Prescription medications and breast cancer:  
how do bisphosphonates and omeprazole affect a woman’s risk of developing breast cancer?

by

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Abstract

**Background:** Breast cancer is the most commonly diagnosed cancer in women. It is a heterogeneous disease having a number of risk factors that vary by menopausal status and disease subtype. There is evidence that some pharmaceutical medications may affect breast cancer risk. This thesis investigated bisphosphonates, which are used to treat osteoporosis, as well as omeprazole, a proton pump inhibitor used to treat gastroesophageal reflux disease, and their association with breast cancer and breast cancer subtypes.

**Methods:** This study utilized questionnaire information provided by participants in the Canadian Breast Cancer Study, a case-control study conducted jointly in Vancouver, British Columbia and Kingston, Ontario. Data from the British Columbia arm of the study was linked to PharmaNet, an administrative pharmaceutical database that records prescriptions dispensed in British Columbia. Multiple imputation and logistic regression were used to model the association between the prescription medications and breast cancer while adjusting for confounders.

**Results:** There was not enough evidence to suggest an association between omeprazole and breast cancer, considered either as a whole (OR: 1.02; 95% CI: 0.68-1.52) or by estrogen receptor status (p-heterogeneity = 0.94). However, there was evidence to suggest long-term bisphosphonate use (≥1025 cumulative Defined Daily Doses) was associated with a decreased risk of invasive breast cancer, relative to no bisphosphonate use (OR: 0.65; 95% CI: 0.45-0.94). The protective effect was not evident for situ disease (OR: 0.89; 95% CI: 0.36-2.19). There was no difference in risk observed by estrogen receptor status (p-heterogeneity = 0.83).
Conclusions: This research showed a protective effect for invasive breast cancer with long-term bisphosphonate use, but the results must be interpreted cautiously due to the potential for confounding by indication. This potential bias has been mitigated somewhat by adjusting for factors strongly associated with bone density, such as BMI, but does not completely eliminate the possibility. The study also had limited power to investigate risk with breast cancer subtypes. In the case of both omeprazole and bisphosphonates, further investigation is warranted to elucidate their relationship with breast cancer.
Lay Summary

Breast cancer is the most diagnosed cancer in women. Researchers often attempt to categorize tumors into subtypes to better predict prognosis or understand causal mechanisms. Tumour subtypes are associated with specific responses to treatment and different clinical outcomes.

Evidence suggests prescription medications taken for reasons other than cancer may affect a woman’s risk of developing breast cancer. This study investigated how bisphosphonates, a class of drug used to treat osteoporosis, and omeprazole, a drug used to treat gastroesophageal reflux disease, affect a woman’s risk of developing breast cancer.

There was no apparent relationship between omeprazole and breast cancer, while long-term use of bisphosphonates may protect women from developing invasive breast cancer. The protective effect was independent of tumour subtype. However, it’s important to remember this is one study among several and the evidence from all studies must be weighed to determine if this is a real effect.
Preface

This thesis uses data from the Canadian Breast Cancer Study, a study on risk factors for breast cancer led by Dr. Kristen Aronson (Queen’s University). The British Columbia arm of the study was led by Dr. John Spinelli (BC Cancer and University of British Columbia). Participants were consented and questionnaire data and biological samples were obtained prior to Brendan Bakos’ involvement.

This study was approved by the University of British Columbia Cancer Agency Research Ethics Board (certificate number H14-01579).

The study design was developed jointly by the student and supervisor. The ethics application and Data Access Request for prescription data and MSP enrollment was completed by B. Bakos. The literature review in Chapter 1 was conducted by B. Bakos. All analyses were performed by B. Bakos with the assistance of Dr. Spinelli. The thesis was completed with the guidance of the thesis supervisory committee, Drs. Spinelli, Rachel Murphy (University of British Columbia), and Trevor Dummer (University of British Columbia).

Preliminary findings of results pertaining to bisphosphonates were disseminated as a poster presentation by B. Bakos at the 4th Canadian Cancer Research Conference, November 5-7, 2017 in Vancouver, British Columbia.
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Abbreviations

Admin          Administrative
AHR            Aryl hydrocarbon receptor
BC             British Columbia
BMI            Body mass index
CBCS           Canadian Breast Cancer Study
CI             Confidence interval
DCIS           Ductal carcinoma in situ
DDD            Defined daily dose
ER             Estrogen receptor
HR             Hazard ratio
IQR            Interquartile range
LCIS           Lobular carcinoma in situ
MET            Metabolic equivalent
MICE           Multiple imputation with chained equations
MSP            Medical Services Plan
OR             Odds ratio
PDD            Prescribed daily dose
PMM            Predictive mean matching
PR             Progesterone receptor
Pub            Publication
RCT            Randomized clinical trial
Acknowledgements

First and foremost I would like to thank my thesis supervisor, Dr. John Spinelli, for his endless patience and expert guidance throughout the duration of this research. I am extremely grateful for his time and commitment to helping me finish this thesis. I would like to thank the participants in the Canadian Breast Cancer Study. I would also like to thank my coworkers in the Cancer Surveillance and Outcomes unit at BC Cancer for their insight and support. And thanks to Population Data BC for their assistance in completing the data access request and support in accessing data. Special thanks are owed to my friend Ramsey Hedayat. Without his support, this work would not be possible.
to my plant, charlie
Chapter 1: Introduction

1.1 Background

Breast cancer is the most commonly diagnosed cancer in women; one in eight women will develop breast cancer in their lifetime, accounting for about a quarter of all projected cancers in women (Canadian Cancer Society’s Advisory Committee on Cancer Statistics, 2017). Several risk factors are known to influence a woman’s risk of developing this disease. These include modifiable risk factors such as use of hormone replacement therapy and maintaining a healthy weight, in addition to non-modifiable ones such as genetic predisposition (Fasching et al., 2011). However, breast cancer is a heterogeneous disease, both on a molecular basis (Cancer Genome Atlas Network, 2012; Perou et al., 2000; Prat & Perou, 2011), and in terms of clinical behaviour (Kurian, Fish, Shema, & Clarke, 2010).

A number of non-invasive breast cancer subtypes have been identified, collectively known as in situ disease. The most common broad classification is ductal carcinoma in situ (DCIS), which accounts for roughly 83% of newly diagnosed in situ breast cancer cases in the US (Ward et al., 2015). Lobular carcinoma in situ (LCIS) is the other major class, with incidence estimated at 3.19 per 100,000 (Li, Anderson, Daling, & Moe, 2002). 11% of women diagnosed with in situ breast cancers develop a subsequent malignant breast cancer (Wong, King, Boileau, Barry, & Golshan, 2017). The causal relationship between LCIS and breast cancer is unclear (Morrow, Schnitt, & Norton, 2015), although evidence suggests it is a precursor to invasive cancer (Begg et al., 2016). DCIS on the other hand, is known to be a precursor lesion from which many invasive breast tumours form (Allred, 2010).

Several invasive breast cancer subtypes have been identified that offer prognostic value (Cheang et al., 2008; Prat et al., 2013). The initial molecular profiling studies of primary breast tumours demonstrated breast cancers could be segregated into biological subtypes based on hierarchical clustering analyses of gene expression (Perou et al., 2000). The exact number of etiological subtypes is still a subject of debate (Anderson, Rosenberg, Prat, Perou, & Sherman, 2014), but clinical subtypes can be broadly classified into Luminal A like, Luminal B like, HER2
overexpressing, and triple negative depending on expression of estrogen receptors (ER), progesterone receptors (PR), HER2, and tumour histology (Curigliano et al., 2017; Goldhirsch et al., 2013). The most important factors in treatment and clinical outcome are the expression of the hormone receptors (ER and PR) and the overexpression of HER2 (Perou & Borresen-Dale, 2011). Not only do these subtypes have implications for the clinical treatment of breast cancer (Curigliano et al., 2017; Goldhirsch et al., 2013), but studies have demonstrated risk factors vary between subtypes (M. E. Barnard, Boeke, & Tamimi, 2015).

Invasive breast cancer has a number of known risk factors. These include age at menarche, parity, age at first birth, time spent breastfeeding, age at menopause, BMI, family history of breast cancer, genetic predisposition, alcohol use, oral contraceptive use, high breast density, and menopausal hormone therapy (M. E. Barnard et al., 2015; Phipps et al., 2012; Vogel, 2008). Risk factors for in situ breast cancers such as high breast density, parity, age at first birth, family history, genetic predisposition, and age at menarche are shared with invasive breast cancers (Mullooly et al., 2017; Ward et al., 2015).

Some modifiable risk factors that have been associated with specific subtypes of invasive breast cancer include high pre-menopausal BMI, use of oral contraceptives, and menopausal hormone therapy use (M. E. Barnard et al., 2015). High pre-menopausal BMI is associated with an increased risk of triple negative breast cancer, while the risk of Luminal A like is decreased. Similarly, greater parity is associated with a lower risk of Luminal A tumours, but may be associated with an increased risk of triple negative breast cancer (M. E. Barnard et al., 2015). Longer time spent breast feeding has been consistently associated with a decreased risk of all breast cancer subtypes, with the exception of HER2, in which the effects are thus far unclear, with the majority of studies being somewhat hampered by sample size (M. E. Barnard et al., 2015).

Risk factors also vary by the menopausal status of the woman at the time of diagnosis. Increased age at menarche was associated with a decreased risk of premenopausal breast cancer, while high parity was associated with a protective effect in postmenopausal breast cancer (Clavel-Chapelon & E3N-EPIC Group, 2002). An age of first birth over 30 increased the breast cancer risk for both
pre and postmenopausal women, although the risk was higher in premenopausal women (Clavel-Chapelon & E3N-EPIC Group, 2002). These differences in risk factors for pre and postmenopausal breast cancer are supported by biological etiological differences (Yamashita, 2015). Clinical roles of PR and Ki67, an antigen which is used as a marker for cell proliferation, indicate an etiological difference between pre and post-menopausal ER-positive breast cancer (Yamashita, 2015). Ultimately, these differences between subtypes and menopausal status serve to illustrate the risks associated with breast cancer are nuanced and complex.

Although prevention strategies for breast cancer are available, these are chiefly lifestyle modifications such as maintaining a healthy bodyweight and reducing alcohol consumption. Other strategies exist, for example prophylactic mastectomy, but the procedure is generally only performed for women at high risk due to the presence of the BRCA1 or BRCA2 mutations (Heisey & Carroll, 2016).

Two pharmaceutical ‘chemopreventive’ agents, tamoxifen and raloxifene, are well documented as primary breast cancer preventive agents (Cuzick et al., 2013). These drugs are selective estrogen receptor modulators, so they are primarily effective in prevention of ER positive breast cancer. However, severe adverse effects such as venous thromboembolism are relatively common, and while the two drugs are effective in reducing breast cancer incidence, they have not been shown to have an impact on mortality due to breast cancer (Prasad & Diener-West, 2015). It has been suggested that the benefits of these chemopreventive agents has been overstated and the harms underappreciated (Prasad & Diener-West, 2015). To date, no safe and effective pharmaceutical chemopreventive agents have been identified that may be widely used for targeted prevention among high risk women. However, there is increasing evidence that some medications prescribed for conditions other than cancer may affect a woman’s risk of getting breast cancer.

