Predictors of Mammographic Density among Women at a High Risk for Breast Cancer

by

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Abstract

Whether mammographic density, a strong predictor of breast cancer risk, is associated with breast cancer risk factors in women at an increased risk of familial breast cancer is unclear. Therefore, this study evaluated the associations between reproductive, hormonal, anthropometric, and lifestyle risk factors and measures of mammographic density (percent density, dense area, non-dense area) among high-risk, BRCA mutation-negative women. Overall, parity and body weight were inversely associated with percent density and positively associated with non-dense area. Increasing height was positively associated with percent density and inversely associated with non-dense area. A later age at smoking and alcohol initiation were positively associated with mammographic density. Among postmenopausal women only, past smoking was inversely associated with mammographic density. In premenopausal women only, an increasing duration of smoking was inversely associated with mammographic density. These findings suggest that mammographic density is associated with several modifiable breast cancer risk factors among this high-risk cohort.
Acknowledgments

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# Table of Contents

Acknowledgments........................................................................................................ iii

Table of Contents........................................................................................................... v

List of Tables ................................................................................................................ viii

List of Figures ................................................................................................................ ix

List of Appendices ......................................................................................................... x

Published Material Not Related to Thesis .................................................................... xi

List of Abbreviations ..................................................................................................... xii

Chapter 1 Introduction .................................................................................................. 1

Chapter 2 Literature Review ......................................................................................... 3

2.1 Breast Cancer ......................................................................................................... 3

2.1.1 Molecular Subtypes and Prognostic Characteristics of Breast Cancer ............ 3

2.1.2 Key Breast Cancer Risk Factors ....................................................................... 4

2.1.2.1 Genetic Susceptibility to Breast Cancer ....................................................... 5

2.1.2.2 The Role of Family History ....................................................................... 6

2.1.2.3 Mammographic Density ............................................................................ 7

2.1.2.4 Benign Breast Diseases ........................................................................... 10

2.1.2.5 Reproductive and Hormonal Factors ....................................................... 11

2.1.2.6 Anthropometric, Lifestyle, and Dietary Factors ..................................... 12

2.1.3 Breast Cancer Management among High-Risk Populations ............................ 13

2.1.3.1 Screening ................................................................................................ 14

2.1.3.2 Primary Prevention Options .................................................................... 15

2.2 Mammographic Density ......................................................................................... 15

2.2.1 Assessment of Mammographic Density .......................................................... 17

2.2.1.1 Area-Based Methods ............................................................................... 18
2.2.1.1 Visual Assessment ................................................................. 18
2.2.1.2 Semi-Automated Methods .................................................. 19
2.2.1.3 Fully Automated Methods .................................................. 20
2.2.1.2 Volumetric-Based Methods ............................................... 21
2.2.1.3 Performance of Mammographic Density Assessment Tools ....... 21

2.2.2 Mammographic Density and Risk of Breast Cancer .................... 23
2.2.2.1 Proposed Biological Mechanisms ........................................ 28
2.2.2.2 Utility of Mammographic Density in Breast Cancer Risk Prediction .... 31
2.2.2.3 Mammographic Density as a Biomarker of Breast Cancer Risk ....... 33
2.2.2.3.1 Genetic Risk Factors and Family History ....................... 35
2.2.2.3.2 Reproductive and Hormonal Risk Factors ...................... 36
2.2.2.3.3 Anthropometric, Dietary, and Lifestyle Risk Factors ........ 37

2.3 The ‘Negative Study’ Cohort ....................................................... 39

Chapter 3 Rationale, Objectives, and Hypotheses .................................. 41
3.1 Rationale ................................................................................. 41
3.2 Objectives .............................................................................. 42
3.3 Hypotheses ............................................................................. 43

Chapter 4 Methodology ..................................................................... 44
4.1 Study Population ....................................................................... 44
4.2 Data Collection .......................................................................... 46
4.3 Subjects Available for Analyses ................................................ 47
4.4 Mammographic Density Assessment ........................................... 51
4.5 Statistical Analyses ................................................................... 52

Chapter 5 Results ............................................................................ 57
5.1 Subject Characteristics ................................................................ 57
5.2 Mammographic Density Measures According to Reproductive and Hormonal Exposures ................................................................. 58
  5.2.1 Premenopausal Women .............................................................. 58
  5.2.2 Postmenopausal Women ............................................................ 59
5.3 Mammographic Density Measures According to Anthropometric and Lifestyle Exposures ........................................................... 59
  5.3.1 Premenopausal Women .............................................................. 59
  5.3.2 Postmenopausal Women ............................................................ 61
Chapter 6 Discussion ........................................................................ 75
  6.1 Discussion .................................................................................. 75
  6.2 Strengths and Limitations .............................................................. 80
  6.3 Implications and Future Directions ................................................ 85
  6.4 Summary of Thesis .................................................................... 88

References ........................................................................................ 90
Appendices ....................................................................................... 109
Copyright Acknowledgements .......................................................... 147
List of Tables

Table 2.1. A summary of mammographic density assessment methods ........................................... 23

Table 2.2. Prospective and nested case-control studies evaluating the association between quantitatively-assessed measures of mammographic density and breast cancer risk in the general population .......................................................................................................................... 26

Table 2.3. Characteristics of breast and ovarian cancer risk prediction assessment tools ............ 32

Table 2.4. Summary of modifiers of mammographic density and premenopausal and postmenopausal breast cancer risk in the general population .......................................................................................................................... 34

Table 5.1. Characteristics at baseline of study population stratified by menopausal status ........ 63

Table 5.2A. Difference in mammographic density measures according to reproductive and hormonal exposures in premenopausal women. .......................................................................................................................... 65

Table 5.2B. Adjusted mean mammographic density measures according to reproductive and hormonal exposures in premenopausal women. .......................................................................................................................... 66

Table 5.2C. Difference in mammographic density measures according to reproductive and hormonal exposures in postmenopausal women. .......................................................................................................................... 67

Table 5.2D. Adjusted mean mammographic density measures according to reproductive and hormonal exposures in postmenopausal women. .......................................................................................................................... 68

Table 5.3A. Difference in mammographic density measures according to anthropometric and lifestyle factors in premenopausal women. .......................................................................................................................... 69

Table 5.3B. Adjusted mean mammographic density measures according to anthropometric and lifestyle factors in premenopausal women. .......................................................................................................................... 70

Table 5.3C. Difference in mammographic density measures according to anthropometric and lifestyle factors in postmenopausal women. .......................................................................................................................... 72

Table 5.3D. Adjusted mean mammographic density measures according to anthropometric and lifestyle factors in postmenopausal women. .......................................................................................................................... 73
List of Figures

Figure 2.1. Forest plot of incidence studies investigating percent mammographic density and breast cancer risk............................................................... 9

Figure 2.2. Forest plot of prevalence studies investigating percent mammographic density and breast cancer risk............................................................... 10

Figure 2.3. Increasing mammographic density as shown on a mammogram......................... 17

Figure 2.4. The measurement of mammographic density using the Cumulus software........... 20

Figure 2.5. A biological hypothesis linking mammographic density and breast cancer risk ...... 29

Figure 2.6. Incidence of breast cancer and breast tissue ageing according to the Pike Model.... 30

Figure 4.1. Subject recruitment of the ‘Negative Study’ among participating genetics clinics across Ontario ............................................................................................................................. 45

Figure 4.2. Flow chart of subjects available for analyses..................................................... 50

Figure 4.3. Distribution of mammographic density measures in premenopausal women......... 55

Figure 4.4. Distribution of mammographic density measures in postmenopausal women ....... 56
List of Appendices

Appendix 1: Invitation Letter to the Proband ................................................................. 110
Appendix 2: Invitation Letter to the Relative ................................................................. 111
Appendix 3: Study Package Letter ................................................................................. 112
Appendix 4: Consent Form ............................................................................................... 113
Appendix 5: Authorization of Medical Release Form ....................................................... 117
Appendix 6: Diet History Questionnaire ............................................................................ 118
Appendix 7: Research Questionnaire for the Risk Factor Analysis for Familial Breast Cancer Study ........................................................................................................ 119
Appendix 8: Follow-Up Questionnaire for the Risk Factor Analysis for Familial Breast Cancer Study ........................................................................................................ 134
Published Material Not Related to Thesis


*Indicates equal authorship
List of Abbreviations

AICR  American Institute for Cancer Research
AJ    Ashkenazi Jewish
AUC   Area under the receiver operating characteristic curve
BBD   Benign breast disease
BC    Breast cancer
BI-RADS Breast Imaging-Reporting and Data System
BMI   Body mass index
BOADICEA Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation
BRCA1 Breast Cancer Susceptibility Gene-1
BRCA2 Breast Cancer Susceptibility Gene-2
BCRAT Breast Cancer Risk Assessment Tool
BV    Breast volume
CAM   Computer-assisted method
CI    Confidence interval
DA    Dense area
DHQ   Diet History Questionnaire
EPIC  European Prospective Investigation into Cancer and Nutrition
ER    Estrogen receptor
FAM   Fully automated method
FDR   First-degree relative
FFDM  Full-field digital mammography
FFTP  First full-term pregnancy
GLM   Generalized linear models
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>HER2</td>
<td>Human epidermal growth factor receptor 2</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>IBIS</td>
<td>International Breast Cancer Intervention Study</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>MD</td>
<td>Mammographic density</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic Equivalent of Task</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MVPA</td>
<td>Moderate-to-vigorous physical activity</td>
</tr>
<tr>
<td>NCC</td>
<td>Nested case-control study</td>
</tr>
<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
</tr>
<tr>
<td>NDA</td>
<td>Non-dense area</td>
</tr>
<tr>
<td>OBSP</td>
<td>Ontario Breast Screening Program</td>
</tr>
<tr>
<td>OC</td>
<td>Oral contraceptive</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly ADP Ribose Polymerase</td>
</tr>
<tr>
<td>PD</td>
<td>Percent density</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor κB</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear factor κB ligand</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SDR</td>
<td>Second-degree relative</td>
</tr>
<tr>
<td>SERM</td>
<td>Selective estrogen-receptor response modulators</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
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<tr>
<td>SFM</td>
<td>Screen-film mammography</td>
</tr>
<tr>
<td>SHBG</td>
<td>Sex hormone binding globulin</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SXA</td>
<td>Single X-Ray Absorptiometry</td>
</tr>
<tr>
<td>TBA</td>
<td>Total breast area</td>
</tr>
<tr>
<td>TNBC</td>
<td>Triple-negative breast cancer</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual assessment scale</td>
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Chapter 1
Introduction

1

Mammographic density is a reflection of the composition of dense and non-dense tissue in the breast and is one of the strongest risk factors for breast cancer in the general population [1]. Extensive mammographic density is associated with greater breast cancer risk [2], and it is also associated with several important reproductive, hormonal, anthropometric, and lifestyle breast cancer risk factors [3]. Given that mammographic density can be modified by these risk factors, it is plausible that these risk factors exert their influence on breast cancer risk through mammographic density [3]. This suggests that mammographic density may act as an intermediate marker of breast cancer risk [3].

Mammographic density is a highly heritable trait between first-degree relatives, and women with one or more first-degree relatives affected with breast cancer have significantly higher mammographic density compared to women in the general population, suggesting that mammographic density is an important risk factor for familial breast cancers [4, 5].

Approximately 25% of all breast cancers have a family history component [6]. Women with a strong family history of breast cancer may qualify for genetic testing for mutations in the \textit{BRCA1} and \textit{BRCA2} genes, however, mutations in these genes only account for 17% to 20% of the familial risk of breast cancer [7]. Women who have a strong family history of breast cancer but belong to \textit{BRCA} mutation-negative families still have a high lifetime risk of breast cancer of approximately 40% by age 70 [8]. These women represent a challenge in the clinic because the etiology behind their breast cancer risk remains unclear, and it is not known if their risk is elevated enough to warrant the surgical and chemopreventive options reserved for \textit{BRCA} mutation carriers [9].

Therefore, there is a need to identify non-surgical, modifiable risk factors that can help reduce the incidence of breast cancer among these high-risk, \textit{BRCA} mutation-negative women. Given the strong risk of breast cancer associated with extensive mammographic density, it is important to determine if the breast cancer risk factors that modify mammographic density in the general population behave similarly among women with a family history of breast cancer.
Utilizing mammographic density as an intermediate marker of breast cancer risk, the overall goal of this thesis was to evaluate the relationships between mammographic density and various reproductive, hormonal, anthropometric, and lifestyle risk factors among a unique cohort of cancer-free, high-risk, BRCA mutation-negative women. The findings from this work will help identify risk factors that modify an important biomarker of breast cancer risk and may help the development of evidence-based prevention strategies that are specifically tailored to this population at an increased risk of disease.
2

2.1 Breast Cancer

Breast cancer remains the most common cancer among women worldwide [10]. In the Canadian population, the lifetime risk of breast cancer is estimated at 12% by the age of 70 [11]. According to the Canadian Cancer Society, breast cancer will account for 26% of all female cancers in 2017, resulting in a projected 25,800 new cancer cases [11]. While the incidence of breast cancer among Canadian women remains high, mortality rates attributable to the disease have declined since the mid-1990’s [11]. This decline is most likely a consequence of the development of mammography-based screening programs, earlier detection of cancers, and improvement in treatment therapies [10, 11]. Despite declines in mortality rates, breast cancer is currently the second most common cause of cancer death in Canadian females, accounting for 13% of all cancer deaths [11]. The estimated lifetime risk of dying of breast cancer among women is 3% [11].

A family history of breast cancer is an important risk factor for approximately 25% of the population, which highlights the role of underlying genetic factors in breast cancer etiology [6, 12]. Approximately 5% to 10% of all breast cancers are due to an inherited genetic predisposition for the disease and are therefore called hereditary breast cancers [6, 7]. The most widely studied and significant contributors to hereditary breast cancers are mutations in the BRCA1 and BRCA2 genes, which each confer very high lifetime risks of breast cancer [12]. In addition, 15% to 20% of all breast cancers involve a family history component, in which a first-, second-, or third-degree relative has been diagnosed with cancer [6]. The exact genetic basis attributable to familial breast cancers is still being investigated. The remaining 70% to 80% of all breast cancers occur sporadically in the general population, that is, among women who do not have a family history of the disease.

2.1.1 Molecular Subtypes and Prognostic Characteristics of Breast Cancer

The classification of molecular subtypes of invasive breast cancer depends on the expression of three receptors in the tumour: 1) the estrogen receptor (ER); 2) the progesterone receptor (PR);
and 3) the HER2 receptor. An estimated 60% of breast cancer cases are of the hormone receptor-positive subtype, in which case either the ER and/or the PR receptor(s) are overexpressed [13]. An estimated 20% of cases are attributed to the overexpression of the human epidermal growth factor receptor 2 (HER2) receptor, identified as the HER2-positive subtype [13]. The third subtype is referred to as basal-like and contributes to the remaining 20% of breast cancer cases [14]. Approximately 85% of all basal-like cases are called triple-negative breast cancer (TNBC) because they are ER-negative, PR-negative, and HER2-negative [14]. Recent studies involving gene expression profiling have further divided the hormone receptor-positive subtypes into two distinct categories, such as luminal A (ER-positive, PR-positive, HER2-negative) and luminal B (ER-positive, PR-positive, HER2-positive) [13].

