14)	0.25

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 15. Characteristics of the study population by sTNFR1 quartiles (Q), Mammograms and Masses Study (2001-2005), n=376

	sTNFR1 (pg/mL)				
Variable	**Q1 (107.6-1526.2)**	**Q2 (1530.7-2348.3)**	**Q3 (2371.0-3361.7)**	**Q4 (3369.7-23442.3)**	**p-value**
Median % mammographic density	34.1	25.0	27.2	21.2	
Median sTNFR1 pg/mL	1070.9	1865.7	2757.3	4322.2	
Median sTNFR2 pg/mL	1813.5	2280.2	2630.8	3243.1	
Mean (SD)					
Age (years)	61 (8)	62 (8)	62 (8)	62 (8)	0.39
Body mass index §	26.5 (5.2)	28.1 (5.2)	28.7 (5.9)	30.3 (7.3)	0.0003
Age at menopause (years)	49 (5)	48 (6)	48 (5)	49 (6)	0.79
Missing	1	2	2	5	
Years since menopause	12 (9)	15 (10)	14 (10)	14 (10)	0.31
Missing	1	2	2	5	
Dense breast area	44.2 (25.3)	37.7 (23.0)	41.7 (29.4)	40.1 (26.9)	0.39
Total breast area	138.2 (60.5)	161.1 (72.1)	165.7 (76.4)	184.9 (87.7)	0.0004
Nondense breast area	94.0 (56.9)	123.4 (70.3)	124.0 (80.5)	144.8 (92.3)	0.0001
Frequency, n (%)					
Enrolled in biopsy clinic	10 (11)	14 (15)	20 (21)	25 (27)	0.03
White	89 (95)	88 (94)	88 (94)	88 (94)	0.99
> High school	76 (81)	69 (76)	67 (73)	62 (69)	0.29
Missing	0	3	2	4	
Ever smoker	39 (41)	40 (43)	38 (40)	39 (41)	0.99
Missing	0	1	0	0	
Current alcohol consumption ≥1/week for ≥6 months	31 (33)	25 (27)	27 (30)	21 (23)	0.47
Missing	1	2	3	3	
Walks for exercise at least 2-3 times/week	57 (61)	56 (61)	60 (65)	46 (51)	0.25
Missing	0	3	2	4	
Age at menarche (years)					0.96
<12	18 (19)	16 (17)	18 (19)	20 (22)	
12-13	57 (61)	55 (59)	55 (59)	56 (60)	
≥14	19 (20)	23 (24)	21 (22)	17 (18)	
Missing	0	0	0	1	
Nulliparous	24 (26)	17 (18)	20 (21)	20 (21)	0.67

Table 15 (continued)

	sTNFR1 (pg/mL)				
Variable	**Q1 (107.6-1526.2)**	**Q2 (1530.7-2348.3)**	**Q3 (2371.0-3361.7)**	**Q4 (3369.7-23442.3)**	**p-value**
Age at first birth ≥30 years or nulliparous	37 (39)	31 (33)	29 (31)	30 (32)	0.6
Ever breastfed	36 (39)	38 (40)	34 (36)	41 (44)	0.76
Missing	1	0	0	0	
Surgical menopause	6 (6)	8 (9)	8 (9)	4 (4)	0.64
Missing	1	2	2	5	
Former hormone therapy use	61 (65)	54 (57)	58 (62)	52 (55)	0.54
Family history of breast cancer †	17 (18)	18 (19)	8 (9)	11 (12)	0.1
Missing	2	1	1	0	
Breast biopsy prior to enrollment	17 (18)	19 (20)	16 (17)	18 (19)	0.94
Missing	0	1	0	0	
Aspirin or other anti-inflammatory agent within 48 hours of blood draw	45 (48)	47 (50)	33 (35)	38 (41)	0.17
Missing	0	0	1	1	
Self-reported current medication use in study questionnaire:					
Any NSAID	42 (45)	45 (48)	33 (35)	30 (32)	0.08
Selective Estrogen Receptor Modulator	9 (10)	6 (6)	9 (10)	7 (7)	0.81
Bisphosphonates	15 (16)	12 (13)	14 (15)	10 (11)	0.72

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 16. Characteristics of the study population by sTNFR2 quartiles (Q), Mammograms and Masses Study (2001-2005), n=376

	sTNFR2 (pg/mL)				
Variable	**Q1 (84.3-1839.0)**	**Q2 (1845.0-2523.8)**	**Q3 (2529.7-3254.7)**	**Q4 (3260.2-8517.8)**	**p-value**
Median % mammographic density	32.4	24.4	27.2	24.3	
Median sTNFR1 pg/mL	1520.3	1989.3	2677.0	3325.0	
Median sTNFR2 pg/mL	1456.5	2232.8	2856.7	3958.2	
Mean (SD)					
Age (years)	60 (7)	61 (7)	63 (9)	64 (8)	0.009
Body mass index §	26.8 (5.3)	28.6 (5.9)	28.4 (6.1)	29.7 (6.7)	0.01
Age at menopause (years)	49 (5)	49 (5)	49 (5)	47 (6)	0.08
Missing	1	3	1	5	
Years since menopause	12 (9)	12 (9)	14 (10)	17 (10)	0.0008
Missing	1	3	1	5	
Dense breast area	42.3 (25.2)	39.6 (24.0)	41.5 (25.1)	40.3 (30.4)	0.90
Total breast area	139.0 (53.8)	170.6 (83.7)	164.4 (70.0)	175.8 (89.0)	0.005
Nondense breast area	96.8 (56.0)	131.0 (86.9)	122.9 (70.8)	135.4 (88.9)	0.003
Frequency, n (%)					
Enrolled in biopsy clinic	13 (14)	19 (20)	17 (18)	20 (21)	0.56
White	87 (93)	87 (93)	90 (96)	89 (95)	0.74
> High school	79 (86)	72 (77)	62 (67)	61 (68)	0.01
Missing	2	1	2	4	
Ever smoker	39 (41)	40 (43)	37 (40)	40 (43)	0.98
Missing	0	0	1	0	
Current alcohol consumption ≥1/week for ≥6 months	33 (35)	29 (31)	22 (24)	20 (22)	0.17
Missing	1	0	4	4	
Walks for exercise at least 2-3 times/week	58 (63)	61 (66)	54 (59)	46 (51)	0.21
Missing	2	1	2	4	
Age at menarche (years)					0.92
<12	19 (20)	19 (20)	15 (16)	19 (20)	
12-13	55 (59)	56 (60)	55 (59)	57 (61)	
≥14	20 (21)	19 (20)	24 (26)	17 (18)	
Missing	0	0	0	1	
Nulliparous	19 (20)	15 (16)	26 (28)	21 (22)	0.27

Table 16 (continued)

	sTNFR2 (pg/mL)				
Variable	**Q1 (84.3-1839.0)**	**Q2 (1845.0-2523.8)**	**Q3 (2529.7-3254.7)**	**Q4 (3260.2-8517.8)**	**p-value**
Age at first birth ≥30 years or nulliparous	32 (34)	31 (33)	36 (38)	28 (30)	0.67
Ever breastfed	45 (48)	30 (32)	39 (41)	35 (37)	0.13
Missing	1	0	0	0	
Surgical menopause	3 (3)	10 (11)	8 (9)	5 (6)	0.19
Missing	1	3	1	5	
Former hormone therapy use	58 (62)	57 (61)	55 (59)	55 (59)	0.96
Family history of breast cancer †	19 (20)	11 (12)	13 (14)	11 (12)	0.31
Missing	0	1	2	1	
Breast biopsy prior to enrollment	19 (20)	15 (16)	22 (23)	14 (15)	0.42
Missing	0	1	0	0	
Aspirin or other anti-inflammatory agent within 48 hours of blood draw	44 (47)	43 (46)	41 (44)	35 (37)	0.53
Missing	0	1	1	0	
Self-reported current medication use in study questionnaire:					
Any NSAID	38 (40)	41 (44)	39 (41)	32 (34)	0.57
Selective Estrogen Receptor Modulator	8 (9)	6 (6)	7 (7)	10 (11)	0.75
Bisphosphonates	16 (17)	9 (10)	7 (7)	19 (20)	0.03

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 17. Correlation between circulating sTNFR levels and percent mammographic density, Mammograms and Masses Study (2001-2005)

	Pearson correlation coefficient*	95% CI	p-value
sTNFR1 pg/mL (n=376)	-0.14	(-0.24, -0.04)	0.007
sTNFR2 pg/mL (n=376)	-0.13	(-0.23, -0.03)	0.01

*Pearson's correlation between the continuous measure of percentage of breast density and the continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2.

Table 18. Correlation between circulating sTNFR levels and dense breast area, Mammograms and Masses Study (2001-2005)

	Pearson correlation coefficient*	95% CI	p-value
sTNFR1 pg/mL (n=376)	-0.05	(-0.15, 0.05)	0.31
sTNFR2 pg/mL (n=376)	-0.02	(-0.12, 0.08)	0.65

*Pearson's correlation between the continuous measure of dense breast area and the continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2.

Table 19. Correlation between circulating sTNFR levels and total breast area, Mammograms and Masses Study (2001-2005)

	Pearson correlation coefficient*	95% CI	p-value
sTNFR1 pg/mL (n=376)	0.19	(0.09, 0.29)	0.0003
sTNFR2 pg/mL (n=376)	0.17	(0.07, 0.27)	0.0009

*Pearson's correlation between the continuous measure of total breast area and the continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2.

Table 20. Correlation between circulating sTNFR levels and nondense breast area, Mammograms and Masses Study (2001-2005)

	Pearson correlation coefficient*	95% CI	p-value
sTNFR1 pg/mL (n=376)	0.20	(0.10, 0.29)	<0.0001
sTNFR2 pg/mL (n=376)	0.17	(0.07, 0.27)	0.0007

*Pearson's correlation between the continuous measure of nondense breast area and the continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2.

Table 21. Relationship between circulating sTNFR levels and percent mammographic density, Mammograms and Masses Study (2001-2005)

	β (%)	SE	p-value	R^2†
sTNFR1 pg/mL				
+Age (n=376)	-3.60	1.39	0.01	0.03
+BMI (n=376)	-0.77	1.26	0.54	0.24
+MV § (n=366)	-1.00	1.23	0.42	0.28
+MV2 * (n=347)	-0.63	1.46	0.66	0.30
sTNFR2 pg/mL				
+Age (n=376)	-0.002	0.001	0.03	0.03
+BMI (n=376)	-0.0003	0.001	0.70	0.24
+MV § (n=366)	-0.0003	0.001	0.68	0.28
+MV2 * (n=347)	-0.0004	0.001	0.65	0.30

BMI=body mass index, MV=multivariable

†R^2 based on regression models of continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2 on the continuous measure of percentage of breast density.

§ Adjusted for the following variables: age (continuous), BMI (continuous), ever had breast biopsy prior to study enrollment (yes/no), nulliparous (yes/no), and current alcohol consumption (yes/no).

* Adjusted for the following variables: age (continuous), BMI (continuous), ever had breast biopsy prior to study enrollment (yes/no), nulliparous (yes/no), current alcohol consumption (yes/no), prior use of hormone therapy (yes/no), post-secondary education (yes/no), aspirin use within 48 hrs of blood draw (yes/no), laboratory technician (1/2), years since menopause (continuous), and site of enrollment (biopsy vs. screening).

Table 22. Relationship between circulating sTNFR levels and dense breast area, Mammograms and Masses Study (2001-2005)

	β (%)	SE	p-value	R^2†
sTNFR1 pg/mL				
+Age (n=376)	-1.81	1.89	0.34	0.005
+BMI (n=376)	-1.45	1.94	0.45	0.007
+MV § (n=366)	-1.73	1.90	0.36	0.09
sTNFR2 pg/mL				
+Age (n=376)	-0.0003	0.001	0.78	0.003
+BMI (n=376)	-0.0001	0.001	0.94	0.006
+MV § (n=366)	-0.0003	0.001	0.82	0.09

BMI=body mass index, MV=multivariable

†R^2 based on regression models of continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2 on the continuous measure of dense breast area.

§ Adjusted for the following variables: age (continuous), BMI (continuous), ever had breast biopsy prior to study enrollment (yes/no), nulliparous (yes/no), and post-secondary education (yes/no).

Table 23. Relationship between circulating sTNFR levels and total breast area, Mammograms and Masses Study (2001-2005)

	β (%)	SE	p-value	R^2†
sTNFR1 pg/mL				
+Age (n=376)	19.58	5.4	0.0003	0.04
+BMI (n=376)	3.08	3.97	0.44	0.51
sTNFR2 pg/mL				
+Age (n=376)	0.01	0.003	0.002	0.03
+BMI (n=376)	0.001	0.002	0.55	0.51

BMI=body mass index

†R^2 based on regression models of continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2 on the continuous measure of total breast area.

Table 24. Relationship between circulating sTNFR levels and nondense breast area, Mammograms and Masses Study (2001-2005)

	β (%)	SE	p-value	R^2†
sTNFR1 pg/mL				
+Age (n=376)	21.39	5.5	0.0001	0.04
+BMI (n=376)	4.54	4.02	0.26	0.51
sTNFR2 pg/mL				
+Age (n=376)	0.01	0.003	0.001	0.03
+BMI (n=376)	0.001	0.002	0.54	0.51

BMI=body mass index

†R^2 based on regression models of continuous levels of log transformed values for sTNFR1 and raw values for sTNFR2 on the continuous measure of nondense breast area.

3.6 LITERATURE CITED

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, and Thun MJ, *Cancer Statistics, 2006.* CA Cancer J Clin, 2006. **56**(2): p. 106-130.

2. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, and Hopper JL, *Heritability of mammographic density, a risk factor for breast cancer.* New England Journal of Medicine, 2002. **347**(12): p. 886-94.

3. Harvey JA and Bovbjerg VE, *Quantitative Assessment of Mammographic Breast Density: Relationship with Breast Cancer Risk.* Radiology, 2004. **230**(1): p. 29-41.

4. Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, and Meyer JE, *Relationship between mammographic and histologic features of breast tissue in women with benign biopsies.* Cancer, 1988. **61**(2): p. 266-71.

5. Fisher ER, Palekar A, Kim WS, and Redmond C, *The histopathology of mammographic patterns.* American Journal of Clinical Pathology, 1978. **69**(4): p. 421-6.

6. Page DL and Winfield AC, *The dense mammogram.* AJR. American Journal of Roentgenology, 1986. **147**(3): p. 487-9.

7. Wellings SR and Wolfe JN, *Correlative studies of the histological and radiographic appearance of the breast parenchyma.* Radiology, 1978. **129**(2): p. 299-306.

8. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, and Paterson AD, *Mammographic breast density as an intermediate phenotype for breast cancer.* The Lancet Oncology, 2005. **6**(10): p. 798-808.

9. Ernster VL, Sacks ST, Peterson CA, and Schweitzer RJ, *Mammographic parenchymal patterns and risk factors for breast cancer.* Radiology, 1980. **134**(3): p. 617-20.

10. de Waard F, Rombach JJ, Collette HJ, and Slotboom B, *Breast cancer risk associated with reproductive factors and breast parenchymal patterns.* J Natl Cancer Inst, 1984. **72**(6): p. 1277-82.

11. Christodoulakos GE, Lambrinoudaki IV, Panoulis KP, Vourtsi AD, Vlachos L, Georgiou E, and Creatsas GC, *The effect of various regimens of hormone replacement therapy on mammographic breast density.* Maturitas, 2003. **45**(2): p. 109-18.

12. *Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial.* Jama, 1995. **273**(3): p. 199-208.

13. Vachon CM, Kuni CC, Anderson K, Anderson VE, and Sellers TA, *Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States).* Cancer Causes and Control, 2000. **11**(7): p. 653-62.

14. Bergkvist L, Tabar L, Bergstrom R, and Adami HO, *Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program.* American Journal of Epidemiology, 1987. **126**(6): p. 1075-81.

15. de Stavola BL, Gravelle IH, Wang DY, Allen DS, Bulbrook RD, Fentiman IS, Hayward JL, and Chaudary MC, *Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study.* International Journal of Epidemiology, 1990. **19**(2): p. 247-54.

16. Grove JS, Goodman MJ, Gilbert FI, Jr., and Mi MP, *Factors associated with mammographic pattern.* British Journal of Radiology, 1985. **58**(685): p. 21-5.

17. Kaufman Z, Garstin WI, Hayes R, Michell MJ, and Baum M, *The mammographic parenchymal patterns of nulliparous women and women with a family history of breast cancer.* Clinical Radiology, 1991. **43**(6): p. 385-8.

18. Gram IT, Funkhouser E, and Tabar L, *Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women.* British Journal of Cancer, 1995. **71**(3): p. 647-50.

19. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao M-S, Khokha R, Martin L, and Boyd N, *The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 343-349.

20. Jeffreys M, Warren R, Gunnell D, McCarron P, and Smith GD, *Life course breast cancer risk factors and adult breast density (United Kingdom).* Cancer Causes and Control, 2004. **15**(9): p. 947-955.

21. Kelsey JL, Gammon MD, and John EM, *Reproductive factors and breast cancer.* Epidemiologic Reviews, 1993. **15**(1): p. 36-47.

22. Writing Group for the Women's Health Initiative I, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* Jama, 2002. **288**(3): p. 321-33.

23. Boyd NF, Lockwood GA, Martin LJ, Byng JW, Yaffe MJ, and Tritchler DL, *Mammographic density as a marker of susceptibility to breast cancer: a hypothesis.* IARC Scientific Publications, 2001. **154**: p. 163-9.

24. Reed MJ and Purohit A, *Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis.* Endocr Rev, 1997. **18**(5): p. 701-15.

25. Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, and Bulun SE, *Estrogen Regulates Expression of Tumor Necrosis Factor Receptors in Breast Adipose Fibroblasts.* J Clin Endocrinol Metab, 2004. **89**(8): p. 4018-4024.

26. Purohit A, Newman S, and Reed M, *The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer.* Breast Cancer Res, 2002. **4**(2): p. 65 - 69.

27. de Jong PC, Blankenstein MA, van de Ven J, Nortier JWR, Blijham GH, and Thijssen JHH, *Importance of local aromatase activity in hormone-dependent breast cancer: a review.* The Breast, 2001. **10**(2): p. 91-99.

28. Morita I, *Distinct functions of COX-1 and COX-2.* Prostaglandins Other Lipid Mediat, 2002. **68-69**: p. 165-75.

29. DuBois RN, *Aspirin and breast cancer prevention: the estrogen connection.* Jama, 2004. **291**(20): p. 2488-9.

30. Gasparini G, Longo R, Sarmiento R, and Morabito A, *Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?* Lancet Oncol, 2003. **4**(10): p. 605-15.

31. Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, and Robertson FM, *Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens.* Cancer Letters, 1999. **140**(1-2): p. 27-35.

32. Zhao Y, Agarwal VR, Mendelson CR, and Simpson ER, *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, 1996. **137**(12): p. 5739-42.

33. Howe LR, Subbaramaiah K, Brown AM, and Dannenberg AJ, *Cyclooxygenase-2: a target for the prevention and treatment of breast cancer.* Endocrine-Related Cancer, 2001. **8**(2): p. 97-114.

34. Hohmann H, Remy R, Brockhaus M, and van Loon A, *Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha).* J Biol Chem, 1989. **264**(25): p. 14927-14934.

35. Bazzoni F and Beutler B, *The tumor necrosis factor ligand and receptor families.* N Engl J Med, 1996. **334**(26): p. 1717-25.

36. Varela LM, Darcy KM, and Ip MM, *The Epidermal Growth Factor Receptor Is Not Required for Tumor Necrosis Factor-{alpha} Action in Normal Mammary Epithelial Cells.* Endocrinology, 1997. **138**(9): p. 3891-3900.

37. Aderka D, *The potential biological and clinical significance of the soluble tumor necrosis factor receptors.* Cytokine Growth Factor Rev, 1996. **7**(3): p. 231-40.

38. Krajcik RA, Massardo S, and Orentreich N, *No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(9): p. 945-6.

39. Verthelyi D and Klinman DM, *Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo.* Immunology, 2000. **100**(3): p. 384-390.

40. White E, Velentgas P, Mandelson MT, Lehman CD, Elmore JG, Porter P, Yasui Y, and Taplin SH, *Variation in mammographic breast density by time in menstrual cycle among women aged 40-49 years.* Journal of the National Cancer Institute, 1998. **90**(12): p. 906-10.

41. Brooks-Asplund EM, Tupper CE, Daun JM, Kenney WL, and Cannon JG, *Hormonal modulation of interleukin-6, tumor necrosis factor and associated receptor secretion in postmenopausal women.* Cytokine, 2002. **19**(4): p. 193-200.

42. Modugno F, Ness RB, and Allen GO, *Alcohol Consumption and the Risk of Mucinous and Nonmucinous Epithelial Ovarian Cancer.* Obstet Gynecol, 2003. **102**(6): p. 1336-1343.

43. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, and Mulvihill JJ, *Projecting individualized probabilities of developing breast cancer for white females who are being examined annually.[comment].* Journal of the National Cancer Institute, 1989. **81**(24): p. 1879-86.

44. Chen Z, Maricic M, Bassford TL, Pettinger M, Ritenbaugh C, Lopez AM, Barad DH, Gass M, and LeBoff MS, *Fracture Risk Among Breast Cancer Survivors: Results From the Women's Health Initiative Observational Study.* Arch Intern Med, 2005. **165**(5): p. 552-558.

45. Byng JW, Boyd NF, Little L, Lockwood G, Fishell E, Jong RA, and Yaffe MJ, *Symmetry of projection in the quantitative analysis of mammographic images.* Eur J Cancer Prev, 1996. **5**(5): p. 319-27.

46. Boyd NF, O'Sullivan B, Fishell E, Simor I, and Cooke G, *Mammographic patterns and breast cancer risk: methodologic standards and contradictory results.* J Natl Cancer Inst, 1984. **72**(6): p. 1253-9.

47. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, and Yaffe MJ, *Mammographic densities and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(12): p. 1133-44.

48. Gram I, Bremnes Y, Ursin G, Maskarinec G, Bjurstam N, and Lund E, *Percentage density, Wolfe's and Tabar's mammographic patterns: agreement and association with risk factors for breast cancer.* Breast Cancer Research, 2005. **7**(5): p. R854 - R861.

49. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, and Yaffe MJ, *Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study.* J Natl Cancer Inst, 1995. **87**(9): p. 670-5.

50. Byrne C, Hankinson SE, Pollak M, Willett WC, Colditz GA, and Speizer FE, *Insulin-like growth factors and mammographic density.* Growth Horm IGF Res, 2000. **10 Suppl A**: p. S24-5.

51. Benichou J, Byrne C, Capece LA, Carroll LE, Hurt-Mullen K, Pee DY, Salane M, Schairer C, and Gail MH, *Secular stability and reliability of measurements of the percentage of dense tissue on mammograms.* Cancer Detection and Prevention, 2003. **27**(4): p. 266-274.

52. Kaaks R, Diorio C, and Brisson J, *Insulin-Like Growth Factor-I and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(12): p. 3019-.

53. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, and Coppack SW, *Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo.* Am J Physiol, 1999. **277**(6 Pt 1): p. E971-5.

54. Hauner H, Bender M, Haastert B, and Hube F, *Plasma concentrations of soluble TNF-alpha receptors in obese subjects.* Int J Obes Relat Metab Disord, 1998. **22**(12): p. 1239-43.

55. Hasegawa Y, Sawada M, Ozaki N, Inagaki T, and Suzumura A, *Increased soluble tumor necrosis factor receptor levels in the serum of elderly people.* Gerontology, 2000. **46**(4): p. 185-8.

56. Whitehouse GH and Leinster SJ, *The variation of breast parenchymal patterns with age.* British Journal of Radiology, 1985. **58**(688): p. 315-8.

57. Whitehead J, Carlile T, Kopecky KJ, Thompson DJ, Gilbert FI, Jr., Present AJ, Threatt BA, Krook P, and Hadaway E, *Wolfe mammographic parenchymal patterns. A study of the masking hypothesis of Egan and Mosteller.* Cancer, 1985. **56**(6): p. 1280-6.

58. van Gils CH, Otten JD, Verbeek AL, and Hendriks JH, *Short communication: breast parenchymal patterns and their changes with age.* British Journal of Radiology, 1995. **68**(814): p. 1133-5.

59. Riza E, dos Santos Silva I, De Stavola B, Perry N, Karadedou-Zafiriadou E, Linos D, Remoundos D-D, and Linos A, *Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece.* European Journal of Cancer, 2005. **41**(4): p. 590-600.

60. McCormack VA, dos Santos Silva I, De Stavola BL, Perry N, Vinnicombe S, Swerdlow AJ, Hardy R, and Kuh D, *Life-course body size and perimenopausal mammographic*

parenchymal patterns in the MRC 1946 British birth cohort. Br J Cancer, 2003. **89**(5): p. 852-9.

61. Lam PB, Vacek PM, Geller BM, and Muss HB, *The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont.* Cancer, 2000. **89**(2): p. 369-75.

62. Modugno F, Ngo D, Allen G, Kuller L, Ness R, Vogel V, Costantino J, and Cauley J, *Breast cancer risk factors and mammographic breast density in women over age 70.* Breast Cancer Research and Treatment, 2005: p. 1-10.

63. Byrne C, Schairer C, Wolfe J, Parekh N, Salane M, Brinton LA, Hoover R, and Haile R, *Mammographic features and breast cancer risk: effects with time, age, and menopause status.* Journal of the National Cancer Institute, 1995. **87**(21): p. 1622-9.

64. Torres-Mejia G, De Stavola B, Allen DS, Perez-Gavilan JJ, Ferreira JM, Fentiman IS, and dos Santos Silva I, *Mammographic Features and Subsequent Risk of Breast Cancer: A Comparison of Qualitative and Quantitative Evaluations in the Guernsey Prospective Studies.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(5): p. 1052-1059.

65. Ursin G, Ma H, Wu AH, Bernstein L, Salane M, Parisky YR, Astrahan M, Siozon CC, and Pike MC, *Mammographic density and breast cancer in three ethnic groups.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(4): p. 332-8.

66. Heng D, Gao F, Jong R, Fishell E, Yaffe M, Martin L, Li T, Stone J, Sun L, Hopper J, and Boyd NF, *Risk Factors for Breast Cancer Associated with Mammographic Features in Singaporean Chinese Women.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(11): p. 1751-1758.