As mentioned above, oral contraceptives are a well-known example of a family of prescription drugs associated with breast cancer. Oral contraceptives increase a woman’s risk of developing breast cancer, however, the increased risk of breast cancer disappears 10 years after use of oral contraceptives has stopped (Collaborative Group on Hormonal Factors in Breast Cancer, 1996).
Other prescription drugs such as statins, a family of drug used to lower cholesterol levels in the blood, have been extensively studied. Some evidence suggests statins may be associated with improved breast cancer survival and recurrence (Van Wyhe, Rahal, & Woodward, 2017). However, meta-analyses and reviews do not provide evidence in support of the idea that statins reduce a woman’s risk of developing breast cancer (Boudreau, Yu, & Johnson, 2010; Islam et al., 2017). Angiotensin-converting enzyme (ACE) inhibitors, a class of drugs used to treat hypertension, also have some evidence of an association with breast cancer. Some studies have found a protective effect, although results have been varied (Fryzek et al., 2006; Li et al., 2013; Raebel et al., 2017). Of primary interest for this thesis are bisphosphonates, a class of drug most commonly used to treat osteoporosis, and omeprazole, a drug that inhibits gastric acid secretion.

Bisphosphonates as a whole can be broadly divided into two classes: non-nitrogen containing (e.g. etidronate) and alkyl-amino (e.g. alendronate), with different modes of action and persistence in the body (Singh et al., 2014). Clinical guidelines suggest the use of the bisphosphonates alendronate, risedronate, and zoledronic acid as first-line therapies for prevention of fractures in menopausal women (Papaioannou et al., 2010). Women who are using steroid medications may also use bisphosphonates (Osteoperosis Canada, 2018), but their use is contra-indicated in pregnancy and, due to their persistence in the body, bisphosphonates carry a teratogenic risk for future pregnancies (McNicholl & Heaney, 2010). There is also some concern of osteonecrosis of the jaw in patients taking oral bisphosphonates for the treatment of osteoporosis, with incidence estimates ranging from 1.04 to 69 per 100,000 patient-years (Khan et al., 2015). Overall bisphosphonates are well tolerated, but their use in premenopausal women should be carefully considered.

Beyond their efficacy in treating osteoporosis, there is substantial biological evidence of bisphosphonates’ anti-tumour activity. A recent Cochrane review highlights a number of studies evaluating their efficacy in preventing bone metastases in women who have breast cancer (O’Carrigan et al., 2017). And, although not officially endorsed by the 2013 St Gallen International Expert Consensus – a panel which meets biennially to discuss and vote on issues
relating to breast cancer care – a number of panelists believed bisphosphonates could be useful for improvement of disease-free survival in post-menopausal women (Goldhirsch et al., 2013).

Several observational studies have suggested an association between bisphosphonates and reduced breast cancer risk, but the literature is inconsistent and no clear consensus has been reached (Table 1, page 6; Cardwell et al., 2012; Chiang et al., 2012; Chlebowski et al., 2010; Fournier et al., 2017; Hue et al., 2014; Monsees, Malone, Tang, Newcomb, & Li, 2011; Newcomb, Trentham-Dietz, & Hampton, 2010; Rennert, Pinchev, & Rennert, 2010; Vestergaard, Fischer, Mele, Mosekilde, & Christiansen, 2011; Vinogradova, Coupland, & Hippisley-Cox, 2013). However, these studies have observed different effects based on the duration of use, for example some studies noted bisphosphonates were only effective with short term use (Chlebowski et al., 2010; Rennert et al., 2010), while others had a protective effect independent of duration (Cardwell et al., 2012; Vestergaard et al., 2011; Vinogradova et al., 2013).

Across the observational studies mentioned above, there was variation in the size of the observed effect. Studies that found evidence of a protective effect observed odds ratios (ORs) or hazard ratios (HRs) ranging from 0.4 to 0.9. An early meta-analysis suggested a pooled risk ratio of 0.85 (95% CI: 0.74 – 0.98), with the protective effect increasing 8% per year of use (Liu et al., 2012). A more recent meta-analysis concluded there was a pooled risk ratio of 0.84 (95% CI: 0.77 – 0.90) across all observational studies and risk ratio of 0.78 (95% CI: 0.64 – 0.96) specific to case-control studies (Ou, Chiu, Wong, Yang, & Yang, 2017). Randomized clinical trials have found no association between bisphosphonates and breast cancer (Hue et al., 2014). Neither alendronate taken orally at 5 mg/day for the first two years and 10 mg/day thereafter with a mean follow-up time of 3.8 years or zoledronic acid given intravenously with a mean follow-up time of 2.8 years had any observed effect on breast cancer risk (Hue et al., 2014). However, these two trials were designed to investigate fracture incidence with use of bisphosphonates and the power to detect reductions in breast cancer incidence was relatively low due to small sample size – less than 2% of participants (165 women) were diagnosed with breast cancer. In the RCT investigating alendronate – most directly relevant to this thesis due to the use of an oral bisphosphonate – the number of breast cancer cases was less than 100 (1.3%) considering both treatment and placebo arms. Further, the exposure period was somewhat shorter than many
Table 1. A summary of key findings from studies that investigated the association between bisphosphonates and breast cancer. See footnote for abbreviations.  

<table>
<thead>
<tr>
<th>Author</th>
<th>Pub Year</th>
<th>Study period</th>
<th>Age</th>
<th>Study type</th>
<th>Sample size (cases)</th>
<th>Effect size OR/HR (95% CI)</th>
<th>Timing of effect</th>
<th>Exposure data source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardwell et al.</td>
<td>2012</td>
<td>1996-2006</td>
<td>40+</td>
<td>Cohort</td>
<td>68098 (870)</td>
<td>0.75 (0.63-0.89)</td>
<td>Any use</td>
<td>Admin</td>
<td>Association less apparent over time.</td>
</tr>
<tr>
<td>Chiang et al.</td>
<td>2012</td>
<td>1998-2009</td>
<td>55+</td>
<td>Cohort</td>
<td>66085 (44)</td>
<td>1.05 (0.97-1.13)</td>
<td>Not sufficient evidence</td>
<td>Admin</td>
<td>Authors state study was underpowered. Looked specifically at alendronate.</td>
</tr>
<tr>
<td>Chlebowski et al.</td>
<td>2010</td>
<td>1993-1998</td>
<td>50+</td>
<td>Cohort</td>
<td>154768 (6276)</td>
<td>0.50 (0.38-0.67)</td>
<td>&lt;2 years</td>
<td>Interview</td>
<td>Null association after 2 years of use.</td>
</tr>
<tr>
<td>Fournier et al.</td>
<td>2017</td>
<td>2004-2011</td>
<td>Born 1925-1950</td>
<td>Cohort</td>
<td>64438 (2407)</td>
<td>0.56 (0.36-0.87)</td>
<td>&lt;1 year since first delivery or null effect</td>
<td>Admin</td>
<td>When dose effect examined with DDD, no association found.</td>
</tr>
<tr>
<td>Hue et al.</td>
<td>2014</td>
<td>1992-1997&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55+</td>
<td>RCT</td>
<td>6194 (103)</td>
<td>1.24 (0.84-1.83)</td>
<td>Not sufficient evidence</td>
<td>RCT</td>
<td>HR for alendronate.</td>
</tr>
<tr>
<td>Hue et al.</td>
<td>2014</td>
<td>2002-2006&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65-89</td>
<td>RCT</td>
<td>7580 (62)</td>
<td>1.15 (0.70-1.89)</td>
<td>Not sufficient evidence</td>
<td></td>
<td>HR for zoledronic acid. Received two doses (once annually for two years)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Admin = Administrative; DDD = defined daily dose; HR = Hazard ratio; Pub = Publication; OR = Odds ratio; RCT = randomized clinical trial
<table>
<thead>
<tr>
<th>Author</th>
<th>Pub Year</th>
<th>Study period</th>
<th>Age</th>
<th>Study type</th>
<th>Sample size (cases)</th>
<th>Effect size OR/HR (95% CI)</th>
<th>Timing of effect</th>
<th>Exposure data source</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monsees et al.</td>
<td>2011</td>
<td>1990-2007</td>
<td>40-79</td>
<td>Case-control</td>
<td>662 (351)</td>
<td>0.40 (0.19-0.85)</td>
<td>≥ 12 months of use</td>
<td>Admin</td>
<td>Looked at alendronate use association with contralateral breast cancer. Protective effect increased with longer use.</td>
</tr>
<tr>
<td>Newcomb et al.</td>
<td>2010</td>
<td>2003-2006</td>
<td>20-69</td>
<td>Case-control</td>
<td>5371 (2396)</td>
<td>0.63 (0.42-0.95)</td>
<td>≥ 25 months of use.</td>
<td>Interview</td>
<td></td>
</tr>
<tr>
<td>Rennert et al.</td>
<td>2010</td>
<td>2000-2006</td>
<td>50+</td>
<td>Case-control</td>
<td>4039 (1832)</td>
<td>0.72 (0.57-0.90)</td>
<td>&gt;1 year of use.</td>
<td>Admin</td>
<td>Risk did not change with longer use.</td>
</tr>
<tr>
<td>Vestergaard et al.</td>
<td>2011</td>
<td>1996-2006</td>
<td>40+</td>
<td>Cohort</td>
<td>348426 (4630)</td>
<td>0.53 (0.38-0.73)</td>
<td>Any use</td>
<td>Admin</td>
<td>HR is for alendronate. No dose response relationship.</td>
</tr>
<tr>
<td>Vinogradova et al.</td>
<td>2013</td>
<td>1997-2011</td>
<td>50+</td>
<td>Case-control</td>
<td>282713 (49933)</td>
<td>0.92 (0.87-0.98)</td>
<td>Any use</td>
<td>Admin</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) All breast cancer cases included in analysis
\(^b\) Authors state this effect is likely explained by healthy screener bias
\(^c\) Randomization and treatment period
observational studies. While RCTs are the gold standard for evidence, these studies are not conclusive due to these limitations. Ultimately, the conflicting nature of the literature surrounding bisphosphonates and breast cancer points to a need for further research.

In contrast, relatively little is known about omeprazole, a commonly prescribed medication used for treatment of gastrointestinal conditions such as dyspepsia and gastroesophageal reflux disease. Omeprazole was also evaluated as an agent that may reduce the risk of breast cancer in an effort to study novel prescription medications with supporting evidence coming from the basic sciences and biologic plausibility. This drug was approved for over-the-counter use in Canada on September 17, 2014 for 14 day treatment with a daily 20mg dose (Health Canada, March 17, 2014; Health Canada, September 16, 2014). However, omeprazole was a prescription medication during the time period of interest for this study. It was typically prescribed in courses of four to eight weeks often followed with a low maintenance dose for an indefinite period of time (Armstrong et al., 2005), although recent clinical trends are pushing toward deprescribing to limit chronic use (Farrell et al., 2017; Naunton, Peterson, Deeks, Young, & Kosari, 2018). To date, no epidemiologic studies have investigated omeprazole’s association with the risk of breast cancer and its potential application as a primary chemopreventive agent. However, there is biologic plausibility and in vitro evidence of omeprazole’s potential to act as a chemopreventive agent for breast cancer.

Omeprazole is a proton pump inhibitor (a class of drug that inhibits gastric acid secretion), but it also targets the aryl hydrocarbon receptor (AHR) which is known to play a role in breast cancer progression (Powell, Goode, & Eltom, 2013). In vitro evidence has suggested omeprazole decreases breast cancer cell invasion and metastases in the ER negative subtype (Jin, Lee, Pfent, & Safe, 2014), likely through targeted interaction with AHR. Further in vitro evidence has indicated AHR could regulate estrogen synthesis and metabolism in bone tissues through cytokine/aromatase signalling (Miki et al., 2017). An RCT demonstrated the aromatase inhibitor anastrazole was effective in primary prevention of postmenopausal breast cancer in high risk women (Cuzick et al., 2014), so it may be the case that AHR inhibitors such as omeprazole could also be useful in primary prevention of high risk breast cancer cases acting on a similar pathway as aromatase inhibitors.
1.2 Study goal and objectives

This study had two primary objectives:

1. Evaluate the association between bisphosphonate use and the risk of breast cancer.
2. Evaluate the association between omeprazole use and the risk of breast cancer.