It is important to classify breast cancers by their molecular subtypes because there seems to be variation in treatment response and prognosis among the subtypes [15]. Generally, luminal A breast cancers are associated with the best prognosis and the lowest grade of cellular differentiation [16]. Both the luminal A and luminal B subtypes respond well to endocrine therapy, such as selective estrogen-receptor response modulators (SERM) and aromatase inhibitors [16]. HER2-positive breast cancers are treated using both cytotoxic chemotherapeutic agents and an anti-HER2 targeted therapy [16]. TNBCs are thought to be the most aggressive form of breast cancer and consequently, are associated with a poorer prognosis and the highest grade of cellular differentiation [14]. The only treatment options available for TNBCs involves cytotoxic chemotherapy and/or Poly ADP Ribose Polymerase (PARP) inhibitors [16].

2.1.2 Key Breast Cancer Risk Factors

Genetic factors and family history are important determinants of risk for a significant proportion of the population [12]. In addition, various modifiable risk factors have been identified and may also influence breast cancer risk, including mammographic density and reproductive, hormonal, dietary, and lifestyle factors [17-19]. Therefore, the variations in breast cancer risk among women may result from complex interactions between age, lifestyle, environmental exposures, genetic factors, and other risk factors. An overview of breast cancer risk factors are discussed in more detail in the following sections.
2.1.2.1 Genetic Susceptibility to Breast Cancer

Deleterious mutations in the *BRCA1* or *BRCA2* genes correspond to very high lifetime risks of breast cancer. The average estimated lifetime risk of breast cancer by age 80 is 72% (range: 65% to 79%) for *BRCA1* mutation carriers, and 69% (range: 61% to 77%) for *BRCA2* mutation carriers [20, 21]. Mutations in these high-penetrance genes are also associated with elevated risks of other cancers, including ovarian, prostate, pancreatic, and melanoma skin cancers [22].

Mutations in the *BRCA* genes account for only 17% to 20% of the familial risk of breast cancer [7]. Mutations in other predisposing genes of high penetrance such as *TP53*, which is responsible for other cancer syndromes involving breast cancer, is thought to contribute to another 5% to 10% of hereditary breast cancers [23]. With the dramatic fall in the cost of genetic testing and the rapid improvement of genetic testing methodologies, at least ten other breast cancer susceptibility genes ranging from low to moderate penetrance have been identified [24]. Collectively, these susceptibility genes may be responsible for 30% of hereditary breast cancer cases [23]. Briefly, the evidence supporting a role of these moderate penetrance genes in breast cancer susceptibility is substantial for the *PALB2, CHEK2*, and *ATM* genes in particular. The risks of breast cancer associated with mutations in these genes range from two- to four-fold [23, 24]. However, the risk of breast cancer associated with *PALB2* mutations has been reported to be as high as five-fold, suggesting that it may be a high penetrance gene [24].

While these other susceptibility genes are associated with lower risks of breast cancer compared to the very high risks associated with mutations in *BRCA1* and *BRCA2*, mutations in these other genes are thought to be modestly prevalent among families with a history of disease. For example, mutations in *CHEK2* and *ATM* are thought to occur in 5% of *BRCA* mutation-negative families [23, 25]. Similarly, previous work from our group evaluated the prevalence of ten breast cancer susceptibility genes among 190 *BRCA* mutation-negative breast cancer patients with a strong family history of the disease [26]. Out of the 17 women that were found to carry deleterious mutations, five (35%) were *CHEK2* mutation carriers, two (12%) were *PALB2* mutation carriers, and one woman (6%) carried an *ATM* mutation [26].

Overall, mutations in the *TP53, PALB2, CHEK2*, and *ATM* genes are probable risk factors for hereditary breast cancer and are likely to be clinically actionable. Carrying a mutation in any of
these genes may warrant the implementation of risk-reducing prevention strategies. The development of multi-gene panel testing has allowed for the simultaneous detection of mutations in these genes for women who have a strong history of breast cancer.

2.1.2.2 The Role of Family History

A family history of breast cancer is a consistent and strong risk factor for the disease. Early studies have shown that women with one affected first-degree relative (e.g., a mother, a sister, or a daughter) have a two-fold increased risk of breast cancer compared to women without a family history, and this risk increases as the number of affected relatives increases [27]. A more recent analysis of three Canadian nested case-control studies similarly showed that having one affected first-degree relative was associated with an odds of developing breast cancer of 1.37 (95% CI 1.10, 1.72) and 2.45 (95% CI 1.30-4.62) for having two or more affected first-degree relatives [5]. In addition to the number of affected relatives, the ages at which relatives are diagnosed is also important for assessing a woman’s risk of disease. A large pooled analysis of 52 epidemiological studies reported that among women at a given age, their breast cancer risk estimates were greater if their affected relatives were younger at their diagnoses, especially if before age 50 [28]. For example, among women who were less than 40 years old, those who had a relative diagnosed before age 40 had a relative risk of breast cancer of 5.7 (95% CI 2.7, 11.8), and those who had a relative diagnosed between age 40 to 49 had a relative risk of 2.9 (95% CI 1.9, 4.4), compared to women with no family history [28].

Metcalfe et al. prospectively evaluated the risk of breast and ovarian cancer among 1,492 unaffected Canadian women who were first-degree relatives of *BRCA* mutation-negative women with breast cancer [8]. These women came from families that had a strong history of breast cancer, defined as two cases between first-degree relatives under the age of 50, or three breast cancer cases at any age but on one side of the family (e.g., paternal or maternal side), and consequently qualified for *BRCA* mutation testing [8]. The study population was found to have a four-fold increased risk of breast cancer, which is equivalent to a lifetime risk of 40% by age 70, in comparison to the general population risk of 12%, but no increased risk for ovarian cancer [8].

As reported previously in Chapter 2, Section 2.1.1.1, mutations in the *BRCA* genes only account for 17% to 20% of the familial risk of breast cancer [7], and another ten breast cancer susceptibility genes may account for another 30% of familial cases [23]. Therefore, a significant
proportion of familial breast cancers remain unexplained. Collectively, these findings not only support a role of unidentified genetic factors in familial breast cancer but also highlight the potential for a role of shared environmental exposures and lifestyle among family members [5].

2.1.2.3 Mammographic Density

Mammographic density is one of the strongest risk factors for breast cancer identified, second to age and carrying a deleterious BRCA mutation, among all women [2, 29]. A comprehensive meta-analysis of epidemiological studies in the general population found that compared to women with little mammographic density (<5% density), women in the highest mammographic density category (≥75% density) had a 4.6-fold increased risk of breast cancer among incidence studies and a 3.67-fold increased risk among prevalence studies [2]. Forest plots summarizing these findings are shown in Figure 2.1 (incidence studies) and Figure 2.2 (prevalence studies).

The population attributable risk of breast cancer due to high mammographic density has been estimated previously [30, 31]. A Canadian study of 1,114 matched case-control pairs from three breast screening programs found that high percent mammographic density (≥50%) accounted for 16% of all breast cancers and 12% of breast cancers detected by screening [30]. Moreover, among women that were younger than the median age of 56 years, percent mammographic density of 50% or more accounted for 26% of all breast cancers and 21% of breast cancers detected by screening [30]. Recently, a larger study of 18,437 cases and 184,309 controls estimated the population attributable risk proportion using mammograms that were visually assessed according to the Breast Imaging Reporting and Data System (BI-RADS) [31]. Briefly, the BI-RADS classification system (described in more detail in Chapter 2, Section 2.2.1.1.1) comprises of four risk categories, where the fourth category represents the highest risk and the first category represents the lowest risk [32]. The authors estimate that 39% of all premenopausal breast cancers could have potentially been prevented if all mammographic density classified as the third and fourth BI-RADS categories were reduced to the second BI-RADS category [31]. In addition, approximately 26% of all postmenopausal breast cancers could have potentially been prevented if all mammographic density classified as the third and fourth BI-RADS categories were reduced to the second BI-RADS category [31]. Based on these findings, a substantial proportion of both premenopausal and postmenopausal breast cancers are attributed to high percent mammographic density.
Dense area (cm$^2$) and non-dense area (cm$^2$), are two other measures of mammographic density and are also breast cancer risk factors. Dense area is positively associated with breast cancer risk and non-dense area is a weak protective factor for breast cancer [33]. The biological basis of the association between mammographic density and breast cancer risk is poorly understood, but it is thought that extensive mammographic density reflects the cumulative exposure of the breast stroma and epithelium to hormonal and growth factors, which stimulate proliferation [3]. More detail on the biological mechanisms linking mammographic density and breast cancer risk is discussed in Chapter 2, Section 2.2.2.1.
Figure 2.1. Forest plot of incidence studies investigating percent mammographic density and breast cancer risk

Footnote to Figure 2.1: Forest plot summary of the relative risk (RR) for increasing categories of percent mammographic density and breast cancer risk from incidence studies conducted in the general population. The squares represent individual effect estimates and 95% CI are represented using lines. The pooled RR is represented with a diamond.

Footnote to Figure 2.2: Forest plot summary of the RR for increasing categories of percent mammographic density and breast cancer risk from prevalence studies conducted in the general population. The squares represent individual effect estimates and 95% CI are represented using lines. The pooled RR is represented with a diamond.


### 2.1.2.4 Benign Breast Diseases

Benign breast disease (BBD) is a collective term for lesions in the breast that appear abnormal or suspicious on a mammogram and are determined to be benign after a biopsy. Benign breast diseases are common, with approximately 80% of the breast biopsies performed each year in the United States being classified as benign [34]. The different types of benign breast diseases vary in their characteristics and some confer greater risks of breast cancers than others. For example,
a recent meta-analysis found that atypical hyperplasia is associated with a four-fold increased risk of breast cancer, which was the highest risk estimate found among the different benign breast diseases [35]. Interestingly, extensive mammographic density is strongly associated with the risk of benign breast disease [29]. For example, a previous study found that women with high mammographic density (≥75% density) had a 12.2-fold increased risk of hyperplasia without atypia and a 9.7-fold increased risk of atypical hyperplasia compared to women with fatty breasts [36]. This suggests a joint effect of these two risk factors on breast cancer risk, but so far there is conflicting evidence to suggest this is the case for mammographic density and atypical hyperplasia: two studies have reported slightly positive findings [37, 38], while two others report negative results [34, 39].

2.1.2.5 Reproductive and Hormonal Factors

In the general population, various menstrual, reproductive, and hormonal factors have been shown to be associated with breast cancer risk, with usually stronger associations among the hormone receptor-positive breast cancer subtypes (i.e., estrogen receptor [ER]-positive and or progesterone receptor [PR]-positive) [19]. There is now substantial evidence that an early age at menarche and a late age at menopause are significantly associated with an increased risk of breast cancer [19]. Parity and a younger age at first live birth exert protective effects against ER-positive and PR-positive breast cancers [40-42]. Similarly, a shorter period of time between menarche and first full-term pregnancy is consistently shown to be inversely associated with breast cancer risk [19, 42]. A longer duration of breastfeeding has been reported to be inversely associated with the risk of all breast cancer subtypes [19, 42], but seems to confer the strongest protection against triple-negative breast cancers (TNBC) [15]. Given that breastfeeding exerts protective effects against both hormone-receptor positive and hormone receptor-negative breast cancers, this suggests that it utilizes a pathway that is independent of parity [42].

Among exogenous hormone usage, current use of estrogen plus progesterone hormone replacement therapy (HRT), but not estrogen alone, in postmenopausal women increases breast cancer risk across all subtypes [15, 19]. In addition, an increased risk of breast cancer associated with oral contraceptive (OC) use has been reported [43, 44], and it has been suggested that this increase in risk may be limited to the triple-negative subtype [44-47]. Two large studies recently confirmed that endogenous hormonal risk factors, such as circulating levels of sex steroid
hormones, are positively associated with increased risks of breast cancer among both premenopausal [48] and postmenopausal women [49]. There is also other evidence suggesting that these increased risks may be stronger for ER-positive and PR-positive breast cancers [50]. Increased levels of prolactin, a hormone necessary for the differentiation of the mammary epithelium, are associated with increased breast cancer risk primarily in postmenopausal women [51, 52] but not in premenopausal women [53, 54]. Similarly, a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort found that increased levels of insulin-like growth factor 1 (IGF-1) were associated with increased risk of hormone receptor-positive breast cancers in both premenopausal and postmenopausal women [55]. Despite these findings, it is important to note that the relationships between these endogenous hormones and breast cancer risk in premenopausal women are not as clear as what has been found among postmenopausal women, due to the difficulty in assessing hormone concentrations throughout the menstrual cycle [48].

2.1.2.6 Anthropometric, Lifestyle, and Dietary Factors

There are several anthropometric, lifestyle, and dietary factors as outlined by a recent report by the American Institute for Cancer Research (AICR) that may also modify breast cancer, although their role in breast cancer etiology is not well-understood [17, 18].

An increasing adult attained height is convincingly associated with breast cancer risk, and likely reflects the factors that affect growth rather than modify breast cancer risk [17, 56]. Epidemiologic data show that a greater body size (e.g., body mass index [BMI] or body weight) confers a protective effect for breast cancer in premenopausal women, but an increased risk of breast cancer in postmenopausal women [57-59]. The mechanisms responsible for the inverse relationship between body size and premenopausal breast cancer risk are unclear. Among postmenopausal women, adipose tissue becomes an important source of endogenous estrogen after menopause, which is known to promote epithelial cell proliferation in the breast, thus possibly contributing to breast carcinogenesis [60, 61]. Increased levels of insulin, IGF-1, and inflammatory cytokines are all associated with adiposity and likely contribute to this relationship as well [62].

Physical activity, particularly moderate-to-vigorous physical activity (MVPA), is likely protective against breast cancer and especially in postmenopausal women [18, 63, 64]. MVPA,
defined as any exercise that increases the heart rate and causes perspiration [65], has been shown to reduce overall body fat and lower breast cancer risk factors, such as sex hormone levels, inflammatory markers, and insulin resistance [66]. MVPA is also thought to lower free sex hormone concentrations by increasing sex hormone binding globulin (SHBG), thereby inhibiting the mitogenic effects of estrogen in the breast [66]. Currently, there is limited evidence of an association between sedentary behaviour and breast cancer risk in both premenopausal and postmenopausal women [18].

The AICR considers both smoking and alcohol as harmful risk factors for breast cancer [17, 18]. Supporting this, studies within large cohorts such as the Nurses’ Health Study have reported modest, positive associations between active smoking and breast cancer risk [67]. Other evidence suggests that this increased risk is greater among women who smoke for a long duration prior to their first live birth [67]. Alcohol may also exert carcinogenic effects on the breast, but the evidence overall is not clear. Like smoking, alcohol may confer a greater risk during the time between menarche and first full-term pregnancy [68], but other studies have suggested that the cumulative exposure to alcohol over the lifetime may be more important for breast cancer risk [69]. While the biological mechanisms remain unclear, alcohol may increase circulating estrogen levels and estrogen receptors in epithelial cells in the breast, consequently increasing their proliferative state [68].

Apart from alcohol, the evidence supporting a role of other dietary factors in breast cancer development is limited [18]. Currently, there is limited evidence suggesting that diets high in non-starchy vegetables and calcium, as well as foods containing carotenoids, may be associated with a decrease in breast cancer risk for both premenopausal and postmenopausal women [18]. In particular, non-starchy vegetables may lower the risk of ER-negative breast cancers [18].