67. Haars G, van Noord PAH, van Gils CH, Grobbee DE, and Peeters PHM, *Measurements of Breast Density: No Ratio for a Ratio.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2634-2640.

68. Khuder SA and Mutgi AB, *Breast cancer and NSAID use: a meta-analysis.* British Journal of Cancer, 2001. **84**(9): p. 1188-92.

69. Harris RE, Beebe-Donk J, Doss H, and Burr Doss D, *Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review).* Oncol Rep, 2005. **13**(4): p. 559-83.

70. Langman MJS, Cheng KK, Gilman EA, and Lancashire RJ, *Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database.* BMJ, 2000. **320**(7250): p. 1642-1646.

71. Neugut A, Rosenberg D, Ahsan H, Jacobson J, Wahid N, Hagan M, Rahman M, Khan Z, Chen L, Pablos-Mendez A, and Shea S, *Association between coronary heart disease and cancers of the breast, prostate, and colon.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(10): p. 869-873.

72. Coogan PF, Rao SR, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD, and Shapiro S, *The Relationship of Nonsteroidal Anti-inflammatory Drug Use to the Risk of Breast Cancer,.* Preventive Medicine, 1999. **29**(2): p. 72-76.

73. Cotterchio M, Kreiger N, Sloan M, and Steingart A, *Nonsteroidal Anti-inflammatory Drug Use and Breast Cancer Risk.* Cancer Epidemiol Biomarkers Prev, 2001. **10**(11): p. 1213-1217.

74. Harris RE, Namboodiri K., Stellman S. D., and Wynder E. L., *Breast Cancer and NSAID Use: Heterogeneity of Effect in a Case-Control Study.* Preventive Medicine, 1995. **24**(2): p. 119-120.

75. Harris RE, Namboodiri KK, and Farrar WB, *Nonsteroidal antiinflammatory drugs and breast cancer.* Epidemiology, 1996. **7**(2): p. 203-5.

76. Meier CR, Schmitz S, and Jick H, *Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer.* Pharmacotherapy, 2002. **22**(3): p. 303-9.

77. Moorman P, Grubber J, Millikan R, and Newman B, *Association between non-steroidal anti-inflammatory drugs (NSAIDs) and invasive breast cancer and carcinoma in situ of the breast.* Cancer Causes and Control, 2003. **14**(10): p. 915-922.

78. Rahme E, Ghosn J, Dasgupta K, Rajan R, and Hudson M, *Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer.* BMC Cancer, 2005. **5**(1): p. 159.

79. Rosenberg L, *Nonsteroidal Anti-inflammatory Drugs and Cancer.* Preventive Medicine, 1995. **24**(2): p. 107-109.

80. Swede H, Mirand AL, Menezes RJ, and Moysich KB, *Association of Regular Aspirin Use and Breast Cancer Risk.* Oncology, 2005. **68**(1): p. 40-47.

81. Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, Subbaramaiah K, Dannenberg AJ, and Neugut AI, *Association of Frequency and Duration of Aspirin Use and Hormone Receptor Status With Breast Cancer Risk.* JAMA, 2004. **291**(20): p. 2433-2440.

82. Zhang Y, Coogan PF, Palmer JR, Strom BL, and Rosenberg L, *Use of Nonsteroidal Antiinflammatory Drugs and Risk of Breast Cancer: The Case-Control Surveillance Study Revisited.* Am J Epidemiol, 2005. **162**(2): p. 165-170.

83. Egan K, Stampfer M, Giovannucci E, Rosner B, and Colditz G, *Prospective study of regular aspirin use and the risk of breast cancer.* J Natl Cancer Inst, 1996. **88**(14): p. 988a-993.

84. Friis S, Sorensen HT, McLaughlin JK, Johnsen SP, Blot WJ, and Olsen JH, *A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin.* Br J Cancer, 2003. **88**(5): p. 684-8.

85. Garcia Rodriguez LA and Gonzalez-Perez A, *Risk of breast cancer among users of aspirin and other anti-inflammatory drugs.* Br J Cancer, 2004. **91**(3): p. 525-9.

86. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, Patel AV, Flanders WD, and Calle EE, *Aspirin and Other Nonsteroidal Anti-inflammatory Drugs and Breast Cancer Incidence in a Large U.S. Cohort.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(1): p. 261-264.

87. Paganini-Hill A, Chao A, Ross RK, and Henderson BE, *Aspirin use and chronic diseases: a cohort study of the elderly.* Bmj, 1989. **299**(6710): p. 1247-50.

88. Sorensen HT, Friis S, Norgard B, Mellemkjaer L, Blot WJ, McLaughlin JK, Ekbom A, and Baron JA, *Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study.* Br J Cancer, 2003. **88**(11): p. 1687-92.

89. Thun M, Namboodiri M, Calle E, Flanders W, and Heath C, Jr, *Aspirin use and risk of fatal cancer.* Cancer Res, 1993. **53**(6): p. 1322-1327.

90. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A, and Ross RK, *Nonsteroidal Anti-Inflammatory Drug Use and Breast Cancer Risk by Stage and Hormone Receptor Status.* J Natl Cancer Inst, 2005. **97**(11): p. 805-812.

91. Harris RE, Kasbari S, and Farrar WB, *Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer.* Oncol Rep, 1999. **6**(1): p. 71-3.

92. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, and McTiernan A, *Breast Cancer and Nonsteroidal Anti-Inflammatory Drugs: Prospective Results from the Women's Health Initiative.* Cancer Res, 2003. **63**(18): p. 6096-6101.

93. Johnson TW, Anderson KE, Lazovich D, and Folsom AR, *Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(12): p. 1586-1591.

94. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, and Hanley JA, *Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage.* Br J Cancer, 2000. **83**(1): p. 112-20.

95. Schreinemachers DM and Everson RB, *Aspirin use and lung, colon, and breast cancer incidence in a prospective study.* Epidemiology, 1994. **5**(2): p. 138-46.

96. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE, *Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial.* JAMA, 2005. **294**(1): p. 47-55.

97. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, and Wallach D, *Variation in serum levels of the soluble TNF receptors among healthy individuals.* Lymphokine and Cytokine Research, 1992. **11**(3): p. 157-9.

98. Maskarinec G, Takata Y, Pagano I, Lurie G, Wilkens LR, and Kolonel LN, *Alcohol consumption and mammographic density in a multiethnic population.* Int J Cancer, 2006. **118**(10): p. 2579-83.

99. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, and Palli D, *Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population.* International Journal of Cancer, 2006. **118**(7): p. 1782-1789.

100. Harrison DA, Duffy SW, Sala E, Warren RML, Couto E, and Day NE, *Deterministic models for breast cancer progression:: Application to the association between mammographic parenchymal pattern and histologic grade of breast cancers.* Journal of Clinical Epidemiology, 2002. **55**(11): p. 1113-1118.

101. Ursin G, Hovanessian-Larsen L, Parisky Y, Pike M, and Wu A, *Greatly increased occurrence of breast cancers in areas of mammographically dense tissue.* Breast Cancer Research, 2005. **7**(5): p. R605 - R608.

4.0 A POLYMORPHISM IN THE TUMOR NECROSIS FACTOR-ALPHA RECEPTOR II GENE, CIRCULATING SOLUBLE TNFR-II, AND MAMMOGRAPHIC DENSITY: THE MAMMOGRAMS AND MASSES STUDY (MAMS)

To be submitted for publication

Gretchen L. Gierach, PhD, MPH,[1] Francesmary Modugno, PhD, MPH,[1, 2] Jane A. Cauley, DrPH, MPH,[1] Joel L. Weissfeld, MD, MPH,[1, 2] John W. Wilson, PhD,[3, 4] Jennifer K. Simpson, PhD,[1] Glenn O. Allen, MPH,[1, 2] Marjorie Romkes, PhD,[5, 6] and Victor G. Vogel, MD, MHS[6]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]National Surgical Adjuvant Breast and Bowel Project Center, University of Pittsburgh, Pittsburgh, PA

[5]Center for Clinical Pharmacology, University of Pittsburgh, Pittsburgh, PA

[6]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA

Running Title: Mammographic density and TNFR2 gene

This work was supported in part by National Institutes of Health grants R25-CA57703, K07-CA80668, and R21-CA95113; by Department of Defense grants DAMD17-02-1-0553 and by PA Department of Health grant P2777693. Additional support was provided by funds received from the NIH/NCRR/GCRC Grant MO1-RR000056.

4.1 ABSTRACT

Background: Studies have demonstrated a strong heritability component for both mammographic density and circulating soluble tumor necrosis factor-alpha (TNF-α) receptor levels. Exactly how mammographic density increases breast cancer risk is unknown, although it has been posited that dense breast areas may reflect exposure to estrogen. TNF-α has a central role in regulating estrogen synthesis in the breast, and circulating TNF receptors may block TNF-α activity. The TNFR2 gene contains a non-synonymous SNP with potential functional significance with respect to circulating sTNFR2 levels.

Methods: We examined the association between percent mammographic density and the TNFR2 –196 M/R polymorphism (T>G) among 376 cancer-free, mostly white, postmenopausal women participating in a cross-sectional study of mammographic density (Pittsburgh, PA, 2001-2005). Women were not taking hormone therapy at the time of blood collection. Percent breast density was calculated using a quantitative method (planimetry). We also evaluated whether plasma levels of sTNFR2 varied by TNFR2 genotype.

Results: The unadjusted mean (SD) percent breast density was higher in women with the TT genotype (32.3% (21.0)) as compared to women with the TG/GG genotypes (26.6% (17.2)), p=0.003. The association remained statistically significant after adjustment for age and body mass index (p=0.03); however, inclusion of additional confounding factors reduced the level of statistical significance (p=0.08). There was no observable difference in circulating sTNFR2 levels between the TNFR2 genotypes, either before or after adjustment for covariates.

Conclusion: Our findings offer little evidence for an independent association between the TNFR2 –196 M/R polymorphism and percent mammographic density among postmenopausal, white women.

4.2 INTRODUCTION

Early detection of breast cancer is critical for reducing mortality. Mammography is currently the primary screening tool available for early detection, with a reduction of ~25% in mortality associated with routine screening in women over age 50 (1). However, mammograms typically miss more than 15% of cancers (2). Notably, over 67% of the decrease in mammographic sensitivity is attributed to breast density (2). The histologic composition of the breast is reflected mammographically by density and parenchymal pattern. The higher the fat content of the breast the lower the radiologic density. Conversely, a high proportion of connective, ductal/epithelial, and glandular tissue increases density (3-6). In addition to its effect on mammographic sensitivity, mammographic density is a known risk factor for breast cancer (7, 8), and the risk associated with mammographic density is greater than that associated with almost all other risk factors for breast cancer (8, 9). Identifying factors that are associated with mammographic density can inform preventative strategies.

Many reproductive and hormonal factors associated with breast cancer (10-13) are also associated with breast density (8, 10-12, 14-23). However, these factors only account for ~20-30% of the variation observed in breast density in the population (9, 14). In fact, studies have demonstrated that breast density has a strong hereditary component (7). A cohort study of families with a history of breast cancer demonstrated evidence for a genetic effect as sister-sister correlations in breast density were significant (r=0.16-0.27) (24), and these results were further clarified in a sib-pair linkage analysis (25). A twin study conducted in Australia and North America estimated that genetic factors likely account for 63% of the unexplained variance in mammographic density in all twins studied (7). In contrast to women at low risk for developing breast cancer, women with known BRCA1 or BRCA2 mutations have been shown to have denser breast tissue (26). In addition, a study of 6146 women in the San Francisco Mammography Registry demonstrated an association between increased breast density with a positive family history of breast cancer (27).

Studies have investigated the association between mammographic density and polymorphisms in specific genes involved in regulating steroid hormone synthesis and

metabolism with varied results (28-38). The gene encoding for tumor necrosis factor-alpha receptor-II (TNFR2) has not yet been studied with respect to mammographic density. The cytokine tumor necrosis factor (TNF)-alpha, secreted by macrophages of the immune system and also by adipocytes, has a central role in regulating estrogen synthesis within both normal and malignant breast tissue (39-41). Moreover, TNF-α induces a range of inflammatory enzymes, including cyclooxygenase (COX)-2. COX-2 cyclizes and oxygenates arachidonic acid, eventually producing prostaglandin E_2 (PGE_2) (42, 43). PGE_2 is believed to drive production of estrogen in the breast, in turn facilitating tumorigenesis (44). TNF-α exerts its effects by binding to two transmembrane cell surface receptors: TNF receptor 1 and TNFR2 (also known as p75, p80, TNFRSF1B) (45, 46), both of which are expressed in virtually all mammalian cells, including mammary epithelial cells (47). TNF-α binds to the two receptors with similar affinity; when engaged, the extracellular domains of the soluble TNF receptors (sTNFRs) may be shed into the circulation (46). These shed sTNFRs can compete for TNF-α with cell surface receptors and thus block TNF-α activity (48).

Whereas the TNFR1 gene does not contain any known functional variants, the TNFR2 gene contains a non-synonymous single nucleotide polymorphism (SNP). The TNFR2 –196 M/R polymorphism (T > G; chromosome 1p36.2, exon 6; results in substitution of methionine by arginine; rs1061622) is located in the extracellular region of the receptor, the region responsible for its proteolytic cleavage and solubilization. Carriers of the G allele have been found to have higher circulating levels of sTNFR2 in previous studies (49, 50). We aimed to examine the association of the TNFR2 –196 M/R polymorphism with mammographic density and with plasma levels of sTNFR2 in cancer-free postmenopausal women.

4.3 METHODS

The Mammograms and Masses Study (MAMS) is a cross-sectional study of correlates of mammographic density. Pre- and postmenopausal women were recruited between 2001 and 2005 through the Magee Womens Hospital Mammographic Screening and Diagnostic Imaging Program in the greater Pittsburgh area (Pennsylvania, USA). Women were excluded from the study if they reported a previous personal history of any cancer (except nonmelanoma skin

cancer). Volunteers arose from two sources: 1) women undergoing outpatient needle breast biopsy through the Breast Biopsy Service at Magee-Womens Hospital (Pittsburgh, PA) and 2) women receiving screening mammography through Magee-Womens Hospital or through a suburban Pittsburgh Magee Womancare Center. To identify and recruit eligible participants, a trained research assistant personally solicited patients visiting the Breast Biopsy Service between September 2001 and May 2005 and women visiting Magee-Womencare Center North (Wexford, PA) and East (Monroeville, PA) between July 2002 and September 2003. To boost recruitment, Magee-Womens Hospital attached study flyers to screening result reports mailed to Magee-Womancare Center patients with negative mammography between November 2003 and April 2005. The procedures followed were in accordance with both the Magee-Womens Hospital and the University of Pittsburgh's Institutional Review Boards, and all participants gave informed consent.

For this ancillary study, only cancer-free postmenopausal women were considered because both cytokine levels (51) and mammographic density (52) vary with the menstrual cycle. In addition, we excluded women who were taking hormone therapy at study enrollment, the time of blood draw, as HT use is related to elevations in mammographic density (8, 12, 23), and the relationship between HT and circulating sTNFR2 levels has not been well-established (53). We further restricted the population to women with completed study questionnaires and available TNFR2 genotype, sTNFR2 and mammographic density results. Of 856 Breast Biopsy Service patients approached, 573 (67%) women lacked a personal cancer history, provided informed consent, completed a personal interview, and provided a blood sample. A subsequent review of breast biopsy pathology reports verified non-breast cancer outcomes in 311 (54% of 573) women and confirmed primary breast cancer in 262 (46% of 573) women; breast cancer cases were excluded from this report. Of the 311 cancer-free women, 109 (35% of 311) were postmenopausal and not taking HT at study enrollment. Of these, 69 (63% of 109) had available questionnaire, TNFR2 genotype, sTNFR2 and mammographic density results. Of approximately 100 Magee-Womancare Center North and East patients approached directly, 86 women lacked a personal cancer history, provided informed consent, completed a personal interview, and provided a blood sample. Subsequent follow-up verified non-breast cancer outcomes in 85 (99%) women and a primary breast cancer in one remaining woman; this breast cancer case was excluded. Of the 85 cancer-free women, 43 (51%) were postmenopausal and not taking HT at

study enrollment; of these, 30 (70%) had available questionnaire, TNFR2 genotype, sTNFR2 and mammographic density results. Finally, mailing study flyers to 21,606 women with negative mammography produced 1,025 responses (5%), including 857 (84% of 1,025) responses from women without a personal cancer history. Of 451 (53% of 857) women providing informed consent and a blood sample, 297 (66% of 451) were postmenopausal and not taking HT at study enrollment. Of the 297 women, 277 (93%) had available questionnaire, TNFR2 genotype, sTNFR2 and mammographic density results. Thus, 376 women, consisting of 69 (18%) from the Breast Biopsy Service, 30 (8%) from Magee-Womancare Center North/East, and 277 (74%) from the mass mailings, were included in the present analysis. The 376 women were similar to the 73 women who were excluded due to missing questionnaire and/or mammographic density results with respect to age and body mass index; however, women excluded from the present study were less likely to attend post-secondary education, to walk for exercise, and to be nulliparous, and they were more likely to have been enrolled in the Breast Biopsy Service. Women excluded from the study due to missing questionnaire data were very similar to the 376 women with respect to mammographic density.

4.3.1 Data collection

At study enrollment, trained clinical staff conducted a personal interview and recorded information on standardized study forms including age, race, menopausal status, history of hormone therapy use, aspirin or other anti-inflammatory drug use in the last 48 hours, weight without shoes or heavy clothing (measured in kilograms with a standard balance beam scale), and height without shoes at full inspiration (measured in centimeters with a stadiometer). Weight and height were used to calculate body mass index (BMI, weight in kilograms divided by height in meters squared).

Lifestyle and reproductive history were obtained through a standardized self-administered take-home questionnaire, including education (high school graduate vs. any post-secondary education), cigarette smoking (current, former, never), number of alcohol drinks per week (among those who reported consuming alcohol ≥1/week for ≥6 months), takes walks for exercise ≥10 minutes without stopping (rarely/1-3 times per month vs. at least 2-3 times per week), parity, breastfeeding duration (never or ≤1 month, 1-12 months, 13+ months), type of

menopause (surgical vs. natural), and number of breast biopsies prior to study enrollment (0, 1, 2+). Current alcohol use was defined as reported consumption of beer, wine, or spirits for ≥1/week for ≥6 months during the year prior to study enrollment. Ethanol exposure in grams/day was calculated and standardized across the different types of alcoholic beverages as previously reported (54). Current alcohol consumption was defined as: no current use, <12 g/day (the equivalent of ~1 alcoholic beverage/day), ≥12 g/day. A family history of breast cancer was defined as a report of breast cancer in a participant's mother or sister. Age at menarche (<12, 12-13, ≥14) and age at first live birth (<30 vs. ≥30 or nulliparous) were categorized according to the Gail Model for 5-year risk of breast cancer (55). Age at menopause was defined according to methods outlined by the Women's Health Initiative (56), where age at menopause was the minimum age at which the participant last had any natural menstrual bleeding, had a bilateral oophorectomy, or began using HT. For a hysterectomized woman without a bilateral oophorectomy, age at menopause was the earliest age at which she began using HT or first had menopausal symptoms. If neither occurred and her age at hysterectomy was 50 years or older, then age at menopause was her age at hysterectomy (56). In this report, one participant has a missing value for her age at menopause because she: 1) did not have a hysterectomy, and 2) has a missing value for age last had any menstrual bleeding, and 3) has a missing value for age of bilateral oophorectomy, and 4) has a missing value for age beginning HT. An additional nine participants have a missing value for their age at menopause because they: 1) had a hysterectomy, but not a bilateral oophorectomy, and 2) had their hysterectomy when <50 years of age, and 3) had a missing value for age beginning HT, and 4) had a missing value for age at which she first experienced menopausal symptoms. All ten women missing age at menopause were age 51 or greater at study enrollment. Years since menopause was calculated by subtracting age at menopause from age at study enrollment.

4.3.2 Non-steroidal anti-inflammatory drug exposure

Non-steroidal anti-inflammatory drug use among MAMS participants was recorded in three different ways. First, at study enrollment, trained clinical staff asked participants if they had used aspirin or another anti-inflammatory agent within the last 48 hours (yes/no). Second, medication use was self-reported in the study questionnaire, with an open-ended question asking

participants to list all medications they were currently taking. Lastly, in February 2005, IRB-approval was obtained to send a follow-up questionnaire to capture medications participants may have taken for pain or inflammation prior to study enrollment. The follow-up survey was sent to all MAMS participants who indicated on their study consent form that they agreed to be contacted by researchers at a future time to answer additional questions. All 376 women included in the present study had agreed to follow-up. To date, 304 (81%) of the follow-up surveys for participants included in this report have been returned, reviewed, and entered into the MAMS database. Each mini-survey was accompanied with a personalized cover letter explaining that investigators were interested in learning about medications taken for pain or inflammation before study enrollment, and each woman's enrollment date was clearly specified in the cover letter and throughout the mini-survey in bold face type. Participants were asked to look at three lists of medications and then to answer the question, "During the year before you joined our study, did you ever take any of these products on a regular basis—that is, for at least once a week, every week, for 6 months or more?" Separate lists were shown for aspirin (e.g. Aspirin, Anacin, Ascriptin, Bayer, Bufferin, Ecotrin, Emprin, Another Aspirin Product), acetaminophen (e.g. Tylenol, Anacin III, Acetaminophen, APAP), and non-aspirin NSAIDs (e.g. Advil, Nuprin, Ibuprofen, Motrin, Naproxen). If the answer was yes, participants were asked to indicate for how many days per week (1, 2-4, or 5-7), on average, they took each type of medication. Participants were also asked for duration of use (in months) for each type of medication. We used information from both questions to create a new variable indicating daily use of each medication for one year or more (yes/no). In this report, "any NSAID" combines the use of aspirin and non-aspirin NSAIDs. After editing study questionnaires for completeness and consistency, a trained research assistant telephoned subjects, when necessary, to retrieve missing information and to resolve inconsistencies.

4.3.3 Mammographic density assessment

Copies of screen-film mammograms completed within ~4.5 months of study enrollment (95% completed within 3 months; mean (SD) =33 (24) days), were assessed by a consultant expert reader (M Salane), initially trained by Wolfe in both Wolfe's method and planimetry (6). With respect to women enrolled through the Breast Biopsy Service, the unaffected side was sent for

evaluation, with the exception of five women, for whom only the affected side was available and assessed. The cranio-caudal view of one breast chosen at random was evaluated for each participant. For two women, only the medio-lateral view was available and assessed. Density measurements from both sides and views have shown a high degree of symmetry (57).

Both a qualitative method, Wolfe's classification, and a quantitative method were used to assess breast density. As Wolfe's classification method is subjective and may vary between observers (58), the quantitative measurements have been deemed more effective in identifying women at increased risk for developing breast cancer (59, 60). Indeed, the majority of studies have shown a stronger association with breast cancer risk for the quantitative methods than for those using Wolfe's classification (8). For this analysis, we therefore chose to examine the quantitative measures only. Using the mammogram image and excluding biopsy scars, Cooper's ligaments, and breast masses, the reader used a wax pencil to outline the entire breast and the portions of breast containing radio-densities. The reader used a compensating polar planimeter (LASICO, Los Angeles, CA) and traced the outline of the entire breast and outlines of dense breast to compute total breast area and dense breast area, respectively. Percentage breast density was calculated by dividing the dense breast area by the total area. Nondense area was calculated by subtracting dense breast area from total breast area. All films were relabeled with a study ID so that the reader remained masked to the participant's identity. We assessed the internal reliability of the reader's readings by randomly sending a masked set of 28 mammograms (8 from the lowest tertile of percent breast density, and 10 each from the remaining two tertiles of percent breast density) for re-review. The intraclass correlation coefficient (ICC) for intra-observer agreement was ρ=0.86 for the continuous measurement of area of dense tissue, ρ=0.99 for total area of the breast, and ρ=0.89 for the measurement of percent breast density. Our ICC estimate for percent breast density is consistent with estimates reported by Boyd et al. (61), who observed an ICC of ρ=0.897 for 150 sets of films in the Canadian National Breast Screening Study, and Byrne et al. (62), who reported an ICC of ρ=0.93 for computer-assisted breast density measurements in the Nurses' Health Study. In addition, the reader's reproducibility in our study is comparable to her reliability as evaluated in the Breast Cancer Detection Demonstration Project (BCDDP), with an ICC of ρ=0.915 (adjusted for case status, age, weight, and film type) for measurement of percent breast density in 193 sets of films (63).

4.3.4 Biological specimen collection

At study enrollment, trained clinical staff drew 40 mL of peripheral blood using standardized phlebotomy procedures; 20 mL was collected with EDTA anticoagulant, which provided 4 mL of buffy coat and 8 mL of plasma. The blood was processed immediately according to standardized protocols at the Magee Womens Hospital Satellite Clinical Research Center. Buffy coat and plasma were separated and placed into individually-labeled 1 mL cryovials and stored at –70°C until analyzed.

4.3.5 Genotyping

DNA extraction and genotyping were completed in the University of Pittsburgh GCRC Pharmacogenetics Core Laboratory (M Romkes). High molecular weight DNA was isolated from EDTA buffy coat specimens using the PureGene DNA Isolation Kit (Gentra Systems, Inc. Minneapolis, MN), according to manufacturer's instructions. The TNFR2 –196 M/R genotypes were determined by restriction fragment length polymorphism (RFLP)-polymerase chain reaction (PCR). The primer sequences used for the amplification of a 242-bp fragment of the TNFR2 exon 6 were as previously published (64): flanking primer forward (F): 5'-ACT CTC CTA TCC TGC CTG CT-3'; and flanking primer reverse (R): 5'-TTC TGG AGT TGG CTG CGT GT-3'. A total of 20ng of genomic DNA was used, along with 200µM of dNTP, 1.5mM of MgCl2, 396nM of primers, and 0.25µl of AmpliTaq Gold. PCR was performed under the following conditions: 94°C for 10 min followed by 40 cycles at 96°C for 1 min, 64°C for 1 min and 72°C for 3 min. A final extension step was carried out at 72°C for 7 min. For the identification of 196M and 196R, 20µl of the PCR product were digested for 2 hours with 10U of Nla III (New England Biolabs) at 37°C followed by electrophoresis on an 8% acrylamide gel for separating the restriction fragments. Gels were visualized on the Fotodyne Gel Documentation System (Hartland, WI). Previously sequenced genomic DNA samples from healthy individuals were used as positive controls for the homozygous wild-type and homozygous mutant genotypes to verify reproducibility of the RFLP-PCR and to confirm accuracy of genotype classifications; these positive controls, along with a negative control containing no genomic DNA, were included with every PCR analysis. Genotype assignments

were reviewed by two independent readers, and any disagreements were resolved by repeated typing. Approximately 5% (n=20) of randomly selected samples were repeated in a masked fashion for verification of the results of the genotyping assays, with an 85% concordance rate. The three discordant results were for TG and GG, which we had determined *a priori* to group together for analysis purposes.