In addition, there were two secondary objectives:

1. Evaluate the association between bisphosphonate use and the risk of breast cancer subtype (ER positive versus ER negative).
2. Evaluate the association between omeprazole use and the risk of breast cancer subtype (ER positive versus ER negative).

1.3 Purpose and rationale

Cancer is a chronic disease that may take a long time to develop. As such, it will often be difficult to detect an effect of chemopreventive agents in short term clinical trials. Population-based studies investigating the association of prescription drugs and breast cancer are necessary to understand unknown adverse or advantageous effects. Due to compelling in-vitro and in-vivo evidence for bisphosphonates and omeprazole, and the lack of randomized controlled trials investigating the questions proposed, a retrospective case control study is an appropriate study design to assess their association with breast cancer. In addition, little work has been done to describe their risk related to breast cancer subtypes.

To that end, this study seeks to investigate the association between bisphosphonates, omeprazole and a woman’s risk of developing both breast cancer and breast cancer subtypes.

1.4 Thesis overview

This thesis is composed of five chapters. The first chapter provides background on breast cancer, breast cancer risk, how prescription drugs may influence this risk, and introduces the purpose and goals of this study. The second chapter describes the methodology used to investigate the
association between the two prescription drugs and breast cancer, while the third chapter presents the results of the analyses. Chapters four and five are dedicated to discussing the results in the context of known literature and the conclusion which summarizes the results and highlights the implications of the research.
Chapter 2: Methods

2.1 Data Sources

2.1.1 Canadian Breast Cancer Study (CBCS) study

The CBCS is a case-control study with 1,142 breast cancer cases and 1,178 controls, from Greater Vancouver, British Columbia (BC), and Kingston, Ontario, which completed data collection in 2010. The data from the population-based BC portion of the study comprise this analysis. Cases aged 40-80 and diagnosed between 2005 and 2009 were recruited from the BC Cancer Registry, and controls were cancer-free individuals recruited from the BC Cancer Breast Screening Program (formerly the BC Screening Mammography Program). The Breast Screening Program is a provincial program in BC that provides screening mammograms to women over the age of 40. Controls were recruited from the same geographic area and frequency-matched to cases in five-year age groups, resulting in 1,003 cases and 1,014 controls. Participation rates were 54% among cases and 57% among controls. Questionnaire information included education, ethnicity, health, medical and reproductive history, family history of cancer, and lifestyle characteristics such as alcohol consumption and physical activity. In addition, participants consented to allow the researchers access to their medical records for information related to breast health and permission to obtain breast tissue blocks that were collected as part of regular care.

2.1.2 Clinical variables

Participant’s tumour subtype information (ER status, behaviour) was obtained from the BC Cancer Registry and the Cancer Agency Information System. Stewardship for information collected specifically for breast cancer patients is by the Breast Cancer Outcomes Unit at BC Cancer.
2.1.3 PharmaNet

PharmaNet is the central database for prescriptions filled in British Columbia (BC Ministry of Health, 2016), and was established in 1995. Prior to its establishment, drug dispensation information was only available for drugs paid for by the PharmaCare program which included individuals over the age of 65 or on income assistance. As a result, comprehensive prescription information at the population level is not available prior to the establishment of PharmaNet.

PharmaNet houses record of every prescription dispensed from community pharmacies in BC. The database also includes prescriptions dispensed by hospital outpatient pharmacies, and medications provided in a physician office, emergency or clinic visit, although it is not currently mandatory for physicians to record medications given during these visits. Drugs dispensed to patients when admitted to hospital or in hospital are not recorded in PharmaNet.

Data from PharmaNet were received through Population Data BC (BC Ministry of Health, 2016). The dataset hosted through Population Data BC contains information on the drug name, as well as the prescription dispense date, quantity, dose strength, and instructions for use. All prescriptions in PharmaNet for alendronate, etidronate, clodronate, pamidronate, zoledronic acid, ibandronate, risedronate, and omeprazole were obtained.

2.1.4 Consolidation File (Medical Services Plan Registration)

The Consolidation File contains a variety of data, such as age, sex, and geo-codes with location of residence for every British Columbia resident covered by provincial medical services plan (MSP). For this study, MSP registration was obtained from the Consolidation File through Population Data BC (British Columbia Ministry of Health, 2015). The MSP data obtained through Population Data BC contained the start and end date of participants enrollment on an annual basis from 1996 to 2010. This information was used to determine the time window in which participants were BC residents and thus their prescription medications were captured by PharmaNet.
2.1.5 Data linkage

The researcher collected data from the CBCS study was linked with pharmaceutical information from PharmaNet and MSP enrollment from the Consolidation File through Population Data BC. The linked data was de-identified and hosted on the Population Data BC Secure Research Environment where the analysis was conducted.

2.2 Study population

The study BC portion of the CBCS study included 2,017 participants (Figure 1). The analysis was restricted to the 1,124 post-menopausal women (581 cases and 543 controls) as risk factors for pre-menopausal and post-menopausal breast cancer are known to differ and because younger women were not likely to have taken bisphosphonates (Figure 1).

As PharmaNet provides information on prescription drug use for women who are based in-province, MSP enrollment was used to determine which women resided in BC and would have their prescriptions captured in PharmaNet. Women diagnosed with breast cancer (cases) who were enrolled in MSP from 1996 – the year PharmaNet began – until one year before diagnosis and women who were breast cancer free at the time of the study (controls) who were enrolled in MSP from 1996 until three years before their interview (controls) date were included in the analysis.²

Although there are two modes of delivery for bisphosphonates: oral and intravenous, during the period of interest, women only took bisphosphonates orally.

² Please see section 2.3.2 (Prescription drug exposure period) on page 16 for an explanation of the different end dates for the exposure period.
Figure 1. A flow chart of participant inclusion in analysis.

2.3 Prescription drug dose

As there are several kinds of bisphosphonates of varying potency and strengths, the dose for each prescription was standardized for comparison. Doses were standardized using the defined daily dose (DDD), established by the World Health Organization (World Health Organization, December 20, 2017). The DDD is defined as “the assumed average maintenance dose per day for a drug used for its main indication in adults.”

The DDD converts the amount of medication used – the quantity dispensed and the strength – into a standard unit, which is the number of DDDs. This can be expressed as a lifetime total or as a smaller unit of time, for example number of DDDs per year. The DDD allows comparison of
different kinds of bisphosphonates with varying strengths/doses. The DDD was used to calculate standard drug usage with the following formula:

\[
\text{# of DDDs} = \frac{\text{Tablets issued} \times \text{Drug strength}}{\text{DDD}}
\]

The drug usage for each prescription dispense date was calculated and summed for all prescriptions of any oral bisphosphonate from 1996 until one year before diagnosis for cases and from 1996 until three years before interview for controls. Prescriptions dispensed after this period were excluded. Drug usage was split into four categories for bisphosphonate exposure – no use, low, moderate, and high – and three categories for omeprazole exposure – no use, low, and high. To classify exposure, the data was divided such that controls were as evenly distributed between categories as possible.

It is worth noting that another metric, known as the Prescribed Daily Dose (PDD), is also commonly used when measuring a population’s use of pharmaceuticals. The PDD is the average daily amount of a drug that is actually prescribed. It can be expressed in a few ways, but in the simplest form it is calculated as:

\[
PDD = \text{Tablets issued} \times \text{Drug strength}
\]

and summed over the years of use. This is problematic, however, as it does not allow different drugs to be summed or compared. Women in the CBCS study used a number of bisphosphonates, such as risedronate and clodronate. As such, the DDD was a more appropriate choice for this study. For consistency, the DDD was also used for omeprazole.

Exposure categories were created by evenly distributing the number of control subjects as much as possible. Low exposure to bisphosphonates was defined as \(\leq 260\) cumulative DDDs, moderate exposure as \(>260\) to 1025 cumulative DDDs and high exposure as \(>1025\) cumulative DDDs. Low exposure to omeprazole was defined as \(\leq 60\) cumulative DDDs while high exposure was defined as \(> 60\) cumulative DDDs.

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Please see section 2.3.2 (Prescription drug exposure period) on page 16 for an explanation of the different end dates for the exposure period.
2.3.1 **Prescription drug exposure duration**

The number of days supplied for each prescription was used to determine the duration of use. For bisphosphonates, duration categories were created to make results more comparable with previous studies, dividing categories by years of use. In the case of omeprazole, there have been no prior studies to establish categories for duration. As such, the duration categories were created such that an even number of controls were divided between each category of omeprazole users.

2.3.2 **Prescription drug exposure period**

Prescriptions within one year of diagnosis date or three years prior to controls interview date were excluded to minimize protopathic bias (breast cancer symptoms leading to use of a drug). Due to difficulty in obtaining permission to contact women through the Screening Mammography Program, accrual of controls did not start until 2007, and continued until 2010. Thus, on average, controls were interviewed approximately two years later than the cases. If the bisphosphonate exposure period was up until one year prior to diagnosis for cases and one year prior to interview for controls, this would mean controls would have an average exposure period two years longer than cases. As a result, they would have the opportunity to receive more prescriptions than cases, resulting in a potential positive bias. To control for this, the end year for controls exposure period was adjusted to two years prior to the interview date, plus one year to minimize the possibility of protopathic bias, so the exposure period was the same between cases and controls.

2.4 **Variables and confounders**

2.4.1 **Outcomes (dependent variables)**

*Breast cancer status*

Breast cancer was analyzed as a binary outcome, where participants with breast cancer (cases) were compared to subjects without (controls).

*Breast cancer by ER status and behaviour*

The subtype comparison was performed as a case-only analysis, with the outcome being whether participants had ER positive (cases) or ER negative (controls) breast cancer (Begg & Zhang,
1994). Other subtypes were of interest (e.g. behaviour or HER2 status), but the sample size was too small to allow analysis. Comparison of breast cancer behaviour was performed in the same manner.