2.1.3 Breast Cancer Management among High-Risk Populations

The identification of women at a high risk for breast cancer, either due to a strong family history of the disease or a deleterious mutation, is crucial in order for early intervention. Currently, there are several prevention strategies made available to these high-risk women, such as intensive screening and primary prevention, to help manage their elevated risk of disease.
2.1.3.1 Screening

Women who are high-risk due to a genetic predisposition (e.g., a BRCA mutation) or a significant family history are eligible for intensive screening programs. In the province of Ontario, the Ontario Breast Screening Program (OBSP) offers biennial mammography to women at average risk, and annual mammography and magnetic resonance imaging (MRI) to women at high risk [70]. Eligible high-risk women are aged 30 to 69 years and are defined as 1) carriers of a predisposing gene mutation (e.g., BRCA1 or BRCA2); 2) are the first-degree relatives of a mutation carrier (e.g., BRCA1, BRCA2) and have declined to be tested themselves; 3) are determined to have a ≥25% lifetime risk of breast cancer, as predicted by computational risk assessment models (e.g., the Tyrer-Cuzick model [71, 72] or the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation [BOADICEA] model [73, 74]) by a genetic counsellor; and 4) have received chest radiation treatment prior to age 30 and for at least eight years [70]. The risk assessment models mentioned here, along with others, will be discussed in more detail in Chapter 2, Section 2.2.2.2.

Briefly, the two main methods of mammography include screen-film mammography (SFM) and full-field digital mammography (FFDM). While both types utilize the same mammography machine, the primary difference is that the images taken using SFM are superimposed onto sheets of x-ray films, while images taken using FFDM are transferred directly onto a computer [75]. Studies have shown that both techniques perform well for the detection of breast cancer, however, FFDM seems to be more accurate among premenopausal women under the age of 50 [76, 77] and for women with high mammographic density [78]. Another advantage to FFDM is that the mammographic images can be easily magnified for a better view of the breast since they are in a digital format [75].

Breast screening using MRI can also be performed as a supplement to mammography [70]. Unlike mammography, MRI can be used to view the internal structures of the breast, which may be ideal for women with high mammographic density [79]. It is generally not recommended that women receive only breast MRIs for cancer screening since they have been shown to miss some cancers that are detected by mammography [79]. MRIs are also associated with a high false-positive rate, which leads to many unnecessary biopsies [79]. For these reasons, breast MRIs are currently reserved for high-risk women in addition to regular mammography screening [79].
2.1.3.2 Primary Prevention Options
Among high-risk populations, primary prevention strategies are limited to chemoprevention (e.g., tamoxifen) and prophylactic surgeries (e.g., bilateral mastectomy, bilateral salpingo-oophorectomy) [80]. Tamoxifen is a selective estrogen receptor antagonist that has been shown to effectively prevent ER-positive breast cancers in the general population [10]. Among BRCA mutation carriers, tamoxifen can effectively reduce the risk of a second breast cancer, but its role in primary prevention among this population is still unclear [81]. According to guidelines set by the National Comprehensive Cancer Network (NCCN), women who have an estimated lifetime risk of breast cancer of ≥20% due to their family history may also be eligible for tamoxifen [82]. While tamoxifen has been shown to exert its protective effects during the course of treatment (usually five years) and for approximately five years after cessation, it does not confer long-term protection and therefore, is not considered superior to prophylactic surgery [80]

Prophylactic bilateral mastectomy remains the most effective form of primary prevention for women who carry a BRCA mutation, with risk reductions of breast cancer estimated at 90% to 95%. Even though mastectomy offers the most protection against breast cancer, uptake is generally low among this population (estimated at only 18%), mainly due to fear of disfigurement and side effects [83]. BRCA mutation carriers are also encouraged to undergo a bilateral salpingo-oophorectomy (the removal of the ovaries and fallopian tubes) to help reduce their elevated risk of ovarian cancer [80]. Among women at an elevated risk of breast cancer due to a family history but no identified genetic predisposition, a prophylactic bilateral mastectomy is an option, however, it is unclear if the risk of breast cancer in this cohort is high enough to warrant this invasive procedure [9]. Moreover, these high-risk women do not have an increased risk for ovarian cancer, therefore, a bilateral salpingo-oophorectomy may also be inappropriate.

2.2 Mammographic Density
Mammographic density is a reflection of the radiologic appearance of breast tissue and is measured using a mammogram. Mammography captures variations in x-ray attenuation of different tissues in the breast: the dense areas of the breast are radiopaque and consequently appear white on a mammogram, while the non-dense areas are radiolucent and appear dark [3, 29]. The dense tissue is primarily composed of fibroglandular tissue (i.e., epithelial and stromal cells), while the non-dense area is mostly composed of adipose tissue [84]. Mammographic
density is typically expressed as a percentage of the mammogram occupied by dense tissue (the amount of dense tissue divided by the total amount of breast tissue) and is referred to as percent mammographic density [3]. The factors that contribute to mammographic density (i.e., the composition of epithelial, stromal, and adipose tissues in the breast) vary widely across women [85]. This concept is illustrated in Figure 2.3.

There is extensive evidence to support mammographic density as an important predictor of breast cancer risk in the general population, based on both prospective and case-control studies showing consistently positive associations between increasing mammographic density and breast cancer risk [2]. These studies are discussed in more detail in Chapter 2, Section 2.2.2. The next section discusses the current methodologies developed for mammographic density assessment.
Footnote to Figure 2.3: Increasing percent mammographic density based on Boyd’s six-category semi-quantitative scale. A: 0%; B: <10%; C: <25%; D: <50%; E: <75%; F: ≥75%.

Reprinted from Lancet Oncology, 6(10), Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, Paterson AD, Mammographic breast density as an intermediate phenotype for breast cancer, 798-808, 2005, with permission from Elsevier.

2.2.1 Assessment of Mammographic Density

Different methods of assessing mammography-derived breast density have evolved over time, and can be grouped into three broad classifications: 1) whether they are area- or volumetric-based; 2) the modality of assessment (i.e., visual, semi-automated, or fully automated); and 3) whether they are qualitative and quantitative [75]. The majority of available methods are area-
based, while volumetric density is still an emerging concept. Each of the different methodologies will be briefly explored in the following sections, based on whether they are area- or volumetric-based. A summary of available mammographic density assessment methods is shown in Table 2.1.

Briefly, Magnetic Resonance Imaging (MRI) [86] and Single X-Ray Absorptiometry (SXA) [87] are two methods that can also assess the composition of the breast without the use of a mammography machine. While studies have shown that the breast density measures produced from these methods are correlated with mammography-derived breast density [88, 89], because these techniques do not utilize mammography, they will not be discussed further.

### 2.2.1.1 Area-Based Methods

Area-based techniques use two-dimensional mammographic images to qualitatively or quantitatively describe the density in the breast [90]. Currently, there are three general categories of area-based methods: 1) Visual Assessment; 2) Semi-Automated Methods; and 3) Fully Automated Methods. The methodology behind each of these techniques, as well as their specific strengths and limitations, will be briefly discussed.

#### 2.2.1.1.1 Visual Assessment

John Wolfe was the first to develop four distinctive categories to describe unique parenchymal patterns in the breast: N1 (predominantly fat), P1 (ductal prominence <25% of the breast), P2 (ductal prominence ≥25% of the breast), and DY (extensive ‘dysplasia’) [91, 92]. Another method developed by Tabár uses categories based on four anatomical characteristics of the breast to develop five different levels of risk [93, 94]. In general, the Tabár categories are comparable to the Wolfe classifications, and they are more distinctive among each other given the anatomical perspective that was used [95]. Taking a quantitative approach, a semi-quantitative visual scale developed by Norman Boyd is based on groups of percent density: A (0%), B (<10%), C (10% to <25%), D (25% to <50%), E (50% to <75%), and F (≥75%) [96].

One of the most widely used visually-assessed classification systems is the Breast Imaging-Reporting and Data System (BI-RADS) system developed by the American College of Radiology [32]. This system describes variations in breast cancer risk using four categories: 1)
almost entirely fatty; 2) scattered fibroglandular densities; 3) heterogeneously dense; and 4) extremely dense [32, 97].

All of the visual methods described have been shown to be associated with breast cancer risk, and the strongest relationships have been reported with the BI-RADS system [1, 98]. However, a primary limitation associated with these visual assessment methods includes their dependence on the expertise of the reader. Even among experienced radiologists, subjectivity and inherent biases across different readers are likely still present [75].

2.2.1.1.2 Semi-Automated Methods

Improving upon the qualitative methods, Byng and colleagues at the University of Toronto developed the Cumulus breast density software [99]. Cumulus is a semi-automated, quantitative, computer-assisted method that uses interactive thresholding to measure mammographic density for both film and digital mammograms [99]. Briefly, using a mammographic image in the cranio-caudal view (i.e., side-view), the reader distinguishes the boundary between the breast tissue and the pectoral muscle. This is essential since pectoral muscle also appears light on a mammogram, and therefore will be included in the dense area calculation if not removed. Next, the reader defines the outer edge of the breast (the red line) in order for the total breast area to be measured (Figure 2.4) [3]. Lastly, the reader uses a sliding scale to capture the dense tissue using thresholds (green line) (Figure 2.4). Once these measurements are defined, a percentage of density (defined as the ratio of the dense area to the total breast area), dense area (cm²), and total breast area (cm²) are calculated. Non-dense area (cm²) is determined by subtracting the dense area from the total breast area.

Cumulus is considered the gold standard for area-based measures of mammographic density [30]. However, given its semi-automated nature and its requirement of a human reader, Cumulus suffers from inherent bias, is labour-intensive, and requires training before use [100]. Madena [101] is another area-based, semi-automated method that is available for research purposes, but it is not used as widely as Cumulus.
Figure 2.4. The measurement of mammographic density using the Cumulus software

Footnote to Figure 2.4: An illustration to show how Cumulus measures percent mammographic density using the craino-caudal view of a mammogram. The red line indicates the outline of the edge of the breast and the green line represents the dense tissue thresholding system.

Reprinted from Lancet Oncology, 6(10), Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, Paterson AD, Mammographic breast density as an intermediate phenotype for breast cancer, 798-808, 2005, with permission from Elsevier.

2.2.1.1.3 Fully Automated Methods

Fully automated, area-based techniques have been developed primarily for the research setting and improve upon the limitations and biases that are associated with visual and semi-automated methods. These methods include AutoDensity [102], ImageJ [103], LIBRA [104], and
MedDensity [105]. These techniques can automatically define the boundary between the breast tissue and pectoral muscle, and distinguish the dense and non-dense tissue in the mammographic image without the use of a trained human reader. Despite these improvements, these methods are still limited by the two-dimensional nature of area-based mammography, in which the degree of compression of the breast can influence its appearance on the mammogram, thereby affecting density measurements [90].

2.2.1.2 Volumetric-Based Methods

Compared to area-based methods, volumetric methods are considered more accurate because area-based methods rely on two-dimensional mammographic images, and therefore, cannot consider the depth (i.e., volume) or overlap of the fibroglandular tissue in the breast [75, 90]. In addition, the density thresholds used by area-based methods define dense tissue dichotomously, that is, by defining pixels as either completely dense (100% dense) or not at all dense (0% dense) [100]. In contrast, volumetric methods have the ability to compute the amount of dense tissue at each pixel in a continuous fashion [100].

All volumetric methods are quantitative and fully-automated, and include CumulusV [106-108], Quantra [109, 110], and Volpara [111, 112]. Briefly, CumulusV uses raw digital mammographic images instead of the screen-film mammograms that are compatible with previous versions of Cumulus [75, 99]. The imaging system relies on internal calibration and the breast thickness and degree of x-ray attenuation in order to calculate volumetric breast density [75, 97]. The Quantra and Volpara systems estimate the thickness of adipose and fibroglandular tissues within each pixel, which are then summed to determine the total breast volume [75]. However, in order to measure breast density, these methods must be used at the time the mammogram is taken, which may be a limitation for use in both the research and in the clinical setting [75].

2.2.1.3 Performance of Mammographic Density Assessment Tools

While the Wolfe grades, Tabár categories, and BI-RADS categories have been shown to be positively associated with risk of breast cancer, quartiles of percent mammographic density measured by Cumulus produces stronger and more consistent associations with breast cancer risk [1, 2]. Even though the BI-RADS system is almost exclusively used by radiologists, it has been shown that the inter-reader reliability among radiologists is poor (κ statistic = 0.56) [113],
compared to reported high inter-reader correlation coefficient for trained readers of Cumulus (more than 0.9) [1].

A study comparing Volpara and Cumulus found a strong agreement between percent density measured by Cumulus and volumetric breast density as determined by Volpara [112]. In addition, a study comparing Cumulus with three volumetric methods (CumulusV, Volpara, and Quantra) found that both Cumulus and CumulusV had significantly higher variability for within-breast percent density measurements compared to Volpara, and Quantra [114]. Studies have also compared Volpara and Quantra to radiologist-assessed BI-RADS [109, 115] and breast density measured using MRI [88], yielding moderate agreement overall.

A study by Eng et al. aimed to compare the validity and reliability of several area-based (i.e., BI-RADS, ImageJ, Cumulus) and volumetric (i.e., Volpara, Quantra) breast density methods, by using each method to measure the percentage of density of digital mammograms [100]. Overall, Volpara and Cumulus were reported to be the most valid, since their distributions of percent density were more strongly associated with breast cancer risk compared to the other methods [100]. In addition, the volumetric methods produced a tighter distribution of percent density values, suggesting that these volume-based methods are more precise than area-based methods [100]. Among the volumetric methods, Volpara was found to report lower percent density values compared to Quantra [100]. Overall, these findings suggest that fully automated, volumetric methods are valid alternatives to the labour-intensive Cumulus [100].
Table 2.1. A summary of mammographic density assessment methods

<table>
<thead>
<tr>
<th>Mode of Assessment</th>
<th>Visual Methods, Area-Based</th>
<th>Semi-quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td><strong>Parenchymal patterns</strong></td>
<td><strong>Qualitative</strong></td>
</tr>
<tr>
<td>Visual Methods, Area-Based</td>
<td>Wolfe patterns</td>
<td>Tabár</td>
</tr>
<tr>
<td>Output</td>
<td>4 Categories</td>
<td>5 Categories</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Semi-Automated Methods, Area-Based</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Output</td>
</tr>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Output</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fully-Automated Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Output</td>
</tr>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Output</td>
</tr>
</tbody>
</table>

*Also assigns a BI-RADS category in addition to continuous percent density

### 2.2.2 Mammographic Density and Risk of Breast Cancer

Extensive mammographic density is strongly and positively associated with breast cancer risk in the general population, among both premenopausal and postmenopausal women. As reported previously in Chapter 2, Section 2.1.1.3, a comprehensive meta-analysis including all epidemiological studies conducted in the general population prior to 2006 found stronger positive associations among incidence studies compared to prevalence studies, and among studies that quantitatively assessed mammographic density compared to qualitative assessment methods [2].

Since the publication of this meta-analysis, Huo et al. [116] reviewed an additional 10 prospective studies [5, 30, 37, 50, 61, 117-121] and eight case-control studies [89, 122-128] that evaluated the association between mammographic density and breast cancer risk. Overall, all studies reported a positive association between increasing mammographic density and breast cancer risk. Of the studies that used percent mammographic density as the exposure, the risk estimates for invasive breast cancer ranged from 2.9- to 4.7-fold when comparing the highest vs.
lowest categories of percent density [30, 50, 89, 123, 126]. Studies by Yaghjyan et al. and Boyd et al. reported the magnitude of the association between percent density and breast cancer risk to persist for 8 to 10 years after the initial mammogram [30, 117]. These findings are important since it has been proposed that mammographic density is a risk factor for breast cancer because the dense tissue hides or “masks” tumours. While masking may occur, these results suggest that it is not the sole mechanism linking mammographic density to breast cancer risk [117].