4.3.6 Soluble TNFR2 assays

We used the laboratory of RP Tracy and commercially available sTNFR1 and sTNFR2 antibody bead kits for human plasma (BioSource International, Camarillo, CA, USA) to measure sTNFR1 and sTNFR2 plasma levels. Multiplex immunoassays, combining the principle of a sandwich immunoassay with fluorescent-bead-based technology, were conducted according to the manufacturer's specifications. To minimize inter-assay variability, all assays were performed with a single lot of sTNFR kits for human plasma. The plates were read on a Bio-Rad Bio-Plex instrument (Hercules, CA) using Bio-Plex Manager Software Version 3.0, with the instrument calibrated using a low RP1 setting, and gates adjusted to 3000 and 10000. The standard curve range was 11,400 to 20 pg/mL for sTNFR2. All samples read well within the standard curve range. Assays were run in duplicate and were preformed by two different technicians, who were masked to the mammographic density and genotyping results. Using control plasma, the laboratory reported a within-assay coefficient of variation of 17.4% for sTNFR2 concentrations. The inter-assay coefficient of variation calculated from the analytic results for 40 masked duplicate plasma samples was 22.4% for sTNFR2 concentrations.

4.3.7 Statistical analysis

Descriptive statistics for all baseline measures were calculated (means for continuous measures and frequencies for nominal variables) to assess the distribution of demographic variables and potential confounding variables. Baseline characteristics were compared across genotypes using the Wilcoxon rank sum test for continuous measures and the chi-squared test for discrete measures. Fisher's exact test for discrete measures was used when expected cell counts are less than five. Allele frequency departures from Hardy-Weinberg equilibrium were tested using the

chi-squared test. Genotypes were modeled in a dichotomous manner, based upon the presence or absence of the variant allele. The two-sample t-test was used to compare mean percent breast density in those women carrying the variant allele and those homozygous for the wild type allele. ANOVA was used to compare means of percent breast density between TNFR2 genotypes while controlling for potential confounding factors. Since age and BMI have been previously shown to be positively associated with circulating sTNFR2 levels (65-67) and negatively associated with percent mammographic density (68-74), the multivariate models comparing mean percent breast density across TNFR2 genotypes were first adjusted for age at enrollment, and then for age and BMI (kg/m^2). Subsequent models added ever smoked cigarettes (yes/no), current alcohol use (yes/no), age at menarche (<12, 12-13, ≥14 years), nulliparity (yes/no), ever breast fed (yes/no), history of breast biopsy prior to study enrollment (yes/no), and aspirin or other anti-inflammatory drug use with 48 hrs of blood draw (yes/no), as these covariates were known to be associated with breast density or with sTNFR2, or they appeared to differ by TNFR2 –196 M/R genotype ($p<0.10$). Although percent mammographic density was the primary focus of this study, the best method of utilizing the information obtained from the dense and nondense components of a mammogram is currently under debate (75). We therefore report the associations between the comparisons across TNFR2 gentotypes for mean dense breast area, total breast area, and nondense breast area for descriptive purposes only, as we did not account for these multiple comparisons *a priori.* To address our secondary aim, examining the effect of the TNFR2 –196 M/R SNP on circulating TNFR2 levels, we used the two-sample t-test for independent samples to compare mean TNFR2 levels in those women carrying the variant allele vs. those homozygous for the wild type allele. Transformations were not necessary to meet t-test and ANOVA assumptions, as both breast density and sTNFR2 were normally distributed. Probability values of ≤ 0.05 were considered statistically significant. All tests of statistical significance were two-tailed. Analyses were performed using SAS software release 8.02 (SAS Institute Inc., Cary, NC).

4.4 RESULTS

Among the 376 MAMS participants in this report, the mean (SD) age was 62 (8) years, ranging from 42-85 (Table 25). The majority of the population was Caucasian (94%) and attended post-secondary education (75%). The mean (SD) years since menopause was 14 (10), ranging from 1-43. The mean (SD) percent breast density was 29.7% (19.5), ranging from 0-94.9%. One hundred sixty three (44%) of this population reported taking aspirin or another anti-inflammatory agent within 48 hours of blood draw, a proportion similar to those who reported current use of aspirin and non-aspirin NSAIDs medications in their original study questionnaire (40%), and slightly less than those who reported any NSAID use at least once a week in the 12 months prior to study enrollment in their follow-up questionnaire (51%) (Table 26).

Previous reports from this population (Paper 2, unpublished data) have shown that percent mammographic density was associated with several breast cancer risk factors in the expected directions (8, 10-12, 14-21, 23, 68-74, 76, 77). For instance, MAMS women with higher percent mammographic density were more likely to be nulliparous and/or have a later age at first birth, to be former hormone therapy users, and to report a history of breast biopsy; and were less likely to have taken aspirin or another anti-inflammatory agent at blood draw than women with lower percent mammographic density. In addition, women with higher percent breast density were younger and had fewer years since menopause, had a lower BMI, were more likely to have attended post-secondary education and to report current consumption of alcohol. In contrast, women with higher sTNFR2 levels had a higher BMI and were older than women with lower sTNFR2 levels (also consistent with previous findings (65-67)). Women with higher sTNFR2 levels had a greater number of years since menopause and were less educated than women with lower sTNFR2 levels (Paper 2, unpublished data).

For the TNFR2 –196 M/R polymorphism, 206 (55%) had the T/T genotype, 134 (36%) had the T/G genotype, and 36 (10%) had the G/G genotype. These proportions diverged from expectations under Hardy-Weinberg equilibrium ($\chi 2 = 4.07$, $p=0.04$), and genotypes (not alleles) were used in subsequent analyses. The minor allele frequency (MAF) in this population was 0.27, which is consistent with that observed in the HapMap European population (MAF=0.25,

HapMap data release #20, January 2006). Table 25 depicts characteristics of the study population by TNFR2 genotype. Women with the TT genotype had a lower BMI; were less likely to breastfeed and for a shorter duration; and were more likely to report current alcohol use and to be nulliparous. These women also tended to be more likely to report a history of breast biopsy, a later age at menarche, and current cigarette smoking compared to women with the TG/GG genotypes. Self-reported medication use by genotype is shown in Table 26. Women with the TT genotype were less likely to report taking aspirin or another anti-inflammatory agent within 48 hours of blood draw (p=0.04). No other self-reported medication use was related to TNFR2 genotype.

Table 27 provides mean percent mammographic density by TNFR2 genotype. The unadjusted mean (SD) percent breast density was higher in women with the TT genotype (32.3% (21.0)) as compared to women with the TG/GG genotypes (26.6% (17.2)), p=0.003. This difference remained statistically significant after adjustment for age and BMI; however, inclusion of additional covariates reduced the level of statistical significance, with an adjusted (least squares) mean percent density for women with the TT genotype of 31.1% vs. 27.9% in women with the TG/GG genotypes, p=0.08. Mean dense breast area did not differ by TNFR2 genotype, while age-adjusted mean total and nondense breast areas were significantly lower in women with the TT genotype (Table 28); these differences were no longer significant after adjustment for additional covariates. There was no observable difference in circulating sTNFR2 levels between the TNFR2 genotypes (Table 29), either before or after adjustment for covariates.

4.5 DISCUSSION

In this study of cancer-free, postmenopausal women, we investigated the association between percent mammographic density and a single-locus allelic variation with potential functional significance (49, 50) in the TNFR2 gene. Compared to carriers of the variant allele, we observed higher mean percent breast density among women homozygous for the TT genotype, although this difference was attenuated after adjusting for potential confounding factors. Further, mean circulating plasma levels of sTNFR2 did not differ by TNFR2 genotype in our population.

To our knowledge, this is the first study to examine the association between the –196 M/R polymorphism and percent mammographic density. Because carriers of the G allele have been found to have higher circulating levels of sTNFR2 in previous studies (49, 50), we chose to evaluate TT genotypes in comparison to TG/GG genotypes. Our initial finding of lower mean percent mammographic density among women with the TG/GG genotypes was consistent with the idea that elevations in circulating sTNFRs in these women (49, 50) may block TNF-α activity (48), thereby preventing induction of COX-2 gene expression (78), resulting in decreased biosynthesis of estrogen and ultimately reducing mammographic density, a marker of breast cancer risk. The differences in mean percent mammographic density remained after adjustment for age and BMI, which was significantly lower among women with the TT genotype, suggesting that the –196 M/R polymorphism influences percent mammographic density independent of an effect on BMI.

Alcohol consumption, nulliparity, history of breast biopsy, and aspirin use within 48 hrs of blood draw were all related to both percent mammographic density and TNFR2 genotype in MAMS; while our findings adjusted for age and BMI seemed biologically plausible, the mean difference in breast density was no longer statistically significant after adjustment for these and other covariates, indicating that the association between percent mammographic density and TNFR2 genotype is not independent of these confounding factors. Instead of focusing on percent mammographic density, investigators have recently argued for examination of the absolute area of dense breast tissue (75, 79). Mean dense breast area did not significantly differ across TNFR2 genotypes, either before or after adjustment for potential confounding factors in our population. In addition, our results are not consistent with the only study to have evaluated the –196 M/R polymorphism and its association with breast cancer risk (80). In this recent study of 113 postmenopausal breast cancer cases and 157 pre- and postmenopausal controls in Tunisia, investigators demonstrated that the –196 M/R heterozygous genotype (TG) was associated with an *increased risk,* rather than having a protective effect on incident breast cancer (OR=2.28, 95% CI: 1.36-3.83) (80). However, these results are to be interpreted with caution, as they might be specific to Tunisians (6 controls and 0 postmenopausal cases had the GG genotype), and the investigators did not attempt to control for potential confounding factors.

Finally, we did not observe an association between the –196 M/R polymorphism and circulating sTNFR2 levels, nor did we observe a significant relationship between circulating

sTNFR2 levels and percent mammographic density in a previous analysis of this population (Paper 2, unpublished data). To date, only one nested case-control study has prospectively examined the relationship between serum levels of sTNFR2 and breast cancer risk (81). The investigators found no association between serum levels of the soluble TNFR2 receptor and breast cancer risk; however, this study had limited power to detect an association in postmenopausal women, with only 61 postmenopausal case-control pairs (81).

Some limitations of this study deserve consideration. Medication use was self-reported, and with the exception of a general question about use of "aspirin or anti-inflammatory agents in the last 48 hours" elicited by trained clinical staff, detailed use and dosage of specific medications were not recorded at the time of blood collection nor were they verified against pill bottles. The lack of ethnic diversity within MAMS reduces the generalizability of our findings to other ethnic groups. In addition, due to the relatively small size of this study and the limited prevalence of some covariates in this population, we were limited in the associations that could be evaluated. For instance, the Women's Health Study reported a significant interaction between cigarette smoking (current, former, never) and current NSAID use with respect to breast cancer risk (82); however, there were only 23 current smokers in our population, 4 of whom were carriers of the variant allele. When current smokers were collapsed into the category with former smokers, ever smoking cigarettes did not differ by mammographic density or TNFR2 genotype in our population. Strengths of the study include the use of a quantitative, highly reproducible measure of mammographic density, and reliable genotyping results. Although we only used a single measure of circulating sTNFR2, measured with fair reproducibility, determination of sTNFR2 concentrations in healthy individuals at time lapses of one year demonstrated that the concentrations of sTNFR2 are stable in each individual (correlation coefficient of 0.90), possibly reflecting genetically determined differences (83); this observation is supported by studies of identical twins, who unlike discordant twins, are more likely to have similar levels of sTNFR2 (83).

In conclusion, studies have demonstrated a strong heritability component for both mammographic density (7, 25-27) and circulating soluble TNF receptor levels (83). While the present results do not offer compelling evidence for an association between the TNFR2 –196 M/R polymorphism with percent mammographic density, and offer no evidence for an

association with circulating sTNFR2 levels in this population, these findings should be replicated in larger populations and with improved measurement of sTNFR2.

Table 25. Characteristics of the study population by genotype, Mammograms and Masses Study (2001-2005)

			TNFR2 –196 M/R Genotype				
Variable	**n=376**	**% or (range)**	**TT (n=206)**	**% or (range)**	**TG/GG (n=170)**	**% or (range)**	**p-value**
Clinic, %							0.83
Biopsy	69	18	169	82	138	81	
Screening clinic	307	82	37	18	32	19	
Age (years), *mean (SD)*	62 (8)	(42-85)	62 (8)	(45-83)	62 (8)	(42-85)	0.89
Race (%)							0.86
White	353	94	193	94	160	94	
Other	23	6	13	6	10	6	
Education level (%)							0.66
High school	93	25	53	26	40	24	
> High school	274	75	149	74	125	76	
Missing	9		4		5		
Body mass index §, *mean (SD)*	28.4 (6.1)	(16.8-46.6)	27.8 (5.9)	(16.8-45.9)	29.1 (6.3)	(17.3-46.6)	0.05
Cigarette Smoking (%)							0.02
Never	219	58	119	58	100	59	
Former	133	35	67	33	66	39	
Current	23	6	19	9	4	2	
Missing	1		1		0		
Cigarette Smoking (%)							0.88
Never	219	58	119	58	100	59	
Ever	156	42	86	42	70	41	
Missing	1		1				

Table 25 (continued)

Variable	n=376	% or (range)	TNFR2 –196 M/R Genotype TT (n=206)	% or (range)	TG/GG (n=170)	% or (range)	p-value
Current alcohol consumption ≥1/week for ≥6 months (%)							0.05
No	263	72	135	67	128	77	
Yes	104	28	65	32	39	23	
Missing	9		6		3		
Current alcohol use ≥ 1/week for ≥ 6 months (%)							0.13
No	263	72	135	68	128	77	
Yes							
<12g/day	68	19	44	22	24	14	
≥12g/day	35	9	20	10	15	9	
Missing	10		7		3		
Walks for exercise (%)							0.59
Rarely/1-3 times/month	148	40	84	42	64	39	
At least 2-3 times/week	219	60	118	58	101	61	
Missing	9		4		5		
Age at menarche (years), %							0.09
<12	72	19	31	15	41	24	
12-13	223	59	128	62	95	56	
≥14	80	21	46	22	34	20	
Missing	1		1		0		
Nulliparous (%)							0.05
No	295	78	154	75	141	83	
Yes	81	21	52	25	29	17	

Table 25 (continued)

			TNFR2 –196 M/R Genotype				
Variable	**n=376**	**% or (range)**	**TT (n=206)**	**% or (range)**	**TG/GG (n=170)**	**% or (range)**	**p-value**
Parity (%)							0.39
Nulliparous	81	21	52	25	29	17	
1	43	11	26	13	17	10	
2	113	30	59	29	54	32	
3	79	21	39	19	40	24	
4	32	9	16	8	16	9	
5+	28	7	14	7	14	8	
Age at first birth (years), *%*							0.16
<30	249	66	130	63	119	70	
≥30 or nulliparous	127	34	76	37	51	30	
Breastfeeding (%)							0.01
Never or ≤1 month	226	60	136	66	90	53	
Ever	149	40	69	34	80	47	
Missing	1		1		0		
Breastfeeding (%)							0.03
Never or ≤1 month	226	60	136	66	90	53	
Ever							
1-12 months	97	26	46	22	51	30	
≥13 months	52	14	23	11	29	17	
Missing	1		1		0		
Age at menopause (years), *mean (SD)*	48 (5)	(26-60)	48 (5)	(30-60)	48 (5)	(26-58)	0.42
Missing	10		5		5		

Table 25 (continued)

			TNFR2 –196 M/R Genotype				
Variable	**n=376**	**% or (range)**	**TT (n=206)**	**% or (range)**	**TG/GG (n=170)**	**% or (range)**	**p-value**
Years since menopause, *mean (SD)*	14 (10)	(1-43)	14 (10)	(1-43)	13 (10)	(1-42)	0.88
Missing	10		5		5		
Surgical menopause (%)							0.26
No	340	93	184	91	156	95	
Yes	26	7	17	8	9	5	
Missing	10		5		5		
Hormone therapy (%)							0.95
Never use	151	40	83	40	68	40	
Past use	225	60	123	60	102	60	
Family history of breast cancer † (%)							0.91
No	318	85	174	85	144	86	
Yes	54	14	30	15	24	14	
Missing	4		2		2		
Breast biopsy < enrollment (%)							0.23
Never	305	81	163	79	142	84	
Ever	70	19	43	21	27	16	
Missing	1		0		1		
Breast biopsy < enrollment (%)							0.09
0	305	81	163	79	142	84	
1	48	13	26	13	22	13	
2+	22	6	17	8	5	3	
Missing	1		0		1		

† History of breast cancer in a mother or sister; § Weight (kg)/height² (m²).

Table 26. Self-reported medication use by genotype in the Mammograms and Masses Study (MAMS), 2001-2005

			TNFR2 –196 M/R Genotype				
Variable	**n=376**	**%**	**TT (n=206)**	**% or (range)**	**TG/GG (n=170)**	**% or (range)**	**p-value**
Aspirin or other anti-inflammatory agent within 48 hours of blood draw							
% yes	163	44	80	39	83	49	0.04
Missing	2		0		2		
Self-reported current medication use in study questionnaire:							
Aspirin, % yes	111	29	59	29	52	31	0.68
Non-aspirin NSAID, % yes	53	14	28	14	25	15	0.76
Acetaminophen, % yes	22	6	10	5	12	7	0.36
Any NSAID, % yes	150	40	79	38	71	42	0.50
Cox-2 inhibitors, % yes	14	4	7	3	7	4	0.71
Selective Estrogen Receptor Modulator (Raloxifene or Tamoxifen), % yes	31	8	15	7	16	9	0.45
Bisphosphonates (Risedronate or Alendronate), % yes	51	14	31	15	20	12	0.35
Self-reported medication use in NSAIDs follow-up questionnaire:							0.19
Aspirin ≥ 1/week in past 12 months, % yes	122	40	61	37	61	44	0.19
Missing	72		40		32		
Aspirin ~daily for 1 year or more, % yes	74	24	38	23	36	26	0.52
Missing	72		40		32		
Non-aspirin NSAID ≥ 1/week in past 12 months, % yes	69	23	36	22	33	24	0.64
Missing	72		40		32		
Non-aspirin NSAID ~daily for 1 year or more, % yes	19	6	11	7	8	6	0.77
Missing	72		40		32		

Table 26 (continued)

			TNFR2 –196 M/R Genotype				
Variable	**n=376**	**%**	**TT (n=206)**	**% or (range)**	**TG/GG (n=170)**	**% or (range)**	**p-value**
Acetaminophen ≥ 1/week in past 12 months, % yes	79	26	40	24	39	28	0.39
Missing	73		40		33		
Acetaminophen ~daily for 1 year or more, % yes	15	5	7	4	8	6	0.52
Missing	73		40		33		
Any NSAID ≥ 1/week in past 12 months, % yes	155	51	80	48	75	54	0.28
Missing	72		40		32		
Any NSAID ~daily for 1 year or more, % yes	88	29	46	28	42	30	0.60
Missing	72		40		32		

Table 27. Percent mammographic density by genotype, Mammograms and Masses Study (2001-2005)

			TNFR2 –196 M/R Genotype							
			TT (n=206)		TG/GG (n=170)					
Variable	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**p1***	**p2**	**p3**	**p4[§]**
% Mammographic density	29.7 (19.5)	(0-94.9)	32.3 (21.0)	(0-94.9)	26.6 (17.2)	(0-84.5)	0.003	0.004	0.03	0.08

*1. P-value for two-sample t-test with unequal variances comparing mean percent mammographic density by genotype.

2. P-value based on analysis of variance (ANOVA) comparing mean % mammographic density by genotype while adjusting for age (continuous).

3. ANOVA adjusting for age (continuous) and BMI (continuous).

4. ANOVA adjusting for age (continuous), BMI (continuous), ever smoked cigarettes (yes/no), current alcohol consumption (yes/no), age at menarche (<12, 12-13, ≥14 years), nulliparous (yes/no), ever breast fed (yes/no), ever had breast biopsy prior to study enrollment (yes/no), and aspirin use within 48 hrs of blood draw (yes/no).

§ Fourteen women are missing from this multivariate model (n=8 with the TT genotype; n=6 with the TG/GG genotype).

Table 28. Dense, total, and nondense breast area by genotype, Mammograms and Masses Study (2001-2005)

			TNFR2 –196 M/R Genotype							
			TT (n=206)		TG/GG (n=170)					
Variable	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**p1***	**p2**	**p3**	**p4[§]**
Dense breast area	40.9 (26.2)	(0-188.1)	42.1 (27.0)	(0-188.1)	39.6 (25.3)	(0-114.7)	0.36	0.36	0.41	0.77
Total breast area	162.5 (76.4)	(31.6-442.0)	155.3 (77.8)	(39.5-442.0)	171.1 (73.8)	(31.6-386.0)	0.04	0.04	0.37	0.31
Nondense breast area	121.5 (78.0)	(3.6-389.0)	113.2 (80.0)	(3.6-389.0)	131.6 (74.4)	(4.9-369.5)	0.02	0.02	0.20	0.26

*1. P-value for two-sample t-test with assumed equal variances comparing mean mammographic area by genotype.

2. P-value based on analysis of variance (ANOVA) comparing mean mammographic area by genotype while adjusting for age (continuous).

3. ANOVA adjusting for age (continuous) and BMI (continuous).

4. ANOVA adjusting for age (continuous), BMI (continuous), ever smoked cigarettes (yes/no), current alcohol consumption (yes/no), age at menarche (<12, 12-13, ≥14 years), nulliparous (yes/no), ever breast fed (yes/no), ever had breast biopsy prior to study enrollment (yes/no), and aspirin use within 48 hrs of blood draw (yes/no).

§ Fourteen women are missing from this multivariate model (n=8 with the TT genotype; n=6 with the TG/GG genotype).

Table 29. Circulating levels of sTNFR2 by genotype, Mammograms and Masses Study (2001-2005)

			TNFR2 –196 M/R Genotype							
			TT (n=206)		TG/GG (n=170)					
Variable	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**p1***	**p2**	**p3**	**p4[§]**
sTNFR2 pg/mL	2662.6 (1202.8)	(84.3-8517.8)	2693.9 (1249.5)	(84.3-7402.7)	2624.6 (1146.2)	(526.5-8517.8)	0.58	0.57	0.33	0.60

*1. P-value for two-sample t-test with assumed equal variances comparing circulating sTNFR2 levels by genotype.

2. P-value based on analysis of variance (ANOVA) comparing circulating sTNFR2 levels by genotype while adjusting for age (continuous).

3. ANOVA adjusting for age (continuous) and BMI (continuous).

4. ANOVA adjusting for age (continuous), BMI (continuous), ever smoked cigarettes (yes/no), current alcohol consumption (yes/no), age at menarche (<12, 12-13, ≥14 years), nulliparous (yes/no), ever breast fed (yes/no), ever had breast biopsy prior to study enrollment (yes/no), and aspirin use within 48 hrs of blood draw (yes/no).

§ Fourteen women are missing from this multivariate model (n=8 with the TT genotype; n=6 with the TG/GG genotype).

4.6 LITERATURE CITED

1. Kerlikowske K, Grady D, Rubin SM, Sandrock C, and Ernster VL, *Efficacy of screening mammography. A meta-analysis.* Jama, 1995. **273**(2): p. 149-54.

2. Buist DSM, Porter PL, Lehman C, Taplin SH, and White E, *Factors Contributing to Mammography Failure in Women Aged 40-49 Years.* J Natl Cancer Inst, 2004. **96**(19): p. 1432-1440.

3. Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, and Meyer JE, *Relationship between mammographic and histologic features of breast tissue in women with benign biopsies.* Cancer, 1988. **61**(2): p. 266-71.

4. Fisher ER, Palekar A, Kim WS, and Redmond C, *The histopathology of mammographic patterns.* American Journal of Clinical Pathology, 1978. **69**(4): p. 421-6.

5. Page DL and Winfield AC, *The dense mammogram.* AJR. American Journal of Roentgenology, 1986. **147**(3): p. 487-9.

6. Wellings SR and Wolfe JN, *Correlative studies of the histological and radiographic appearance of the breast parenchyma.* Radiology, 1978. **129**(2): p. 299-306.

7. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, and Hopper JL, *Heritability of mammographic density, a risk factor for breast cancer.* New England Journal of Medicine, 2002. **347**(12): p. 886-94.

8. Harvey JA and Bovbjerg VE, *Quantitative Assessment of Mammographic Breast Density: Relationship with Breast Cancer Risk.* Radiology, 2004. **230**(1): p. 29-41.

9. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, and Paterson AD, *Mammographic breast density as an intermediate phenotype for breast cancer.* The Lancet Oncology, 2005. **6**(10): p. 798-808.

10. Ernster VL, Sacks ST, Peterson CA, and Schweitzer RJ, *Mammographic parenchymal patterns and risk factors for breast cancer.* Radiology, 1980. **134**(3): p. 617-20.

11. de Waard F, Rombach JJ, Collette HJ, and Slotboom B, *Breast cancer risk associated with reproductive factors and breast parenchymal patterns.* J Natl Cancer Inst, 1984. **72**(6): p. 1277-82.

12. Christodoulakos GE, Lambrinoudaki IV, Panoulis KP, Vourtsi AD, Vlachos L, Georgiou E, and Creatsas GC, *The effect of various regimens of hormone replacement therapy on mammographic breast density.* Maturitas, 2003. **45**(2): p. 109-18.

13. *Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial.* Jama, 1995. **273**(3): p. 199-208.

14. Vachon CM, Kuni CC, Anderson K, Anderson VE, and Sellers TA, *Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States).* Cancer Causes and Control, 2000. **11**(7): p. 653-62.

15. Bergkvist L, Tabar L, Bergstrom R, and Adami HO, *Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program.* American Journal of Epidemiology, 1987. **126**(6): p. 1075-81.