2.4.2 Covariates/confounders

All potential confounders were derived from questionnaire data from the CBCS study (Table 2). The age of the women was calculated as the age of diagnosis for cases, and the age two years prior to interview for the controls in order to control for the differing exposure periods. For physical activity, lifetime leisure-time, household, and occupational physical activity data were collected using a self-report questionnaire adapted from the Lifetime Total Physical Activity Questionnaire (Friedenreich, Courneya, & Bryant, 1998). The intensity for leisure and household was mild, moderate, and vigorous, while occupational questions also included a sedentary level of intensity. Intensity was described only by the body’s response e.g. elevated heart rate. No sample activities were given to describe intensity levels. The intensity was used to derive a metabolic equivalent (MET) which is a measure for expressing the energy cost of physical activity (Jette, Sidney, & Blumchen, 1990). A MET value of 1.5, 3.3, 4, and 8 was assigned to sedentary, mild, moderate, and vigorous intensity, respectively (Ainsworth et al., 2011; Boyle et al., 2015). MET-hours per week was calculated based on the time participants spent performing each activity along with the intensity and averaged over the course of their life.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Missing (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Participants age at entry into the study</td>
<td>0.0</td>
</tr>
<tr>
<td>Age at first birth</td>
<td>Participants age at first birth if they had a child.</td>
<td>0.0</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>Ever/never</td>
<td>0.0</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>Participants age when they began menstruating.</td>
<td>1.2</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>The number of months spent breast feeding.</td>
<td>0.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>The number of pack-years participants smoked.</td>
<td>0.0</td>
</tr>
<tr>
<td>Variable</td>
<td>Description</td>
<td>Value</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td>First-degree family history of breast cancer. Yes/no.</td>
<td>0.0</td>
</tr>
<tr>
<td>Education</td>
<td>Highest level of education participants achieved, categorized as less than high school, high school, certificate/diploma, or undergraduate and above.</td>
<td>0.1</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Classified as European, East Asian, or other.</td>
<td>0.0</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td>Ever/never</td>
<td>0.0</td>
</tr>
<tr>
<td>Max body mass index (BMI)</td>
<td>Maximum self-reported BMI of participants.</td>
<td>6.0</td>
</tr>
<tr>
<td>BMI two years prior to study</td>
<td>Participants’ self-reported BMI two years prior to the interview.</td>
<td>8.2</td>
</tr>
<tr>
<td>Leisure physical activity</td>
<td>Physical activity related to sports and leisure as lifetime average MET-hrs/week</td>
<td>1.6</td>
</tr>
<tr>
<td>Household physical activity</td>
<td>Physical activity involving housework as lifetime average MET-hrs/week</td>
<td>1.2</td>
</tr>
<tr>
<td>Occupational physical activity</td>
<td>Physical activity on the job as lifetime average MET-hours/week</td>
<td>4.1</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Alcohol intake; average number of drinks per week. A drink was defined as one glass of wine, one bottle of beer, or one ounce of spirits.</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### 2.4.3 Missing data and imputation

For this study, max BMI, BMI two years prior to the study, physical activity, alcohol consumption, age at menarche, education, and months spent breast feeding were imputed using multiple imputation with chained equations (MICE), implementing the predictive mean matching (PMM) method of the mice package in R (Little, 1988; van Buuren & Groothuis-Oudshoorn, 2011). PMM follows the distribution of known data and confines imputed values within the limits of the known data, preventing observations from being absurdly high or low, as is possible with other regression-based imputation methods. This method is preferred to simple imputation methods such as replacement by the mean as it will produce less biased estimates in instances where the distribution is skewed.
Single imputation PMM may be used to obtain unbiased estimates, but it will result in high variance and poor standard errors. Multiple imputation is used over single imputation methods as it reduces bias and produces confidence intervals that more accurately reflect the uncertainty in the estimate. MICE involves regression switching which has been shown to perform better than other multiple imputation methods, such as the NORM algorithm which assumes a joint multivariate distribution (Bernaards, Belin, & Schafer, 2007; Yu, Burton, & Rivero-Arias, 2007).

PMM is further appealing as it is semi-parametric and offers some robustness in the event the imputation model is misspecified, which is not the case for parametric methods such as linear regression imputation. PMM also performs well when normality assumptions are violated or imputation involves semicontinuous data (White, Royston, & Wood, 2011; van Buuren, 2012; Vink, Frank, Pannekoek, & van Buuren, 2014). However, there are some scenarios where it may perform poorly, in particular if missing data is abundant and there are few complete cases. This particular issue is not of concern in this dataset, which had only 0.9% of predictor variables missing.

Previously it was shown that there was no practical benefit to having more than five imputed datasets for the sake of imputation efficiency (Schafer, 1999), but more recent recommendations state the number of imputed datasets should be equal to at least the percent of missing data to ensure stable estimates (White, Royston, & Wood, 2011). For example, if 13% of the dataset was missing, at least 13 imputed sets should be used, but more may be required for the sake of reproducibility. While only 0.9% of regression variables were missing for women included in the analysis (with a maximum missing of 8.2% for BMI two years prior to the study), 20 imputed datasets were used to ensure stable results as the size of the dataset did not impose any practical limitations on the imputation time. 30 iterations were used in generating the imputed datasets to allow convergence of imputed estimates.

To perform regression, each of the imputed datasets must be used separately and then results pooled. Pooling averages the estimates from each model and calculates the total variance across all sets by Rubin’s rules. The variance is calculated by accounting for the uncertainty in the missing information (J. Barnard & Rubin, 1999).
All variables (listed in Table 2) were used for imputation. Other variables were also included in the prediction matrix in an effort to improve imputation accuracy, including whether a participant was a smoker, parous, and how many children they had.

For number of months spent breast feeding, values were imputed for each pregnancy where missing and the total number of months breast feeding calculated after imputation. Similarly, as participants were able to specify multiple activities for physical activity in the questionnaire, missing values were imputed for each specification of leisure, household, and occupational physical activity then summed to get cumulative MET-hours per week per year of life.

2.5 Statistical Analysis

Data cleaning and statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., North Carolina) and R 3.5.0 (R Core Team, Vienna).

As outcomes were binary, unconditional multivariate logistic regression was performed with the imputed datasets to model the association between pharmaceutical medication use and the likelihood of breast cancer (considered as a whole), behaviour, or ER-status, with the odds ratio (OR) as the measure of association. Potential confounders were considered as independent variables. All potential confounders were entered into the model in order to obtain adjusted ORs for bisphosphonate and omeprazole association with breast cancer. The decision of whether a variable was a potential confounder was determined using clinical and scientific evidence and observing a change in the magnitude of the OR of exposure variables by 10% or greater upon inclusion of the potential confounder and all other identified confounders. As exposure variables were analyzed as categorical, the sum of a 10% change for each of the n-1 categories within the exposure variable was used. In the case of bisphosphonate exposure, which had 4 categories, a sum of change for each parameter estimate greater than 30% was used, while in the case of omeprazole with 3 levels, a sum of change greater than 20% was used. The model was refined with the change in estimate procedure, removing variables with the smallest impact on the exposure OR until removal of any single variable resulted in a change greater than the threshold. All variables in Table 2 were considered as a potential confounder for model selection.
Tests for trend across dose levels were conducted by creating a continuous variable with assigned values equal to the median level among controls within each category and adjusting for confounders identified using the change in estimate approach.

In the CBCS, controls were recruited from a screening program and therefore healthy screener bias was a concern. However, 92.5% of post-menopausal women with breast cancer had received a mammogram prior to diagnosis so a sensitivity analysis was not considered necessary as the vast majority of cases had been screened.

2.5.1 Ethical considerations and disclaimer

Ethical approval had already been received for the original study from the University of British Columbia/BC Cancer Agency Research Ethics Board (certificate number H04-60142). Consent provided by participants for researchers to access their medical records. Ethics approval for this study was also obtained from the University of British Columbia Cancer Agency Research Ethics Board (certificate number H14-01579).

All inferences, opinions, and conclusions drawn in this thesis are those of the author, and do not reflect the opinions or policies of the Data Stewards.
Chapter 3: Results

3.1 Participant characteristics

1124 women from the CBCS study were postmenopausal and included in the case-control investigation of the association between pharmaceutical prescriptions and breast cancer risk. Characteristics of the 1124 women who were eligible for the main bisphosphonate analysis are shown in Table 3. The median age was 61 with the majority of participants identifying as European. However, a larger number of women with breast cancer identified as East Asian. Controls tended to have higher levels of education and were more likely to have used oral contraceptives. Fewer controls had a family history of breast cancer. BMI, both two years prior to study entry and maximum, were comparable between cases and controls. Ranges for physical activity were similar, although controls had higher median household and leisure physical activity. The median period of possible exposure time was 10 years for both cases and controls after the adjustment noted in section 2.3.2 on page 16.

Table 3. Characteristics for participants involved in the CBCS study who met inclusion criteria.

<table>
<thead>
<tr>
<th>Participant characteristic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 581</td>
<td>N = 543</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In situ</td>
<td>113 (19.4)</td>
<td>--</td>
</tr>
<tr>
<td>ER negative</td>
<td>74 (12.7)</td>
<td>--</td>
</tr>
<tr>
<td>ER positive</td>
<td>373 (64.1)</td>
<td>--</td>
</tr>
<tr>
<td>Invasive, unknown</td>
<td>21 (3.6)</td>
<td>--</td>
</tr>
<tr>
<td>ER status</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>Age (median, Interquartile Range)</td>
<td>56-69</td>
<td>54-66</td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>119 (20.5)</td>
<td>102 (18.8)</td>
</tr>
<tr>
<td>24-29</td>
<td>134 (22.1)</td>
<td>120 (22.1)</td>
</tr>
<tr>
<td>29+</td>
<td>124 (21.3)</td>
<td>99 (18.2)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>135 (23.2)</td>
<td>162 (29.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>69 (11.9)</td>
<td>60 (11.0)</td>
</tr>
<tr>
<td>Highest level of education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than high school</td>
<td>91 (15.7)</td>
<td>36 (6.6)</td>
</tr>
<tr>
<td>High school</td>
<td>140 (24.1)</td>
<td>132 (24.3)</td>
</tr>
<tr>
<td>Diploma or certificate</td>
<td>165 (28.4)</td>
<td>165 (30.4)</td>
</tr>
<tr>
<td>Participant characteristic</td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>N = 581</td>
<td>N = 543</td>
</tr>
<tr>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undergraduate or higher</td>
<td>184 (31.7)</td>
<td>210 (38.7)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>365 (62.8)</td>
<td>426 (78.4)</td>
</tr>
<tr>
<td>East Asian</td>
<td>126 (21.7)</td>
<td>55 (10.1)</td>
</tr>
<tr>
<td>Other</td>
<td>90 (15.5)</td>
<td>62 (11.4)</td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>122 (21.0)</td>
<td>75 (13.8)</td>
</tr>
<tr>
<td>No</td>
<td>459 (79.0)</td>
<td>468 (86.2)</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>248 (42.7)</td>
<td>250 (46.0)</td>
</tr>
<tr>
<td>No</td>
<td>333 (57.3)</td>
<td>293 (54.0)</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>307 (52.8)</td>
<td>330 (60.8)</td>
</tr>
<tr>
<td>No</td>
<td>274 (47.2)</td>
<td>213 (39.2)</td>
</tr>
<tr>
<td>Maximum BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>26.6</td>
<td>25.7</td>
</tr>
<tr>
<td>23.8-30.4</td>
<td>23.3-29.0</td>
<td></td>
</tr>
<tr>
<td>BMI two years prior to study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>25.0</td>
<td>23.9</td>
</tr>
<tr>
<td>22.5-28.6</td>
<td>21.8-27.3</td>
<td></td>
</tr>
<tr>
<td>Pack years smoked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0-6.5</td>
<td>0-7.0</td>
<td></td>
</tr>
<tr>
<td>Cumulative months breast feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1-8</td>
<td>0-8</td>
<td></td>
</tr>
<tr>
<td>Age at menarche</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>12-14</td>
<td>12-14</td>
<td></td>
</tr>
<tr>
<td>Leisure physical activity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>5.8</td>
<td>10.1</td>
</tr>
<tr>
<td>1.6-14.3</td>
<td>3.3-23.7</td>
<td></td>
</tr>
<tr>
<td>Household physical activity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>64.6</td>
<td>70.2</td>
</tr>
<tr>
<td>21.7-120.8</td>
<td>30.5-127.6</td>
<td></td>
</tr>
<tr>
<td>Occupational physical activity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>19.1</td>
<td>18.6</td>
</tr>
<tr>
<td>9.8-28.3</td>
<td>9.9-29.2</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median, IQR)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0-1</td>
<td>0-4</td>
<td></td>
</tr>
</tbody>
</table>

*Lifetime average MET-hours per week
3.2 Bisphosphonates

3.2.1 Bisphosphonate exposure association with breast cancer risk

Of 543 controls, 105 (19.3%) had used a bisphosphonate at any time (Table 5.). In comparison, of 581 cases, 101 (17.4%) had used a bisphosphonate at any time. 22 women (10.7% of users) had only one bisphosphonate prescription. Low exposure to bisphosphonates was defined as ≤260 cumulative DDDs, moderate exposure as >260 to 1025 cumulative DDDs and high exposure as >1025 cumulative DDDs. Of bisphosphonate users, the majority of cases (60.4%) and controls (60.0%) used bisphosphonate for more than two years.