Absolute dense area (cm$^2$) and non-dense area (cm$^2$) are also breast cancer risk factors in addition to percent mammographic density. Dense area reflects the fibroglandular tissue, while non-dense area corresponds to the amount of fat tissue in the breast [40]. Several prospective studies have shown that dense area is positively associated with breast cancer risk, while non-dense area is inversely associated with breast cancer risk, for both pre- and postmenopausal women [40, 61, 117, 129]. Findings from Yaghjyan et al. were the first to confirm that, like percent density, the associations between dense area and non-dense area and breast cancer also persist for up to 10 years after the mammogram [117]. A recent meta-analysis of 13 case-control studies found that while dense area is a strong risk factor for breast cancer, the risk estimates for percent density were larger in magnitude, and it is, therefore, a stronger risk factor [33]. The findings for non-dense area and breast cancer risk were less consistent, with most studies reporting an inverse relationship, but the association was attenuated in several studies after adjustment for dense area [33]. Possible reasons for the observed negative correlation between dense and non-dense area may include measurement error, in which the dense area is misclassified as non-dense area, or collinearity between the two measures [33]. Based on the heterogeneity across these studies, the authors concluded that non-dense area is a weak, protective factor for breast cancer [33]. Therefore, percent mammographic density is the strongest predictor of the three measures, since it incorporates both dense area and non-dense area [33].

The characteristics of epidemiological studies prospectively investigating the relationships between quantitatively-assessed measures of mammographic density and breast cancer risk in the general population are summarized in Table 2.2.
Whether mammographic density is associated with breast cancer subtypes [130-139] has also been reviewed [116]. Because mammographic density may be influenced by exogenous and endogenous hormones, it was postulated that women with higher mammographic density were more likely to be diagnosed with ER-positive and PR-positive breast cancers [116]. Despite the biological plausibility of this hypothesis, results are conflicting. One prospective cohort study by Conroy et al. found mean percent density was significantly greater for ER-positive and PR-positive tumours [131], while two case-control studies found mammographic density was significantly associated with ER-negative tumours [130, 132]. In contrast, a cohort study by Heusinger et al. found percent density was inversely associated with ER expression and positively associated with PR expression [133]. One case-control study found higher percent density was associated with TNBC [134]. Apart from these findings, the remaining literature has found mammographic density to either be positively associated with all breast cancer subtypes [135, 136] or not associated with any breast cancer subtypes [137-139].
Table 2.2. Prospective and nested case-control studies evaluating the association between quantitatively-assessed measures of mammographic density and breast cancer risk in the general population

<table>
<thead>
<tr>
<th>Study, Year [ref]</th>
<th>Study design, Country</th>
<th>BC Cases: Controls</th>
<th>Follow-up</th>
<th>MD measure(s); MD Method; Comparison</th>
<th>Subgroup Analyses</th>
<th>Main Finding: mean, OR, RR, HR (95%CI); P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boyd, 1995 [96]</td>
<td>NCC, Canada</td>
<td>354 cases: 354 controls</td>
<td>1980-1997</td>
<td>PD; Cumulus; ≥75% density vs. 0% density</td>
<td>All</td>
<td>4.04 (2.12, 7.69); 0.0001</td>
</tr>
<tr>
<td>Byrne, 1995 [140]</td>
<td>NCC, United States</td>
<td>1,880 cases: 2,152 controls</td>
<td>1974-1989</td>
<td>PD; VAS; ≥75% density vs. 0% density</td>
<td>All</td>
<td>4.35 (3.1-6.1); &lt;0.001</td>
</tr>
<tr>
<td>Kato, 1995 [141]</td>
<td>NCC, United States</td>
<td>52 cases: 195 controls (pre) 91 cases: 178 controls (post)</td>
<td>1985-1991</td>
<td>PD, DA, BV; VAS; highest vs. lowest tertile</td>
<td>All</td>
<td>PD: 3.6 (1.4-9.1); &lt;0.01 DA: 4.6 (1.6-12.9); &lt;0.01 BV: 1.9 (0.6-6.0); n.s.</td>
</tr>
<tr>
<td>Maskarinec, 2000</td>
<td>NCC, United States</td>
<td>647 cases: 647 controls</td>
<td>1991-1997</td>
<td>PD, DA; Cumulus; highest vs. lowest quintile</td>
<td>All</td>
<td>PD: 1.8 (1.1-3.0); n.s. DA: 1.8 (1.2-2.6); 0.03</td>
</tr>
<tr>
<td>van Gils, 2000</td>
<td>NCC, Netherlands</td>
<td>129 cases: 517 controls</td>
<td>1985-1994</td>
<td>PD; FAM; &lt;5% density vs. 5-25% density and &gt;25% density</td>
<td>All</td>
<td>5-25% density: 2.6 (1.4-4.8) &gt;25% density: 2.9 (1.6-5.6)</td>
</tr>
<tr>
<td>Maskarinec, 2005</td>
<td>NCC, United States</td>
<td>607 cases: 667 controls</td>
<td>1993-2000</td>
<td>PD, DA, TBA; CAM; means in cases vs. controls</td>
<td>All</td>
<td>PD: 39.6% vs. 29.7%; &lt;0.001 DA: 37.3 cm² vs. 28.4 cm²; &lt;0.001 TBA: 107.4 cm² vs. 115.7 cm²; 0.01</td>
</tr>
<tr>
<td>Torres-Mejía, 2005</td>
<td>Cohort, United Kingdom</td>
<td>111 cases: 3100 controls</td>
<td>1986-2003</td>
<td>PD, DA, NDA, TBA, BV; Cumulus; highest vs. lowest quartile</td>
<td>All</td>
<td>PD: 3.49 (1.69, 7.18); 0.001 DA: 2.69 (1.40, 5.16); 0.003 NDA: 0.56 (0.29, 1.11); n.s. TBA: 1.03 (0.54, 1.96); n.s. BV: 0.73 (0.38, 1.40); n.s.</td>
</tr>
<tr>
<td>Tamimi, 2007 [50]</td>
<td>NCC, United States</td>
<td>253 cases: 520 controls</td>
<td>1989-1998</td>
<td>PD; Cumulus; highest vs. lowest quartile</td>
<td>Postmenopausal</td>
<td>3.8 (2.2, 6.6); &lt;0.001</td>
</tr>
<tr>
<td>Boyd, 2007 [30]</td>
<td>3 NCCs, Canada</td>
<td>1114 cases: 1114 controls</td>
<td>1984-1999</td>
<td>PD; Cumulus; &gt;75% density vs. &lt;10% density</td>
<td>All</td>
<td>4.7 (3.0, 7.4); &lt;0.001</td>
</tr>
<tr>
<td>Lokate, 2011 [118]</td>
<td>NCC, Netherlands</td>
<td>358 cases: 859 controls</td>
<td>2001-2006</td>
<td>PD, DA, NDA; Cumulus; highest vs. lowest quintile</td>
<td>Postmenopausal</td>
<td>PD: 1.8 (1.0, 2.9); 0.002 DA: 2.8 (1.7, 4.8); &lt;0.001 NDA: 2.4 (1.3, 4.2); &lt;0.001</td>
</tr>
</tbody>
</table>
### Table 2.2, continued

<table>
<thead>
<tr>
<th>Study, Year [ref]</th>
<th>Study design, Country</th>
<th>BC Cases: Controls</th>
<th>Follow-up</th>
<th>MD measure(s); MD Method; Comparison</th>
<th>Subgroup Analyses</th>
<th>Main Finding: mean, OR, RR, HR (95% CI); P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pettersson, 2011 [61]</td>
<td>NCC, United States</td>
<td>464 cases: 988 controls (pre) 960 cases: 1662 controls (post)</td>
<td>1990-2004</td>
<td>PD, DA, NDA; Cumulus; highest tertile vs. lowest tertile (pre); highest vs. lowest quintile (post)</td>
<td></td>
<td>Premenopausal PD: 2.72 (1.93, 3.83) 1 DA: 2.01 (1.45, 2.77) 1 NDA: 0.51 (0.36, 0.72) 1 PD: 3.28 (2.41, 4.45) 1 DA: 2.19 (1.65, 2.89) 1 NDA: 0.46 (0.34, 0.62) 1</td>
</tr>
<tr>
<td>Yaghjyan, 2013 [117]</td>
<td>NCC, United States</td>
<td>1,028 cases: 1,780 controls</td>
<td>1989-2004</td>
<td>PD, DA, NDA; Cumulus; ≥50% density vs. &lt;10% density</td>
<td>Postmenopausal</td>
<td>PD: 3.37 (2.45, 4.65); &lt;0.0001 DA: 1.94 (1.53, 2.47); &lt;0.0001 NDA: 0.50 (0.38, 0.65); &lt;0.0001</td>
</tr>
<tr>
<td>Baglietto, 2014 [129]</td>
<td>NCC, Australia</td>
<td>590 cases: 1,695 controls</td>
<td>1990-2007</td>
<td>PD, DA, NDA; Cumulus; DA and NDA: Highest vs. lowest quintile PD: &gt;50% density vs. &lt;5% density</td>
<td>All</td>
<td>DA: 2.73 (1.95, 3.83) 1 NDA: 0.48 (0.33, 0.71) 1 PD: 4.43 (2.64, 7.41) 1</td>
</tr>
<tr>
<td>Yaghjyan, 2015 [40]</td>
<td>NCC, United States</td>
<td>1,044 cases: 1,794 controls</td>
<td>1989-2004</td>
<td>PD, DA, DNA; Cumulus; PD: ≥50% density vs. &lt;10% density DA and NDA: highest vs. lowest quartile</td>
<td>All</td>
<td>PD: 3.36 (2.44, 4.63); &lt;0.0001 DA: 1.96 (1.55, 2.48); &lt;0.0001 NDA: 0.51 (0.39, 0.66); &lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: BC, breast cancer. BV, breast volume (cm³). CAM, computer-assisted method. DA, dense area (cm²). FAM, fully-automated method. HR, hazard ratio. PD, percent density (%). NCC, nested-case control. NDA, non-dense area (cm²). OR, odds ratio. RR, relative risk. TBA, total breast area (cm²). VAS, visual assessment scale. n.s. = not significant

1No P-value indicated
2.2.2.1 Proposed Biological Mechanisms

While the evidence supporting an association between mammographic density and breast cancer risk is unequivocal, the biological mechanisms explaining this relationship are not well-understood [1]. As previously described in Chapter 2, Section 2.2, fibroglandular tissue (primarily epithelial and stromal cells) comprises the dense tissue, while non-dense tissue is composed of fat. Given that breast cancers are thought to originate from the dense, epithelial tissue, it has been postulated that the number and proliferative state of these epithelial cells may not only affect the mammographic appearance of the breast, but also influence the probability of genetic damage, thereby leading to carcinogenesis [3, 84].

The breast stroma produces collagen, which has confirmed mechanical properties that can assist with tumour invasion [3]. In addition, some stromal fibroblasts are pre-adipocytes that can differentiate into adipocytes under the correct stimuli, further complicating the delicate relationship between the breast tissue components [84, 85]. Interestingly, single fibroblasts taken from high mammographic density environments have been shown to accumulate less fat in their surroundings compared to fibroblasts from low mammographic density environments, suggesting that fibroblasts in mammographically-dense breasts have a different phenotype [85].

Bridging these concepts together is a possible hypothesis proposed by Martin and Boyd [84] (Figure 2.5). Briefly, the presence of extensive mammographic density may create an environment that facilitates epithelial cell proliferation, thus leading to carcinogenesis. In addition, endogenous breast mitogens, such as IGF-1 and prolactin, are thought to also stimulate epithelial cell proliferation. The release or inhibition of these mitogens is influenced by genes, age, exogenous hormone use, and other breast cancer risk factors. Ultimately, the cumulative exposure of the breast stroma and epithelium to these mitogens may be responsible for the increased risk of breast cancer that is strongly associated with mammographic density [84]. Lastly, genetic damage to either the breast stroma or epithelium due to the activity of mutagens can also initiate carcinogenesis [84].
Figure 2.5. A biological hypothesis linking mammographic density and breast cancer risk

Footnote to Figure 2.5: The epithelial, stromal and fat cells in the breast are responsible for variations in mammographic density and are closely related to each other. Stromal cells produce collagen, and some stromal cells are pre-adipocytes that can differentiate into adipocytes. Both stromal and epithelial cells, the components of dense tissue, are susceptible to proliferative signals via endogenous mitogens, such as IGF-1 and prolactin. The release of these mitogens is modified by a woman’s age, genes, hormones, and other risk factors. Genetic damage to the breast stroma and epithelium through mutagenesis can also initiate carcinogenesis.


Mammographic density declines with increasing age due to the natural process of lobular involution [1]. During involution, the breast epithelial tissue experiences atrophy and is replaced with fat tissue [29]. What is seemingly paradoxical, however, is that breast cancer incidence increases with age [84, 146]. This contradictory relationship can be explained by a concept
proposed by Pike et al., called ‘Pike’s Paradox’ [147], which is based on the idea that ‘breast tissue ageing’ is more relevant for age-specific breast cancer risk as opposed to the chronological age of breast tissue. Breast tissue ageing reflects the kinetics of the breast epithelium and its accrual of genetic damage over time. As shown in Figure 2.6, the rate of breast tissue ageing is the most rapid at menarche, decreases with each full-term pregnancy, slows further during the perimenopausal period, and is the slowest after menopause. Therefore, it is thought that breast cancer risk accumulates the fastest during the period between menarche and first full-term pregnancy [19].

**Figure 2.6. Incidence of breast cancer and breast tissue ageing according to the Pike Model**

Footnote to Figure 2.6: The Pike Model uses the concept of breast tissue ageing to explain the paradoxical decline of mammographic density but increases in breast cancer risk associated with increasing age. The rate of breast tissue ageing is the greatest at menarche and slows down with each full term first-pregnancy. During the perimenopausal period, the rate of breast tissue ageing slows further and is the slowest at menopause. ‘b’ represents a one-time increase in risk that is associated with first full-term pregnancy (FFTP).

*Reprinted from Lancet Oncology, 6(10), Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, Paterson AD, Mammographic breast density as an intermediate phenotype for breast cancer, 798-808, 2005, with permission from Elsevier.*
2.2.2.2 Utility of Mammographic Density in Breast Cancer Risk Prediction

The lifetime risk of breast cancer due to an inherited deleterious germline mutation in the BRCA1 or BRCA2 genes is approximately 40% to 87% by age 70 [7]. Moreover, even among BRCA mutation-negative families with a strong history of the disease, the risk of breast cancer remains high compared to the general population [8]. Therefore, the timely identification of individuals and families at increased risk is crucial in order for early intervention and prevention of disease [10, 148]. At this time, several computational risk prediction models are available to not only identify individuals who are at high risk for carrying an inherited genetic predisposition for disease, but to also help clinicians and genetic counsellors determine who will benefit from primary prevention options such as tamoxifen and prophylactic surgery [149].