16. de Stavola BL, Gravelle IH, Wang DY, Allen DS, Bulbrook RD, Fentiman IS, Hayward JL, and Chaudary MC, *Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study.* International Journal of Epidemiology, 1990. **19**(2): p. 247-54.

17. Grove JS, Goodman MJ, Gilbert FI, Jr., and Mi MP, *Factors associated with mammographic pattern.* British Journal of Radiology, 1985. **58**(685): p. 21-5.

18. Kaufman Z, Garstin WI, Hayes R, Michell MJ, and Baum M, *The mammographic parenchymal patterns of nulliparous women and women with a family history of breast cancer.* Clinical Radiology, 1991. **43**(6): p. 385-8.

19. Gram IT, Funkhouser E, and Tabar L, *Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women.* British Journal of Cancer, 1995. **71**(3): p. 647-50.

20. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao M-S, Khokha R, Martin L, and Boyd N, *The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 343-349.

21. Jeffreys M, Warren R, Gunnell D, McCarron P, and Smith GD, *Life course breast cancer risk factors and adult breast density (United Kingdom).* Cancer Causes and Control, 2004. **15**(9): p. 947-955.

22. Kelsey JL, Gammon MD, and John EM, *Reproductive factors and breast cancer.* Epidemiologic Reviews, 1993. **15**(1): p. 36-47.

23. Writing Group for the Women's Health Initiative I, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* Jama, 2002. **288**(3): p. 321-33.

24. Pankow JS, Vachon CM, Kuni CC, King RA, Arnett DK, Grabrick DM, Rich SS, Anderson VE, and Sellers TA, *Genetic analysis of mammographic breast density in adult women: evidence of a gene effect.* Journal of the National Cancer Institute, 1997. **89**(8): p. 549-56.

25. Vachon CM, King RA, Atwood LD, Kuni CC, and Sellers TA, *Preliminary sibpair linkage analysis of percent mammographic density.* Journal of the National Cancer Institute, 1999. **91**(20): p. 1778-9.

26. Huo Z, Giger ML, Olopade OI, Wolverton DE, Weber BL, Metz CE, Zhong W, and Cummings SA, *Computerized analysis of digitized mammograms of BRCA1 and BRCA2 gene mutation carriers.* Radiology, 2002. **225**(2): p. 519-26.

27. Ziv E, Shepherd J, Smith-Bindman R, and Kerlikowske K, *Mammographic breast density and family history of breast cancer.* J Natl Cancer Inst, 2003. **95**(7): p. 556-8.

28. Haiman CA, Bernstein L, Berg D, Ingles SA, Salane M, and Ursin G, *Genetic determinants of mammographic density.* Breast Cancer Research, 2002. **4**(3): p. R5.

29. Haiman CA, Hankinson SE, De Vivo I, Guillemette C, Ishibe N, Hunter DJ, and Byrne C, *Polymorphisms in steroid hormone pathway genes and mammographic density.* Breast Cancer Research and Treatment, 2003. **77**(1): p. 27-36.

30. Hong CC, Thompson HJ, Jiang C, Hammond GL, Tritchler D, Yaffe M, and Boyd NF, *Val158Met Polymorphism in catechol-O-methyltransferase gene associated with risk factors for breast cancer.* Cancer Epidemiology, Biomarkers and Prevention, 2003. **12**(9): p. 838-47.

31. Hong CC, Thompson HJ, Jiang C, Hammond GL, Tritchler D, Yaffe M, and Boyd NF, *Association between the T27C polymorphism in the cytochrome P450 c17alpha (CYP17) gene and risk factors for breast cancer.* Breast Cancer Res Treat, 2004. **88**(3): p. 217-30.

32. Lai JH, Vesprini D, Zhang W, Yaffe MJ, Pollak M, and Narod SA, *A Polymorphic Locus in the Promoter Region of the IGFBP3 Gene Is Related to Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(4): p. 573-582.

33. Lillie EO, Bernstein L, Ingles SA, Gauderman WJ, Rivas GE, Gagalang V, Krontiris T, and Ursin G, *Polymorphism in the Androgen Receptor and Mammographic Density in Women Taking and Not Taking Estrogen and Progestin Therapy.* Cancer Res, 2004. **64**(4): p. 1237-1241.

34. Lord S, Mack W, Van Den Berg D, Pike M, Ingles S, Haiman C, Wang W, Parisky Y, Hodis H, and Ursin G, *Polymorphisms in genes involved in estrogen and progesterone metabolism and mammographic density changes in women randomized to postmenopausal hormone therapy: results from a pilot study.* Breast Cancer Research, 2005. **7**(3): p. R336 - R344.

35. Maskarinec G, Lurie G, Williams AE, and Le Marchand L, *An investigation of mammographic density and gene variants in healthy women.* Int J Cancer, 2004. **112**(4): p. 683-8.

36. Mulhall C, Hegele RA, Cao H, Tritchler D, Yaffe M, and Boyd NF, *Pituitary Growth Hormone and Growth Hormone-Releasing Hormone Receptor Genes and Associations with Mammographic Measures and Serum Growth Hormone.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2648-2654.

37. van Duijnhoven FJB, Bezemer ID, Peeters PHM, Roest M, Uitterlinden AG, Grobbee DE, and van Gils CH, *Polymorphisms in the Estrogen Receptor {alpha} Gene and Mammographic Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2655-2660.

38. van Duijnhoven FJB, Peeters PHM, Warren RML, Bingham SA, Uitterlinden AG, van Noord PAH, Monninkhof EM, Grobbee DE, and van Gils CH, *Influence of Estrogen Receptor {alpha} and Progesterone Receptor Polymorphisms on the Effects of Hormone Therapy on Mammographic Density.* Cancer Epidemiol Biomarkers Prev, 2006. **15**(3): p. 462-467.

39. Reed MJ and Purohit A, *Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis.* Endocr Rev, 1997. **18**(5): p. 701-15.

40. Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, and Bulun SE, *Estrogen Regulates Expression of Tumor Necrosis Factor Receptors in Breast Adipose Fibroblasts.* J Clin Endocrinol Metab, 2004. **89**(8): p. 4018-4024.

41. Purohit A, Newman S, and Reed M, *The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer.* Breast Cancer Res, 2002. **4**(2): p. 65 - 69.

42. Morita I, *Distinct functions of COX-1 and COX-2.* Prostaglandins Other Lipid Mediat, 2002. **68-69**: p. 165-75.

43. DuBois RN, *Aspirin and breast cancer prevention: the estrogen connection.* Jama, 2004. **291**(20): p. 2488-9.

44. Gasparini G, Longo R, Sarmiento R, and Morabito A, *Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?* Lancet Oncol, 2003. **4**(10): p. 605-15.

45. Hohmann H, Remy R, Brockhaus M, and van Loon A, *Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha).* J Biol Chem, 1989. **264**(25): p. 14927-14934.

46. Bazzoni F and Beutler B, *The tumor necrosis factor ligand and receptor families.* N Engl J Med, 1996. **334**(26): p. 1717-25.

47. Varela LM, Darcy KM, and Ip MM, *The Epidermal Growth Factor Receptor Is Not Required for Tumor Necrosis Factor-{alpha} Action in Normal Mammary Epithelial Cells.* Endocrinology, 1997. **138**(9): p. 3891-3900.

48. Aderka D, *The potential biological and clinical significance of the soluble tumor necrosis factor receptors.* Cytokine Growth Factor Rev, 1996. **7**(3): p. 231-40.

49. Glenn CL, Wang WY, Benjafield AV, and Morris BJ, *Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor.* Human Molecular Genetics, 2000. **9**(13): p. 1943-9.

50. Tolusso B, Sacco S, Gremese E, La Torre G, Tomietto P, and Ferraccioli GF, *Relationship between the tumor necrosis factor receptor II (TNF-RII) gene polymorphism and sTNF-RII plasma levels in healthy controls and in rheumatoid arthritis.* Human Immunology, 2004. **65**(12): p. 1420-1426.

51. Verthelyi D and Klinman DM, *Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo.* Immunology, 2000. **100**(3): p. 384-390.

52. White E, Velentgas P, Mandelson MT, Lehman CD, Elmore JG, Porter P, Yasui Y, and Taplin SH, *Variation in mammographic breast density by time in menstrual cycle among women aged 40-49 years.* Journal of the National Cancer Institute, 1998. **90**(12): p. 906-10.

53. Brooks-Asplund EM, Tupper CE, Daun JM, Kenney WL, and Cannon JG, *Hormonal modulation of interleukin-6, tumor necrosis factor and associated receptor secretion in postmenopausal women.* Cytokine, 2002. **19**(4): p. 193-200.

54. Modugno F, Ness RB, and Allen GO, *Alcohol Consumption and the Risk of Mucinous and Nonmucinous Epithelial Ovarian Cancer.* Obstet Gynecol, 2003. **102**(6): p. 1336-1343.

55. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, and Mulvihill JJ, *Projecting individualized probabilities of developing breast cancer for white females who are being examined annually.[comment].* Journal of the National Cancer Institute, 1989. **81**(24): p. 1879-86.

56. Chen Z, Maricic M, Bassford TL, Pettinger M, Ritenbaugh C, Lopez AM, Barad DH, Gass M, and LeBoff MS, *Fracture Risk Among Breast Cancer Survivors: Results From the Women's Health Initiative Observational Study.* Arch Intern Med, 2005. **165**(5): p. 552-558.

57. Byng JW, Boyd NF, Little L, Lockwood G, Fishell E, Jong RA, and Yaffe MJ, *Symmetry of projection in the quantitative analysis of mammographic images.* Eur J Cancer Prev, 1996. **5**(5): p. 319-27.

58. Boyd NF, O'Sullivan B, Fishell E, Simor I, and Cooke G, *Mammographic patterns and breast cancer risk: methodologic standards and contradictory results.* J Natl Cancer Inst, 1984. **72**(6): p. 1253-9.

59. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, and Yaffe MJ, *Mammographic densities and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 1998. **7**(12): p. 1133-44.

60. Gram I, Bremnes Y, Ursin G, Maskarinec G, Bjurstam N, and Lund E, *Percentage density, Wolfe's and Tabar's mammographic patterns: agreement and association with risk factors for breast cancer.* Breast Cancer Research, 2005. **7**(5): p. R854 - R861.

61. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, and Yaffe MJ, *Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study.* J Natl Cancer Inst, 1995. **87**(9): p. 670-5.

62. Byrne C, Hankinson SE, Pollak M, Willett WC, Colditz GA, and Speizer FE, *Insulin-like growth factors and mammographic density.* Growth Horm IGF Res, 2000. **10 Suppl A**: p. S24-5.

63. Benichou J, Byrne C, Capece LA, Carroll LE, Hurt-Mullen K, Pee DY, Salane M, Schairer C, and Gail MH, *Secular stability and reliability of measurements of the percentage of dense tissue on mammograms.* Cancer Detection and Prevention, 2003. **27**(4): p. 266-274.

64. Al-Ansari AS, Ollier WER, Villarreal J, Ordi J, Teh L-S, and Hajeer AH, *Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus.* Tissue Antigens, 2000. **55**(1): p. 97-99.

65. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, and Coppack SW, *Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo.* Am J Physiol, 1999. **277**(6 Pt 1): p. E971-5.

66. Hauner H, Bender M, Haastert B, and Hube F, *Plasma concentrations of soluble TNF-alpha receptors in obese subjects.* Int J Obes Relat Metab Disord, 1998. **22**(12): p. 1239-43.

67. Hasegawa Y, Sawada M, Ozaki N, Inagaki T, and Suzumura A, *Increased soluble tumor necrosis factor receptor levels in the serum of elderly people.* Gerontology, 2000. **46**(4): p. 185-8.

68. Whitehouse GH and Leinster SJ, *The variation of breast parenchymal patterns with age.* British Journal of Radiology, 1985. **58**(688): p. 315-8.

69. Whitehead J, Carlile T, Kopecky KJ, Thompson DJ, Gilbert FI, Jr., Present AJ, Threatt BA, Krook P, and Hadaway E, *Wolfe mammographic parenchymal patterns. A study of the masking hypothesis of Egan and Mosteller.* Cancer, 1985. **56**(6): p. 1280-6.

70. van Gils CH, Otten JD, Verbeek AL, and Hendriks JH, *Short communication: breast parenchymal patterns and their changes with age.* British Journal of Radiology, 1995. **68**(814): p. 1133-5.

71. Riza E, dos Santos Silva I, De Stavola B, Perry N, Karadedou-Zafiriadou E, Linos D, Remoundos D-D, and Linos A, *Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece.* European Journal of Cancer, 2005. **41**(4): p. 590-600.

72. McCormack VA, dos Santos Silva I, De Stavola BL, Perry N, Vinnicombe S, Swerdlow AJ, Hardy R, and Kuh D, *Life-course body size and perimenopausal mammographic parenchymal patterns in the MRC 1946 British birth cohort.* Br J Cancer, 2003. **89**(5): p. 852-9.

73. Lam PB, Vacek PM, Geller BM, and Muss HB, *The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont.* Cancer, 2000. **89**(2): p. 369-75.

74. Modugno F, Ngo D, Allen G, Kuller L, Ness R, Vogel V, Costantino J, and Cauley J, *Breast cancer risk factors and mammographic breast density in women over age 70.* Breast Cancer Research and Treatment, 2005: p. 1-10.

75. Kaaks R, Diorio C, and Brisson J, *Insulin-Like Growth Factor-I and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(12): p. 3019-.

76. Maskarinec G, Takata Y, Pagano I, Lurie G, Wilkens LR, and Kolonel LN, *Alcohol consumption and mammographic density in a multiethnic population.* Int J Cancer, 2006. **118**(10): p. 2579-83.

77. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, and Palli D, *Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population.* International Journal of Cancer, 2006. **118**(7): p. 1782-1789.

78. Howe LR, Subbaramaiah K, Brown AM, and Dannenberg AJ, *Cyclooxygenase-2: a target for the prevention and treatment of breast cancer.* Endocrine-Related Cancer, 2001. **8**(2): p. 97-114.

79. Haars G, van Noord PAH, van Gils CH, Grobbee DE, and Peeters PHM, *Measurements of Breast Density: No Ratio for a Ratio.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2634-2640.

80. Mestiri S, Bouaouina N, Ben Ahmed S, and Chouchane L, *A functional polymorphism of the tumor necrosis factor receptor-II gene associated with the survival and relapse prediction of breast carcinoma.* Cytokine, 2005. **30**(4): p. 182-187.

81. Krajcik RA, Massardo S, and Orentreich N, *No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk.* Cancer Epidemiol Biomarkers Prev, 2003. **12**(9): p. 945-6.

82. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE, *Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial.* JAMA, 2005. **294**(1): p. 47-55.

83. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, and Wallach D, *Variation in serum levels of the soluble TNF receptors among healthy individuals.* Lymphokine and Cytokine Research, 1992. **11**(3): p. 157-9.

5.0 GENERAL DISCUSSION

Breast cancer is the most common cancer in women around the world; in the United States this year, it is expected that breast cancer alone will account for 31% (212,920) of all new cancer cases among women and 40,970 women are expected to die from the disease (1). Breast density is a known risk factor for breast cancer (2, 3), and the risk associated with mammographic density is greater than that associated with almost all other risk factors for breast cancer (3, 4). Therefore, understanding factors that affect breast density and their underlying mechanism is an important research question. Endogenous and exogenous estrogen exposures have been associated with breast density (3, 5) and have been implicated in breast cancer (6-9). Most breast tumors are initially dependent on estrogen for survival; paradoxically, the highest incidence of breast cancer occurs in postmenopausal women when ovarian production of estrogens is minimal. In postmenopausal women, estrogens continue to be produced in non-ovarian sites, such as adipose tissue, as well as in normal and cancerous breast tissues (10). In fact, postmenopausal breast cancer is largely estrogen receptor (ER) or progesterone receptor (PR) positive, and is more responsive to anti-estrogen therapy and aromatase inhibitors, even after controlling for stage and other prognostic factors (11). In addition, recent studies have demonstrated a protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs) with respect to postmenopausal breast cancer risk, suggesting that inflammation and cytokines may play a role in postmenopausal breast cancer (12-14).

Cytokines may be associated with breast cancer risk independently. However, it is also biologically plausible that cytokines may alter breast cancer risk through their relationship with breast density; no prior study has investigated this possibility. Hence, we sought explore to the association between inflammation and breast cancer risk in two populations of postmenopausal women: the Study of Osteoporotic Fractures (SOF), and the Mammograms and Masses Study (MAMS).

5.1 ARTICLE 1

In the first article, we tested reported use of NSAIDs for their effect on incident breast cancer within the Study of Osteoporotic Fractures (SOF) cohort. We further investigated whether the relationship between NSAIDs and breast cancer incidence differed by hormone receptor status and tumor type at diagnosis. The Study of Osteoporotic Fractures is a multi-center, prospective cohort study of white women recruited from four U.S. centers, 1986-1988. Complete NSAID medication and breast cancer risk factor information was available for 6695 women, mean (SD) age 73 (5) years. During a mean (SD) of 13.2 (3.8) years of follow-up, there were no differences in the risk of incident breast cancer (n=372 cases) by use of aspirin, non-aspirin NSAID, or any NSAID, before and after adjustment for potential confounding factors. Further, we observed no difference in breast cancer risk by frequency and duration of NSAID use.

Our results are consistent with seven prior large prospective studies finding no association (15-21), and with null results from a randomized controlled trial of alternate-day low-dose aspirin (22). However, our results are not consistent with five prospective studies demonstrating a protective effect from use of NSAIDs (12, 23-26) and one suggesting an increased risk (27). Although the reason for these differing results is unclear, one explanation may be due to differences in exposure assessment across studies. In SOF, a medication inventory was not conducted until visit 4, at which time patients were asked to bring all medications to the clinic for verification of use; however, the questions regarding frequency and duration of aspirin and non-aspirin NSAID use were only asked at visit two. Further, at visit two only non-prescription NSAID use was captured. This is an important limitation, especially given that a recent case-control study observed a 71% reduction in breast cancer risk associated with use of selective COX-2 inhibitors (28). If in SOF users of prescription NSAIDs were included in the referent group (i.e. non-NSAID users), results would be biased toward the null. Our results do not support a protective effect of nonprescription NSAIDs among older postmenopausal, white women.

5.2 ARTICLE 2

The second article describes an ancillary study we conducted within the Mammograms and Masses Study (MAMS), an ongoing cross-sectional study of correlates of breast density. In 376 MAMS participants, we measured plasma levels of two soluble receptors for TNF-alpha (sTNFR1 and sTNFR2) and examined their association with percent mammographic breast density. We chose to measure receptors for the cytokine TNF-alpha, because TNF-alpha is believed to have a central role in regulating estrogen synthesis in the breast (29-31). Circulating TNF receptors may block TNF-α activity (32), thereby preventing induction of COX-2 gene expression (33, 34) and ultimately estrogen biosynthesis in the breast. In MAMS, we found that mean percent mammographic density was lower among women in the highest quartiles of circulating levels of sTNFR1 and sTNFR2. After adjustment for BMI, the inverse association initially observed between the circulating sTNFRs and percent mammographic density disappeared.

This is the first study to examine the association between circulating sTNFRs and percent mammographic density. Our initial findings of an inverse correlation between the sTNFRs and percent breast density were consistent with the idea that circulating sTNFRs may block TNF-α activity, thereby preventing induction of COX-2 gene expression, resulting in decreased biosynthesis of estrogen and ultimately reducing mammographic density, a marker of breast cancer risk. However, since body mass index correlates strongly and positively with both the nondense and total breast areas (35), and thus correlates inversely with percent breast density (36-38), potential confounding by adiposity is of particular concern when studying factors, such as circulating sTNFRs, which are positively correlated with BMI. We therefore examined the absolute area of dense breast tissue, instead of percent breast density (35, 39). Indeed, circulating sTNFRs were not associated with dense breast area, both before and after adjustment for potential confounding factors in our population. Interestingly, MAMS participants who reported use of aspirin or another anti-inflammatory agent within 48 hours of blood collection were significantly more likely to have lower percent mammographic density. Consistent with this biologic mechanism is the epidemiologic evidence supporting and association between use of NSAIDs and reduced breast cancer risk (reviewed in (40) and (14)). Thus, in spite of the lack

of an independent association between sTNFRs and mammographic density in this study, we cannot rule out the hypothesis that inflammation plays a role in mammographic density.

5.3 ARTICLE 3

Our interest in the third article was to expand upon our ancillary study in MAMS, by evaluating a non-synonymous SNP in the TNFR2 gene, known as the –196 M/R polymorphism (T to G transition), with respect to both percent mammographic density and circulating sTNFR2 levels. Compared to carriers of the variant allele, we observed higher mean percent breast density among women homozygous for the TT genotype, although this difference was attenuated after adjusting for potential confounding factors.

To our knowledge, this is the first study to examine the association between the –196 M/R polymorphism and percent mammographic density. Because carriers of the G allele have been found to have higher circulating levels of sTNFR2 in previous studies (41, 42), we chose to evaluate TT genotypes in comparison to TG/GG genotypes. Our initial finding of lower mean percent mammographic density among women with the TG/GG genotypes was consistent with the idea that elevations in circulating sTNFRs in these women (41, 42) may block TNF-α activity (32), thereby preventing induction of COX-2 gene expression (34), resulting in decreased biosynthesis of estrogen and ultimately reducing mammographic density, a marker of breast cancer risk. The differences in mean percent mammographic density remained after adjustment for age and BMI, which was significantly lower among women with the TT genotype, suggesting that the –196 M/R polymorphism influences percent mammographic density independent of an effect on BMI. However, inclusion of additional confounding factors reduced this difference ($p=0.08$). Further, mean circulating plasma levels of sTNFR2 did not differ by TNFR2 genotype in our population, failing to confirm the previous literature that demonstrated functional significance for this polymorphism. Since, in MAMS, the inter-assay coefficient of variation calculated from the analytic results for 40 masked duplicate plasma samples did not indicate good reproducibility for sTNFR2 concentrations (CV=22.4%), the lack of association observed in our study may be due to inadequate measurement of sTNFR2. In fact, studies have demonstrated a strong heritability component for both mammographic density (2, 43-45) and

circulating soluble TNF receptor levels (46). Hence, while the present results do not offer compelling evidence for an association between the TNFR2 –196 M/R polymorphism with percent mammographic density, and offer no evidence for an association with circulating sTNFR2 levels in this population, these findings should be replicated in larger populations and with improved sTNFR2 measures (i.e. enzyme-linked immunosorbent assay).

5.4 SUMMARY AND FUTURE DIRECTIONS

This project has provided us with a unique opportunity to explore the potential association between inflammation and breast cancer risk in two populations of postmenopausal women. We used two different exposures known to be associated with inflammation (NSAID use and cytokines) and tested their association with incident breast cancer and mammographic density, a well-established risk factor for breast cancer. Despite the evidence that NSAIDs consistently inhibit COX-2 (34), resulting in decreased biosynthesis of estrogen and thereby hindering breast tumor cell growth *in vitro* and in animal models (28, 47-50), our results do not support a protective effect of nonprescription NSAIDs among older postmenopausal women participating in the Study of Osteoporotic Fractures. However, given the potential public health impact should NSAIDs be successful chemopreventive agents for breast cancer, these findings warrant further investigation in larger populations with carefully defined exposure assessment, for instance in a randomized clinical trial. Furthermore, as we did not assess prescription NSAID use, we could not rule out the possibility that other NSAIDs, such as selective COX-2 inhibitors, might reduce the risk of breast cancer. Finally, evidence suggests that inter-individual variation in metabolism of NSAIDs occurs by two major enzymes, CYP2C9 and UGT1A6, which may explain some of the inconsistencies seen in the epidemiologic literature (51-53). No prior study has investigated the association between breast cancer risk (and breast density) with both NSAID use *and* genotypic variations in NSAID metabolizing genes.

We did not observe strong support for an independent association between sTNFRs and the TNFR2 polymorphism with respect to percent mammographic density among postmenopausal women in the Mammograms and Masses Study. However, we did confirm that percent mammographic density is associated with several breast cancer risk factors (3, 9, 36-38,

54-70). For instance, MAMS women with higher percent mammographic density were more likely to be nulliparous and/or have a later age at first birth, to be former hormone therapy users, and to report a history of breast biopsy than women with lower percent mammographic density. In addition, women with higher percent breast density were younger and had fewer years since menopause, had a lower BMI, were more likely to have attended post-secondary education and to report current consumption of alcohol. In addition, we confirmed that women with higher sTNFR levels had a higher BMI and were older than women with lower sTNFR levels (71-73). While not the primary aim of the study, recent aspirin use reported at blood collection was associated with lower percent mammographic density.

While the mechanism by which mammographic density increases breast cancer risk remains unknown, it is thought that dense areas may be associated with increased cell proliferation (74). A recent retrospective study of diagnostic mammograms from women diagnosed with DCIS demonstrated that DCIS lesions occurred overwhelmingly in areas of mammographically dense tissue, suggesting that some characteristic of the dense tissue is *directly* influencing the carcinogenic process in the breast (75). Thus, we hope our findings will encourage other investigators to further examine potential mechanisms by which inflammation may be related to mammographic density and breast cancer risk.

5.5 LITERATURE CITED

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, and Thun MJ, *Cancer Statistics, 2006.* CA Cancer J Clin, 2006. **56**(2): p. 106-130.

2. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, and Hopper JL, *Heritability of mammographic density, a risk factor for breast cancer.* New England Journal of Medicine, 2002. **347**(12): p. 886-94.

3. Harvey JA and Bovbjerg VE, *Quantitative assessment of mammographic breast density: relationship with breast cancer risk.* Radiology, 2004. **230**(1): p. 29-41.

4. Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, and Paterson AD, *Mammographic breast density as an intermediate phenotype for breast cancer.* The Lancet Oncology, 2005. **6**(10): p. 798-808.

5. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, and Minkin S, *The association of breast mitogens with mammographic densities.* British Journal of Cancer, 2002. **87**(8): p. 876-82.

6. The Endogenous Hormones and Breast Cancer Collaborative Group, *Endogenous Sex Hormones and Breast Cancer in Postmenopausal Women: Reanalysis of Nine Prospective Studies.* JNCI Cancer Spectrum, 2002. **94**(8): p. 606-616.

7. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, and Cummings SR, *Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group.[comment].* Annals of Internal Medicine., 1999. **130**(4 Pt 1): p. 270-7.