There was a non-significant association between high bisphosphonate exposure and breast cancer risk after adjusting for confounders when considering in situ and invasive breast cancers together (OR: 0.60; 95% CI: 0.33-1.06; p-trend = 0.06; Table 5.). The strongest effect was observed for high bisphosphonate exposure (OR: 0.60; 95% CI: 0.33-1.06). The non-significant protective effect remained when examining dose as a continuous variable (OR: 0.91; 95% CI: 0.81-1.02). The effect was attenuated when examining duration (OR: 0.97; 95 CI%: 0.89-1.04).

Table 4. Estimated ORs, 95% CI, p-values, and p-trend from logistic regression investigating the association between bisphosphonate exposure and breast cancer.

<table>
<thead>
<tr>
<th>Bisphosphonate exposure</th>
<th>Cases</th>
<th>Controls</th>
<th>OR*</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>480 (82.6)</td>
<td>438 (80.7)</td>
<td>Ref</td>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>Ever</td>
<td>101 (17.4)</td>
<td>105 (19.3)</td>
<td>0.75</td>
<td>0.54-1.06</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>480 (82.6)</td>
<td>438 (80.7)</td>
<td>Ref</td>
<td></td>
<td>0.06‡</td>
</tr>
<tr>
<td>Low</td>
<td>42 (7.2)</td>
<td>35 (6.4)</td>
<td>0.86</td>
<td>0.52-1.42</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>36 (6.2)</td>
<td>35 (6.4)</td>
<td>0.79</td>
<td>0.46-1.33</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>23 (4.0)</td>
<td>35 (6.4)</td>
<td>0.60</td>
<td>0.33-1.06</td>
<td></td>
</tr>
<tr>
<td>Numeric dose</td>
<td>581 (100.0)</td>
<td>543 (100.0)</td>
<td>0.91</td>
<td>0.81-1.02</td>
<td>0.10</td>
</tr>
<tr>
<td>(365 DDDs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>480 (82.6)</td>
<td>438 (80.7)</td>
<td>Ref</td>
<td></td>
<td>0.41‡</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>25 (4.3)</td>
<td>26 (4.8)</td>
<td>0.83</td>
<td>0.46-1.51</td>
<td></td>
</tr>
<tr>
<td>1-2 years</td>
<td>15 (2.6)</td>
<td>16 (2.9)</td>
<td>0.68</td>
<td>0.31-1.47</td>
<td></td>
</tr>
<tr>
<td>2+ years</td>
<td>61 (10.5)</td>
<td>63 (11.6)</td>
<td>0.74</td>
<td>0.49-1.12</td>
<td></td>
</tr>
<tr>
<td>Numeric duration</td>
<td>581 (100.0)</td>
<td>543 (100.0)</td>
<td>0.97</td>
<td>0.89-1.04</td>
<td>0.39</td>
</tr>
</tbody>
</table>
### Bisphosphonate exposure association with invasive breast cancer risk

When considering tumour invasiveness, 15.2% of women with an invasive tumour used bisphosphonate at any time, compared with 26.5% of women with an in situ tumour (Error! reference source not found.). Most women with in situ breast cancer had moderate bisphosphonate exposure, while women with invasive breast cancer had low bisphosphonate exposure.

There was evidence to suggest an association between bisphosphonate use at any time and decreased odds of invasive breast cancer (OR: 0.65; 95% CI: 0.45-0.94). A non-significant increase in risk of in situ breast cancer was observed with ‘ever’ bisphosphonate exposure (OR: 1.18; 95% CI: 0.70-1.98). Women with the highest exposure to bisphosphonates had the strongest protective effect for invasive breast cancer (OR: 0.52; 95% CI: 0.27-0.98; Table 5). There was also evidence of a linear dose-response (p-trend = 0.02). In contrast, moderate bisphosphonate exposure was associated with an increased risk of in situ breast cancer (OR: 1.77; 95% CI: 0.87-3.57), while low and high exposure were associated with a decreased risk (low exposure OR: 0.87; 95% CI: 0.37-2.08; high exposure OR: 0.89; 95% CI: 0.36-2.19).

Women with long-term exposure (2+ years category) experienced a significant protective effect for invasive breast cancer (OR: 0.61; 95% CI: 0.39-0.97). There was a non-significant dose-response with duration (p-trend = 0.27). A non-significant reduction in invasive breast cancer was observed when using continuous duration (OR: 0.92; 95% CI: 0.85-1.01). A non-significant increase in risk of in situ breast cancer was observed for all durations of bisphosphonate exposure.

<table>
<thead>
<tr>
<th>Bisphosphonate exposure (1 year)</th>
<th>Cases N (%)</th>
<th>Controls N (%)</th>
<th>OR*</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
</table>

*Adjusted for age, BMI two years prior to study entry, highest level of education, and ethnicity
‡ p-trend
Table 5. Estimated ORs, 95% CI, p-values, and p-trend from logistic regression investigating the association between bisphosphonate exposure and breast cancer, stratified by tumour invasiveness.

<table>
<thead>
<tr>
<th>Bisphosphonate exposure</th>
<th>Cases N (%)</th>
<th>OR* 95% CI</th>
<th>p-value</th>
<th>Cases N (%)</th>
<th>OR* 95% CI</th>
<th>p-value</th>
<th>p-heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>438 (80.7)</td>
<td>397 (84.8)</td>
<td>Ref</td>
<td>0.02</td>
<td>83 (73.5)</td>
<td>Ref</td>
<td>0.53 0.02</td>
</tr>
<tr>
<td>Ever</td>
<td>105 (19.3)</td>
<td>71 (15.2)</td>
<td>0.65 0.45-0.94</td>
<td>30 (26.5)</td>
<td>1.18 0.70-1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>438 (80.6)</td>
<td>397 (84.8)</td>
<td>Ref</td>
<td>0.02‡</td>
<td>83 (73.5)</td>
<td>Ref</td>
<td>0.79‡ 0.02</td>
</tr>
<tr>
<td>Low</td>
<td>35 (6.4)</td>
<td>34 (7.3)</td>
<td>0.83 0.49-1.42</td>
<td>8 (7.1)</td>
<td>0.87 0.37-2.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>35 (6.4)</td>
<td>21 (4.5)</td>
<td>0.58 0.32-1.06</td>
<td>15 (13.3)</td>
<td>1.77 0.87-3.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>35 (6.4)</td>
<td>16 (3.4)</td>
<td>0.52 0.27-0.98</td>
<td>7 (6.2)</td>
<td>0.89 0.36-2.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numeric dose</td>
<td>543 (100.0)</td>
<td>468 (100.0)</td>
<td>0.86 0.75-0.98</td>
<td>113 (100.0)</td>
<td>1.05 0.90-1.23</td>
<td>0.56 0.01</td>
<td></td>
</tr>
<tr>
<td>(365 DDDs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>438 (80.7)</td>
<td>397 (84.8)</td>
<td>Ref</td>
<td>0.27‡</td>
<td>83 (73.5)</td>
<td>Ref</td>
<td>0.73‡ 0.50</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>26 (4.8)</td>
<td>19 (4.1)</td>
<td>0.76 0.40-1.46</td>
<td>† 1.19</td>
<td>0.46-3.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 years</td>
<td>16 (2.9)</td>
<td>11 (2.4)</td>
<td>0.62 0.27-1.42</td>
<td>† 1.01</td>
<td>0.31-3.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2+ years</td>
<td>63 (11.6)</td>
<td>41 (8.8)</td>
<td>0.61 0.39-0.97</td>
<td>† 1.22</td>
<td>0.66-2.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numeric duration</td>
<td>543 (100.0)</td>
<td>468 (100.0)</td>
<td>0.92 0.85-1.01</td>
<td>113 (100.0)</td>
<td>1.08 0.97-1.21</td>
<td>0.18 0.006</td>
<td></td>
</tr>
<tr>
<td>(1 year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age, BMI two years prior to study entry, highest level of education, and ethnicity

‡ p-trend

†Suppressed due to small numbers.
3.2.3  Bisphosphonate exposure association with estrogen receptor status

Due to limited sample size, bisphosphonate exposure in the ER status analysis was limited to ever/never exposure and continuous dose and duration. The majority of cases were ER positive (Table 6). The proportion using bisphosphonates was similar between ER positive and ER negative breast cancer cases (15.3% and 13.5%, respectively).

In the investigation of ER-status, there was no evidence of an association between bisphosphonate exposure and ER positive subtype (OR: 0.92; 95% CI: 0.42-2.00; Table 6). The effect was similar when examining duration of use (OR: 0.89; 95% CI: 0.75-1.06) and dose (OR: 0.93; 95% CI: 0.71-1.23).
Table 6. Estimated ORs, 95% CI, and p-values from logistic regression investigating the association between bisphosphonate exposure and ER status.

<table>
<thead>
<tr>
<th>Bisphosphonate exposure</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>OR*</th>
<th>95% CI</th>
<th>p-value</th>
<th>ER negative Cases N (%)</th>
<th>OR*</th>
<th>95% CI</th>
<th>p-value</th>
<th>p-heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>438 (80.7)</td>
<td>316 (84.7)</td>
<td>Ref</td>
<td></td>
<td>0.03</td>
<td>64 (86.4)</td>
<td>Ref</td>
<td>0.30</td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td>Ever</td>
<td>105 (19.3)</td>
<td>57 (15.3)</td>
<td>0.64</td>
<td>0.44-0.96</td>
<td>0.02</td>
<td>10 (13.5)</td>
<td>0.67</td>
<td>0.32-1.42</td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Numeric dose</td>
<td>543 (100.0)</td>
<td>373 (100.0)</td>
<td>0.84</td>
<td>0.73-0.97</td>
<td>0.02</td>
<td>74 (100.0)</td>
<td>0.87</td>
<td>0.66-1.16</td>
<td>0.34</td>
<td>0.05</td>
</tr>
<tr>
<td>(365 DDDs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numeric duration</td>
<td>543 (100.0)</td>
<td>373 (100.0)</td>
<td>0.91</td>
<td>0.82-1.00</td>
<td>0.05</td>
<td>74 (100.0)</td>
<td>0.98</td>
<td>0.83-1.16</td>
<td>0.81</td>
<td>0.20</td>
</tr>
<tr>
<td>(1 year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, maximum BMI, highest level of education, cumulative months breast feeding, age at first pregnancy, and oral contraceptive use.
3.3 Omeprazole

3.3.1 Omeprazole exposure association with breast cancer risk

Of the 543 controls, 57 (10.5%) had used omeprazole at any time (Table 7). Of 581 cases, 74 (12.7%) had used omeprazole at any time. Low exposure to omeprazole was defined as ≤ 60 cumulative DDDs while high exposure was defined as > 60 cumulative DDDs. Omeprazole users were similarly split between duration categories, with 6.4% in both low and high exposure for cases and roughly 5.2% in both high and low for controls.

Considering breast cancer as a whole, there was no evidence of an association between omeprazole use and breast cancer risk (Table 7). Users of omeprazole had a very slight and non-significant increased risk of breast cancer (OR: 1.02; 95% CI: 0.68-1.62;). No dose response was observed (p-trend = 0.71). The highest risk was found for high omeprazole exposure, although it was not significant (OR: 1.10; 95% CI: 0.64-1.93). There was little difference between dose (OR: 1.01; 95% CI 0.88-1.15) and duration (OR: 0.99; 95% CI: 0.85-1.16), neither exposure measure suggested there was an association between omeprazole use and breast cancer.