BRCAPRO is the most commonly used tool for predicting an individual’s risk of carrying a BRCA mutation [150]. The Breast Cancer Risk Assessment Tool (BCRAT) [151], also known as the Gail model, is used worldwide to predict breast cancer risk for women in the general population [3]. This model considers various breast cancer risk factors, including age, age at menarche, age at first live birth, and the number of first-degree relatives affected with breast cancer [3], and has been validated among the general population [152]. The International Breast Cancer Intervention Study (IBIS) model [71, 72], also known as the Tyrer-Cuzick model, incorporates similar risk factors, except it also includes benign breast diseases, hormone replacement therapy use, and height and weight [148]. It includes a more complex family history component compared to the Gail model but is restricted to breast and ovarian cancer diagnoses in close relatives. This model was initially developed for use in the high-risk International Breast Cancer Intervention Study (IBIS-1) [153, 154], and has been well validated in similar high-risk populations [155-158]. For larger families where relevant cancer diagnoses are among distant relatives (i.e., second- or third-degree relatives), the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) [73, 74] model may be more appropriate. In addition to incorporating the pathological characteristics of breast cancer tumours, the BOADICEA model also considers other cancer diagnoses in addition to breast and ovarian cancer, such as pancreatic and prostate cancer [74]. A summary of the available breast cancer risk prediction models is shown in Table 2.3.
Table 2.3. Characteristics of breast and ovarian cancer risk prediction assessment tools

<table>
<thead>
<tr>
<th>Name of Model</th>
<th>Eligibility Criteria</th>
<th>Parameters Used</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer Risk Assessment Tool (BCRAT)/ Gail model [151]</td>
<td>All women including the general population</td>
<td>Age; age at menarche; age at first live birth; number of benign breast biopsies; number of affected FDRs with breast cancer</td>
<td>Breast cancer risk</td>
</tr>
<tr>
<td>BRCAPRO [150]</td>
<td>Individuals and/or families with suspected hereditary breast and ovarian cancer</td>
<td>Age; family history of breast and ovarian cancer in FDRs and SDRs and ages at diagnoses; AJ ancestry</td>
<td>Probability of carrying a BRCA mutation</td>
</tr>
<tr>
<td>Tyrer-Cuzick model/International Breast Cancer Intervention Study (IBIS) model [72]</td>
<td>Unaffected women with a family history of breast or ovarian cancer</td>
<td>Age; age at menarche; age at first live birth; height; weight; duration of HRT use; number of benign breast biopsies; BBD; number of affected FDRs with breast and ovarian cancer; ages of diagnoses; MD; AJ ancestry</td>
<td>Breast and ovarian cancer risk; probability of carrying a BRCA mutation</td>
</tr>
<tr>
<td>Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) [74]</td>
<td>All women with a familial or personal history of breast or ovarian cancer</td>
<td>Age; relatives with breast, ovarian, prostate, pancreatic cancer; ages of diagnoses; breast cancer pathology; AJ ancestry; previous BRCA genetic testing in the individual or in relatives</td>
<td>Breast and ovarian cancer risk; probability of carrying a BRCA mutation</td>
</tr>
</tbody>
</table>

Abbreviations: FDRs, first-degree relatives. SDRs, second-degree relatives. AJ, Ashkenazi Jewish. HRT, hormone replacement therapy. BBD, benign breast disease. MD, mammographic density.

Given that mammographic density is more strongly associated with breast cancer risk compared to the other breast cancer risk factors included in risk prediction models, it is crucial that it is also included in an individual’s risk assessment. Previous attempts at including BI-RADS-assessed mammographic density resulted in minimal improvement in risk prediction [159-164]. Recent studies have incorporated quantitative measures of mammographic density into the Gail and IBIS models [148, 158, 165]. Warwick et al. found that after adjusting percent density for age and
BMI, its inclusion into the IBIS model predicted breast cancer risk better than the IBIS model alone (area under the receiver operating characteristic curve [AUC] = 0.62 for percent density and IBIS together vs. 0.52 for IBIS alone; \( P = 0.002 \)) [148]. Following this study, Brentnall et al. were the first to evaluate the inclusion of mammographic density into the Gail and IBIS models in a prospective screening setting [165]. Including percent density into both models provided more information compared to the models run alone (AUC = 0.59 vs. 0.55 for the Gail model and 0.61 vs. 0.57 for the IBIS model), but whether these values were significantly different from each other was not reported [165]. It is expected that volumetric-based assessments of mammographic density will help improve the predictive accuracy of these models [10]. The latest version of IBIS (version 8) [72], was released in March 2017 and includes mammographic density, in which the user can indicate the percentage of density or the corresponding BI-RADS category, based on the method of assessment (i.e., Volpara-derived percent mammographic density, visually assessed percent mammographic density, or BI-RADS).

### 2.2.2.3 Mammographic Density as a Biomarker of Breast Cancer Risk

Several well-established breast cancer risk factors have been found to be associated with mammographic density to some extent. All breast cancer risk factors must employ some type of effect on the breast; therefore, it is plausible these risk factors are exerting their effects on breast cancer risk, at least partially, by influencing the fibroglandular and adipose tissue in the breast that contributes to mammographic density [84, 146]. Based on this concept, if mammographic density can be modified by these risk factors, it is possible that changes in mammographic density can modify breast cancer risk. Exploring this further, the relationships between mammographic density and the primary genetic, reproductive, hormonal, anthropometric, lifestyle, and dietary risk factors for breast cancer will be discussed. A summary of these relationships is shown in Table 2.4.
Table 2.4. Summary of modifiers of mammographic density and premenopausal and postmenopausal breast cancer risk in the general population

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Mammographic Density&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Breast Cancer Risk</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Premenopausal</td>
<td>Postmenopausal</td>
</tr>
<tr>
<td><strong>Menstrual/Reproductive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing age at menarche</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Increasing age at menopause</td>
<td>↑</td>
<td>N/A</td>
<td>↑</td>
</tr>
<tr>
<td>Parity</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>?</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Increasing age at first birth</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Exogenous Hormones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Hormone Replacement Therapy&lt;sup&gt;2&lt;/sup&gt;</td>
<td>↑</td>
<td>N/A</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing height</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Increasing body weight&lt;sup&gt;*&lt;/sup&gt;</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Increasing body mass index</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Lifestyle/Dietary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>?</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Smoking</td>
<td>?</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Alcohol&lt;sup&gt;*&lt;/sup&gt;</td>
<td>?</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Chemopreventive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamoxifen&lt;sup&gt;*&lt;/sup&gt;</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

Legend: ↓: decrease in mammographic density/breast cancer risk; ↑: increase in mammographic density/breast cancer risk; ?: unclear association with mammographic density/breast cancer risk. N/A: not applicable.

<sup>1</sup>The relationships between each exposure and mammographic density were established using both area-based and volumetric-based mammographic density measurements, except for the exposures denoted with an asterisk (*), which are only based on area-based measurements.

<sup>2</sup>Hormone replacement therapy containing combined estrogen and progesterone only.
2.2.2.3.1 Genetic Risk Factors and Family History

Mammographic density is a heritable trait, with early studies by Wolfe et al. showing high congruity in parenchymal patterns between mother-daughter pairs and sister pairs compared to age-matched controls [166]. Following these findings, two large twin studies based in Australia and North America found that the correlation of percent density was 0.61 for monozygotic pairs in Australia, 0.67 for monozygotic pairs in North America, 0.25 for dizygotic pairs in Australia, and 0.27 for dizygotic pairs in North America [4]. The authors estimated that heritability of percent density accounted for 60% of the variation in percent density in the Australian cohort, 67% in the North American cohort, and 63% among all twin pairs combined [4]. Similarly, another twin study conducted within the California Twin Program cohort found mammographic density was more strongly correlated among monozygotic twins compared to dizygotic twins [167]. Overall, these results demonstrate an underlying genetic basis to the inheritance of mammographic density. It is estimated that genetic factors contribute to approximately 25% in dizygotic twins, 30% in mother-daughter and sister-pairs, and 60-70% in monozygotic twins, of the variance in mammographic density [158, 168].

Given that mammographic density and familial breast cancers likely share genetic factors, it is plausible that mammographic density may mediate the risk of breast cancer that is associated with family history. Supporting this, Martin et al. found that women with at least one or two or more affected first-degree relatives with breast cancer had 3.1% and 7.0% higher mammographic density compared to women in the general population, respectively [5]. The increase in mammographic density associated with having a family history of the disease was significantly associated with an increased risk of breast cancer [5]. Based on these findings, the authors estimate that percent mammographic density explained 14% of the association between having a first-degree family history and breast cancer risk [5]. Among a multiethnic cohort of Caucasian and Asian women [169], the odds ratio for every 10% increase in percent density was higher among women with a family history of breast cancer (OR = 1.30, 95% CI 1.13, 1.49) than among women from the general population (OR = 1.14, 95% CI 1.09, 1.20). While these trends were observed in both ethnic groups, the results were stronger for Caucasian women [169].

While mammographic density is associated with an increased risk of breast cancer among BRCA mutation carriers, the distribution of mammographic density among this high-risk population is not significantly different compared to non-carrier women from BRCA mutation-positive
families (i.e., at low-to-average risk of breast cancer) [170-172]. Therefore, mammographic density likely acts as a risk factor for breast cancer independently from the BRCA genes among this high-risk population [171].

Some of the SNPs associated with breast cancer risk are also associated with mammographic density measures [3, 116, 173-175]. A previous study reported an association between a SNP located in the LSP1 loci with dense area and percent density [173], which was confirmed by a cross-sectional study that also identified a novel association between a SNP in the RAD51L1 gene and dense area [174]. Further, a large study by Stone et al. used data from over ten thousand women to evaluate the associations between mammographic density measures and 77 common breast cancer susceptibility variants [175]. Findings from this study confirmed previous associations and identified novel relationships with the following genes: EBF1, MIR1972-2:FTO, 2p24.1 and dense area and percent density; MLK1 and dense area and non-dense area; NTN4 and non-dense area [175]. Collectively, there may be an 18% overlap between susceptibility variants associated with mammographic density and breast cancer risk [175].

2.2.2.3.2 Reproductive and Hormonal Risk Factors

The relationships between reproductive risk factors and mammographic density have been well-defined. Menopause has consistently been shown to be associated with a reduction in dense area and an increase in non-dense area in the breast, which overall results in a lower percentage of mammographic density [146]. Lobular involution of the breast epithelium is thought to be a prime contributor to menopause-related reductions in mammographic density [1, 29]. Briefly, lobular involution is an age-related process where epithelial terminal duct lobules in the breast atrophy and are replaced with adipose tissue [176].

In the general population, nulliparous women have consistently higher mammographic density compared to parous women, and linear, inverse trends between the number of live births and mammographic density have been observed among large cohort studies [1, 41, 42]. For example, it has been estimated that every live birth results in a 2% reduction in percent density [84]. A later age at first full-term pregnancy has been found to be positively associated with percent mammographic density [42, 177]. Age at menarche is thought to be inversely associated with mammographic density [146] but many studies do not report an association [178, 179]. However, a study by Yaghjyan et al. reported that a longer time interval between menarche and
first-full term birth was positively associated with percent density among postmenopausal women [42]. The relationship between breastfeeding and mammographic density is less consistent, with some studies reporting null associations [179, 180] and others reporting positive associations, which have only been observed among premenopausal women [42, 178]. This suggests that the changes in mammographic density attributable to breastfeeding are transient. Other reproductive and menstrual-associated factors including endometriosis [181] and the stages of the menstrual cycle [182, 183] do not seem to be associated with mammographic density. Overall, the influence of age, parity, and menopausal status are thought to account for 20% to 30% of the variance in percent mammographic density in the general population [1].

Oral contraceptive use is associated with higher mammographic density, mostly due to changes in dense area [184]. Among postmenopausal women, the use of combined hormone replacement therapy (HRT), that is, containing both estrogen and progesterone, is associated with increased mammographic density, while HRT containing only estrogen is not [40, 184-186]. Tamoxifen, a selective estrogen receptor antagonist, has been shown to effectively reduce mammographic density, which in turn has been linked to a reduction in breast cancer risk [187], the recurrence of ER-positive breast cancers [188], and improved breast cancer survival [189, 190]. In addition, other literature supports the use of changes in mammographic density as a marker of treatment response [191]. Briefly, levels of circulating sex hormones such as estrogen, testosterone, and progesterone do not seem to be associated with mammographic density [50, 84, 192], suggesting that they influence breast cancer risk independently of mammographic density. Most studies have observed a positive association with sex hormone binding globulin and percent mammographic density and dense area [84, 193], but some have found null results [192]. A recent study found that among postmenopausal women, prolactin was positively associated with percent mammographic density and inversely associated with non-dense area [52]. Moreover, circulating IGF-1 levels are positively associated with percent mammographic density among both premenopausal and postmenopausal women [57, 84].

2.2.2.3.3 Anthropometric, Dietary, and Lifestyle Risk Factors
Adult attained height is positively associated with mammographic density in some studies [60, 194, 195], but others have reported null associations [57, 196-198]. Percent density and dense area are inversely associated with body size (measured as BMI or weight), while non-dense area is positively associated with body size [57, 61]. This is because as body weight increases, the
amount of adipose tissue in the breast increases, and this results in a lower percentage of mammographic density [57]. In addition, the inverse association between dense area and body size may be due to excess body size increasing the rate of differentiation of stromal pre-adipocytes into adipocytes in the breast [116].

A limited number of studies have reported an inverse association between physical activity and mammographic density [199, 200], and a meta-analysis of over 20 studies reported a null association [201]. Across epidemiological studies, there is wide variation in the duration, type (e.g., recreational, leisure, occupational, household physical activity), and intensity (e.g., vigorous, moderate, light) of activities, as well as how physical activity is assessed (e.g., questionnaires, accelerometers) [201]. Furthermore, the presence of misclassification bias, especially among studies using questionnaires to measure physical activity levels, is a likely contributor to these inconsistent findings [199-201]. More objective measures of physical activity such as accelerometers may minimize this, but their high costs make them unsuitable for large epidemiological studies [201]. For these reasons, it is difficult to elucidate the relationship between physical activity and mammographic density.

Evidence supporting a role of dietary factors and mammographic density is limited. Briefly, a low-fat, high-carbohydrate trial among women with ≥50% mammographic density reported significant reductions in mammographic density after two years of follow-up, particularly among women who recently became postmenopausal [202]. In the same cohort, significant reductions in total or saturated fat intake and cholesterol intake were significantly associated with lower dense area [203]. However, these findings could not be replicated among a larger group of women who recently underwent menopause [204]. Cross-sectional studies have reported inverse associations between vitamin D and calcium [205-208], vegetable intake and olive oil consumption [207], and positive associations between animal protein and red meat [209, 210], sugar-sweetened beverages [211], and high glycemic load foods [212] with mammographic density. Null associations between mammographic density and fibre intake [213, 214], circulating carotenoids [215], and erythrocyte omega-3 and omega-6 polyunsaturated fatty acids [216] have been reported. Some studies in Asian cohorts have observed inverse associations between dietary phytoestrogens and mammographic density, but these findings have not been replicated in other populations [217, 218].
There is substantial evidence to suggest a positive relationship between alcohol and mammographic density, specifically with the dense area in the breast [219]. The probable mechanisms behind this association include the positive effects of alcohol on endogenous estrogen, aromatase activity, and constituents of the growth hormone-insulin-like growth factor axis, all of which can increase the proliferation of dense epithelial and stromal cells in the breast [219]. The literature reviewing smoking and mammographic density is mixed, but most studies report an inverse association with percent mammographic density, and mainly in postmenopausal women [220]. Only one prior study directly evaluated the relationships between smoking and dense area and non-dense area among postmenopausal women [221]. While the authors reported a positive trend between percent density and increasing pack-years ($P$ trend = 0.06), they did not detect any clear associations with dense area and non-dense area [221]. A possible mechanism that may explain the inverse association between smoking and percent density involves tobacco’s role as an anti-estrogenic agent by inhibiting the conversion of androgens into estrogens [220].