8. Collaborative Group on Hormonal Factors in Breast C, *Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. [comment][erratum appears in Lancet 1997 Nov 15;350(9089):1484].* Lancet., 1997. **350**(9084): p. 1047-59.

9. Writing Group for the Women's Health Initiative I, *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* Jama, 2002. **288**(3): p. 321-33.

10. Miller WR and Forrest AP, *Oestradiol synthesis by a human breast carcinoma.* Lancet, 1974. **2**(7885): p. 866-8.

11. Abeloff MD, Lichter AS, Niederhuber JE, Pierce LJ, and Love RR, *Breast*, in *Clinical Oncology*, Abeloff MD, et al., Editors. 2000, Churchill Livingstone: Philadelphia.

12. Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, Loar A, Rodabough RJ, White E, and McTiernan A, *Breast Cancer and Nonsteroidal Anti-Inflammatory Drugs: Prospective Results from the Women's Health Initiative.* Cancer Res, 2003. **63**(18): p. 6096-6101.

13. Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, Subbaramaiah K, Dannenberg AJ, and Neugut AI, *Association of Frequency and Duration of Aspirin Use and Hormone Receptor Status With Breast Cancer Risk.* JAMA, 2004. **291**(20): p. 2433-2440.

14. Harris RE, Beebe-Donk J, Doss H, and Burr Doss D, *Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review).* Oncol Rep, 2005. **13**(4): p. 559-83.

15. Egan K, Stampfer M, Giovannucci E, Rosner B, and Colditz G, *Prospective study of regular aspirin use and the risk of breast cancer.* J Natl Cancer Inst, 1996. **88**(14): p. 988a-993.

16. Friis S, Sorensen HT, McLaughlin JK, Johnsen SP, Blot WJ, and Olsen JH, *A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin.* Br J Cancer, 2003. **88**(5): p. 684-8.

17. Garcia Rodriguez LA and Gonzalez-Perez A, *Risk of breast cancer among users of aspirin and other anti-inflammatory drugs.* Br J Cancer, 2004. **91**(3): p. 525-9.

18. Jacobs EJ, Thun MJ, Connell CJ, Rodriguez C, Henley SJ, Feigelson HS, Patel AV, Flanders WD, and Calle EE, *Aspirin and Other Nonsteroidal Anti-inflammatory Drugs and Breast Cancer Incidence in a Large U.S. Cohort.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(1): p. 261-264.

19. Paganini-Hill A, Chao A, Ross RK, and Henderson BE, *Aspirin use and chronic diseases: a cohort study of the elderly.* Bmj, 1989. **299**(6710): p. 1247-50.

20. Sorensen HT, Friis S, Norgard B, Mellemkjaer L, Blot WJ, McLaughlin JK, Ekbom A, and Baron JA, *Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study.* Br J Cancer, 2003. **88**(11): p. 1687-92.

21. Thun M, Namboodiri M, Calle E, Flanders W, and Heath C, Jr, *Aspirin use and risk of fatal cancer.* Cancer Res, 1993. **53**(6): p. 1322-1327.

22. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, and Buring JE, *Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial.* JAMA, 2005. **294**(1): p. 47-55.

23. Harris RE, Kasbari S, and Farrar WB, *Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer.* Oncol Rep, 1999. **6**(1): p. 71-3.

24. Johnson TW, Anderson KE, Lazovich D, and Folsom AR, *Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2002. **11**(12): p. 1586-1591.

25. Sharpe CR, Collet JP, McNutt M, Belzile E, Boivin JF, and Hanley JA, *Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage.* Br J Cancer, 2000. **83**(1): p. 112-20.

26. Schreinemachers DM and Everson RB, *Aspirin use and lung, colon, and breast cancer incidence in a prospective study.* Epidemiology, 1994. **5**(2): p. 138-46.

27. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A, and Ross RK, *Nonsteroidal Anti-Inflammatory Drug Use and Breast Cancer Risk by Stage and Hormone Receptor Status.* J Natl Cancer Inst, 2005. **97**(11): p. 805-812.

28. Harris R, Beebe-Donk J, and Alshafie G, *Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors.* BMC Cancer, 2006. **6**(1): p. 27.

29. Reed MJ and Purohit A, *Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis.* Endocr Rev, 1997. **18**(5): p. 701-15.

30. Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, and Bulun SE, *Estrogen Regulates Expression of Tumor Necrosis Factor Receptors in Breast Adipose Fibroblasts.* J Clin Endocrinol Metab, 2004. **89**(8): p. 4018-4024.

31. Purohit A, Newman S, and Reed M, *The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer.* Breast Cancer Res, 2002. **4**(2): p. 65 - 69.

32. Aderka D, *The potential biological and clinical significance of the soluble tumor necrosis factor receptors.* Cytokine Growth Factor Rev, 1996. **7**(3): p. 231-40.

33. Gasparini G, Longo R, Sarmiento R, and Morabito A, *Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?* Lancet Oncol, 2003. **4**(10): p. 605-15.

34. Howe LR, Subbaramaiah K, Brown AM, and Dannenberg AJ, *Cyclooxygenase-2: a target for the prevention and treatment of breast cancer.* Endocrine-Related Cancer, 2001. **8**(2): p. 97-114.

35. Kaaks R, Diorio C, and Brisson J, *Insulin-Like Growth Factor-I and Mammographic Breast Density.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(12): p. 3019-.

36. McCormack VA, dos Santos Silva I, De Stavola BL, Perry N, Vinnicombe S, Swerdlow AJ, Hardy R, and Kuh D, *Life-course body size and perimenopausal mammographic*

parenchymal patterns in the MRC 1946 British birth cohort. Br J Cancer, 2003. **89**(5): p. 852-9.

37. Lam PB, Vacek PM, Geller BM, and Muss HB, *The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont.* Cancer, 2000. **89**(2): p. 369-75.

38. Modugno F, Ngo D, Allen G, Kuller L, Ness R, Vogel V, Costantino J, and Cauley J, *Breast cancer risk factors and mammographic breast density in women over age 70.* Breast Cancer Research and Treatment, 2005: p. 1-10.

39. Haars G, van Noord PAH, van Gils CH, Grobbee DE, and Peeters PHM, *Measurements of Breast Density: No Ratio for a Ratio.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(11): p. 2634-2640.

40. Khuder SA and Mutgi AB, *Breast cancer and NSAID use: a meta-analysis.* British Journal of Cancer, 2001. **84**(9): p. 1188-92.

41. Glenn CL, Wang WY, Benjafield AV, and Morris BJ, *Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor.* Human Molecular Genetics, 2000. **9**(13): p. 1943-9.

42. Tolusso B, Sacco S, Gremese E, La Torre G, Tomietto P, and Ferraccioli GF, *Relationship between the tumor necrosis factor receptor II (TNF-RII) gene polymorphism and sTNF-RII plasma levels in healthy controls and in rheumatoid arthritis.* Human Immunology, 2004. **65**(12): p. 1420-1426.

43. Vachon CM, King RA, Atwood LD, Kuni CC, and Sellers TA, *Preliminary sibpair linkage analysis of percent mammographic density.* Journal of the National Cancer Institute, 1999. **91**(20): p. 1778-9.

44. Huo Z, Giger ML, Olopade OI, Wolverton DE, Weber BL, Metz CE, Zhong W, and Cummings SA, *Computerized analysis of digitized mammograms of BRCA1 and BRCA2 gene mutation carriers.* Radiology, 2002. **225**(2): p. 519-26.

45. Ziv E, Shepherd J, Smith-Bindman R, and Kerlikowske K, *Mammographic breast density and family history of breast cancer.* J Natl Cancer Inst, 2003. **95**(7): p. 556-8.

46. Aderka D, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, and Wallach D, *Variation in serum levels of the soluble TNF receptors among healthy individuals.* Lymphokine and Cytokine Research, 1992. **11**(3): p. 157-9.

47. Ip MM, Mazzer C, Watson D, and Ip C, *The effect of eicosanoid synthesis inhibitors on DMBA-induced rat mammary carcinogenesis.* Proc Am Assoc Cancer Res, 1989. **30**: p. 182.

48. Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, and Robertson FM, *Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens.* Cancer Letters, 1999. **140**(1-2): p. 27-35.

49. Lee PP and Ip MM, *Regulation of proliferation of rat mammary tumor cells by inhibitors of cyclooxygenase and lipoxygenase.* Prostaglandins Leukot Essent Fatty Acids, 1992. **45**: p. 21-31.

50. Steele VE, Moon RC, Lubet RA, Grubbs CJ, Reddy BS, Wargovich M, McCormick DL, Pereira MA, Crowell JA, Bagheri D, and et al., *Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program.* J Cell Biochem Suppl, 1994. **20**: p. 32-54.

51. Martin JH, Begg EJ, Kennedy MA, Roberts R, and Barclay ML, *Is cytochrome P450 2C9 genotype associated with NSAID gastric ulceration?* Br J Clin Pharmacol, 2001. **51**(6): p. 627-30.

52. Miners JO and Birkett DJ, *Cytochrome P4502C9: an enzyme of major importance in human drug metabolism.* Br J Clin Pharmacol, 1998. **45**(6): p. 525-38.

53. Ciotti M, Marrone A, Potter C, and Owens IS, *Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications.* Pharmacogenetics, 1997. **7**(6): p. 485-95.

54. de Waard F, Rombach JJ, Collette HJ, and Slotboom B, *Breast cancer risk associated with reproductive factors and breast parenchymal patterns.* J Natl Cancer Inst, 1984. **72**(6): p. 1277-82.

55. Vachon CM, Kuni CC, Anderson K, Anderson VE, and Sellers TA, *Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States).* Cancer Causes and Control, 2000. **11**(7): p. 653-62.

56. Ernster VL, Sacks ST, Peterson CA, and Schweitzer RJ, *Mammographic parenchymal patterns and risk factors for breast cancer.* Radiology, 1980. **134**(3): p. 617-20.

57. Bergkvist L, Tabar L, Bergstrom R, and Adami HO, *Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program.* American Journal of Epidemiology, 1987. **126**(6): p. 1075-81.

58. de Stavola BL, Gravelle IH, Wang DY, Allen DS, Bulbrook RD, Fentiman IS, Hayward JL, and Chaudary MC, *Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study.* International Journal of Epidemiology, 1990. **19**(2): p. 247-54.

59. Grove JS, Goodman MJ, Gilbert FI, Jr., and Mi MP, *Factors associated with mammographic pattern.* British Journal of Radiology, 1985. **58**(685): p. 21-5.

60. Kaufman Z, Garstin WI, Hayes R, Michell MJ, and Baum M, *The mammographic parenchymal patterns of nulliparous women and women with a family history of breast cancer.* Clinical Radiology, 1991. **43**(6): p. 385-8.

61. Gram IT, Funkhouser E, and Tabar L, *Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women.* British Journal of Cancer, 1995. **71**(3): p. 647-50.

62. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao M-S, Khokha R, Martin L, and Boyd N, *The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer.* Cancer Epidemiol Biomarkers Prev, 2005. **14**(2): p. 343-349.

63. Jeffreys M, Warren R, Gunnell D, McCarron P, and Smith GD, *Life course breast cancer risk factors and adult breast density (United Kingdom).* Cancer Causes and Control, 2004. **15**(9): p. 947-955.

64. Christodoulakos GE, Lambrinoudaki IV, Panoulis KP, Vourtsi AD, Vlachos L, Georgiou E, and Creatsas GC, *The effect of various regimens of hormone replacement therapy on mammographic breast density.* Maturitas, 2003. **45**(2): p. 109-18.

65. Maskarinec G, Takata Y, Pagano I, Lurie G, Wilkens LR, and Kolonel LN, *Alcohol consumption and mammographic density in a multiethnic population.* Int J Cancer, 2006. **118**(10): p. 2579-83.

66. Whitehouse GH and Leinster SJ, *The variation of breast parenchymal patterns with age.* British Journal of Radiology, 1985. **58**(688): p. 315-8.

67. Whitehead J, Carlile T, Kopecky KJ, Thompson DJ, Gilbert FI, Jr., Present AJ, Threatt BA, Krook P, and Hadaway E, *Wolfe mammographic parenchymal patterns. A study of the masking hypothesis of Egan and Mosteller.* Cancer, 1985. **56**(6): p. 1280-6.

68. van Gils CH, Otten JD, Verbeek AL, and Hendriks JH, *Short communication: breast parenchymal patterns and their changes with age.* British Journal of Radiology, 1995. **68**(814): p. 1133-5.

69. Riza E, dos Santos Silva I, De Stavola B, Perry N, Karadedou-Zafiriadou E, Linos D, Remoundos D-D, and Linos A, *Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece.* European Journal of Cancer, 2005. **41**(4): p. 590-600.

70. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, and Palli D, *Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population.* International Journal of Cancer, 2006. **118**(7): p. 1782-1789.

71. Mohamed-Ali V, Goodrick S, Bulmer K, Holly JM, Yudkin JS, and Coppack SW, *Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo.* Am J Physiol, 1999. **277**(6 Pt 1): p. E971-5.

72. Hauner H, Bender M, Haastert B, and Hube F, *Plasma concentrations of soluble TNF-alpha receptors in obese subjects.* Int J Obes Relat Metab Disord, 1998. **22**(12): p. 1239-43.

73. Hasegawa Y, Sawada M, Ozaki N, Inagaki T, and Suzumura A, *Increased soluble tumor necrosis factor receptor levels in the serum of elderly people.* Gerontology, 2000. **46**(4): p. 185-8.

74. Harrison DA, Duffy SW, Sala E, Warren RML, Couto E, and Day NE, *Deterministic models for breast cancer progression:: Application to the association between mammographic parenchymal pattern and histologic grade of breast cancers.* Journal of Clinical Epidemiology, 2002. **55**(11): p. 1113-1118.

75. Ursin G, Hovanessian-Larsen L, Parisky Y, Pike M, and Wu A, *Greatly increased occurrence of breast cancers in areas of mammographically dense tissue.* Breast Cancer Research, 2005. **7**(5): p. R605 - R608.

6.0 PUBLIC HEALTH SIGNIFICANCE

In these collective studies, we attempted to explore whether breast cancer risk may be significantly decreased by reducing inflammation. It is well known that mammograms provide early detection of breast cancer and a 20-30% reduction in breast carcinoma mortality. However, breast density has been shown to affect mammographic sensitivity and specificity, with greater breast density being associated with a reduction in the technical performance of mammography. Breast density is also predictive of breast cancer risk. Extensive areas of light appearing, mammographically dense tissue on a mammogram have been associated with a 4-6 fold increase in breast cancer risk after adjusting for other known risk factors for the disease. Thus, women who are at higher risk of the disease may not be as well served by mammograms.

Increased understanding of factors that affect breast density and their underlying mechanisms is needed, and inflammatory cytokines may be involved. While we did not observe a reduction in incident breast cancer with NSAID use, we did find an inverse association between aspirin use and breast density. Such findings could provide the impetus for investigating NSAIDs as potential chemopreventive agents for breast cancer. Confirmation of this finding in additional studies would have important pubic health implications.

APPENDIX A

NONSTEROIDAL ANTI-INFLAMMATORY DRUG USE AND BREAST CANCER IN OLDER WOMEN: THE STUDY OF OSTEOPOROTIC FRACTURES

Table 30. Visit 2 analysis: Incident breast cancer among participants in the Study of Osteoporotic Fractures, 1986-2003

Total Cohort	**9704**
Total with available Visit 2 data	**9339**
Prevalent breast cancer self-reported in first annual questionnaire (year 1)	470
Additional prevalent breast cancer diagnosed before visit 2	52
Censored before visit 2	1
No available data on breast cancer status during follow-up	113
Confirmed cases	443
Non-cases	8260
Total with confirmed breast cancer information, eligible for V2 analysis	**8703**
Total with missing covariate data	2008
Aspirin or Ibuprofen at visit 2	438
Potential confounding factors at visit 2	1570
Final sample for V2 analysis	**6695**

Table 31. Characteristics of women by breast cancer status in the Study of Osteoporotic Fractures

	Controls (n=6323)		Cases (n=372)		
Variable	**n**	%	**n**	%	**p-value**
Clinic*					0.34
A	1670	26	111	30	
B	1540	24	95	25	
C	1412	22	77	21	
D	1701	27	89	24	
Age (±SD), years	73 (5)		72 (4)		0.0004
Age (years)					
≤72	3307	52	221	59	0.008
73+	3016	48	151	41	
Education*					0.17
<High school	1362	21	69	19	
High school graduate	4961	78	303	81	
Family history of breast cancer*					0.03
No	5500	87	309	83	
Yes	823	13	63	17	
Age at first Menses					0.22
<12	745	12	49	14	
12-13	3337	54	204	57	
14+	2086	34	106	30	
Missing	155		13		
Parity*					0.99
Nulliparous	1179	19	67	18	
1	871	14	51	14	
2	1726	27	102	27	
3	1284	20	79	21	
4	660	10	37	10	
5+	603	9	36	10	
Nulliparous*					0.76
No	5144	81	305	82	
Yes	1179	19	67	18	
Age at first live birth					0.78
≤20	791	15	45	15	
>20	4344	85	259	85	
Missing	1188		68		
Age (years) at menopause*					0.63
≤40	550	10	30	9	
41-45	1017	19	65	21	
46-50	2034	38	110	35	
≥51	1726	32	108	34	
Missing	996		59		
Surgical menopause*					0.18
No	5555	88	318	85	
Yes	768	12	54	14	

Table 31 (continued)

Variable	Controls (n=6323) n	%	Cases (n=372) n	%	p-value
Estrogen only therapy (Oral)*					0.003
Never use	3645	58	190	51	
Past use	1777	28	106	28	
Current use	901	14	76	20	
Estrogen only therapy (Any Current)*					0.001
No	5422	86	296	80	
Yes	901	14	76	20	
Average no. of alcoholic drinks/week (±SD)*	2 (4)		2 (4)		0.96
Average no. of alcoholic drinks/week*					0.59
None	1830	29	102	27	
<1	2134	34	131	35	
1-2	780	12	50	13	
2-7	1041	16	65	17	
>7	538	9	24	6	
Cigarette Smoking					0.09
Never	3802	60	245	66	
Former	2026	32	102	27	
Current	495	8	25	7	
Walks for exercise*					0.37
No	3006	47	168	45	
Yes	3317	52	204	55	
Body mass index §					0.07
<25	2849	45	150	40	
25+	3474	55	222	60	
Hypertension*					0.3
Never	3944	62	242	65	
Ever	2379	38	130	35	
Hip bone mineral density (±SD), g/cm²	0.76 (0.13)		0.79 (0.12)		<0.0001
Stroke					0.7
Never	6020	96	358	96	
Ever	262	4	14	4	
Missing	41		0		
Heart Attack					0.52
Never	5076	93	299	94	
Ever	377	7	19	6	
Missing	870		54		
Mammogram at visit 3					0.03
No	839	20	36	15	
Yes	3275	80	209	85	
Missing	2209		127		

***Information collected at baseline clinical visit.**

† History of breast cancer in a mother or sister.

§ Weight (kg)/height² (m²).

Table 32. Clinical characteristics of breast cancer cases by regular aspirin use, SOF (1986-1993)

	Regular use of asiprin in past 12 mos					Daily use of aspirin for at least 1 year				
	No (n=229)		Yes (n=143)			No (n=297)		Yes (n=75)		
Characteristics	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Age at diagnosis, mean (SD), y	78 (5)		79 (6)		0.38	78 (5)		78 (6)		0.75
Estrogen receptor status, No. (%)†					§					§
Positive	168	73	101	71		211	71	58	77	
Negative	20	9	17	12		32	11	5	7	
Borderline	0	0	1	1		1	0.3	0	0	
Unknown	41	18	24	17		53	18	12	16	
Estrogen receptor status, No. (%)†					0.34					0.26
Positive ‖	168	89	102	86		212	87	58	8	
Negative	20	11	17	14		32	13	5	92	
missing	41		24			53		12		
Progesterone receptor status, No. (%)†					§					§
Positive	131	57	78	55		169	57	40	53	
Negative	49	21	39	27		66	22	22	29	
Borderline	5	2	2	1		6	2	1	1	
Unknown	44	19	24	17		56	19	12	16	
Progesterone receptor status, No. (%)†					0.24					0.24
Positive ‖	136	74	80	67		175	73	41	65	
Negative	49	26	39	33		66	27	22	35	
missing	44		24			56		12		
Cancer stage at diagnosis, No. (%)‡					0.92					0.88
O (in situ)	33	14	19	13		41	14	11	15	
I	127	55	79	55		167	56	39	52	
II (no nodes)	27	12	14	10		35	12	6	8	
II (+ nodes)	23	10	13	9		25	8	11	15	
III	6	3	8	6		11	4	3	4	
IV	1	0	3	2		4	1	0	0	
Unknown	12	5	7	5		14	5	5	7	

†Women with unknown estrogen receptor status were excluded in statistical tests.

‡The p value compares women with stage II cancer or greater at diagnosis with other cases. Women with unknown cancer stage at diagnosis were excluded from this analysis.

‖ Borderline recoded to positive; § Cell sizes too sparse to use chi square test to compare clinical characteristic by medication use.

Table 33. Clinical characteristics of breast cancer cases by regular non-aspirin NSAID use, SOF (1986-1993)

	Regular use of a non-aspirin NSAID in past 12 months					Daily use of a non-aspirin NSAID for at least 1 year				
	No (n=273)		Yes (n=99)			No (n=323)		Yes (n=49)		
Characteristics	**n**	**%**	**n**	**%**	**p-value**	**n**	**%**	**n**	**%**	**p-value**
Age at diagnosis, mean (SD), y	78 (5)		79 (6)		0.47	78 (5)		78 (6)		0.37
Estrogen receptor status, No. (%)†					§					§
Positive	194	71	75	76		231	71	38	78	
Negative	30	11	7	7		32	10	5	10	
Borderline	1	0	0	0		1	0	0	0	
Unknown	48	17	17	17		59	18	6	12	
Estrogen receptor status, No. (%)†					0.25					0.93
Positive ‖	195	87	75	91		232	88	38	88	
Negative	30	13	7	8		32	12	5	12	
missing	48		17			59		6		
Progesterone receptor status, No. (%)†					§					§
Positive	149	54	60	61		181	56	28	57	
Negative	68	25	20	20		73	23	15	31	
Borderline	5	2	2	2		7	2	0	0	
Unknown	51	19	17	17		62	19	6	12	
Progesterone receptor status, No. (%)†					0.29					0.35
Positive ‖	154	69	62	76		188	72	28	65	
Negative	68	31	20	24		73	28	15	35	
missing	51		17			62		6		
Cancer stage at diagnosis, No. (%)‡					0.87					§
O (in situ)	40	15	12	12		48	15	4	8	
I	151	55	55	55		179	55	27	55	
II (no nodes)	31	11	10	10		34	11	7	14	
II (+ nodes)	30	11	6	6		33	10	3	6	
III	8	3	6	6		12	4	2	4	
IV	1	0	3	3		3	1	1	2	
Unknown	12	4	7	7		14	4	5	10	

†Women with unknown estrogen receptor status were excluded in statistical tests.

‡The p value compares women with stage II cancer or greater at diagnosis with other cases. Women with unknown cancer stage at diagnosis were excluded from this analysis.

‖ Borderline recoded to positive; § Cell sizes too sparse to use chi square test to compare clinical characteristic by medication use.

Table 34. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use by estrogen receptor (ER) status in the Study of Osteoporotic Fractures, 1986-1993

	Cases	Unadjusted*		Adjusted**		Adjusted***	
Risk Factor	ER+ (ER+/PR+ or ER+/PR-) (n=270)	HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Aspirin ≥ 1/week in past 12 months							
No	168	1.00		1.00		1.00	
Yes	102	0.95	0.74, 1.22	0.95	0.75, 1.22	0.93	0.72, 1.19
Aspirin frequency: §							
No regular use	168	1.00		1.00		1.00	
1-4 days/week	44	0.99	0.71, 1.38	0.98	0.71, 1.37	0.97	0.69, 1.35
5-7 days/week	51	0.86	0.63, 1.18	0.87	0.63, 1.19	0.83	0.61, 1.14
Missing	7						
Aspirin ~daily for 1 year or more §							
No	212	1.00		1.00		1.00	
Yes	58	1.00	0.75, 1.34	1.01	0.75, 1.35	0.97	0.72, 1.30
Aspirin duration: §							
No regular use	212	1.00		1.00		1.00	
< 5 years	29	0.91	0.62, 1.35	0.92	0.62, 1.35	0.89	0.60, 1.32
5+ years	29	1.12	0.76, 1.65	1.13	0.76, 1.66	1.07	0.72, 1.58
Missing	0						
Non-aspirin NSAID ≥ 1/week in past 12 months §							
No	195	1.00		1.00		1.00	
Yes	75	1.07	0.82, 1.40	1.07	0.82, 1.40	1.02	0.78, 1.34
Non-aspirin NSAID frequency: §							
No regular use	195	1.00		1.00		1.00	
1-4 days/week	28	1.21	0.81, 1.80	1.21	0.81, 1.79	1.18	0.79, 1.76
5-7 days/week	45	1.00	0.72, 1.39	1.01	0.73, 1.39	0.94	0.68, 1.31
Missing	2						
Non-aspirin NSAID ~daily for 1 year or more §							
No	232	1.00		1.00		1.00	
Yes	38	1.10	0.78, 1.55	1.10	0.78, 1.55	1.03	0.72, 1.45
Non-aspirin NSAID duration: §							
No regular use	232	1.00		1.00		1.00	
< 5 years	26	1.13	0.75, 1.70	1.14	0.76, 1.70	1.09	0.72, 1.63
5+ years	12	1.06	0.59, 1.89	1.05	0.59, 1.88	0.94	0.52, 1.68
Missing	0						

Table 34 (continued)

Risk Factor	Cases ER+ (ER+/PR+ or ER+/PR-) (n=270)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Any NSAIDs ≥ 1/week in past 12 months							
No	129	1.00		1.00		1.00	
Yes	141	0.92	0.73, 1.17	0.93	0.73, 1.18	0.88	0.69, 1.13
Any NSAIDs frequency: §							
No regular use	129	1.00		1.00		1.00	
1-4 days/week	75	0.86	0.65, 1.15	0.87	0.65, 1.16	0.81	0.61, 1.09
5-7 days/week	60	0.97	0.71, 1.31	0.96	0.71, 1.31	0.93	0.69, 1.27
Missing	6						
Any NSAIDs ~daily for 1 year or more §							
No	189	1.00		1.00		1.00	
Yes	81	0.98	0.76, 1.27	0.99	0.76, 1.28	0.94	0.72, 1.22
NSAIDs duration: §							
No regular use	189	1.00		1.00		1.00	
< 5 years	45	0.95	0.69, 1.32	0.96	0.69, 1.32	0.92	0.66, 1.28
5+ years	36	1.03	0.72, 1.47	1.04	0.73, 1.48	0.96	0.67, 1.38
Missing	0						
Acetaminophen ≥ 1/week in past 12 months §							
No	211	1.00		1.00		1.00	
Yes	58	0.95	0.71, 1.27	0.94	0.71, 1.26	0.92	0.68, 1.23
Missing	1						
Acetaminophen frequency: §							
No regular use	211	1.00		1.00		1.00	
1-4 days/week	36	0.98	0.69, 1.40	0.98	0.69, 1.40	0.95	0.67, 1.36
5-7 days/week	14	0.70	0.41, 1.20	0.70	0.41, 1.21	0.69	0.40, 1.18
Missing	9						
Acetaminophen ~daily for 1 year or more § †							
No	261	1.00		1.00		1.00	
Yes	9						
Missing	0						

Table 34 (continued)

Risk Factor	Cases ER+ (ER+/PR+ or ER+/PR-) (n=270)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Acetaminophen duration: § †							
No regular use	261	1.00		1.00		1.00	
< 5 years	4						
5+ years	5						
Missing	0						

*Proportional hazards regression models.
**Age-adjusted hazard ratio
***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.
‡HR, hazard ratio; CI, confidence interval.
§ Too few hormone receptor-negative breast cancer cases to estimate HR associated with medication use.
† Too few hormone receptor-positive breast cancer cases to estimate HR associated with medication use.