Table 7. Estimated ORs, 95% CI, and p-values from logistic regression investigating the association between omeprazole exposure and breast cancer.

<table>
<thead>
<tr>
<th>Omeprazole exposure</th>
<th>Cases N (%)</th>
<th>Controls N (%)</th>
<th>OR*</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>507 (87.3)</td>
<td>486 (89.5)</td>
<td>Ref</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>Ever</td>
<td>74 (12.7)</td>
<td>57 (10.5)</td>
<td>1.02</td>
<td>0.68-1.52</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>507 (87.3)</td>
<td>486 (89.5)</td>
<td>Ref</td>
<td></td>
<td>0.71‡</td>
</tr>
<tr>
<td>Low</td>
<td>36 (6.2)</td>
<td>31 (5.7)</td>
<td>0.94</td>
<td>0.54-1.62</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>38 (6.5)</td>
<td>26 (4.8)</td>
<td>1.10</td>
<td>0.64-1.93</td>
<td></td>
</tr>
<tr>
<td>Numeric dose (365 DDDs)</td>
<td>581 (100.0)</td>
<td>543 (100.0)</td>
<td>1.01</td>
<td>0.88-1.15</td>
<td>0.92</td>
</tr>
<tr>
<td>None</td>
<td>507 (87.3)</td>
<td>486 (89.5)</td>
<td>Ref</td>
<td></td>
<td>0.85‡</td>
</tr>
<tr>
<td>&gt;0 - ≤ 2 months</td>
<td>37 (6.4)</td>
<td>28 (5.2)</td>
<td>1.10</td>
<td>0.63-1.93</td>
<td></td>
</tr>
<tr>
<td>&gt;2 months</td>
<td>37 (6.4)</td>
<td>29 (5.3)</td>
<td>0.95</td>
<td>0.55-1.63</td>
<td></td>
</tr>
<tr>
<td>Numeric duration (1 year)</td>
<td>581 (100.0)</td>
<td>543 (100.0)</td>
<td>0.99</td>
<td>0.85-1.16</td>
<td>0.94</td>
</tr>
</tbody>
</table>
*Adjusting for age, BMI two years prior to study entry, highest level of education, ethnicity, cumulative months breast feeding, age at menarche, age at first pregnancy, leisure physical activity, household physical activity, and family history of breast cancer.

‡ p-trend

### 3.3.2 Omeprazole exposure association with invasive breast cancer risk

Women with in situ breast cancer were more likely to have used omeprazole than women with invasive breast cancer (Table 8). 21.2% of women with in situ breast cancer used omeprazole at any time, with 9.7% using for more than 2 months.

Similar results to breast cancer as a whole were seen when considering only invasive breast cancer cases (Table 8). 365 DDDs of omeprazole exposure showed no significant association with invasive breast cancer (OR: 1.02; 95% CI: 0.90-1.17). Low omeprazole exposure had the largest effect size with a protective effect for invasive breast cancer, although it was not significant (OR: 0.66; 95% CI: 0.35-1.22).

Notably, the opposite association was observed between categorical and continuous omeprazole dose. There was a significantly increased risk of in situ breast cancer (OR: 1.81; 95% CI: 1.01-3.23), but this effect was only significant for short term users of omeprazole (OR: 2.36; 95% CI: 1.09-5.11). When considering continuous omeprazole dose, there was a non-significant protective association with in situ breast cancer (OR: 0.85; 95% CI: 0.60-1.20).
Table 8. Estimated ORs, 95% CI, and p-values from logistic regression investigating the association between omeprazole exposure and breast cancer, stratified by tumour invasiveness.

<table>
<thead>
<tr>
<th>Omeprazole exposure</th>
<th>Invasive</th>
<th></th>
<th></th>
<th></th>
<th>In situ</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>p-heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
<td>OR*</td>
<td>95% CI</td>
<td>p-value</td>
<td>Cases</td>
<td>OR*</td>
<td>95% CI</td>
<td>p-value</td>
<td>Cases</td>
<td>OR*</td>
</tr>
<tr>
<td>Never</td>
<td>438 (80.7)</td>
<td>418 (89.3)</td>
<td>Ref</td>
<td>0.25</td>
<td>89 (78.8)</td>
<td>Ref</td>
<td>0.05</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>105 (19.3)</td>
<td>50 (10.7)</td>
<td>0.77</td>
<td>0.49-1.20</td>
<td>24 (21.2)</td>
<td>1.81</td>
<td>1.01-3.23</td>
<td>0.11</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>438 (80.6)</td>
<td>418 (89.3)</td>
<td>Ref</td>
<td>0.75‡</td>
<td>89 (78.8)</td>
<td>Ref</td>
<td>0.29‡</td>
<td>0.40</td>
<td>0.08‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>35 (6.4)</td>
<td>23 (4.9)</td>
<td>0.66</td>
<td>0.35-1.22</td>
<td>13 (11.5)</td>
<td>2.03</td>
<td>0.95-4.34</td>
<td>0.34</td>
<td>0.11</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>High</td>
<td>35 (6.4)</td>
<td>27 (5.8)</td>
<td>0.91</td>
<td>0.50-1.65</td>
<td>11 (9.7)</td>
<td>1.59</td>
<td>0.71-3.57</td>
<td>0.43</td>
<td>0.34</td>
<td>0.08‡</td>
<td>0.08‡</td>
</tr>
<tr>
<td>Numeric dose</td>
<td>543 (100.0)</td>
<td>468 (100.0)</td>
<td>1.02</td>
<td>0.90-1.17</td>
<td>0.72</td>
<td>113 (100.0)</td>
<td>0.85</td>
<td>0.62-1.18</td>
<td>0.34</td>
<td>0.40</td>
<td>0.08‡</td>
</tr>
<tr>
<td>Numeric duration</td>
<td>543 (100.0)</td>
<td>468 (100.0)</td>
<td>1.01</td>
<td>0.87-1.18</td>
<td>0.88</td>
<td>113 (100.0)</td>
<td>0.85</td>
<td>0.60-1.20</td>
<td>0.34</td>
<td>0.43</td>
<td>0.08‡</td>
</tr>
</tbody>
</table>

*Adjusting for age, BMI two years prior to study entry, highest level of education, ethnicity, cumulative months breast feeding, age at menarche, age at first pregnancy, leisure physical activity, household physical activity, and family history of breast cancer.

‡ p-trend
3.3.3 Omeprazole exposure association with estrogen receptor status

There was no evidence of an association between omeprazole exposure and ER positive breast cancer (OR: 0.81; 95% CI: 0.51-1.29; Table 9). A similar null effect was seen when investigating dose (OR: 1.04; 95% CI: 0.90-1.20) and duration (OR: 1.02; 95% CI: 0.87-1.02). Similar non-significant associations were observed when investigating omeprazole exposure and ER negative breast cancer (OR: 0.90; 95% CI: 0.39-2.08). Estimates were similar for dose (OR: 1.13; 95% CI: 0.90-1.41) and duration (OR: 1.13; 95% CI: 0.88-1.44).
Table 9. Estimated ORs, 95% CI, and p-values from logistic regression investigating the association between omeprazole exposure and ER status

<table>
<thead>
<tr>
<th>Omeprazole exposure</th>
<th>Controls N (%)</th>
<th>Cases N (%)</th>
<th>ER positive</th>
<th></th>
<th></th>
<th>Cases N (%)</th>
<th>ER negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>OR* 95% CI</td>
<td>p-value</td>
<td>N (%)</td>
<td>OR* 95% CI</td>
<td>p-value</td>
<td>p-heterogeneity</td>
</tr>
<tr>
<td>Never</td>
<td>438 (80.7)</td>
<td>332 (89.0)</td>
<td>Ref</td>
<td>66 (86.5)</td>
<td>Ref</td>
<td>0.37</td>
<td>0.94</td>
</tr>
<tr>
<td>Ever</td>
<td>105 (19.3)</td>
<td>41 (11.0)</td>
<td>0.81 0.51-1.29</td>
<td>8 (13.5)</td>
<td>0.90 0.39-2.08</td>
<td>0.81</td>
<td>0.70</td>
</tr>
<tr>
<td>Numeric dose</td>
<td>543 (100.0)</td>
<td>373 (100.0)</td>
<td>1.04 0.90-1.20</td>
<td>74 (100.0)</td>
<td>1.13 0.90-1.41</td>
<td>0.31</td>
<td>0.64</td>
</tr>
<tr>
<td>(365 DDDs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numeric duration</td>
<td>543 (100.0)</td>
<td>373 (100.0)</td>
<td>1.02 0.87-1.20</td>
<td>74 (100.0)</td>
<td>1.13 0.88-1.44</td>
<td>0.35</td>
<td>0.64</td>
</tr>
<tr>
<td>(1 year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusting for age, max BMI, BMI two years prior to study entry, highest level of education, ethnicity, HRT use, smoking (pack years), age at menarche, cumulative months breast feeding, oral contraceptive use, family history of breast cancer, leisure physical activity, occupational physical activity, and alcohol consumption.
Chapter 4: Discussion

4.1 Bisphosphonates

4.1.1 Summary of main findings

This study provided evidence suggesting long-term bisphosphonate use is associated with a decreased risk of invasive breast cancer. There was not enough evidence to suggest there was an association between short-term bisphosphonate use and invasive breast cancer, but there was evidence of a significant linear dose-response trend. This study contributes to a growing body of literature investigating the association between bisphosphonate use and breast cancer which has had inconsistent results.

The results presented in this thesis should be interpreted cautiously due to the small sample size and the strong potential for confounding by indication. That is, the reason women take bisphosphonates – low bone density – is itself associated with a decreased risk in breast cancer. This reduced risk is likely because low bone density is often caused by low levels of estrogen, a risk factor for breast cancer. Confounding by indication is particularly likely given that women are recommended to take a ‘drug holiday’ from bisphosphonates unless they are at high risk for a fracture (Papaioannou et al., 2010), which could likely be due to low bone density. This means that the women the lowest bone density will naturally take the highest cumulative dose of bisphosphonates. This has been an acknowledged challenge of studies conducted previously (Dreyfuss, 2010). Low bone density should ideally be directly controlled for. When not available, bone density can be controlled for somewhat through strongly correlated proxies, as was done in this thesis with confounders such as BMI.

With respect to the potential for confounding by indication, it is encouraging there was not enough evidence to suggest there was an association between bisphosphonate use and ER-status in this analysis (OR: 0.71; 95% CI: 0.38 – 1.35). If the observed effect were due to low bone density and hence low estrogen exposure, one might expect the protective effect to be more evident in ER positive breast cancer. Further, bone density tends to be highly correlated with BMI which is controlled for in this study (Morin, Leslie, & Manitoba Bone Density Program, 2009). No study has reported a difference between ER positive and ER negative breast cancer,
although Monsees et al. restricted analyses to ER positive breast cancers and noted a protective effect (Monsees et al., 2011). Unfortunately, the investigation of the association between bisphosphonate exposure and ER-status was somewhat limited by sample size and this analysis was restricted to a binary exposure variable (ever/never). There was only evidence of an association between bisphosphonates and case status after long-term exposure so it is possible there could be a differential effect between ER positive and ER negative breast cancer that was masked by grouping all bisphosphonate exposure levels together. To date, there is no evidence to suggest bisphosphonates have a differential effect for ER positive and ER negative breast cancers.