2.3 The ‘Negative Study’ Cohort

Our group previously reported that women who have a strong family history of breast cancer (defined as two cases between first-degree relatives under age 50, or three cases at any age on one side of the family), but do not have an identified $BRCA1$ or $BRCA2$ mutation in their affected relatives, face a lifetime risk of breast cancer of 40% by age 70 [8]. While this risk estimate is not as high as the risk faced by $BRCA$ mutation carriers, it is significantly elevated in comparison to the estimated 12% lifetime risk in the general population (i.e., women who do not have a family history of the disease) [11]. While prevention strategies at the clinical level have expanded to include these high-risk women, such as by offering risk-reducing surgery (e.g., prophylactic mastectomy), intensive breast screening (e.g., annual mammography, MRI) and chemoprevention (e.g., tamoxifen), there is no consensus for whether these women should follow the same preventive measures as women who carry a $BRCA$ mutation [9].

Based on this, Kotsopoulos and colleagues initiated the ‘Risk Factor Analysis for Familial Breast Cancer’ study (i.e., the ‘Negative Study’) at the Women’s College Research Institute. The goal of this longitudinal, prospective cohort study is to evaluate whether genetic (e.g., other breast cancer predisposing genes) and non-genetic (e.g., reproductive, hormonal and lifestyle) factors
influence risk in this under-studied population, as well as to investigate how biomarkers of breast cancer (e.g., mammographic density) are implicated in cancer development. There is also a special emphasis on evaluating the impact of modifiable risk factors [9]. The findings from this study will help develop prevention and screening strategies for these high-risk women.

The ‘Negative Study’ consists of women with a strong family history of breast cancer but no \textit{BRCA} mutation in their affected relatives. Briefly, participants are eligible for this study if 1) there is no \textit{BRCA} mutation found in their family; 2) they have a strong family history of breast cancer; and 3) they have no personal history of cancer. A strong family history entails two breast cancers in first-degree relatives under the age of 50 or three cases at any age on one side of the family. Most importantly, this study population is unique and under-studied, given that breast cancer preventive measures and risk factors for breast cancer development have not been evaluated to the same extent in this high-risk population in comparison to the general population and among \textit{BRCA} mutation carriers. Therefore, this study represents the first research initiative aimed at studying breast cancer risk factors exclusively in this high-risk population.
Chapter 3
Rationale, Objectives, and Hypotheses

3

3.1 Rationale

Mammographic density is the strongest risk factor for breast cancer in the general population, second to age and carrying a genetic predisposition to the disease [29]. High percent mammographic density (≥50%) is common in the general population and may contribute to a substantial proportion of breast cancers of approximately 16% to 39% [29-31]. Mammographic density is associated with various reproductive, hormonal, and lifestyle factors that are also implicated in breast cancer development. Moreover, mammographic density can be modified by the same risk factors that modify breast cancer risk [3]. Thus, it is plausible that these risk factors exert their influence on breast cancer risk through mammographic density, suggesting that mammographic density may act as an intermediate marker of breast cancer risk [3]. Therefore, the identification of risk factors that can modify mammographic density can help clarify its role in breast cancer development, and help reduce breast cancer incidence [3].

Mammographic density is a partially heritable trait, and there is substantial evidence to suggest that it is also implicated in familial breast cancers [5]. Despite this, the majority of evidence regarding the etiology of mammographic density is based on research conducted in the general population. Given that mammographic density is associated with both genetic and non-genetic breast cancer risk factors in the general population, it is important to clarify the role of mammographic density among high-risk populations, where our understanding of modifiable breast cancer risk factors is particularly limited. Of interest for this study is a population of women who face an elevated lifetime risk for breast cancer due to a strong family history of the disease, but have no BRCA mutation identified in their families (will be referred to as “high-risk women” hereafter). It is unknown if the risk factors that modify mammographic density in the general population behave similarly in these high-risk women.

The overall goal of the current study is to evaluate the relationship between various breast cancer risk factors and measures of mammographic density (e.g., percent density, dense area, and non-dense area) in high-risk women enrolled into the prospective, ‘Negative Study’ using a cross-
sectional approach. Due to the small number of breast cancer cases in this longitudinal cohort study, mammographic density will be used as an intermediate marker of breast cancer risk. Percent mammographic density, dense area, and non-dense area will be measured using Cumulus, which is an area-based, computer-assisted method that is considered to be the gold standard of mammographic density assessment [100]. The relationships between the exposures of interest and the measures of mammographic density will be evaluated among premenopausal and postmenopausal women separately since mammographic density is known to decline after menopause [84].

This work will help identify breast cancer risk factors that may influence mammographic density, an important biomarker of breast cancer risk, in an understudied high-risk population. Furthermore, the findings from this work may contribute to the identification of evidence-based prevention strategies that are specifically tailored to this unique study population.

3.2 Objectives
1. To evaluate the relationship between reproductive factors (i.e., age at menarche, parity, breastfeeding) and percent mammographic density, dense area, and non-dense area in high-risk premenopausal and postmenopausal women.

2. To evaluate the relationship between hormonal factors (i.e., oral contraceptive use, hormone replacement therapy use) and percent mammographic density, dense area, and non-dense area in high-risk premenopausal and postmenopausal women.

3. To evaluate the relationship between anthropometric measures (i.e., body weight, height) and percent mammographic density, dense area, and non-dense area in high-risk premenopausal and postmenopausal women.

4. To evaluate the relationship between lifestyle factors (i.e., physical activity, smoking, alcohol) and percent mammographic density, dense area, and non-dense area in high-risk premenopausal and postmenopausal women.
3.3 Hypotheses

1. Percent mammographic density and dense area will be positively associated with an earlier age at menarche, nulliparity, and a shorter duration of breastfeeding. Non-dense area will be positively associated with parity and a greater duration of breastfeeding.

2. Percent mammographic density and dense area will be positively associated with oral contraceptive and hormone replacement therapy use.

3. Percent mammographic density and dense area will be positively associated with height, while non-dense area will be inversely associated with height. Percent mammographic density and dense area will be inversely associated with body weight, while non-dense area will be positively associated with body weight.

4. Percent mammographic density and dense area will be positively associated with alcohol consumption, while non-dense area will be inversely associated with alcohol consumption. Percent mammographic density and dense area will be inversely associated with smoking, while non-dense area will be positively associated with smoking. Percent mammographic density and non-dense area will be inversely associated with physical activity.
Chapter 4
Methodology

4

4.1 Study Population

Potentially eligible women were identified from a longitudinal prospective cohort study called the ‘Negative Study’ based at the Familial Breast Cancer Research Unit, Women’s College Hospital (Toronto, Canada) and previously described in detail in Kotsopoulos et al. [9]. Briefly, participants were recruited from six participating genetic counselling clinics throughout Southern Ontario based on pedigree review. Participants were eligible if 1) there is no found BRCA mutation; 2) they have a strong family history of breast cancer; and 3) they have no personal history of cancer. A strong family history entails two breast cancers in first-degree relatives under the age of 50 or three cases of breast cancer at any age on one side of the family. Moreover, participants must not have had a bilateral mastectomy prior to enrollment.

These women come from families who initially sought genetic testing for BRCA mutations due to a strong family history of breast cancer. BRCA mutation detection in the affected relatives was performed using a range of techniques, as genetic testing methodologies have evolved over time, but all nucleotide sequences were confirmed by Sanger direct sequencing. Pedigrees from the genetic counselling clinics were reviewed retrospectively to identify families that meet the eligibility criteria. If a family was eligible, the proband (defined as the first family member to undergo genetic testing) was contacted and if interested, was invited to share the study information with her cancer-free, first-degree relatives. This study was approved by the institutional ethics review boards of the six host institutions and all study subjects provided written informed consent upon enrollment. A flow diagram of subject recruitment for the ‘Negative Study’ is shown in Figure 4.1.
Figure 4.1. Subject recruitment of the ‘Negative Study’ among participating genetics clinics across Ontario

*Eligibility Criteria
Age 18 to 65
First-degree relative with breast cancer
No BRCA mutations in affected relatives
Strong family history
No personal history of cancer
No bilateral mastectomy

Negative Study Cohort
Enrolled participants (n = 244)
Lost to follow-up (n = 5)
No longer eligible (n = 3)
Withdrew from study (n = 2)
Completed follow-up 1 (n = 168); follow-up 2 (n = 68); follow-up 3 (n = 28)
4.2 Data Collection
The ‘Negative Study’ currently has 244 participants and enrollment has been on-going since 2009. At the time of enrollment, all participants are mailed an initial study package and are instructed to sign a consent form, complete a baseline research questionnaire, complete the first Canadian version of the Diet History Questionnaire (DHQ) [222], and provide biological samples in the form of blood, urine, and toenails. In addition, participants sign a medical release form for review of any breast imaging (e.g., MRIs, mammograms), as well as pathology reports. A follow-up questionnaire is mailed biennially to participants in order to update information on their family and personal medical histories, as well as to ascertain incident cancers.

The baseline and follow-up research questionnaires collect similar information, including detailed information regarding family history, reproductive and medical histories, preventive surgery and breast screening, and lifestyle factors such as body weight, physical activity, smoking, and alcohol. Specifically, the questionnaires inquire about parity (e.g., number of live births), duration of breastfeeding, exogenous hormone use (e.g., oral contraceptives and hormone replacement therapy), current height and weight, the average number of alcoholic drinks consumed per week (0-3, 4-9, 10-20 and 20 or more), and their smoking habits (number of packs of cigarettes smoked per week). ‘Ever’ alcohol drinkers were defined as those who reported consuming at least one alcoholic beverage within the last six months.

Physical activity levels were measured using an assessment tool that was initially developed for use within the Nurses’ Health Study [223]. The physical activity questionnaire involved the completion of a checklist, where participants reported the length of time (0 to ≥11 hours) they spent engaged in various physical activities per week during the last 12 months, including walking, jogging, swimming, tennis, aerobics and weightlifting. A Metabolic Equivalent of Task (MET) score was assigned to each physical activity to describe the intensity, using established criteria by Ainsworth et al. [224]. MET values for walking were assigned based on the reported walking pace (average: 3.0 METs). Next, a total weekly activity score was calculated by summing all MET values for each participant. In order to investigate the independent effect of moderate-to-vigorous physical activity (MVPA), a second score that included only activities at the moderate and vigorous intensity levels was also created.
The physical activity portion of the questionnaires was previously validated using 325 Nurses’ Health Study participants [225]. In this study, participants first completed the physical activity questionnaire, and then completed four 1-week activity recalls and four 7-day activity diaries for over a year [225]. Afterwards, participants completed the physical activity questionnaire for a second time, yielding a correlation of 0.64 for the total physical activity score [225]. Although the rest of our research questionnaires have not been previously validated, these questionnaires were obtained and minimally adapted from an on-going international collaborative study of BRCA mutation carriers that includes more than 55 participation centres in 11 countries. Furthermore, these questionnaires have been used in over 60 studies of BRCA mutation carriers [226-234].

The collection, storage and retrieval of all data pertaining to this study is based at the primary institution (Women’s College Research Institute, Toronto, Canada) and is outlined in more detail in Kotsopoulos et al. [9]. In order to maintain the confidentiality of patient information, each participant is assigned a unique study ID upon enrollment. Names, date of birth, and other identifying information are removed from the study materials and are replaced with the study ID number in order to identify the data. All epidemiological data is entered into a Microsoft Access Database that is stored on a secure server that is only accessible to study personnel. In addition, physical copies of the research questionnaires, medical release forms, mammographic images, and other confidential study materials are locked securely in cabinets. The biological specimens are stored in -80°C freezers which are equipped with alarms and are continuously monitored for deviations in temperature. Identifying information is removed from all biological samples and is replaced with the study ID number prior to storage.

4.3 Subjects Available for Analyses

Women were eligible for inclusion in the current analyses if they were between the ages of 18 to 65 years at the time of enrollment and reported having at least one mammogram. Based on the cross-sectional approach of this study, one mammogram was requested for each participant. Mammographic images were requested by faxing a request to the appropriate hospital or imaging centre by using the authorization for medical release forms signed by the participants. Given the strong family history of disease among the study population, most participants who undergo mammography receive exams regularly (i.e., annually or biennially) and start earlier than the
general population’s recommendation of age 50 (average age of first mammogram within the cohort: 37.8 years [range: 21 to 52 years]). Therefore, it was important to carefully consider which mammogram should be requested for each participant. For all participants who reported having a mammogram, the mammogram that was performed closest to the date of their baseline research questionnaire was requested. However, some participants did not begin regular mammography screening at the time of their baseline questionnaire. Instead, these women reported undergoing their first mammogram at the time of their first follow-up questionnaire or later. For these participants, this initial mammogram was requested, and the follow-up questionnaire closest in time to this mammogram was used to ascertain risk factor information. Since the follow-up questionnaires collect similar information to the baseline questionnaire, comprehensive risk factor information was extracted for all women, regardless of which questionnaire was used.

At the time the current study was initiated, 191 participants reported having at least one mammogram. Of these women, mammographic images from 173 participants were successfully obtained from the corresponding hospitals and imaging centres. The remaining images (n = 18) were not obtained due to various reasons, such as, participants did not respond to our inquiry of their mammogram location (n = 4); the images could not be located from the reported imaging centre (n = 4); the imaging location refused to copy images (n = 1); and the participant was living abroad and logistic reasons prevented the retrieval of her images (n = 1). Moreover, three participants had undergone mammography at least eight years prior to their baseline research questionnaire and had not undergone mammography since that time. Given the extensive length of time between their previous imaging and their research questionnaires, these participants were not included in the analyses. Lastly, imaging requests for five participants are still in progress.

Of the 173 mammographic images obtained, a total of 157 images were successfully quantified and included in the analyses. A detailed description of the mammographic density quantification process is presented in the next section. Eleven of the mammographic images obtained were in a compressed format, and consequently, were not compatible with the mammographic density quantification software. The remaining five images are ready to be included in the next batch of images for quantification.
Of the 157 participants included in the analyses (97 premenopausal and 60 postmenopausal), the majority of women (n = 131) reported having a mammogram at their baseline questionnaire. The remaining 26 women reported their first mammogram after their baseline questionnaire. Consequently, the follow-up questionnaire closest in time to their initial mammogram was used (Follow-up 1: n = 17; Follow-up 2: n = 8; Follow-up 3: n = 1). A flow diagram of subject selection for the analyses is summarized in Figure 4.2.
Figure 4.2. Flow chart of subjects available for analyses

Women with a strong family history of breast cancer but no identified BRCA mutation in their affected relatives  
\[ n = 244 \]

Subjects excluded (\( n = 53 \))  
- BRCA mutation found in family (\( n = 1 \))  
- Breast cancer at baseline (\( n = 2 \))  
- No previous mammogram (\( n = 50 \))

Subjects with a previous mammogram (\( n = 191 \))

Subjects excluded (\( n = 18 \))  
- Unknown location of mammogram (\( n = 4 \))  
- Hospital could not locate images (\( n = 4 \))  
- Mammogram ≥8 years prior to first questionnaire (\( n = 3 \))  
- Requests in progress (\( n = 5 \))  
- Imaging location refused to copy images (\( n = 1 \))  
- Logistically challenging to request images (\( n = 1 \))

Mammographic images obtained (\( n = 173 \))

Subjects excluded (\( n = 16 \))  
- Images incompatible with Cumulus software (\( n = 11 \))  
- Images waiting for next read (\( n = 5 \))

Data available for analysis  
- Percent Density: \( n = 157 \)  
- Non-Dense Area: \( n = 144^1 \)  
- Dense Area: \( n = 144^1 \)

Footnote to Figure 4.2: Flow chart for eligible subjects available for analysis.