Table 35. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use by progesterone receptor status (PR+) in SOF (1986-1993)

	Cases	Unadjusted*		Adjusted**		Adjusted***	
Risk Factor	PR+ (ER+/PR+ or ER-/PR+) (n=216)	HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Aspirin ≥ 1/week in past 12 months							
No	136	1.00		1.00		1.00	
Yes	80	0.92	0.70, 1.22	0.93	0.70, 1.22	0.89	0.67, 1.18
Aspirin frequency: §							
No regular use	136	1.00		1.00		1.00	
1-4 days/week	40	1.11	0.78, 1.58	1.10	0.77, 1.57	1.08	0.76, 1.53
5-7 days/week	35	0.73	0.50, 1.06	0.74	0.51, 1.07	0.70	0.48, 1.01
Missing	5						
Aspirin ~daily for 1 year or more §							
No	175	1.00		1.00		1.00	
Yes	41	0.86	0.61, 1.21	0.87	0.62, 1.22	0.82	0.58, 1.15
Aspirin duration: §							
No regular use	175	1.00		1.00		1.00	
< 5 years	21	0.81	0.51, 1.27	0.81	0.51, 1.27	0.78	0.49, 1.22
5+ years	20	0.94	0.59, 1.49	0.94	0.59, 1.50	0.87	0.55, 1.39
Missing	0						
Non-aspirin NSAID ≥ 1/week in past 12 months §							
No	154	1.00		1.00		1.00	
Yes	62	1.12	0.83, 1.50	1.12	0.83, 1.50	1.06	0.78, 1.42
Non-aspirin NSAID frequency: §							
No regular use	154	1.00		1.00		1.00	
1-4 days/week	26	1.42	0.94, 2.15	1.42	0.93, 2.14	1.38	0.91, 2.10
5-7 days/week	34	0.96	0.66, 1.39	0.96	0.66, 1.39	0.89	0.61, 1.29
Missing	2						
Non-aspirin NSAID ~daily for 1 year or more §							
No	188	1.00		1.00		1.00	
Yes	28	1.00	0.67, 1.49	1.00	0.67, 1.49	0.91	0.61, 1.37
Non-aspirin NSAID duration: §							
No regular use	188	1.00		1.00		1.00	
< 5 years	18	0.97	0.60, 1.57	0.97	0.60, 1.58	0.92	0.56, 1.49
5+ years	10	1.09	0.57, 2.05	1.08	0.57, 2.05	0.93	0.49, 1.77
Missing	0						
Any NSAIDs ≥ 1/week in past 12 months							
No	102	1.00		1.00		1.00	
Yes	114	0.94	0.72, 1.23	0.95	0.73, 1.24	0.89	0.68, 1.17

Table 35 (continued)

Risk Factor	Cases PR+ (ER+/PR+ or ER-/PR+) (n=216)	Unadjusted* HR‡	Unadjusted* 95% CI	Adjusted** HR‡	Adjusted** 95% CI	Adjusted*** HR‡	Adjusted*** 95% CI
Any NSAIDs frequency: §							
No regular use	102	1.00		1.00		1.00	
1-4 days/week	55	0.80	0.58, 1.11	0.81	0.58, 1.12	0.74	0.53, 1.03
5-7 days/week	55	1.12	0.80, 1.55	1.11	0.80, 1.55	1.07	0.77, 1.49
Missing	4						
Any NSAIDs ~daily for 1 year or more §							
No	158	1.00		1.00		1.00	
Yes	58	0.84	0.62, 1.14	0.85	0.63, 1.15	0.78	0.58, 1.07
NSAIDs duration: §							
No regular use	158	1.00		1.00		1.00	
< 5 years	32	0.81	0.56, 1.19	0.82	0.56, 1.20	0.77	0.53, 1.13
5+ years	26	0.89	0.59, 1.35	0.90	0.59, 1.36	0.81	0.53, 1.23
Missing	0						
Acetaminophen ≥ 1/week in past 12 months §							
No	167	1.00		1.00		1.00	
Yes	48	0.99	0.72, 1.36	0.99	0.72, 1.36	0.95	0.69, 1.31
Missing	1						
Acetaminophen frequency: §							
No regular use	167	1.00		1.00		1.00	
1-4 days/week	34	1.17	0.81, 1.69	1.17	0.81, 1.69	1.13	0.78, 1.64
5-7 days/week	7	0.44	0.21, 0.94	0.45	0.21, 0.95	0.43	0.20, 0.91
Missing	8						
Acetaminophen ~daily for 1 year or more § †							
No	211	1.00		1.00		1.00	
Yes	5						
Missing	0						
Acetaminophen duration: § †							
No regular use	211	1.00		1.00		1.00	
< 5 years	2						
5+ years	3						
Missing	0						

*Proportional hazards regression models.

**Age-adjusted hazard ratio

***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.

‡HR, hazard ratio; CI, confidence interval.

§ Too few hormone receptor-negative breast cancer cases to estimate HR associated with medication use.

† Too few hormone receptor-positive breast cancer cases to estimate HR associated with medication use.

Table 36. Estimated relative hazard of breast cancer associated with history of NSAID and acetaminophen use by progesterone receptor status (PR-) in SOF (1986-1993)

	Cases	Unadjusted*		Adjusted**		Adjusted***	
Risk Factor	PR- (ER-/PR- or ER+/PR-) (n=88)	HR‡	95% CI	HR‡	95% CI	HR‡	95% CI
Aspirin ≥ 1/week in past 12 months							
No	49	1.00		1.00		1.00	
Yes	39	1.25	0.82, 1.90	1.25	0.82, 1.91	1.24	0.81, 1.89
Aspirin frequency: §							
No regular use	49	1.00		1.00		1.00	
1-4 days/week	12	0.93	0.49, 1.74	0.92	0.49, 1.73	0.90	0.48, 1.70
5-7 days/week	24	1.38	0.85, 2.25	1.40	0.86, 2.28	1.39	0.85, 2.27
Missing	3						
Aspirin ~daily for 1 year or more §							
No	66	1.00		1.00		1.00	
Yes	22	1.21	0.75, 1.97	1.23	0.76, 1.99	1.22	0.75, 1.98
Aspirin duration: §							
No regular use	66	1.00		1.00		1.00	
< 5 years	11	1.11	0.58, 2.10	1.11	0.59, 2.11	1.10	0.58, 2.09
5+ years	11	1.35	0.72, 2.57	1.38	0.73, 2.60	1.37	0.72, 2.62
Missing	0						
Non-aspirin NSAID ≥ 1/week in past 12 months §							
No	68	1.00		1.00		1.00	
Yes	20	0.82	0.50, 1.36	0.83	0.50, 1.36	0.79	0.48, 1.32
Non-aspirin NSAID frequency: §							
No regular use	68	1.00		1.00		1.00	
1-4 days/week	4						
5-7 days/week	15						
Missing	1						
Non-aspirin NSAID ~daily for 1 year or more §							
No	73	1.00		1.00		1.00	
Yes	15	1.38	0.79, 2.40	1.38	0.79, 2.41	1.35	0.77, 2.38
Non-aspirin NSAID duration: §							
No regular use	73	1.00		1.00		1.00	
< 5 years	12						
5+ years	3						
Missing	0						
Any NSAIDs ≥ 1/week in past 12 months							
No	40	1.00		1.00		1.00	
Yes	48	1.02	0.67, 1.55	1.02	0.67, 1.55	0.99	0.65, 1.52

Table 36 (continued)

	Cases	Unadjusted*		Adjusted**		Adjusted***	
Risk Factor	**PR- (ER-/PR- or ER+/PR-) (n=88)**	**HR‡**	**95% CI**	**HR‡**	**95% CI**	**HR‡**	**95% CI**
Any NSAIDs frequency: §							
No regular use	40	1.00		1.00		1.00	
1-4 days/week	32	1.18	0.74, 1.88	1.20	0.75, 1.91	1.16	0.72, 1.87
5-7 days/week	13	0.68	0.36, 1.27	0.68	0.36, 1.26	0.66	0.35, 1.24
Missing	3						
Any NSAIDs ~daily for 1 year or more §							
No	55	1.00		1.00		1.00	
Yes	33	1.37	0.89, 2.10	1.38	0.90, 2.13	1.39	0.90, 2.16
NSAIDs duration: §							
No regular use	55	1.00		1.00		1.00	
< 5 years	20	1.44	0.86, 2.40	1.46	0.87, 2.43	1.46	0.87, 2.45
5+ years	13	1.27	0.70, 2.33	1.29	0.70, 2.36	1.30	0.71, 2.41
Missing	0						
Acetaminophen ≥ 1/week in past 12 months §							
No	71	1.00		1.00		1.00	
Yes	17	0.83	0.49, 1.40	0.82	0.48, 1.40	0.83	0.49, 1.41
Missing	0						
Acetaminophen frequency: §							
No regular use	71	1.00		1.00		1.00	
1-4 days/week	8						
5-7 days/week	7						
Missing	2						
Acetaminophen ~daily for 1 year or more § †							
No	83	1.00		1.00		1.00	
Yes	5						
Missing	0						
Acetaminophen duration: § †							
No regular use	83	1.00		1.00		1.00	
< 5 years	2						
5+ years	3						
Missing	0						

*Proportional hazards regression models.

**Age-adjusted hazard ratio

***Data were controlled for age, current use of estrogen therapy, BMI, surgical menopause, total hip BMD, smoking, family history of breast cancer, study center, walking for exercise, nulliparity, and hypertension.

‡HR, hazard ratio; CI, confidence interval.

§ Too few hormone receptor-negative breast cancer cases to estimate HR associated with medication use.

† Too few hormone receptor-positive breast cancer cases to estimate HR associated with medication use.

APPENDIX B

THE MAMMOGRAMS AND MASSES STUDY (MAMS)

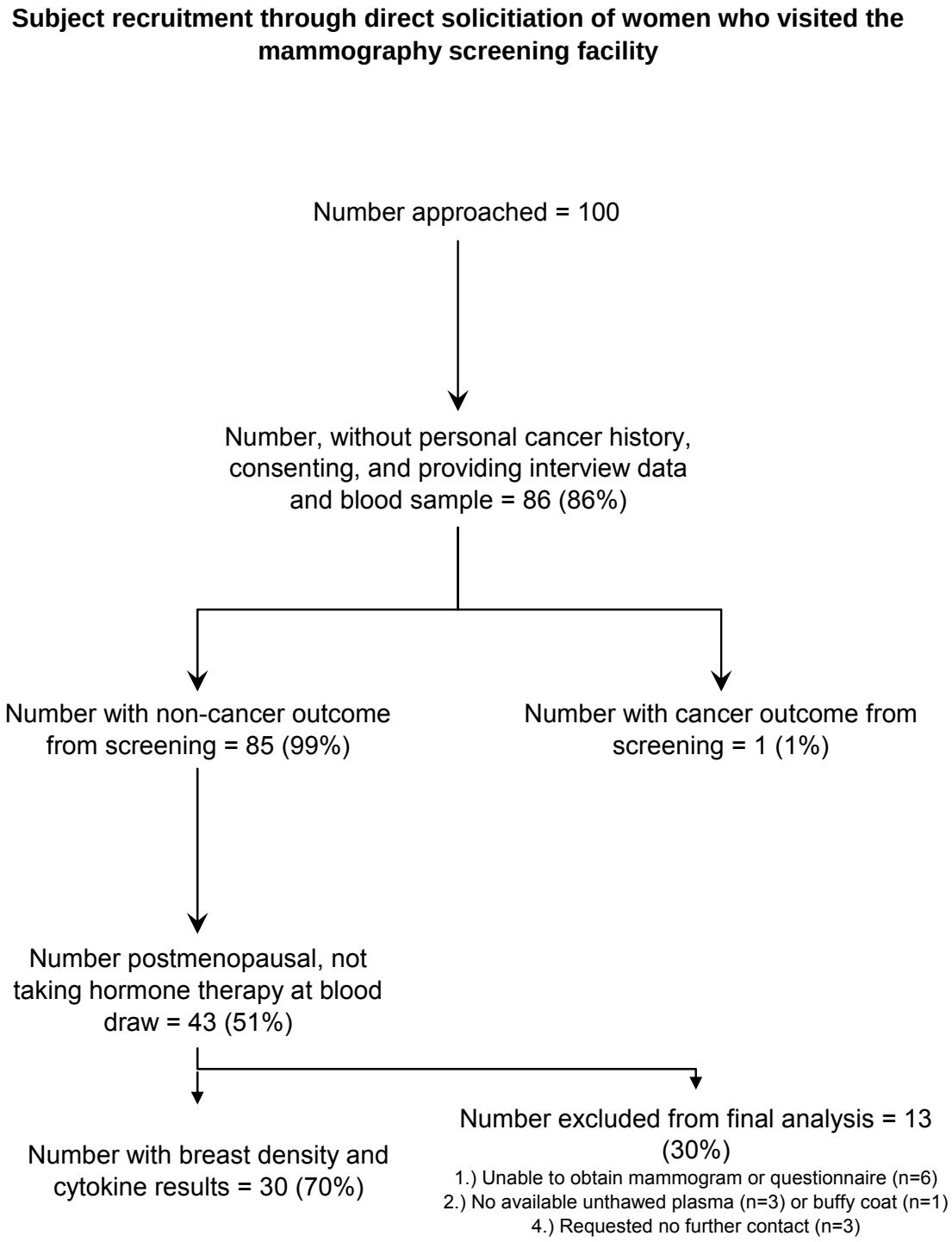


Figure 2. Recruitment in the mammography screening facility, MAMS

Subject recruitment through direct solicitiation of women who visited the breast biopsy facility or surgical clinic

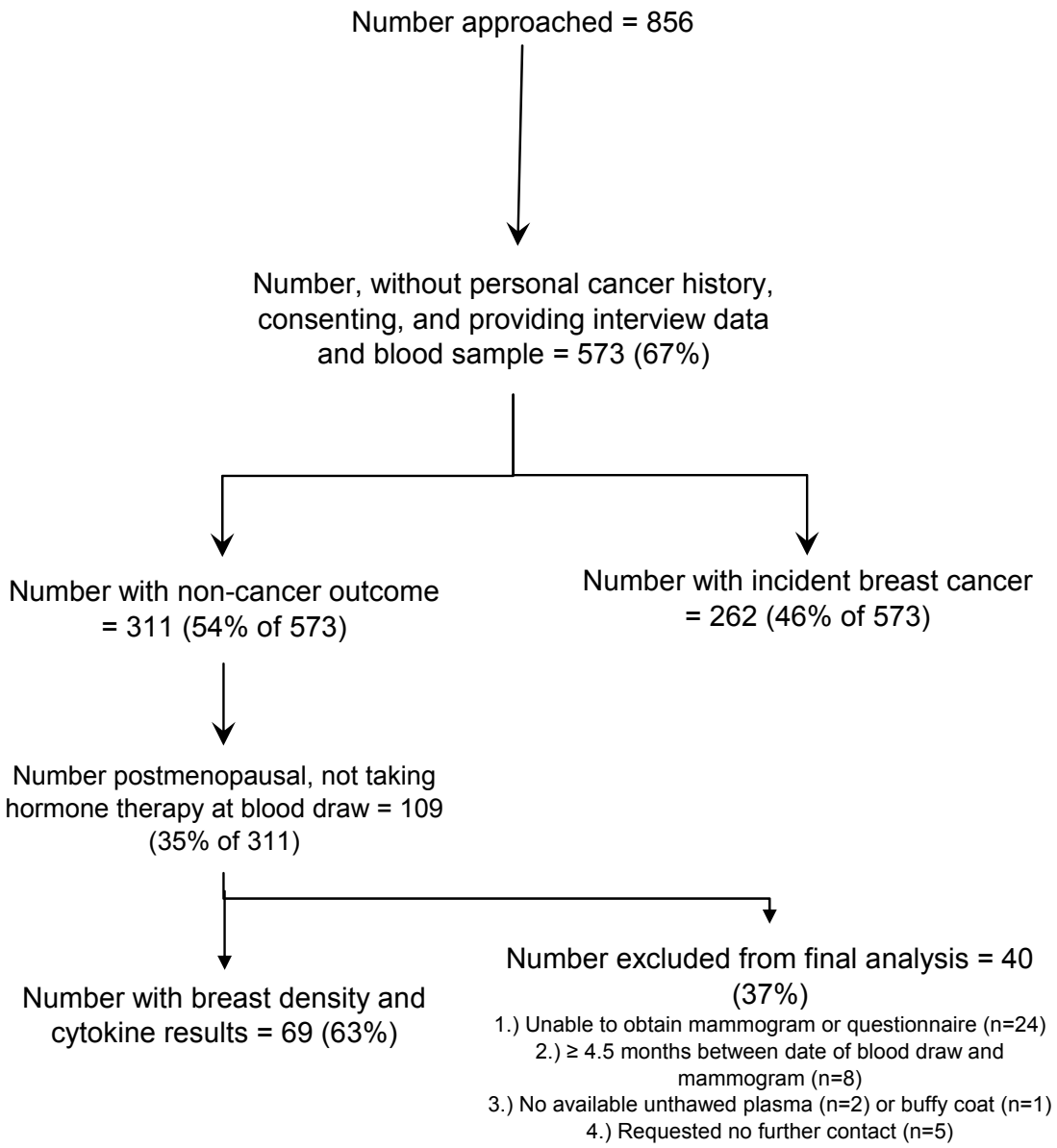


Figure 3. Recruitment in the biopsy facility or surgical clinic, MAMS

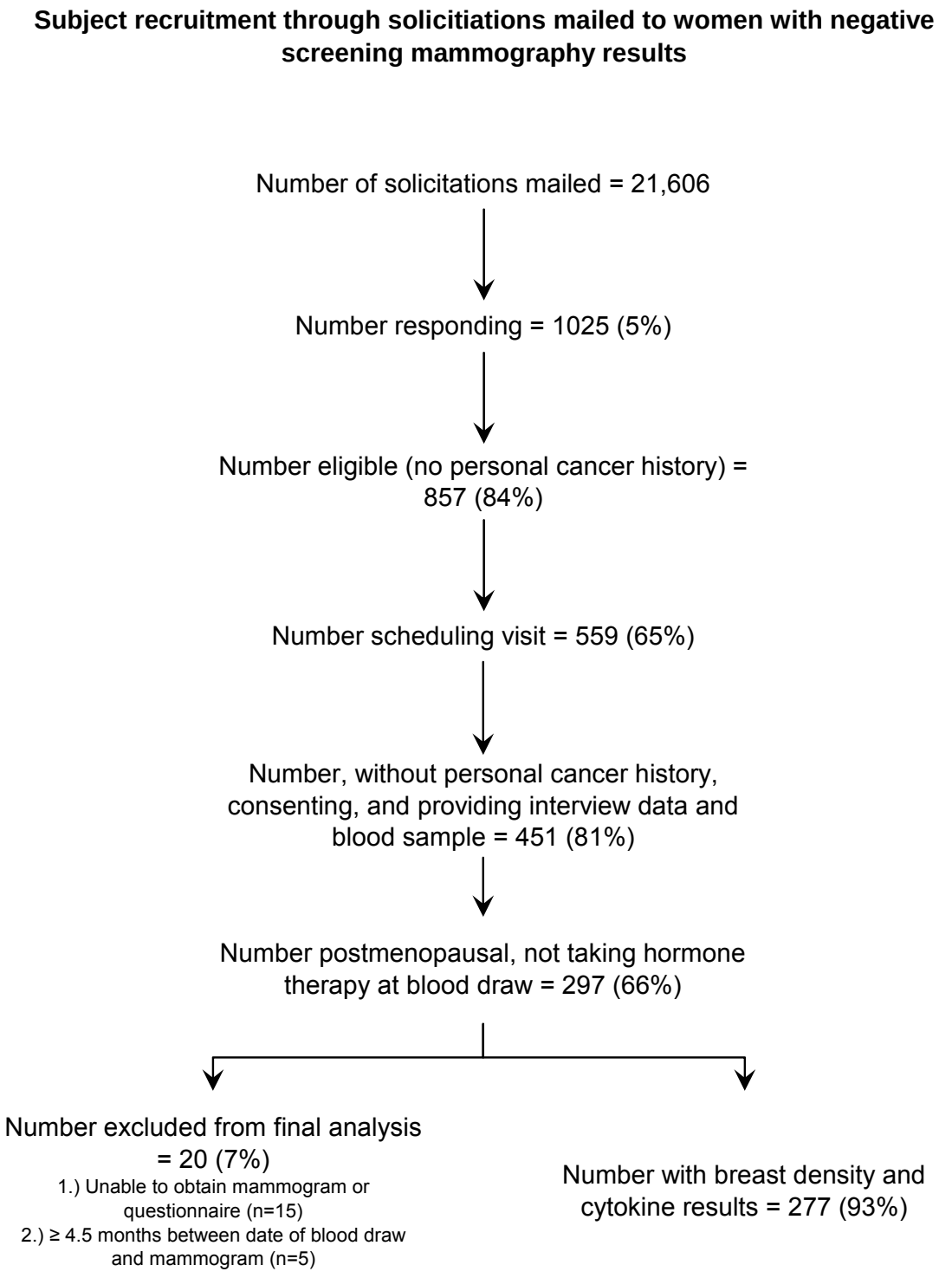
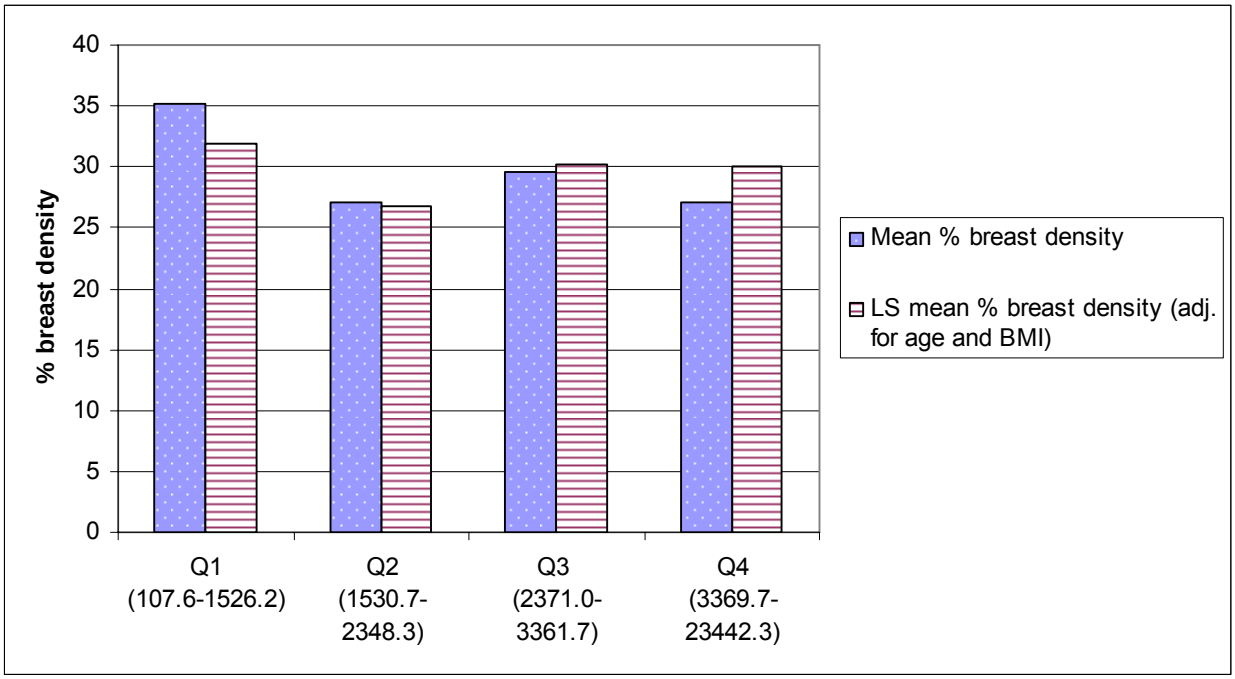
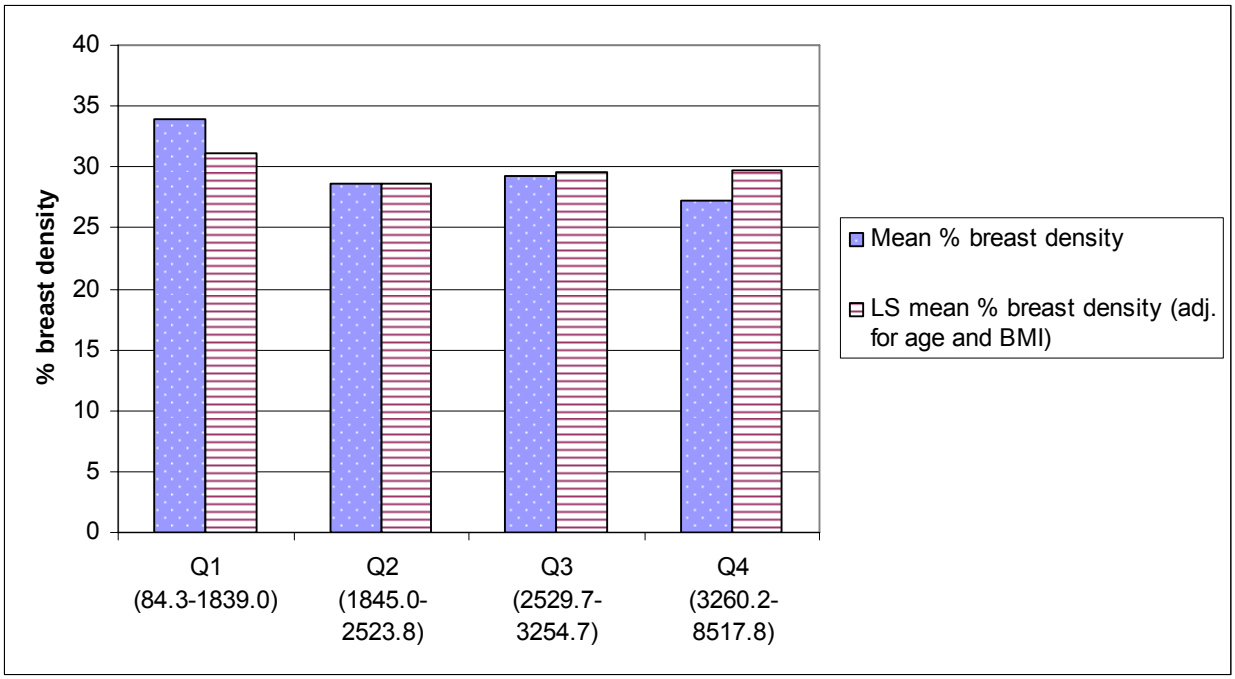


Figure 4. Recruitment by flyer, MAMS



LS=least squares mean; Q=quartile

Figure 5. Mean % mammographic density by quartiles of sTNFR1, unadjusted and adjusted for age and BMI



LS=least squares mean; Q=quartile

Figure 6. Mean % mammographic density by quartiles of sTNFR2, unadjusted and adjusted for age and BMI

BIBLIOGRAPHY

Abeloff, M. D., A. S. Lichter, et al. (2000). Breast. Clinical Oncology. M. D. Abeloff, J. O. Armitage, A. S. Lichter and J. E. Niederhuber. Philadelphia, Churchill Livingstone.