The efficacy of bisphosphonates in preventing bone metastases in early breast cancer has been well documented, indicated by a recent Cochrane review (O’Carrigan et al., 2017). In this case, bisphosphonates work by inhibiting osteoclast-mediated bone resorption. However, the mechanism by which bisphosphonates could potentially prevent primary breast cancer is less clear. There is in vivo evidence suggesting use of zoledronate in monotherapy promotes tumour cell apoptosis (Foroni et al., 2014). This has been reinforced by in vitro studies suggesting zoledronate acts in a non-hormone dependent manner (Gschwantler-Kaulich et al., 2017), which is consistent with findings of this analysis. In vitro and in silico evidence suggests bisphosphonates may act on human epidermal growth factor (HER) (Yuen et al., 2014). To that end, future studies with larger sample sizes may seek to investigate bisphosphonates protective effect in HER2 positive breast cancer.

4.1.2 Comparison to other studies

Most observational studies found evidence to suggest a protective effect (Cardwell et al., 2012; Chlebowski et al., 2010; Monsees et al., 2011; Newcomb et al., 2010; Rennert et al., 2010; Vestergaard et al., 2011; Vinogradova et al., 2013), but an investigation of alendronate use in women with osteoporosis did not find evidence of an association (Chiang et al., 2012) and the most recent observational study did not find sufficient evidence of an association (Fournier et al., 2017). Of the above studies, the recent study by Fournier et al. best controlled for confounders. Only Chlebowski et al. has directly adjusted for bone mineral density measured by dual-energy

4 For a summary of key findings of studies, please refer to Table 1 on page 6.
x-ray absorptiometry (Chlebowski et al., 2010), arguably the most important biometric measure to control for, which was not possible in this study.

The protective effect of bisphosphonates on invasive breast cancer risk in this study was similar to many previously reported effect sizes, with an estimated OR of 0.52 (95% CI: 0.27 – 0.98) for high bisphosphonate exposure relative to none. Notably, this effect was not as strong for in situ breast cancer (OR: 0.89, 95% CI: 0.36-2.19). One proposed hypothesis for the effect of bisphosphonates is the slowed progression of in situ or preclinical disease to invasive cancer (Chlebowski et al., 2010), which the results from this study seem to support.

The observed effect sizes of this study, noted above, are comparable with other studies. When a protective effect was noted the effect size ranged from 0.4 to 0.92. This thesis utilized a quantifiable measure of drug dose (cumulative number of DDDs), while the majority of studies used only duration as the unit of measure (Chiang et al., 2012; Chlebowski et al., 2010; Monsees et al., 2011; Newcomb et al., 2010; Rennert et al., 2010; Vestergaard et al., 2011; Vinogradova et al., 2013). One would generally expect duration to tend towards the null when compared to cumulative DDD as it would fail to differentiate between differences in dose.

Among observational studies examining bisphosphonate dose (Cardwell et al., 2012; Fournier et al., 2017), Fournier et al. did not find evidence for a dose-response relationship, while Cardwell et al. did not perform any formal test for trend. Cardwell et al. only found evidence of an effect with short-term use – less than 730 DDDs or approximately two years supply – although notably the study had poor control for confounders and poor management of missing data, overlooking the potential utility of imputation to help deal with the issue as this thesis has done.

The study conducted by Fournier et al. had the most comprehensive control for covariates associated with bone mineral density, such as physical activity, Fracture Risk Assessment Tool (FRAX) score, history of fracture, and reimbursements for osteoporosis treatments, but also suffered from a lack of power (Fournier et al., 2017). Only Chlebowski et al. has directly addressed this issue by adjusting for bone mineral density, although exposure data was obtained
through interviews and is therefore less reliable than this thesis, which utilized administrative data (Chlebowski et al., 2010).

A minor point of consideration for the study conducted by Fournier et al. was the potential for misclassification in bisphosphonate exposure as the data is prescription based and therefore pertains to delivery rather than intake, although that weakness is shared with this analysis and is unlikely to have much effect outside of participants with only one prescription where stoppage from side effects seems most likely. In this study, only 10.7% of bisphosphonate users in this analysis had one prescription, suggesting that most women would have utilized their prescriptions fully as the most likely reason for early termination would be side effects.

The use of duration and dose in the Fournier et al. study was valuable, however, the categories for dose are irregular and there was no explanation as to how the categories were constructed in the methods. The authors state there was a null effect, although an evident protective effect with short term use was explained away as a healthy screener bias (HR: 0.56; 95% CI: 0.36-0.87). There was no evidence of a differential effect for invasive and in situ disease. However, the study looks underpowered as evidenced by the small number of cases in the highest dose category (n=22). Further, the study utilized more categories than this thesis and did not report an analysis of the continuous duration. In general, continuous predictors are preferred as categorization can lead to loss of information and bias. With that said, it often comes with the tradeoff of interpretability. In the study by Fournier et al. comparison to other studies is somewhat difficult given the irregular choice of categories.

The other observational study which did not find evidence to suggest an association between bisphosphonates and breast cancer, conducted by Chiang et al., was noted to be underpowered (Chiang et al., 2012). The primary strength of the study by Chiang et al. was the use of administrative data and analysis of dose, as in this thesis. However, there was little control for confounders. The study effectively adjusted only for age and comorbidities which women were already matched on. The study did not find any evidence of an effect with greater than 731 DDDs or two years of use (HR: 0.94; 95% CI: 0.80-1.09). The use of dose and duration is somewhat confused in this study, as there is typically a difference between the two in this thesis.
and other studies – namely duration is for length of use while cumulative DDDs refer to duration times dose received. 731 DDDs would only be considered moderate use in this thesis, which also did not find a significant association at this level. The different result may also be due to study population, as the study by Chiang et al. was conducted with a Taiwanese population which has different ethnic distributions and may vary in prescription practices.

In an effort to make this thesis more comparable to other studies, duration was categorized by years of use: no exposure, ≤ 1 year, ≤ 2 years, and >2 years of use. Consistent with the findings by dose, users with the longest duration had the strongest protective effect for invasive breast cancer (OR: 0.61; 95% CI: 0.39-0.97). However, the linear test for trend was not significant for duration (p = 0.27). These results were similar to the study by Chlebowski et al., which found evidence for a protective effect in invasive breast cancer (OR: 0.68; 95% CI: 0.52-0.89) (Chlebowski et al., 2010). However, bisphosphonate use was associated with an increased risk of in situ breast cancer (excluding LCIS) and the timing of the effect found by Chlebowski et al. differed from that found in this thesis. The study found evidence of a significant reduction of breast cancer risk with less than 2 years since the start of bisphosphonate use (HR: 0.50, 95% CI: 0.38-0.67), but a non-significant reduction thereafter (2 to 5 years HR: 0.86, 95% CI: 0.64-1.17; >5 years HR: 0.83; 95% CI: 0.53-1.27). Duration, as defined by Chlebowski et al., was different than used in this thesis, being defined as time since first use, and is not necessarily related to actual intake. The null effect may be related to sample size issues, but it may also suggest bisphosphonates slow progression of breast cancer rather than prevent it entirely. Any bisphosphonate exposure was associated with an increased risk of in situ breast cancer (HR: 1.59; 95% CI: 1.09-2.33; Chlebowski et al., 2010). This result was similar to this analysis, which found that ever bisphosphonate use was also associated with a non-significant increased risk of in situ breast cancer (OR: 1.18; 95% CI: 0.70-1.98). These results may also suggest bisphosphonates slow progression of in situ breast cancer to invasive breast cancer as noted above.

Chlebowski et al. had a large overall sample size of 154,768, but only 2,816 bisphosphonate users. The sample population was diverse and baseline differences between bisphosphonate users and non-users were evident, whereas the CBCS case-control study used in this thesis had a much
more homogenous population. Beyond the study design difference – Chlebowski et al. utilized a cohort design – the primary distinction between the studies was in the collection of exposure data. Chlebowski et al. collected bisphosphonate usage data via interview which is much more susceptible to misclassification bias than the administrative data used in this thesis. It is possible the difference in effect timing was due to the influence of recall bias. However, the study by Chlebowski et al. was strong in the sense that it was the sole study to possess information on bone mineral density and thus had direct control of one of the primary confounders in assessing the association bisphosphonate use and breast cancer. To that end, the consistency in the protective effect evident for invasive breast cancer between this study and the only conducted by Chlebowski et al. is encouraging as it suggests confounding by indication is being reasonably controlled.

The study by Chlebowski et al. noted bisphosphonate exposure was associated with an increased risk of in situ breast cancer (HR: 1.59; 95% CI: 1.09-2.33) (Chlebowski et al., 2010). This result was similar to this analysis, which found that ever bisphosphonate use was also associated with a non-significant increased risk of in situ breast cancer (OR: 1.18; 95% CI: 0.70-1.98). These results may suggest bisphosphonates slow progression of in situ breast cancer to invasive breast cancer as noted above.

Other studies have found evidence for a short-term effect similar to Chelebowski et al. (Newcomb et al., 2010; Vestergaard et al., 2011), however, the effect did not diminish with long-term use in the study conducted by Newcomb et al., indicating a similar long-term effect for invasive breast cancer, as in this thesis. Newcomb et al. also found significant evidence of a dose-response relationship between bisphosphonate use and invasive breast cancer risk, while Vestergaard et al. did not find evidence of a dose-response relationship. Unlike the study conducted by Chlebowski et al. and Newcomb et al., Vestergaard et al. and used administrative data and thus have more reliable exposure data, similar to this thesis.

Vestergaard et al. had a unique component to their study in that it examined the effect of individual bisphosphonates such as alendronate and etidronate. In instances where the sample size was reasonable, it was noted women taking specific bisphosphonates had an increased risk
of breast cancer prior to taking the drug, but a decreased risk after taking the drug. This seems to provide evidence against the confounding by indication hypothesis.

Notably, the study by Vestergaard et al. was designed as a cohort, which differs to the case-control design of this thesis. Controls in the cohort study (non-bisphosphonate exposed women) tended to be generally healthier than cases in terms of comorbidities and prescription patterns (Vestergaard et al., 2011). Control of confounders was also quite weak in this study, failing to control for important breast cancer risk factors such as BMI and age at menopause, which the CBCS data allowed this thesis to investigate as a potential confounder. Vestergaard et al. did not differentiate between invasive and in situ breast cancers, which may help to explain the small effect size for etidronate (HR: 0.80; 95% CI: 0.73-0.89) The primary strength of the study by Vestergaard et al. was in the large sample size for alendronate and etidronate users and the administrative nature of the data. The sample size was larger than the study presented in this thesis, but it was similar in that it also used administrative data. This is in contrast to the study by Newcomb et al., which derived exposure data from interviews and may suffer from recall and misclassification bias.

The study conducted by Newcomb et al. was the first to present evidence suggesting an association between bisphosphonate use and primary breast cancer prevention. However, it was arguably one of the weakest in terms of control for confounders and poor exposure data relative to other studies as well as generally lacking transparency for the methodology – owing somewhat to the nature of short communications in journals. The authors reported an interaction between BMI and bisphosphonate use, with a significant protective effect for invasive breast cancer with ever and current use of bisphosphonates limited to women who had a BMI between 25-29.9 kg·m\(^2\). There was a non-significant increase in risk among users with a BMI greater than 30 kg·m\(^2\), although examination of confidence intervals suggests the study may have been underpowered which would make detection of an interaction effect difficult.