\(^1\)Due to a subset of images not having an accurate pixel size, 144 subjects were included in the non-dense area and dense area analyses.
4.4 Mammographic Density Assessment

As described in Chapter 2, Section 2.2.1.1.2, the Cumulus breast density software (version 4) (University of Toronto, Toronto, Canada) was used in order to quantify mammographic density. Cumulus is an area-based, semi-automated and computer-assisted thresholding method and is currently considered to be the gold standard for measuring mammographic density [100]. Like other quantitative methods, Cumulus has been shown to measure percent density more accurately than qualitative methods such as the Wolfe grades and the BI-RADS classification system [235].

The cranio-caudal view of the right breast of each mammographic image was used to measure percent density, dense area, and non-dense area. Since mammographic density has been shown to be strongly correlated between breasts [236], we chose to read the right breast and maintained consistency across all images. All images were de-identified and were renamed using the unique study ID belonging to each participant. Approximately 13 mammographic images were initially films. Nine of these films were digitized by the respective imaging centres. The remaining four films were digitized with a pixel spacing of 252 μm/pixel using the iCAD scanner and Fulcrum software at the Sunnybrook Health Sciences Centre. For one participant, only a mammogram of the left breast was available. For two participants, the laterality of their mammographic images was not indicated, so one cranio-caudal image was selected to be read.

The mammographic images were read in three different batches by two readers. Reader 1 (M.Y.) read the first two batches (Number of images [n] Batch 1: n = 61; Batch 2: n = 39) and Reader 2 (O.M.) read Batch 3 (n = 57). In all three batches, the images were randomized and 15% of the images were randomly repeated using a random number generating program. The two batches read by Reader 1 were read once, while the third batch read by Reader 2 was read twice with a time period of one week in between the reads. The within-person intraclass correlation coefficients (within-batch variation) of Batch 1 and Batch 2 read by Reader 1 were 0.96 and 0.98, respectively. The within-person intraclass correlation coefficients for Batch 3 read by Reader 2 were 0.78 for the first read and 0.82 for the second read (mean intraclass correlation = 0.80). Of the repeated images in Batch 3, the mean reliability among the images belonging to premenopausal women and postmenopausal women was 0.78 and 0.75, respectively. The interclass correlation coefficient (between-batch variation) for the two attempts of Batch 3 was 0.93. For all images that were read more than once (i.e., due to repeats),
the values of the measures of mammographic density were averaged and the mean value was used. A subset of the images read by Reader 1 (n = 56) were also read by Reader 2, yielding an interclass correlation coefficient of 0.86.

The dense area (cm\(^2\)) and non-dense area (cm\(^2\)) of each mammographic images were also determined since both of these measures have also been shown to be associated with breast cancer risk [33]. The pixel spacing of each image (range: 49 to 252 μm/pixel) was converted into centimetres squared (cm\(^2\)) to get the area of each pixel. Next, the dense area and the total breast area were converted into centimetres squared (cm\(^2\)) using the area of each pixel. The non-dense area was determined by subtracting the dense area by the total breast area. A subset of the images (n = 13) were in a format that would not allow for an accurate pixel size to be obtained, and consequently, these images could not be assessed for these additional measures.

4.5 Statistical Analyses

To carry out the study objectives, a cross-sectional analysis was performed. All exposures were analyzed continuously, as well as dichotomously using high vs. low categories based on the median distribution in the entire cohort, or among users for the oral contraceptive, smoking, and alcohol variables (<13 and ≥13 years for age at menarche, <11 and ≥11 months for breastfeeding, <9 and ≥9 years for duration of oral contraceptive use, <16 and ≥16 for age at first use of cigarettes, <3 and ≥3 packs for packs of cigarettes smoked per week, <14 and ≥14 years for duration of smoking, <18 and ≥18 years for age at first use of alcohol, and <2 and ≥2 drinks for number of alcoholic drinks consumed per week). For the anthropometric variables, the 75\(^{th}\) percentile was used (<170.2 and ≥170.2 cm for height and <77.1 and ≥77.1 kg for weight) because of their strong correlation with mammographic density. The mean MET-hours per week of total physical activity in the cohort was 34.6 MET-hours per week (range: 0 to 211 MET-hours per week), which is equivalent to 5.0 METs of activity per day, or about one hour of low-impact aerobics [224]. Therefore, the 75\(^{th}\) percentile for the physical activity variables were chosen (<43.1 and ≥43.1 MET-hours per week for Total Weekly Activity and <27.0 and ≥27.0 MET-hours per week for Moderate-to-Vigorous Physical Activity) in order to help maximize the investigation of high physical activity and mammographic density.
Before analyses were conducted, all mammographic density measures were graphed using histograms and were tested for normality using visual assessment of the residual plots, and using the Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling normality tests. Since percent density, dense area, and non-dense area were all found to be non-normally distributed, all values were square root-transformed to improve normality. The distributions of percent density, dense area, and non-dense area before and after square-root transformation are shown in Figure 4.3 (premenopausal women) and Figure 4.4 (postmenopausal women). Among postmenopausal women, an extremely high value for percent density (77.2%; approximately 4 standard deviations above the mean) was observed, even after applying the square-root transformation. This participant was excluded from all analyses, thus 59 postmenopausal women were included in the final analyses.

All analyses were performed in premenopausal and postmenopausal women separately because mammographic density declines after menopause [84]. Differences in descriptive characteristics were evaluated using the Student’s t-test for continuous variables and the \( \chi^2 \)–test for categorical variables. Generalized Linear Models (GLM) were used to obtain the beta-estimates, least-square adjusted means, and the 95% confidence interval (CI) of percent density, dense area, and non-dense area. The reported \( \beta \)-coefficients, least-square means, 95% confidence intervals, and \( P \) values are from regression models using square-root transformed percent density, dense area, and non-dense area values. Further, the least-square means and corresponding 95% confidence intervals of the mammographic density values were back-transformed to help with the interpretation of findings. Evaluating possible effect modification by BMI was planned \textit{a priori} for all analyses, however, due to the small sample size, there was insufficient power to do this.

Backward selection was performed \textit{a priori} in order to determine the model that best suited the data. All eligible covariates were included in the initial model, in which backward selection using the PROC GLMSELECT procedure in SAS was used to determine the most relevant covariates. This procedure systematically removes the variable that produces the largest significance level one at a time and stops once all the variables remaining in the model have a significance level below the default significance level to stay of 0.15. All covariates that were selected by the backward selection procedure, as well as any covariates that are known to be associated with mammographic density, were retained in the final model. All models adjusted for age (continuous) and BMI (continuous) at the time of the mammogram, as well as for parity
(continuous). Depending on the exposure, other covariates were included in the model. For example, the parity exposures (total live births and breastfeeding) were additionally adjusted for age at first live birth (continuous). Moreover, the height and weight models were not adjusted for BMI; instead, they were mutually adjusted for each other (continuous). The physical activity and alcohol analyses were additionally adjusted for smoking status (never, former, current). The smoking analyses were additionally adjusted for alcohol consumption (drinks per week). Single imputation was performed when data was missing, in which case, the median value of the exposure was imputed.

Some of the study subjects were related to each other, which may violate the assumption of independent observations that is associated with linear regression. Therefore, we conducted a sensitivity analysis by excluding one of the subjects in each pair of relatives if both women had the same menopausal status, in order to determine if having both relatives in the model significantly influenced the results.

All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA). All $P$ values were based on two-sided tests and were considered statistically significant if $P \leq 0.05$. 
Figure 4.3. Distribution of mammographic density measures in premenopausal women

Footnote to Figure 4.3: Untransformed percent density, dense area, non-dense area (A-C); Square-root transformed percent density, dense area, non-dense area (D-F). Curves represent the normal distribution. The sample size, mean, and standard deviation of the normal is displayed in the top-right corner of each image.
Figure 4.4. Distribution of mammographic density measures in postmenopausal women

Footnote to Figure 4.4: Untransformed percent density, dense area, non-dense area (A-C); Square-root transformed percent density, dense area, non-dense area (D-F). Curves represent the normal distribution. The sample size, mean, and standard deviation of the normal is displayed in the top-right corner of each image.
Chapter 5
Results

5.1 Subject Characteristics

A total of 156 women were included in the current study. The average percentage of mammographic density was 27.5% (range: 0.2%-79.7%) among premenopausal women and 14.0% (range: 0.7%-58.3%) among postmenopausal women. The baseline characteristics of the study sample are shown in Table 5.1 by menopausal status. On average, postmenopausal women were older in age ($P < 0.0001$), had a lower mean percent density ($P < 0.0001$), a lower mean absolute dense area ($P = 0.0001$), and a greater mean non-dense area ($P = 0.04$) compared to premenopausal women. Postmenopausal women also tended to be shorter in height ($P = 0.02$) and reported a greater mean BMI at age 18 ($P = 0.03$) and at age 30 ($P = 0.03$) compared to premenopausal women. Premenopausal and postmenopausal women were significantly different with respect to parity, as the mean number of live births was 2.3 among postmenopausal women and 1.9 among premenopausal women ($P = 0.005$). In addition, postmenopausal women tended to be younger in age at their first live birth compared to premenopausal women (25.5 years vs. 30.2 years; $P < 0.001$). Premenopausal women were more likely to breastfeed for a longer duration (15.1 months vs. 9.3 months; $P = 0.01$).

Among postmenopausal women, 49% of women reported ever smoking, compared to 34% of ever smokers among premenopausal women ($P = 0.06$). Among smokers, postmenopausal women reported smoking a higher mean number of packs of cigarettes per week compared to premenopausal women (4.2 packs vs. 2.8 packs; $P = 0.01$). Age at menarche, current BMI, nulliparity, oral contraceptive use, alcohol consumption, and physical activity levels were not significantly different between premenopausal and postmenopausal women ($P \geq 0.15$).

We also evaluated whether there were any significant differences in basic characteristics between the 157 women included in the analyses and the remaining participants enrolled in the ’Negative Study’ who were not included (n = 87). Of these 87 participants, three women were no longer
eligible for the study due to a breast cancer diagnosis at baseline (n = 2) and a BRCA mutation was identified in the family after enrollment (n = 1). Of the remaining 84 women, 83% (n = 70) were premenopausal while 17% (n = 14) were postmenopausal. Forty percent of these women had previously undergone mammography (n = 34), of which 41% (n = 14) were postmenopausal. Sixty percent (n = 50) of the women had not undergone mammography, of which all were premenopausal.

Among all premenopausal women, those who were included in the study were significantly older (43.8 years vs. 32.5 years; $P < 0.0001$) and were most likely to be parous (73% vs. 29%; $P < 0.0001$) compared to women who were not included. Among parous women, there were no significant differences between the mean number of live births between women included in the study and those who were not included (1.9 births vs. 2.1 births; $P = 0.46$). Lastly, current BMI did not significantly differ between women who were included in the study and those who were not included (25.8 kg/m$^2$ vs. 24.2 kg/m$^2$; $P = 0.07$).

Among all postmenopausal women, those who were included in the study did not significantly differ from the women who were not included by age (56.4 years vs. 55.4 years; $P = 0.54$), parity (77% vs. 79%; $P = 0.88$), or by current BMI (26.5 kg/m$^2$ vs. 25.9 kg/m$^2$; $P = 0.66$). Among parous women, those who were included in the study tended to have a greater mean number of live births compared to women who were not included (2.3 births vs. 1.6 births; $P = 0.004$).

### 5.2 Mammographic Density Measures According to Reproductive and Hormonal Exposures

#### 5.2.1 Premenopausal Women

The β-coefficients and their respective 95% CI and $P$-values for the multivariate linear regression analyses for the associations between reproductive and hormonal exposures and the three mammographic density measures among premenopausal women are shown in Table 5.2A. Table 5.2B shows the relationships of the same reproductive and hormonal exposures presented as dichotomous variables and the mammographic density measures estimated using adjusted least square means. After covariate adjustment, an increasing number of live births among parous women was associated with significantly lower percent density ($\beta = -0.66; 95\% \text{ CI} -1.31, -0.01; P = 0.05$) (Table 5.2A). Women in the highest category of parity had an adjusted mean percent density of 19.2% compared to an adjusted mean percent density of 31.4% among women.
in the lowest parity category \((P = 0.07)\) (Table 5.2B). There was also some suggestion of an inverse association between parity and dense area. Women who had two live births had a mean adjusted dense area of 24.1 cm\(^2\) compared to 38.7 cm\(^2\) among women in the lowest parity category \((P = 0.06)\) (Table 5.2B). There were no other significant associations observed \((P \geq 0.08)\) (Tables 5.2A-B).

Sensitivity analyses excluding one woman from one premenopausal sister-pair did not significantly change the results (data not shown).

### 5.2.2 Postmenopausal Women

The \(\beta\)-coefficients and their respective 95\% CI and \(P\)-values for the multivariate linear regression analyses for the associations between reproductive and hormonal exposures and the three mammographic density measures among postmenopausal women are shown in Table 5.2C. Table 5.2D shows the relationships of the same reproductive and hormonal exposures presented as dichotomous variables and the mammographic density measures estimated using adjusted least square means. Among parous women, women in the highest category of parity had an adjusted mean percent density of 10.0\% compared to 18.8\% among women in the lowest category of parity \((P = 0.06)\) (Table 5.2D). Although not significant, women in the highest category of parity had an adjusted mean dense area of 14.3 cm\(^2\) compared to 24.3 cm\(^2\) among women in the lowest category of parity \((P = 0.08)\) (Table 5.2D). Lastly, a positive association was observed among parity and non-dense area. Women in the highest category of parity had a higher adjusted mean non-dense area of 146.9 cm\(^2\) compared to 95.3 cm\(^2\) among women in the lowest category of parity \((P = 0.03)\) (Table 5.2D). There were no other significant associations observed \((P \geq 0.09)\) (Tables 5.2C-D).

Sensitivity analyses excluding one woman from each of the two postmenopausal sister-pairs did not significantly change the results (data not shown).

### 5.3 Mammographic Density Measures According to Anthropometric and Lifestyle Exposures

#### 5.3.1 Premenopausal Women

The \(\beta\)-coefficients and their respective 95\% CI and \(P\)-values for the multivariate linear regression analyses for the associations between anthropometric and lifestyle exposures and the three
mammographic density measures among premenopausal women are shown in Table 5.3A. Table 5.3B shows the relationships of the same anthropometric and lifestyle exposures presented as dichotomous variables and the mammographic density measures estimated using adjusted least square means. An increasing body weight was associated with significantly lower percent density ($\beta = -0.05; 95\% \text{ CI} -0.08, -0.03; P < 0.0001$) (Table 5.3A). Women with a high body weight had an adjusted mean percent density of 13.9\% compared to 28.4\% among women with a low body weight ($P = 0.0003$) (Table 5.3B). An increasing body weight was also associated with significantly greater non-dense area ($\beta = 0.13; 95\% \text{ CI} 0.10, 0.17; P < 0.0001$) (Table 5.3A). Women with a high body weight had an adjusted mean non-dense area of 177.9 cm$^2$ compared to 82.0 cm$^2$ among women with a low body weight ($P < 0.0001$) (Table 5.3B). An increasing height was modestly associated with greater percent density ($\beta = 0.05; 95\% \text{ CI} -0.00, 0.10; P = 0.06$), and was associated with significantly lower non-dense area ($\beta = -0.12; 95\% \text{ CI} -0.21, -0.04; P = 0.005$) (Table 5.3A).