Adams, E. F., B. Rafferty, et al. (1991). "Interleukin 6 is secreted by breast fibroblasts and stimulates 17 beta-oestradiol oxidoreductase activity of MCF-7 cells: possible paracrine regulation of breast 17 beta-oestradiol levels." Int J Cancer **49**(1): 118-21.

Aderka, D. (1996). "The potential biological and clinical significance of the soluble tumor necrosis factor receptors." Cytokine Growth Factor Rev **7**(3): 231-40.

Aderka, D., H. Engelmann, et al. (1992). "Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors." J Exp Med **175**(2): 323-9.

Aderka, D., H. Engelmann, et al. (1992). "Variation in serum levels of the soluble TNF receptors among healthy individuals." Lymphokine and Cytokine Research **11**(3): 157-9.

Aderka, D., H. Englemann, et al. (1991). "Increased serum levels of soluble receptors for tumor necrosis factor in cancer patients." Cancer Research **51**(20): 5602-7.

Aiello, E. J., D. S. M. Buist, et al. (2005). "Association between Mammographic Breast Density and Breast Cancer Tumor Characteristics." Cancer Epidemiol Biomarkers Prev **14**(3): 662-668.

Aiello, E. J., S. S. Tworoger, et al. (2005). "Associations among Circulating Sex Hormones, Insulin-Like Growth Factor, Lipids, and Mammographic Density in Postmenopausal Women." Cancer Epidemiol Biomarkers Prev **14**(6): 1411-1417.

Al-Ansari, A. S., W. E. R. Ollier, et al. (2000). "Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus." Tissue Antigens **55**(1): 97-99.

Alberg, A. J. and S. Singh (2001). "Epidemiology of breast cancer in older women: implications for future healthcare." Drugs & Aging **18**(10): 761-72.

Amir, H., C. Makwaya, et al. (2001). "Breast cancer during the HIV epidemic in an African population." Oncology Reports **8**(3): 659-61.

Atkinson, C., R. Warren, et al. (1999). "Mammographic patterns as a predictive biomarker of breast cancer risk: effect of tamoxifen." Cancer Epidemiol Biomarkers Prev **8**(10): 863-6.

Band, P. R., N. D. Le, et al. (2002). "Carcinogenic and endocrine disrupting effects of cigarette smoke and risk of breast cancer.[comment]." Lancet **360**(9339): 1044-9.

Barton, A., S. John, et al. (2001). "Association between rheumatoid arthritis and polymorphism of tumor necrosis factor receptor II, but not tumor necrosis factor receptor I, in Caucasians." Arthritis and Rheumatism **44**(1): 61-5.

Bazzoni, F. and B. Beutler (1996). "The tumor necrosis factor ligand and receptor families." N Engl J Med **334**(26): 1717-25.

Beltinger, C. P., P. S. White, et al. (1996). "Physical mapping and genomic structure of the human TNFR2 gene." Genomics **35**(1): 94-100.

Benichou, J., C. Byrne, et al. (2003). "Secular stability and reliability of measurements of the percentage of dense tissue on mammograms." Cancer Detection and Prevention **27**(4): 266-274.

Bergkvist, L., L. Tabar, et al. (1987). "Epidemiologic determinants of the mammographic parenchymal pattern. A population-based study within a mammographic screening program." American Journal of Epidemiology **126**(6): 1075-81.

Berube, S., C. Diorio, et al. (2004). "Vitamin D, calcium, and mammographic breast densities." Cancer Epidemiol Biomarkers Prev **13**(9): 1466-72.

Boyd, N. F., J. W. Byng, et al. (1995). "Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study." J Natl Cancer Inst **87**(9): 670-5.

Boyd, N. F., G. S. Dite, et al. (2002). "Heritability of mammographic density, a risk factor for breast cancer." New England Journal of Medicine **347**(12): 886-94.

Boyd, N. F., C. Greenberg, et al. (1997). "Effects at two years of a low-fat, high-carbohydrate diet on radiologic features of the breast: results from a randomized trial. Canadian Diet and Breast Cancer Prevention Study Group." J Natl Cancer Inst **89**(7): 488-96.

Boyd, N. F., G. A. Lockwood, et al. (1998). "Mammographic densities and breast cancer risk." Cancer Epidemiol Biomarkers Prev **7**(12): 1133-44.

Boyd, N. F., G. A. Lockwood, et al. (2001). "Mammographic density as a marker of susceptibility to breast cancer: a hypothesis." IARC Scientific Publications **154**: 163-9.

Boyd, N. F., B. O'Sullivan, et al. (1982). "Mammographic signs as risk factors for breast cancer." Br J Cancer **45**(2): 185-93.

Boyd, N. F., B. O'Sullivan, et al. (1984). "Mammographic patterns and breast cancer risk: methodologic standards and contradictory results." J Natl Cancer Inst **72**(6): 1253-9.

Boyd, N. F., J. M. Rommens, et al. (2005). "Mammographic breast density as an intermediate phenotype for breast cancer." The Lancet Oncology **6**(10): 798-808.

Boyd, N. F., J. Stone, et al. (2002). "The association of breast mitogens with mammographic densities." British Journal of Cancer **87**(8): 876-82.

Bright, R. A., A. S. Morrison, et al. (1988). "Relationship between mammographic and histologic features of breast tissue in women with benign biopsies." Cancer **61**(2): 266-71.

Brisson, J., B. Brisson, et al. (2000). "Tamoxifen and mammographic breast densities." Cancer Epidemiol Biomarkers Prev **9**(9): 911-5.

Brisson, J., F. Merletti, et al. (1982). "Mammographic features of the breast and breast cancer risk." Am J Epidemiol **115**(3): 428-37.

Brisson, J., A. S. Morrison, et al. (1984). "Height and weight, mammographic features of breast tissue, and breast cancer risk." Am J Epidemiol **119**(3): 371-81.

Brisson, J., R. Verreault, et al. (1989). "Diet, mammographic features of breast tissue, and breast cancer risk." Am J Epidemiol **130**(1): 14-24.

Brooks-Asplund, E. M., C. E. Tupper, et al. (2002). "Hormonal modulation of interleukin-6, tumor necrosis factor and associated receptor secretion in postmenopausal women." Cytokine **19**(4): 193-200.

Brueggemeier, R. W., A. L. Quinn, et al. (1999). "Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens." Cancer Letters **140**(1-2): 27-35.

Buist, D. S. M., P. L. Porter, et al. (2004). "Factors Contributing to Mammography Failure in Women Aged 40-49 Years." J Natl Cancer Inst **96**(19): 1432-1440.

Byng, J. W., N. F. Boyd, et al. (1996). "Automated analysis of mammographic densities." Phys Med Biol **41**(5): 909-23.

Byng, J. W., N. F. Boyd, et al. (1996). "Symmetry of projection in the quantitative analysis of mammographic images." Eur J Cancer Prev **5**(5): 319-27.

Byng, J. W., M. J. Yaffe, et al. (1998). "Analysis of mammographic density and breast cancer risk from digitized mammograms." Radiographics **18**(6): 1587-98.

Byrne, C., G. A. Colditz, et al. (2000). "Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density." Cancer Research **60**(14): 3744-8.

Byrne, C., S. E. Hankinson, et al. (2000). "Insulin-like growth factors and mammographic density." Growth Horm IGF Res **10 Suppl A**: S24-5.

Byrne, C., C. Schairer, et al. (1995). "Mammographic features and breast cancer risk: effects with time, age, and menopause status." Journal of the National Cancer Institute **87**(21): 1622-9.

Carlile, T., K. J. Kopecky, et al. (1985). "Breast cancer prediction and the Wolfe classification of mammograms." Jama **254**(8): 1050-3.

Cauley, J. A., S. R. Cummings, et al. (1990). "Prevalence and determinants of estrogen replacement therapy in elderly women." Am J Obstet Gynecol **163**(5 Pt 1): 1438-44.

Cauley, J. A., F. L. Lucas, et al. (1999). "Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group.[comment]." Annals of Internal Medicine. **130**(4 Pt 1): 270-7.

Cauley, J. A., F. L. Lucas, et al. (1996). "Bone mineral density and risk of breast cancer in older women: the study of osteoporotic fractures. Study of Osteoporotic Fractures Research Group." Jama **276**(17): 1404-8.

Chandrasekharan, N. V., H. Dai, et al. (2002). "From the Cover: COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure, and expression." PNAS **99**(21): 13926-13931.

Chaudary, M. A., I. H. Gravelle, et al. (1983). "Breast parenchymal patterns in women with bilateral primary breast cancer." Br J Radiol **56**(670): 703-6.

Chen, W. Y., G. A. Colditz, et al. (2002). "Use of postmenopausal hormones, alcohol, and risk for invasive breast cancer.[summary for patients in Ann Intern Med. 2002 Nov 19;137(10):I43; PMID: 12435234]." Annals of Internal Medicine Online **137**(10): 798-804.

Chen, Z., M. Maricic, et al. (2005). "Fracture Risk Among Breast Cancer Survivors: Results From the Women's Health Initiative Observational Study." Arch Intern Med **165**(5): 552-558.

Chow, C. K., D. Venzon, et al. (2000). "Effect of tamoxifen on mammographic density." Cancer Epidemiol Biomarkers Prev **9**(9): 917-21.

Christodoulakos, G. E., I. V. Lambrinoudaki, et al. (2003). "The effect of various regimens of hormone replacement therapy on mammographic breast density." Maturitas **45**(2): 109-18.

Christodoulakos, G. E., I. V. Lambrinoudaki, et al. (2006). "The effect of low dose hormone therapy on mammographic breast density." Maturitas **54**: 78-85.

Ciotti, M., A. Marrone, et al. (1997). "Genetic polymorphism in the human UGT1A6 (planar phenol) UDP-glucuronosyltransferase: pharmacological implications." Pharmacogenetics **7**(6): 485-95.

Clavel-Chapelon, F. and M. Gerber (2002). "Reproductive factors and breast cancer risk. Do they differ according to age at diagnosis?" Breast Cancer Research & Treatment **72**(2): 107-15.

"Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report," 1998. NIH Publication No. 98-4083.

Collaborative Group on Hormonal Factors in Breast, C. (1996). "Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies.[comment]." Lancet **347**(9017): 1713-27.

Collaborative Group on Hormonal Factors in Breast, C. (1997). "Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. [comment][erratum appears in Lancet 1997 Nov 15;350(9089):1484]." Lancet. **350**(9084): 1047-59.

Collaborative Group on Hormonal Factors in Breast, C. (2002). "Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease.[comment]." Lancet **360**(9328): 187-95.

Constantin, A., P. Dieude, et al. (2004). "Tumor necrosis factor receptor II gene polymorphism and severity of rheumatoid arthritis." Arthritis Rheum **50**(3): 742-7.

Coogan, P. F., S. R. Rao, et al. (1999). "The Relationship of Nonsteroidal Anti-inflammatory Drug Use to the Risk of Breast Cancer,." Preventive Medicine **29**(2): 72-76.

Cook, N. R., I. M. Lee, et al. (2005). "Low-Dose Aspirin in the Primary Prevention of Cancer: The Women's Health Study: A Randomized Controlled Trial." JAMA **294**(1): 47-55.

Cotterchio, M., N. Kreiger, et al. (2001). "Nonsteroidal Anti-inflammatory Drug Use and Breast Cancer Risk." Cancer Epidemiol Biomarkers Prev **10**(11): 1213-1217.

Crandall, C., S. Palla, et al. (2005). "Positive association between mammographic breast density and bone mineral density in the Postmenopausal Estrogen/Progestin Interventions Study." Breast Cancer Research **7**(6): R922 - R928.

Cummings, S. R., D. M. Black, et al. (1990). "Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group." JAMA **263**(5): 665-8.

Cuzick, J., J. Warwick, et al. (2004). "Tamoxifen and Breast Density in Women at Increased Risk of Breast Cancer." J Natl Cancer Inst **96**(8): 621-628.

Danforth, D. N., Jr. and M. K. Sgagias (1993). "Tumour necrosis factor-alpha modulates oestradiol responsiveness of MCF-7 breast cancer cells in vitro." J Endocrinol **138**(3): 517-28.

de Jong, P. C., M. A. Blankenstein, et al. (2001). "Importance of local aromatase activity in hormone-dependent breast cancer: a review." The Breast **10**(2): 91-99.

de Stavola, B. L., I. H. Gravelle, et al. (1990). "Relationship of mammographic parenchymal patterns with breast cancer risk factors and risk of breast cancer in a prospective study." International Journal of Epidemiology **19**(2): 247-54.

de Waard, F., J. J. Rombach, et al. (1984). "Breast cancer risk associated with reproductive factors and breast parenchymal patterns." J Natl Cancer Inst **72**(6): 1277-82.

Deapen, D., L. Liu, et al. (2002). "Rapidly rising breast cancer incidence rates among Asian-American women." International Journal of Cancer **99**(5): 747-50.

Deb, S., S. Amin, et al. (2004). "Estrogen Regulates Expression of Tumor Necrosis Factor Receptors in Breast Adipose Fibroblasts." J Clin Endocrinol Metab **89**(8): 4018-4024.

Dieudé, P., E. Petit, et al. (2002). "Association between tumor necrosis factor receptor II and familial, but not sporadic, rheumatoid arthritis: Evidence for genetic heterogeneity." Arthritis & Rheumatism **46**(8): 2039-2044.

Diorio, C., M. Pollak, et al. (2005). "Levels of C-Peptide and Mammographic Breast Density." Cancer Epidemiol Biomarkers Prev **14**(11): 2661-2664.

Diorio, C., M. Pollak, et al. (2005). "Insulin-Like Growth Factor-I, IGF-Binding Protein-3, and Mammographic Breast Density." Cancer Epidemiol Biomarkers Prev **14**(5): 1065-1073.

Dite, G., J. Wark, et al. (2006). "Is there a positive association between mammographic density and bone mineral density?" Breast Cancer Research **8**(1): 401.

Dite, G. S., J. D. Wark, et al. (2005). "Is there overlap between the genetic determinants of mammographic density and bone mineral density?" Cancer Epidemiol Biomarkers Prev **14**(9): 2266-8.

Dror, Y., T. Hahn, et al. (1998). "Soluble tumor necrosis factor receptor levels in familial Mediterranean fever." Journal of Rheumatology **25**(12): 2481-2.

DuBois, R. N. (2004). "Aspirin and breast cancer prevention: the estrogen connection." Jama **291**(20): 2488-9.

Duncan, L. J., N. G. Coldham, et al. (1994). "The interaction of cytokines in regulating oestradiol 17 beta-hydroxysteroid dehydrogenase activity in MCF-7 cells." J Steroid Biochem Mol Biol **49**(1): 63-8.

"Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial." (1995). Jama **273**(3): 199-208.

Egan, K., M. Stampfer, et al. (1996). "Prospective study of regular aspirin use and the risk of breast cancer." J Natl Cancer Inst **88**(14): 988a-993.

Egan, R. L. and R. C. Mosteller (1977). "Breast cancer mammography patterns." Cancer **40**(5): 2087-90.

Ellison, R. C., Y. Zhang, et al. (2001). "Exploring the Relation of Alcohol Consumption to Risk of Breast Cancer. ." American Journal of Epidemiology **154**(8): 740-747.

Ernster, V. L., S. T. Sacks, et al. (1980). "Mammographic parenchymal patterns and risk factors for breast cancer." Radiology **134**(3): 617-20.

Erren, T. C. (2001). "A meta-analysis of epidemiologic studies of electric and magnetic fields and breast cancer in women and men." Bioelectromagnetics **Suppl**(5): S105-19.

Fabris, M., B. Tolusso, et al. (2002). "Tumor necrosis factor-alpha receptor II polymorphism in patients from southern Europe with mild-moderate and severe rheumatoid arthritis." J Rheumatol **29**(9): 1847-50.

Fisher, B., J. P. Costantino, et al. (1998). "Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study." Journal of the National Cancer Institute **90**(18): 1371-88.

Fisher, E. R., A. Palekar, et al. (1978). "The histopathology of mammographic patterns." American Journal of Clinical Pathology **69**(4): 421-6.

Friedenreich, C. M. and M. R. Orenstein (2002). "Physical activity and cancer prevention: etiologic evidence and biological mechanisms." Journal of Nutrition **132**(11 Suppl): 3456S-3464S.

Friis, S., H. T. Sorensen, et al. (2003). "A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin." Br J Cancer **88**(5): 684-8.

Gadducci, A., M. Ferdeghini, et al. (1995). "Serum Levels of Tumor Necrosis Factor (TNF), Soluble Receptors for TNF (55- and 75-kDa sTNFr), and Soluble CD14 (sCD14) in Epithelial Ovarian Cancer." Gynecologic Oncology **58**(2): 184-188.

Gadducci, A., M. Ferdeghini, et al. (1996). "Serum levels of soluble receptors for tumor necrosis factor (p55 and p75 sTNFr) in endometrial cancer." Anticancer Res **16**(5B): 3125-8.

Gail, M. H., L. A. Brinton, et al. (1989). "Projecting individualized probabilities of developing breast cancer for white females who are being examined annually.[comment]." Journal of the National Cancer Institute **81**(24): 1879-86.

Gammon, M. D., M. S. Wolff, et al. (2002). "Environmental toxins and breast cancer on Long Island. II. Organochlorine compound levels in blood." Cancer Epidemiology Biomarkers & Prevention **11**(8): 686-97.

Garcia Rodriguez, L. A. and A. Gonzalez-Perez (2004). "Risk of breast cancer among users of aspirin and other anti-inflammatory drugs." Br J Cancer **91**(3): 525-9.

Gasparini, G., R. Longo, et al. (2003). "Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents?" Lancet Oncol **4**(10): 605-15.

Gierach, G. and V. Vogel (2004). Epidemiology of Breast Cancer. Advanced Therapy of Breast Disease, 2nd Edition. S. E. Singletary, G. L. Robb and G. N. Hortobagyi. Hamilton, Ontario, BC Decker, Inc.**:** 58-74.

Glenn, C. L., W. Y. Wang, et al. (2000). "Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor." Human Molecular Genetics **9**(13): 1943-9.

Gram, I., Y. Bremnes, et al. (2005). "Percentage density, Wolfe's and Tabar's mammographic patterns: agreement and association with risk factors for breast cancer." Breast Cancer Research **7**(5): R854 - R861.

Gram, I. T., E. Funkhouser, et al. (1995). "Reproductive and menstrual factors in relation to mammographic parenchymal patterns among perimenopausal women." British Journal of Cancer **71**(3): 647-50.

Gravelle, I. H., J. C. Bulstrode, et al. (1986). "A prospective study of mammographic parenchymal patterns and risk of breast cancer." Br J Radiol **59**(701): 487-91.

Greendale, G. A., S. L. Palla, et al. (2005). "The Association of Endogenous Sex Steroids and Sex Steroid Binding Proteins with Mammographic Density: Results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study." Am J Epidemiol **162**(9): 826-34.

Grosen, E. A., G. A. Granger, et al. (1993). "Measurement of the soluble membrane receptors for tumor necrosis factor and lymphotoxin in the sera of patients with gynecologic malignancy." Gynecol Oncol **50**(1): 68-77.

Grove, J. S., M. J. Goodman, et al. (1985). "Factors associated with mammographic pattern." British Journal of Radiology **58**(685): 21-5.

Guo, Y. P., L. J. Martin, et al. (2001). "Growth factors and stromal matrix proteins associated with mammographic densities." Cancer Epidemiology, Biomarkers and Prevention **10**(3): 243-8.

Haars, G., P. A. H. van Noord, et al. (2005). "Measurements of Breast Density: No Ratio for a Ratio." Cancer Epidemiol Biomarkers Prev **14**(11): 2634-2640.

Habel, L. A., J. J. Dignam, et al. (2004). "Mammographic Density and Breast Cancer After Ductal Carcinoma In Situ." J Natl Cancer Inst **96**(19): 1467-1472.

Haiman, C. A., L. Bernstein, et al. (2002). "Genetic determinants of mammographic density." Breast Cancer Research **4**(3): R5.

Haiman, C. A., S. E. Hankinson, et al. (2003). "Polymorphisms in steroid hormone pathway genes and mammographic density." Breast Cancer Research and Treatment **77**(1): 27-36.

Hancock, S. L., M. A. Tucker, et al. (1993). "Breast cancer after treatment of Hodgkin's disease." Journal of the National Cancer Institute **85**(1): 25-31.

Hankinson, S. E., G. A. Colditz, et al. (1997). "A prospective study of oral contraceptive use and risk of breast cancer (Nurses' Health Study, United States)." Cancer Causes & Control **8**(1): 65-72.

Hankinson, S. E., W. C. Willett, et al. (1998). "Circulating concentrations of insulin-like growth factor-I and risk of breast cancer.[comment]." Lancet **351**(9113): 1393-6.

Harris, R., J. Beebe-Donk, et al. (2006). "Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors." BMC Cancer **6**(1): 27.

Harris, R. E., J. Beebe-Donk, et al. (2005). "Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review)." Oncol Rep **13**(4): 559-83.

Harris, R. E., R. T. Chlebowski, et al. (2003). "Breast Cancer and Nonsteroidal Anti-Inflammatory Drugs: Prospective Results from the Women's Health Initiative." Cancer Res **63**(18): 6096-6101.

Harris, R. E., S. Kasbari, et al. (1999). "Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer." Oncol Rep **6**(1): 71-3.

Harris, R. E., Namboodiri K., et al. (1995). "Breast Cancer and NSAID Use: Heterogeneity of Effect in a Case-Control Study." Preventive Medicine **24**(2): 119-120.

Harris, R. E., K. K. Namboodiri, et al. (1996). "Nonsteroidal antiinflammatory drugs and breast cancer." Epidemiology **7**(2): 203-5.

Harrison, D. A., S. W. Duffy, et al. (2002). "Deterministic models for breast cancer progression:: Application to the association between mammographic parenchymal pattern and histologic grade of breast cancers." Journal of Clinical Epidemiology **55**(11): 1113-1118.

Harvey, J., C. Scheurer, et al. (2005). "Hormone replacement therapy and breast density changes." Climacteric **8**(2): 185-92.

Harvey, J. A. and V. E. Bovbjerg (2004). "Quantitative Assessment of Mammographic Breast Density: Relationship with Breast Cancer Risk." Radiology **230**(1): 29-41.

Hasegawa, Y., M. Sawada, et al. (2000). "Increased soluble tumor necrosis factor receptor levels in the serum of elderly people." Gerontology **46**(4): 185-8.

Hauner, H., M. Bender, et al. (1998). "Plasma concentrations of soluble TNF-alpha receptors in obese subjects." Int J Obes Relat Metab Disord **22**(12): 1239-43.

Heller, R. A., K. Song, et al. (1992). "The p70 tumor necrosis factor receptor mediates cytotoxicity." Cell **70**(1): 47-56.

Heng, D., F. Gao, et al. (2004). "Risk Factors for Breast Cancer Associated with Mammographic Features in Singaporean Chinese Women." Cancer Epidemiol Biomarkers Prev **13**(11): 1751-1758.

Higuchi, M. and B. B. Aggarwal (1993). "P80 form of the human tumor necrosis factor receptor is involved in DNA fragmentation." FEBS Letters **331**(3): 252-5.

Hilakivi-Clarke, L., A. Cabanes, et al. (2002). "Do estrogens always increase breast cancer risk?" Journal of Steroid Biochemistry & Molecular Biology **80**(2): 163-74.

Hill, D., S. Preston-Martin, et al. (2002). "Medical radiation, family history of cancer, and benign breast disease in relation to breast cancer risk in young women, USA." Cancer Causes Control **13(8)**: 711-8.

Hohmann, H., R. Remy, et al. (1989). "Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha)." J Biol Chem **264**(25): 14927-14934.