The study by Monsees et al. came to similar conclusions as that of this analysis and the study by Newcomb et al., finding evidence of a dose-response relationship and the strongest protective effect with long-term bisphosphonate use (Monsees et al., 2011). Intriguingly, given a similar
sample size, the study by Monsees et al. found a protective effect with one (OR: 0.38; 95% CI: 0.19-0.75) and two years (OR: 0.36; 95% CI: 0.14-0.88) of bisphosphonate use for ER positive breast cancer (Monsees et al., 2011). Although a there was a strong association with two years or greater use and invasive breast cancer noted in this thesis (OR 0.52; 95% CI 0.27-0.98), there was not enough evidence to suggest there was an effect with one to two years of use (OR: 0.62; 95% CI: 0.27-1.42). The differences could perhaps be due to the focus on ER positive breast cancer, although this thesis did not note any significant difference in the association of bisphosphonates with ER status. The study had strong control of confounders, but notably didn’t differentiate between pre and post-menopausal women, which would likely dilute the observed effect. Breast cancer etiology is known to differ for ER-positive tumours depending on women’s menopausal status, making any lack of control for this important factor is concerning (Yamashita, 2015).

Vinogradova et al. also did not control for menopausal status and found a weak protective effect of bisphosphonates for women who were ever users (OR: 0.92; 95% CI: 0.87-0.98) (Vinogradova et al., 2013). The effect was non-duration dependent. A slightly stronger effect was observed when bisphosphonate use was considered as at least two prescriptions (OR: 0.90, 95% CI: 0.85-0.96), although the effect was only evident with short term use. The small effect sizes may be explained by the lack of differentiation between in situ and invasive breast cancer, as well as the absence of menopausal status. The study’s primary strength was in the use of administrative data to measure exposure, similar to this thesis, and the reasonable sample size. However, control of confounders was not ideal. Many confounders were not consistently recorded and there was a lack of data on physical activity, which is controlled for in this analysis.

Rennert et al. have also controlled for ‘sports activity’, and found a significant reduction in breast cancer with longer than one year of bisphosphonate use (OR: 0.72; 95% CI: 0.57-0.90) and any use (OR: 0.68; 95% CI: 0.56-0.82) (Rennert et al., 2010). The results were similar when the analysis was restricted to invasive breast cancer (>1 year use, OR: 0.70; 95% CI: 0.55-0.91). To that end, the effects are similar to this study, which saw an effect when considering any bisphosphonate use, but specifically with long-term use. The study by Rennert et al. had reasonable control of confounders, although it failed to control for menopausal status. The
analyses adjusted for fruit and vegetable intake, which was unique to this study. Intriguingly, high fruit and vegetable intake was associated with lower odds of bisphosphonate use.

The RCT which investigated breast cancer incidence with oral alendronate use did not find evidence of an effect (Hue et al., 2014). Participants were given 5 mg/day for the first two years and 10 mg/day after that. The mean follow up time was 3.8 year. Although suggestive of an overall dose-response effect given the significance of the trend for invasive breast cancer (p = 0.02), there was not enough evidence found in this thesis to say there was an association between moderate bisphosphonate use – the dose the average participant would have received in the RCT – and breast cancer. To that end, it’s plausible the results of the RCT and this thesis are not incongruent. Although typically the gold standard, the study was not designed to have sufficient power to investigate the relationship between bisphosphonates and breast cancer. The sample size and dose duration in the alendronate RCT presented by Hue et al. are somewhat unsatisfactory in light of the evidence presented in this thesis. The results of the RCT may also be consistent with the hypothesis that bisphosphonates merely slow the progression of in situ cancers to invasive.

4.2 Omeprazole
4.2.1 Summary of main findings
To the author’s knowledge, this is the first study to evaluate the association between omeprazole and breast cancer. In vitro evidence suggested omeprazole decreased breast cancer cell invasion and metastases in ER negative breast cancer (Jin et al., 2014), likely by binding to the AHR. The possibility of omeprazole being a chemopreventive agent in primary breast cancer was explored, but we did not observe an association between omeprazole and breast cancer. The use of proton pump inhibitors at half-dose as a maintenance treatment of gastroesophageal reflux disease was common and recommended in Canada during the study period (Armstrong et al., 2005), but it may be the case that these low maintenance doses are not high enough to have an effect on breast cancer.

The potential utility of an agent that could be effective in treating ER negative breast tumours is exciting, which were the implications of the aforementioned in vitro evidence, as this subtype is
more challenging to treat and with worse prognoses. This analysis found that the effect of omeprazole use was significantly different between in-situ and invasive cancer (p-heterogeneity = 0.003), and a non-significant protective effect with invasive breast cancer (OR: 0.77; 95% CI: 0.50-1.20). There was not enough evidence to suggest the effect of omeprazole differed by ER status in this study (p-heterogeneity = 0.94). Intriguingly, there was evidence of an increased risk of in situ breast cancer with omeprazole use (OR: 1.81; 95% CI: 1.01-3.23), although this effect was only statistically significant for women with less than two months of use (OR: 2.36; 95%: 1.09-5.11).

It may be that omeprazole is associated with an increased risk of in situ breast cancer, but the results are difficult to interpret in any meaningful way given the difference between continuous and categorical exposure variables. This may suggest a complicated relationship between omeprazole and in situ breast cancer, or, more likely, the results are due to random variation.

Biologically, it is plausible and it may be that omeprazole prevents cell invasion, thus stopping progression of in situ to invasive breast cancer (Jin et al., 2014). Recent in vitro evidence has indicated AHR could regulate estrogen synthesis and metabolism in bone tissues through cytokine/aromatase signalling (Miki et al., 2017). An RCT has demonstrated the aromatase inhibitor anastrazole was effective in primary prevention of postmenopausal breast cancer in high risk women (Cuzick et al., 2014). Future research may seek to evaluate the use of AHR inhibitors such as omeprazole in primary prevention of high risk postmenopausal breast cancer if used as an adjuvant agent with aromatase inhibitors. Alternatively, omeprazole may help prevent metastases (Hanieh, 2015; Jin et al., 2014), similar to bisphosphonates. Future epidemiological studies may seek to investigate the effect of omeprazole or other AHR inhibitors for their efficacy in prevention of metastases.

### 4.3 Strengths and limitations

One of the primary strengths of this research is the administrative nature of the data. Information on the prescribed drug type, strength, and indication for use is complete, detailed, and eliminates any chance of recall or non-response bias. This data allowed for a more accurate calculation of exposure. Naturally, it cannot be confirmed whether the drugs were taken, merely that they were
prescribed. The possibility of misclassification bias still exists as this study does not have any information on adherence, although it is significantly reduced. Misclassification is also possible as PharmaNet does have some exclusions such as inpatient care, but omeprazole and oral bisphosphonates are not likely to be delivered in that setting. A further strength of this study is the long observation period for the exposure.

Another strength is the ability to adjust for potential confounding factors. The CBCS study provided comprehensive data on a wide range of factors associated with breast cancer. In the case of the bisphosphonate analysis, information on factors associated with bone mineral density and osteoporosis, such as physical activity and alcohol consumption, was available and controlled for in the analysis. Many other studies have lacked comprehensive information on confounders, and more specifically, confounders related to bone mineral density. However, some confounders in this study were not ideal.

Physical, household, and occupational physical activity appear to be particularly susceptible to misclassification bias. No particular examples were given in the questionnaire to illustrate the appropriate level of intensity and, as a result, will vary according to the individual’s perception of the task. As the only guidance was in reference to sweating, heart rate, and effort, women with varying levels of fitness will experience the same tasks quite differently. This misclassification will apply to other covariates as well, although likely not to the same extent. With that said, the questionnaire used for physical activity was validated which should mitigate the risk of misclassification (Kobayashi et al., 2013). As with any retrospective study, recall error is also likely to contribute to misclassification. This is likely to bias results towards the null.

A major strength of this study is in the comprehensive data provided by participants in the questionnaire for the CBCS. This allowed for restriction to postmenopausal women, which few other studies have done, and control for number of pack years smoked, which no other study has done. Although direct information on bone density was not available for this study, the risk of confounding by indication has been mitigated somewhat by adjusting for potential factors associated with bone density such as BMI, smoking, and HRT use when constructing models to
investigate the association between bisphosphonate exposure and breast cancer. This study is unique in using both maximum BMI and recent BMI as a potential confounder.

A major limitation of this study was the potential for confounding by indication in the bisphosphonate analysis. That is, the reason women would take bisphosphonates – low bone density – may be associated with a decreased risk of breast cancer. This has been mitigated somewhat by adjusting for characteristics associated with bone density such as BMI, but the study lacked precise information on bone mineral density to best control for confounding by indication. Further, it may be the case that other factors that allow women to tolerate bisphosphonates could explain the association between their use and the protective effect in invasive breast cancers.

The sample size was also a limiting factor for parts of this study. The subtype analysis was limited in both the exposure and subtype categorization. Analyses of additional subtypes beyond ER status and tumour behavior (invasive vs in situ) should be done in future studies.

A unique problem in this thesis was the atypical nature of enrollment of the case-control study. With controls being consistently enrolled later than cases, special consideration was required in order to avoid a positive bias given the longer exposure period. This posed a substantial methodological challenge. In adjusting the exposure period to end year two years prior to study entry, there was no way to know if controls were post-menopausal at this time. As such, the possibility for misclassification of menopausal status exists.
Chapter 5: Conclusion

5.1 Bisphosphonates

This study contributes to a somewhat contradictory body of literature surrounding bisphosphonate use and the risk of breast cancer. It reinforces the association of long-term use with a decreased risk of breast cancer, although the results are to be interpreted with caution. Bisphosphonates are a relatively safe and well tolerated drug, so it may have use as a chemopreventive agent for primary prevention, in addition to its current use in preventing bone metastases. Further, bisphosphonates use as a potential primary prevention drug has the potential to be useful for researchers in the basic sciences who may seek to develop novel drugs for cancer prevention based on the structure of bisphosphonates. Future epidemiologic studies designed specifically for assessing the long-term efficacy of bisphosphonates in slowing the progression of in situ breast cancer to invasive breast cancer will be necessary to fully evaluate the potential benefit of this intervention. Studies with data on bone mineral density would also be extremely beneficial in clarifying the association between bisphosphonates and breast cancer.

5.2 Omeprazole

This study did not find evidence to suggest omeprazole was associated with breast cancer. Future work will require larger sample sizes and may seek to investigate an enantiomer of omeprazole, esomeprazole. A growing body of literature suggests esomeprazole may itself have anti-cancer properties or enhance the effect of other antitumour treatments (Goh, Sleptsova-Freidrich, & Petrovic, 2014; Wang et al., 2015).

As with many prescription medications, drug interactions are something of which to be cautious. Some evidence has emerged suggesting proton pump inhibitors – a class of drugs of which omeprazole is a part of – have an adverse association with patient outcomes when used in conjunction with other drugs (Shamliyan, Middleton, & Borst, 2017). More comprehensive studies with larger sample sizes investigating drug interactions may be required to fully appreciate the relationship between prescription medications such as omeprazole and breast cancer.
5.3 Summary
This study investigated the association between of omeprazole and bisphosphonates with breast cancer and separately for ER positive and ER negative breast cancer. The noted protective effect of long-term bisphosphonate use for breast cancer as a whole contributes to a growing body of literature investigating this drug’s relationship with breast cancer. To the author’s knowledge, this is the first population-level investigation of the association between omeprazole and breast cancer risk factors, and there was not enough evidence to suggest an association.

5.4 Implications and future work
Cancer is a disease with a long latency period. As such, it will often be difficult to detect the effect of potential chemopreventive drugs in randomized clinical trials. Population-based studies investigating the association of prescription drugs and breast cancer that collect important breast cancer risk factors are necessary to understand unknown adverse or advantageous effects. To that end, this study is a valuable contribution to a growing body of literature that will help guide decision making and suggest possible areas of research for the basic sciences to investigate preventative breast cancer interventions. Further epidemiologic studies with larger sample sizes should investigate drug interactions and their association with breast cancer or to investigate more specific and better defined breast cancer subtypes.


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doi:10.1093/jnci/djr399


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