Hong, C. C., H. J. Thompson, et al. (2003). "Val158Met Polymorphism in catechol-O-methyltransferase gene associated with risk factors for breast cancer." Cancer Epidemiology, Biomarkers and Prevention **12**(9): 838-47.

Hong, C. C., H. J. Thompson, et al. (2004). "Association between the T27C polymorphism in the cytochrome P450 c17alpha (CYP17) gene and risk factors for breast cancer." Breast Cancer Res Treat **88**(3): 217-30.

Horn-Ross, P. L., E. M. John, et al. (2001). "Phytoestrogen Consumption and Breast Cancer Risk in a Multiethnic Population - The Bay Area Breast Cancer Study." American Journal of Epidemiology **154**(5): 434-441.

Hoshaw, S. J., P. J. Klein, et al. (2001). "Breast implants and cancer: causation, delayed detection, and survival." Plastic & Reconstructive Surgery **107**(6): 1393-407.

Hotamisligil, G. S., P. Arner, et al. (1997). "Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance." Diabetes **46**(3): 451-5.

Howe, L. R., K. Subbaramaiah, et al. (2001). "Cyclooxygenase-2: a target for the prevention and treatment of breast cancer." Endocrine-Related Cancer **8**(2): 97-114.

Hube, F., M. Birgel, et al. (1999). "Expression pattern of tumour necrosis factor receptors in subcutaneous and omental human adipose tissue: role of obesity and non-insulin-dependent diabetes mellitus." European Journal of Clinical Investigation **29**(8): 672-8.

Huo, Z., M. L. Giger, et al. (2002). "Computerized analysis of digitized mammograms of BRCA1 and BRCA2 gene mutation carriers." Radiology **225**(2): 519-26.

Il'yasova, D., L. H. Colbert, et al. (2005). "Circulating Levels of Inflammatory Markers and Cancer Risk in the Health Aging and Body Composition Cohort." Cancer Epidemiol Biomarkers Prev **14**(10): 2413-2418.

Ininns, E. K., M. Gatanaga, et al. (1992). "Growth of the endometrial adenocarcinoma cell line AN3 CA is modulated by tumor necrosis factor and its receptor is up-regulated by estrogen in vitro." Endocrinology **130**(4): 1852-6.

Innes, K. E. and T. E. Byers (1999). "Preeclampsia and breast cancer risk." Epidemiology **10**(6): 722-32.

Ip, M. M., C. Mazzer, et al. (1989). "The effect of eicosanoid synthesis inhibitors on DMBA-induced rat mammary carcinogenesis." Proc Am Assoc Cancer Res **30**: 182.

Irwin, M., E. Aiello, et al. (2005). "Pre-diagnosis physical activity and mammographic density in breast cancer survivors." Breast Cancer Research and Treatment: 1-8.

Jablonska, E., M. Kiluk, et al. (1998). "Soluble TNF receptors release by polymorphonuclear cells and the serum levels in breast cancer patients before and after treatment." Neoplasma **45**(1): 17-20.

Jacobs, E. J., M. J. Thun, et al. (2005). "Aspirin and Other Nonsteroidal Anti-inflammatory Drugs and Breast Cancer Incidence in a Large U.S. Cohort." Cancer Epidemiol Biomarkers Prev **14**(1): 261-264.

Jeffreys, M., R. Warren, et al. (2004). "Life course breast cancer risk factors and adult breast density (United Kingdom)." Cancer Causes and Control **15**(9): 947-955.

Jemal, A., R. Siegel, et al. (2006). "Cancer Statistics, 2006." CA Cancer J Clin **56**(2): 106-130.

Johnson, J. W. (2001). "Transactions of the sixty-third annual meeting of the South Atlantic Association of Obstetricians and Gynecologyists. The millennial mark: presidential address." American Journal of Obstetrics & Gynecology **185**(2): 261-7.

Johnson, T. W., K. E. Anderson, et al. (2002). "Association of Aspirin and Nonsteroidal Anti-inflammatory Drug Use with Breast Cancer." Cancer Epidemiol Biomarkers Prev **11**(12): 1586-1591.

Kaaks, R., C. Diorio, et al. (2005). "Insulin-Like Growth Factor-I and Mammographic Breast Density." Cancer Epidemiol Biomarkers Prev **14**(12): 3019-.

Kaaks, R., E. Lundin, et al. (2002). "Prospective study of IGF-I, IGF-binding proteins, and breast cancer risk, in northern and southern Sweden." Cancer Causes & Control **13**(4): 307-16.

Kadar, D. (1998). Anti-inflammatory analgesics. Principles of Medical Pharmacology. H. Kalant and W. H. E. Roschlau. New York, Oxford University Press**:** 410-30.

Kaminska, J., M. P. Nowacki, et al. (2005). "Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I--an independent prognostic factor." Tumour Biol **26**(4): 186-94.

Kato, I., C. Beinart, et al. (1995). "A nested case-control study of mammographic patterns, breast volume, and breast cancer (New York City, NY, United States)." Cancer Causes Control **6**(5): 431-8.

Kaufman, Z., W. I. Garstin, et al. (1991). "The mammographic parenchymal patterns of nulliparous women and women with a family history of breast cancer." Clinical Radiology **43**(6): 385-8.

Kelsey, J. L., M. D. Gammon, et al. (1993). "Reproductive factors and breast cancer." Epidemiologic Reviews **15**(1): 36-47.

Kerlikowske, K., D. Grady, et al. (1995). "Efficacy of screening mammography. A meta-analysis." Jama **273**(2): 149-54.

Kerlikowske, K., J. Shepherd, et al. (2005). "Are Breast Density and Bone Mineral Density Independent Risk Factors for Breast Cancer?" J Natl Cancer Inst **97**(5): 368-374.

Kharfi, A., Y. Labelle, et al. (2003). "Deficient expression of tumor necrosis factor receptor type 2 in the endometrium of women with endometriosis." Am J Reprod Immunol **50**(1): 33-40.

Khuder, S. A. and A. B. Mutgi (2001). "Breast cancer and NSAID use: a meta-analysis." British Journal of Cancer **84**(9): 1188-92.

Komata, T., N. Tsuchiya, et al. (1999). "Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus." Tissue Antigens **53**(6): 527-33.

Krajcik, R. A., S. Massardo, et al. (2003). "No association between serum levels of tumor necrosis factor-alpha (TNF-alpha) or the soluble receptors sTNFR1 and sTNFR2 and breast cancer risk." Cancer Epidemiol Biomarkers Prev **12**(9): 945-6.

Lacey, J. V., Jr., S. S. Devesa, et al. (2002). "Recent trends in breast cancer incidence and mortality." Environmental & Molecular Mutagenesis **39**(2-3): 82-8.

Lai, J. H., D. Vesprini, et al. (2004). "A Polymorphic Locus in the Promoter Region of the IGFBP3 Gene Is Related to Mammographic Breast Density." Cancer Epidemiol Biomarkers Prev **13**(4): 573-582.

Lam, P. B., P. M. Vacek, et al. (2000). "The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont." Cancer **89**(2): 369-75.

Langman, M. J. S., K. K. Cheng, et al. (2000). "Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database." BMJ **320**(7250): 1642-1646.

Lee, P. P. and M. M. Ip (1992). "Regulation of proliferation of rat mammary tumor cells by inhibitors of cyclooxygenase and lipoxygenase." Prostaglandins Leukot Essent Fatty Acids **45**: 21-31.

Li, T., L. Sun, et al. (2005). "The Association of Measured Breast Tissue Characteristics with Mammographic Density and Other Risk Factors for Breast Cancer." Cancer Epidemiol Biomarkers Prev **14**(2): 343-349.

Lillie, E. O., L. Bernstein, et al. (2004). "Polymorphism in the Androgen Receptor and Mammographic Density in Women Taking and Not Taking Estrogen and Progestin Therapy." Cancer Res **64**(4): 1237-1241.

Lipworth, L., H. O. Adami, et al. (1996). "Serum steroid hormone levels, sex hormone-binding globulin, and body mass index in the etiology of postmenopausal breast cancer." Epidemiology **7**(1): 96-100.

Lopez, P., L. Van Horn, et al. (2003). "Physical inactivity and percent breast density among Hispanic women." International Journal of Cancer **107**(6): 1012-6.

Lord, S., W. Mack, et al. (2005). "Polymorphisms in genes involved in estrogen and progesterone metabolism and mammographic density changes in women randomized to postmenopausal hormone therapy: results from a pilot study." Breast Cancer Research **7**(3): R336 - R344.

Macdiarmid, F., D. Wang, et al. (1994). "Stimulation of aromatase activity in breast fibroblasts by tumor necrosis factor alpha." Mol Cell Endocrinol **106**(1-2): 17-21.

Madhusudan, S., M. Foster, et al. (2004). "A Phase II Study of Etanercept (Enbrel), a Tumor Necrosis Factor {alpha} Inhibitor in Patients with Metastatic Breast Cancer." Clin Cancer Res **10**(19): 6528-6534.

Marshall, S. F., L. Bernstein, et al. (2005). "Nonsteroidal Anti-Inflammatory Drug Use and Breast Cancer Risk by Stage and Hormone Receptor Status." J Natl Cancer Inst **97**(11): 805-812.

Martin, J. H., E. J. Begg, et al. (2001). "Is cytochrome P450 2C9 genotype associated with NSAID gastric ulceration?" Br J Clin Pharmacol **51**(6): 627-30.

Masala, G., D. Ambrogetti, et al. (2006). "Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population." International Journal of Cancer **118**(7): 1782-1789.

Maskarinec, G., G. Lurie, et al. (2004). "An investigation of mammographic density and gene variants in healthy women." Int J Cancer **112**(4): 683-8.

Maskarinec, G. and L. Meng (2000). "A case-control study of mammographic densities in Hawaii." Breast Cancer Res Treat **63**(2): 153-61.

Maskarinec, G., I. Pagano, et al. (2005). "Mammographic Density and Breast Cancer Risk." Am. J. Epidemiol.: kwi270.

Maskarinec, G., Y. Takata, et al. (2006). "Alcohol consumption and mammographic density in a multiethnic population." Int J Cancer **118**(10): 2579-83.

Maskarinec, G., A. E. Williams, et al. (2003). "Mammographic densities in a one-year isoflavone intervention." European Journal of Cancer Prevention **12**(2): 165-9.

Maskarinec, G., A. E. Williams, et al. (2003). "A cross-sectional investigation of breast density and insulin-like growth factor I." International Journal of Cancer **107**(6): 991-6.

McCormack, V. A., I. dos Santos Silva, et al. (2003). "Life-course body size and perimenopausal mammographic parenchymal patterns in the MRC 1946 British birth cohort." Br J Cancer **89**(5): 852-9.

Medvedev, A. E., T. Espevik, et al. (1996). "Distinct Roles of the Two Tumor Necrosis Factor (TNF) Receptors in Modulating TNF and Lymphotoxin alpha Effects." J Biol Chem **271**(16): 9778-9784.

Meier, C. R., S. Schmitz, et al. (2002). "Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer." Pharmacotherapy **22**(3): 303-9.

Meilahn, E. N., B. De Stavola, et al. (1998). "Do urinary oestrogen metabolites predict breast cancer? Guernsey III cohort follow-up." British Journal of Cancer **78**(9): 1250-5.

Melbye, M., J. Wohlfahrt, et al. (1997). "Induced abortion and the risk of breast cancer.[comment]." New England Journal of Medicine **336**(2): 81-5.

Mestiri, S., N. Bouaouina, et al. (2005). "A functional polymorphism of the tumor necrosis factor receptor-II gene associated with the survival and relapse prediction of breast carcinoma." Cytokine **30**(4): 182-187.

Miller, W. R. and A. P. Forrest (1974). "Oestradiol synthesis by a human breast carcinoma." Lancet **2**(7885): 866-8.

Miners, J. O. and D. J. Birkett (1998). "Cytochrome P4502C9: an enzyme of major importance in human drug metabolism." Br J Clin Pharmacol **45**(6): 525-38.

Modugno, F., R. B. Ness, et al. (2003). "Alcohol Consumption and the Risk of Mucinous and Nonmucinous Epithelial Ovarian Cancer." Obstet Gynecol **102**(6): 1336-1343.

Modugno, F., D. Ngo, et al. (2005). "Breast cancer risk factors and mammographic breast density in women over age 70." Breast Cancer Research and Treatment: 1-10.

Mohamed-Ali, V., S. Goodrick, et al. (1999). "Production of soluble tumor necrosis factor receptors by human subcutaneous adipose tissue in vivo." Am J Physiol **277**(6 Pt 1): E971-5.

Moorman, P., J. Grubber, et al. (2003). "Association between non-steroidal anti-inflammatory drugs (NSAIDs) and invasive breast cancer and carcinoma in situ of the breast." Cancer Causes and Control **14**(10): 915-922.

Morimoto, L., E. White, et al. (2002). "Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States)." Cancer Causes Control **13(8)**: 741-51.

Morita, C., T. Horiuchi, et al. (2001). "Association of tumor necrosis factor receptor type II polymorphism 196R with Systemic lupus erythematosus in the Japanese: molecular and functional analysis." Arthritis Rheum **44**(12): 2819-27.

Morita, I. (2002). "Distinct functions of COX-1 and COX-2." Prostaglandins Other Lipid Mediat **68-69**: 165-75.

Mulhall, C., R. A. Hegele, et al. (2005). "Pituitary Growth Hormone and Growth Hormone-Releasing Hormone Receptor Genes and Associations with Mammographic Measures and Serum Growth Hormone." Cancer Epidemiol Biomarkers Prev **14**(11): 2648-2654.

Muti, P., H. L. Bradlow, et al. (2000). "Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women." Epidemiology **11**(6): 635-40.

Neugut, A., D. Rosenberg, et al. (1998). "Association between coronary heart disease and cancers of the breast, prostate, and colon." Cancer Epidemiol Biomarkers Prev **7**(10): 869-873.

Newcomb, P. A., A. Trentham-Dietz, et al. (2001). "Fracture History and Risk of Breast and Endometrial Cancer." American Journal of Epidemiology **153**(11): 1071-1078.

Noh, J. J., G. Maskarinec, et al. (2006). "Mammographic densities and circulating hormones: A cross-sectional study in premenopausal women." The Breast **15**(1): 20-28.

Paganini-Hill, A., A. Chao, et al. (1989). "Aspirin use and chronic diseases: a cohort study of the elderly." Bmj **299**(6710): 1247-50.

Page, D. L. and A. C. Winfield (1986). "The dense mammogram." AJR. American Journal of Roentgenology **147**(3): 487-9.

Pankow, J. S., C. M. Vachon, et al. (1997). "Genetic analysis of mammographic breast density in adult women: evidence of a gene effect." Journal of the National Cancer Institute **89**(8): 549-56.

Pawluczyk, O., B. J. Augustine, et al. (2003). "A volumetric method for estimation of breast density on digitized screen-film mammograms." Med Phys **30**(3): 352-64.

Peral, B., J. L. San Millan, et al. (2002). "Comment: the methionine 196 arginine polymorphism in exon 6 of the TNF receptor 2 gene (TNFRSF1B) is associated with the polycystic ovary syndrome and hyperandrogenism." Journal of Clinical Endocrinology and Metabolism **87**(8): 3977-83.

Pfeilschifter, J., R. Koditz, et al. (2002). "Changes in Proinflammatory Cytokine Activity after Menopause." Endocr Rev **23**(1): 90-119.

Pike, M. C., M. D. Krailo, et al. (1983). "'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer." Nature **303**(5920): 767-70.

Purohit, A., L. J. Duncan, et al. (1997). "Paracrine control of oestrogen production in breast cancer." Endocr Relat Cancer **4**: 323-330.

Purohit, A., S. Newman, et al. (2002). "The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer." Breast Cancer Res **4**(2): 65 - 69.

Rahme, E., J. Ghosn, et al. (2005). "Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer." BMC Cancer **5**(1): 159.

Rainsford, K. D. (2003). "Discovery, mechanisms of action and safety of ibuprofen." Int J Clin Pract Suppl(135): 3-8.

Reed, M. J. and A. Purohit (1997). "Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis." Endocr Rev **18**(5): 701-15.

Reed, M. J. and A. Purohit (2001). "Aromatase regulation and breast cancer." Clinical Endocrinology **54**(5): 563-71.

Riza, E., I. dos Santos Silva, et al. (2001). "Urinary estrogen metabolites and mammographic parenchymal patterns in postmenopausal women." Cancer Epidemiol Biomarkers Prev **10**(6): 627-34.

Riza, E., I. dos Santos Silva, et al. (2005). "Correlates of high-density mammographic parenchymal patterns by menopausal status in a rural population in Northern Greece." European Journal of Cancer **41**(4): 590-600.

Rosenberg, L. (1995). "Nonsteroidal Anti-inflammatory Drugs and Cancer." Preventive Medicine **24**(2): 107-109.

Roubidoux, M. A., J. E. Bailey, et al. (2004). "Invasive Cancers Detected after Breast Cancer Screening Yielded a Negative Result: Relationship of Mammographic Density to Tumor Prognostic Factors." Radiology **230**(1): 42-48.

Saftlas, A. F., R. N. Hoover, et al. (1991). "Mammographic densities and risk of breast cancer." Cancer **67**(11): 2833-8.

Sala, E., R. Warren, et al. (1998). "Mammographic parenchymal patterns and mode of detection: implications for the breast screening programme." J Med Screen **5**(4): 207-12.

Santee, S. M. and L. B. Owen-Schaub (1996). "Human tumor necrosis factor receptor p75/80 (CD120b) gene structure and promoter characterization." Journal of Biological Chemistry **271**(35): 21151-9.

Sato, R., K. J. Helzlsouer, et al. (2002). "Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer." Cancer Epidemiology Biomarkers & Prevention **11**(5): 451-7.

Schreinemachers, D. M. and R. B. Everson (1994). "Aspirin use and lung, colon, and breast cancer incidence in a prospective study." Epidemiology **5**(2): 138-46.

"SEER*Stat Database: 9 SEER Incidence Registries for Public-Use, National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, Released April 2003, based on the November 2002 data (for the years 1973-2000)." Retrieved November 11, 2004.

Sellers, T. A., L. H. Kushi, et al. (2001). "Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women." Epidemiology **12**(4): 420-8.

Sharpe, C. R., J. P. Collet, et al. (2000). "Nested case-control study of the effects of non-steroidal anti-inflammatory drugs on breast cancer risk and stage." Br J Cancer **83**(1): 112-20.

Sites, C. K., M. J. Toth, et al. (2002). "Menopause-related differences in inflammation markers and their relationship to body fat distribution and insulin-stimulated glucose disposal." Fertil Steril **77**(1): 128-35.

Smith-Warner, S. A., D. Spiegelman, et al. (2001). "Types of dietary fat and breast cancer: a pooled analysis of cohort studies." International Journal of Cancer **92**(5): 767-74.

Sorensen, H. T., S. Friis, et al. (2003). "Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study." Br J Cancer **88**(11): 1687-92.

Soysal, S., M. E. Soysal, et al. (2006). "The basal and TRH stimulated levels of prolactin in low risk climacteric patients with increased breast density: A matched pair case control trial." Maturitas **In Press**.

Steele, V. E., R. C. Moon, et al. (1994). "Preclinical efficacy evaluation of potential chemopreventive agents in animal carcinogenesis models: methods and results from the NCI Chemoprevention Drug Development Program." J Cell Biochem Suppl **20**: 32-54.

Suijkerbuijk, K. P. M., F. J. B. Van Duijnhoven, et al. (2006). "Physical Activity in Relation to Mammographic Density in the Dutch Prospect-European Prospective Investigation into Cancer and Nutrition Cohort." Cancer Epidemiol Biomarkers Prev **15**(3): 456-460.

Swede, H., A. L. Mirand, et al. (2005). "Association of Regular Aspirin Use and Breast Cancer Risk." Oncology **68**(1): 40-47.

Tabar, L. and P. B. Dean (1982). "Mammographic parenchymal patterns. Risk indicator for breast cancer?" Jama **247**(2): 185-9.

Tamimi, R. M., S. E. Hankinson, et al. (2005). "Endogenous Sex Hormone Levels and Mammographic Density among Postmenopausal Women." Cancer Epidemiol Biomarkers Prev **14**(11): 2641-2647.

Terry, M. B., M. D. Gammon, et al. (2004). "Association of Frequency and Duration of Aspirin Use and Hormone Receptor Status With Breast Cancer Risk." JAMA **291**(20): 2433-2440.

Tesarova, P., J. Kvasnicka, et al. (2000). "Soluble TNF and IL-2 receptors in patients with breast cancer." Med Sci Monit **6**(4): 661-7.

The Endogenous Hormones and Breast Cancer Collaborative Group (2002). "Endogenous Sex Hormones and Breast Cancer in Postmenopausal Women: Reanalysis of Nine Prospective Studies." JNCI Cancer Spectrum **94**(8): 606-616.

Thun, M., M. Namboodiri, et al. (1993). "Aspirin use and risk of fatal cancer." Cancer Res **53**(6): 1322-1327.

Tolusso, B., S. Sacco, et al. (2004). "Relationship between the tumor necrosis factor receptor II (TNF-RII) gene polymorphism and sTNF-RII plasma levels in healthy controls and in rheumatoid arthritis." Human Immunology **65**(12): 1420-1426.

Torres-Mejia, G., B. De Stavola, et al. (2005). "Mammographic Features and Subsequent Risk of Breast Cancer: A Comparison of Qualitative and Quantitative Evaluations in the Guernsey Prospective Studies." Cancer Epidemiol Biomarkers Prev **14**(5): 1052-1059.

Ursin, G., L. Hovanessian-Larsen, et al. (2005). "Greatly increased occurrence of breast cancers in areas of mammographically dense tissue." Breast Cancer Research **7**(5): R605 - R608.

Ursin, G., H. Ma, et al. (2003). "Mammographic density and breast cancer in three ethnic groups." Cancer Epidemiol Biomarkers Prev **12**(4): 332-8.

Vacek, P. M. and B. M. Geller (2004). "A Prospective Study of Breast Cancer Risk Using Routine Mammographic Breast Density Measurements." Cancer Epidemiol Biomarkers Prev **13**(5): 715-722.

Vachon, C. M., R. A. King, et al. (1999). "Preliminary sibpair linkage analysis of percent mammographic density." Journal of the National Cancer Institute **91**(20): 1778-9.

Vachon, C. M., C. C. Kuni, et al. (2000). "Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States)." Cancer Causes and Control **11**(7): 653-62.

Vachon, C. M., L. H. Kushi, et al. (2000). "Association of diet and mammographic breast density in the Minnesota breast cancer family cohort." Cancer Epidemiol Biomarkers Prev **9**(2): 151-60.

van den Brandt, P. A., D. Spiegelman, et al. (2000). "Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk." American Journal of Epidemiology **152**(6): 514-27.

van Duijnhoven, F. J. B., I. D. Bezemer, et al. (2005). "Polymorphisms in the Estrogen Receptor {alpha} Gene and Mammographic Density." Cancer Epidemiol Biomarkers Prev **14**(11): 2655-2660.

van Duijnhoven, F. J. B., P. H. M. Peeters, et al. (2006). "Influence of Estrogen Receptor {alpha} and Progesterone Receptor Polymorphisms on the Effects of Hormone Therapy on Mammographic Density." Cancer Epidemiol Biomarkers Prev **15**(3): 462-467.

van Gils, C. H., J. H. Hendriks, et al. (1999). "Changes in mammographic breast density and concomitant changes in breast cancer risk." European Journal of Cancer Prevention **8**(6): 509-15.

van Gils, C. H., J. D. Otten, et al. (1995). "Short communication: breast parenchymal patterns and their changes with age." British Journal of Radiology **68**(814): 1133-5.

Varela, L. M., K. M. Darcy, et al. (1997). "The Epidermal Growth Factor Receptor Is Not Required for Tumor Necrosis Factor-{alpha} Action in Normal Mammary Epithelial Cells." Endocrinology **138**(9): 3891-3900.

Verthelyi, D. and D. M. Klinman (2000). "Sex hormone levels correlate with the activity of cytokine-secreting cells in vivo." Immunology **100**(3): 384-390.

Vogel, V. G. (1998). Breast cancer risk factors and preventive approaches to breast cancer. Cancer in Women. S. S. Kavanagh J, Einhorn N, DePetrillo AD, Eds. Cambridge, MA, Blackwell Scientific Publications, Inc.**:** 58-91.

Wellings, S. R. and J. N. Wolfe (1978). "Correlative studies of the histological and radiographic appearance of the breast parenchyma." Radiology **129**(2): 299-306.

White, E., P. Velentgas, et al. (1998). "Variation in mammographic breast density by time in menstrual cycle among women aged 40-49 years." Journal of the National Cancer Institute **90**(12): 906-10.

Whitehead, J., T. Carlile, et al. (1985). "Wolfe mammographic parenchymal patterns. A study of the masking hypothesis of Egan and Mosteller." Cancer **56**(6): 1280-6.

Whitehouse, G. H. and S. J. Leinster (1985). "The variation of breast parenchymal patterns with age." British Journal of Radiology **58**(688): 315-8.

Wolfe, J. N. (1976). "Risk for breast cancer development determined by mammographic parenchymal pattern." Cancer **37**(5): 2486-92.

Wolfe, J. N., A. F. Saftlas, et al. (1987). "Mammographic parenchymal patterns and quantitative evaluation of mammographic densities: a case-control study." AJR. American Journal of Roentgenology **148**(6): 1087-92.

Writing Group for the Women's Health Initiative, I. (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**(3): 321-33.

Zhang, Y., P. F. Coogan, et al. (2005). "Use of Nonsteroidal Antiinflammatory Drugs and Risk of Breast Cancer: The Case-Control Surveillance Study Revisited." Am J Epidemiol **162**(2): 165-170.

Zhao, Y., V. R. Agarwal, et al. (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**(12): 5739-42.

Ziv, E., J. Cauley, et al. (2001). "Association between the T29-->C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: The Study of Osteoporotic Fractures." JAMA **285**(22): 2859-63.

Ziv, E., J. Shepherd, et al. (2003). "Mammographic breast density and family history of breast cancer." J Natl Cancer Inst **95**(7): 556-8.

Zmuda, J. M., J. A. Cauley, et al. (2001). "Bone mass and breast cancer risk in older women: differences by stage at diagnosis." Journal of the National Cancer Institute **93**(12): 930-6.