Among women who ever smoked, an increasing age at smoking initiation was associated with greater percent density ($\beta = 0.17; 95\% \text{ CI} 0.00, 0.33; P = 0.05$) and greater dense area ($\beta = 0.32; 95\% \text{ CI} 0.15, 0.49; P = 0.0001$) (Table 5.3A). Women who started smoking at age 16 or later had a higher adjusted mean dense area of 33.3 cm$^2$ compared to 18.6 cm$^2$ among women who started smoking earlier ($P = 0.06$) (Table 5.3B). An increasing number of packs of cigarettes smoked per week was associated with significantly lower dense area ($\beta = -0.40; 95\% \text{ CI} -0.77, -0.03; P = 0.04$) (Table 5.3A). Women who reported smoking at least three packs of cigarettes per week had lower adjusted mean percent density (17.4\% vs. 27.6\%; $P = 0.07$) and lower adjusted mean dense area (16.5 cm$^2$ vs. 37.3 cm$^2$; $P = 0.01$) compared to women who smoked less packs per week (Table 5.3B). An increasing duration of smoking was associated with significantly lower percent density ($\beta = -0.08; 95\% \text{ CI} -0.13, -0.02; P = 0.01$) and lower dense area ($\beta = -0.13; 95\% \text{ CI} -0.19, -0.07; P < 0.0001$) (Table 5.3A). Women who smoked for at least 14 years had significantly lower adjusted mean percent density (15.8\% vs. 32.9\%; $P = 0.01$) and lower adjusted mean dense area (17.0 cm$^2$ vs. 43.9 cm$^2$; $P = 0.001$) compared to women who smoked for a shorter duration (Table 5.3B).

Alcohol user status (never/former/current) was not associated with the three measures of mammographic density ($P \geq 0.36$) (Tables 5.3A-B). An increasing age of first use of alcohol was associated with significantly higher percent density ($\beta = 0.15; 95\% \text{ CI} 0.4, 3.0; P = 0.03$).
and dense area ($\beta = 0.20; 95\% \text{ CI } 0.04, 0.36; P = 0.01$) (**Table 5.3A**). Women who started using alcohol at age 18 had a lower adjusted mean non-dense area of 95.6 cm$^2$ compared to 124.5 cm$^2$ among women started using alcohol earlier ($P = 0.04$) (**Table 5.3B**).

Physical activity was not significantly associated with the mammographic density measures ($P \geq 0.44$) (**Tables 5.3A-B**).

Sensitivity analyses excluding one woman from one premenopausal sister-pair did not significantly change the results (data not shown).

### 5.3.2 Postmenopausal Women

The $\beta$-coefficients and their respective 95% CI and $P$-values for the multivariate linear regression analyses for the associations between anthropometric and lifestyle exposures and the three mammographic density measures among postmenopausal women are shown in **Table 5.3C**.

**Table 5.3D** shows the relationships of the same anthropometric and lifestyle exposures presented as dichotomous variables and the mammographic density measures estimated using adjusted least square means. An increasing body weight was associated with significantly lower percent density ($\beta = -0.04; 95\% \text{ CI } -0.07, -0.02; P = 0.002$) and significantly higher non-dense area ($\beta = 0.13; 95\% \text{ CI } 0.08, 0.18; P < 0.0001$) (**Table 5.3C**). Women with a high body weight had a lower adjusted mean percent density (8.0% vs. 13.6%; $P = 0.04$) and higher non-dense area (186.3 cm$^2$ vs. 118.9 cm$^2$; $P = 0.0005$) compared to women with a low body weight (**Table 5.3D**). Although not significant, an increasing height was associated with lower non-dense area ($\beta = -0.09; 95\% \text{ CI } -0.20, 0.02; P = 0.09$) (**Table 5.3C**). When the relationship of height and non-dense area was evaluated using adjusted least square means, women with greater height were found to have a significantly lower adjusted mean non-dense area of 106.3 cm$^2$ compared to 144.6 cm$^2$ among women with a lower height ($P = 0.02$) (**Table 5.3D**).

Women who formerly smoked had significantly higher percent density ($\beta = 1.13; 95\% \text{ CI } 0.41, 1.84; P = 0.003$), higher dense area ($\beta = 1.12; 95\% \text{ CI } 0.24, 1.99; P = 0.01$), and lower non-dense area ($\beta = 1.35; 95\% \text{ CI } -2.66, -0.03; P = 0.05$) compared to women who never smoked (**Table 5.3C-D**). When smoking status was evaluated using adjusted least square means, former smokers had significantly higher adjusted mean percent density (17.3% vs. 9.2%; $P = 0.003$) and adjusted mean dense area (22.8 cm$^2$ vs. 13.4 cm$^2$; $P = 0.01$), and significantly lower non-dense
area (116.8 cm² vs. 147.7 cm²; $P = 0.05$) compared to never smokers (Table 5.3D). Current smokers did not have significantly different measures of mammographic density measures compared to never smokers ($P \geq 0.51$) (Table 5.3C-D).

Alcohol user status was not associated with the measures of mammographic density ($P \geq 0.32$) (Table 5.3C). Among alcohol users, an increasing age of first use of alcohol was associated with higher percent density ($\beta = 0.12; 95\% \text{ CI} 0.04, 0.19; P = 0.003$) and higher dense area ($\beta = 0.17; 95\% \text{ CI} 0.08, 0.25; P = 0.0002$) (Table 5.3C).

Increasing levels of total weekly activity were associated with higher non-dense area ($\beta = 0.02; 95\% \text{ CI} 0.00, 0.03; P = 0.05$) (Table 5.3C). Moderate-to-vigorous physical activity was not significantly associated with the mammographic density measures ($P \geq 0.10$) (Tables 5.3C-D).

Sensitivity analyses excluding one woman from each of the two postmenopausal sister-pairs did not significantly change the results (data not shown).
### Table 5.1. Characteristics at baseline of study population stratified by menopausal status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Premenopausal Women N = 97</th>
<th>Postmenopausal Women N = 59</th>
<th>( p^i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at questionnaire, mean (range)</td>
<td>43.3 (27.0-58.3)</td>
<td>56.6 (43.2-68.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at mammogram, mean (range)</td>
<td>43.9 (29.2-59.0)</td>
<td>56.5 (41.8-68.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Years between questionnaire and mammogram, mean (range)</td>
<td>0.9 (0-5.6)</td>
<td>0.6 (0-2.0)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Percent Density (%)</strong>, mean (range)</td>
<td>27.5 (0.2-79.7)</td>
<td>14.0 (0.7-58.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Dense Area (cm(^2))</strong>, mean (range)</td>
<td>33.2 (0.6-152.8)</td>
<td>18.4 (1.6-111.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Non-Dense Area (cm(^2))</strong>, mean (range)</td>
<td>116.1 (16.6-496.1)</td>
<td>143.0 (50.7-348.5)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Height (cm)</strong>, mean (range)</td>
<td>166.5 (149.9-188.0)</td>
<td>164.0 (152.0-175.3)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>BMI (kg/m(^2))</strong>, mean (range)</td>
<td>Current 25.8 (18.0-48.0)</td>
<td>26.7 (18.4-42.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>At 18 years 21.4 (15.7-44.3)</td>
<td>20.4 (16.9-26.6)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>At 30 years 23.7 (18.0-46.3)</td>
<td>22.3 (17.7-34.9)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>At 40 years 24.3 (18.8-42.9)</td>
<td>24.2 (17.7-36.8)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Greater BMI 27.2 (18.3-49.6)</td>
<td>28.5 (20.6-42.0)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Age at menarche, mean (range)</td>
<td>12.8 (8.0-16.0)</td>
<td>13.1 (11.0-16.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>Parity(^2), n (%)</td>
<td>71 (73)</td>
<td>46 (77)</td>
<td></td>
</tr>
<tr>
<td>Nulliparous 26 (27)</td>
<td>14 (23)</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>1 21 (30)</td>
<td>6 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 35 (49)</td>
<td>22 (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3 15 (21)</td>
<td>18 (39)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Mean (range) 1.9 (1-3)</td>
<td>2.3 (1-4)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Age at first birth, mean (range)</td>
<td>30.2 (18.0-42.0)</td>
<td>25.5 (15.0-37.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breastfeeding, mean months (range)(^i)</td>
<td>15.1 (0-87)</td>
<td>9.3 (0-39)</td>
<td>0.01</td>
</tr>
<tr>
<td>Age at menopause,(^4), mean (range)</td>
<td>N/A</td>
<td>46.4 (26-56)</td>
<td>N/A</td>
</tr>
<tr>
<td>Hormone therapy use, n (%)(^4)</td>
<td>Never</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>N/A</td>
<td>43 (73)</td>
<td></td>
</tr>
<tr>
<td>Years of HRT use, mean (range)</td>
<td>6.8 (0.3-23.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive use, n (%)</td>
<td>Never</td>
<td>10 (10)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Ever</td>
<td>87 (90)</td>
<td>53 (91)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Years of OC use, mean (range)</td>
<td>9.6 (0.5-34.0)</td>
<td>9.6 (0.08-30.0)</td>
<td>0.92</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>Never</td>
<td>4 (4)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Ever</td>
<td>93 (96)</td>
<td>55 (92)</td>
<td>0.68</td>
</tr>
<tr>
<td>Current</td>
<td>73 (79)</td>
<td>46 (84)</td>
<td>0.87</td>
</tr>
<tr>
<td>Drinks consumed per week, mean (range)</td>
<td>3.9 (2-15)</td>
<td>4.7 (2-20)</td>
<td>0.15</td>
</tr>
</tbody>
</table>
### Table 5.1, continued

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Premenopausal Women N = 97</th>
<th>Postmenopausal Women N = 59</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>64 (66)</td>
<td>30 (51)</td>
<td>ref</td>
</tr>
<tr>
<td>Ever</td>
<td>33 (34)</td>
<td>29 (49)</td>
<td>0.06</td>
</tr>
<tr>
<td>Current</td>
<td>7 (20)</td>
<td>7 (22)</td>
<td>0.17</td>
</tr>
<tr>
<td>Number of packs smoked per week, mean (range)</td>
<td>2.8 (1-7)</td>
<td>4.2 (1-9)</td>
<td>0.01</td>
</tr>
<tr>
<td>Physical Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Across the lifespan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET-hours per week grade 7-8, mean (range)</td>
<td>56.3 (7.3-140.8)</td>
<td>54.8 (10.3-152.0)</td>
<td>0.82</td>
</tr>
<tr>
<td>MET-hours per week grade 9-12, mean (range)</td>
<td>58.0 (10.3-148.3)</td>
<td>53.6 (10.3-159.5)</td>
<td>0.52</td>
</tr>
<tr>
<td>MET-hours per week ages 18-22, mean (range)</td>
<td>54.3 (11.8-127.3)</td>
<td>44.5 (7.3-130.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>MET-hours per week ages 23-29, mean (range)</td>
<td>52.3 (7.3-118.3)</td>
<td>48.1 (17.3-143.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>MET-hours per week ages 30-34, mean (range)</td>
<td>49.7 (10.3-148.3)</td>
<td>50.6 (14.8-143.0)</td>
<td>0.89</td>
</tr>
<tr>
<td>Within the last year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total physical activity MET-hours per week, mean (range)</td>
<td>36.2 (0-162.6)</td>
<td>30.5 (0-211.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>MVPA MET-hours per week, mean (range)</td>
<td>23.3 (0-147.6)</td>
<td>19.1 (0-141.0)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*P* values were calculated using the Student’s *t*-test for continuous variables and the chi-square test for categorical variables.

1Parity includes live births only.
3Among parous women only.
4Among postmenopausal women only.
Metabolic Equivalent of Task. Includes walking and moderate and vigorous physical activity.
6Moderate to vigorous physical activity.
7Fisher’s exact test was used instead of the chi-square test when the expected number of observations were <5
Table 5.2A. Difference in mammographic density measures according to reproductive and hormonal exposures among premenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Percent Density (%)</th>
<th>Dense Area (cm²)</th>
<th>Non-Dense Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>β-Estimate¹ (95% CI)</td>
<td>P²</td>
</tr>
<tr>
<td>Age at menarche, years</td>
<td>97</td>
<td>-0.05 (-0.33, 0.22)</td>
<td>0.69</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>26</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Parous</td>
<td>71</td>
<td>-0.35 (-1.15, 0.44)</td>
<td>0.38</td>
</tr>
<tr>
<td>Total live births³</td>
<td>71</td>
<td>-0.66 (-1.31, -0.01)</td>
<td>0.05</td>
</tr>
<tr>
<td>Breastfeeding³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>4</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>66</td>
<td>-0.16 (-2.22, 1.90)</td>
<td>0.85</td>
</tr>
<tr>
<td>Breastfeeding duration, months³</td>
<td>71</td>
<td>0.00 (-0.03, 0.04)</td>
<td>0.92</td>
</tr>
<tr>
<td>OC use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>87</td>
<td>-0.2 (-1.35, 0.96)</td>
<td>0.73</td>
</tr>
<tr>
<td>Duration of OC use, years</td>
<td>87</td>
<td>-0.03 (-0.09, 0.02)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

¹β-Estimates and 95% confidence intervals are from analyses using untransformed mammographic density measures.
²P-values are from analyses using square root-transformed mammographic density measures.
³Among parous women only.
All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The parity (total live births) and breastfeeding models were additionally adjusted for age at first birth (continuous). OC, oral contraceptives.
Table 5.2B. Adjusted mean mammographic density measures according to reproductive and hormonal exposures among premenopausal women

<table>
<thead>
<tr>
<th>Age at menarche</th>
<th>Percent Density (%)</th>
<th>Dense Area (cm²)</th>
<th>Non-Dense Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean¹ PD</td>
<td>95% CI</td>
</tr>
<tr>
<td>&lt;13 years</td>
<td>32</td>
<td>25.2</td>
<td>19.4-31.8</td>
</tr>
<tr>
<td>≥13 years</td>
<td>65</td>
<td>23.4</td>
<td>19.4-27.7</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>26</td>
<td>26.6</td>
<td>20.1-34.1</td>
</tr>
<tr>
<td>Parous</td>
<td>71</td>
<td>23.1</td>
<td>19.3-27.2</td>
</tr>
<tr>
<td>Total live births³</td>
<td>1</td>
<td>31.4</td>
<td>22.9-41.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20.2</td>
<td>14.9-26.3</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>19.2</td>
<td>11.5-28.9</td>
</tr>
<tr>
<td>Breastfeeding³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>4</td>
<td>24.8</td>
<td>9.1-48.3</td>
</tr>
<tr>
<td>Ever</td>
<td>66</td>
<td>22.9</td>
<td>18.8-27.5</td>
</tr>
<tr>
<td>Breastfeeding duration³</td>
<td>&lt;11 months</td>
<td>30</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>≥11 months</td>
<td>40</td>
<td>23.7</td>
</tr>
<tr>
<td>OC use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10</td>
<td>25.7</td>
<td>15.9-38.0</td>
</tr>
<tr>
<td>Ever</td>
<td>87</td>
<td>23.8</td>
<td>20.3-27.5</td>
</tr>
<tr>
<td>Duration of OC use</td>
<td>&lt;9 years</td>
<td>40</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>≥9 years</td>
<td>47</td>
<td>22.1</td>
</tr>
</tbody>
</table>

¹Adjusted least-square means and 95% confidence intervals are from analyses using untransformed mammographic density measures.  
²P-values are from analyses using square root-transformed mammographic density measures.  
³Among parous women only.  
All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The parity (total live births) and breastfeeding models were additionally adjusted for age at first birth (continuous). OC, oral contraceptives.
| Table 5.2C. Difference in mammographic density measures according to reproductive and hormonal exposures among postmenopausal women |
|---|---|---|---|---|---|---|
| | Percent Density (%) | Dense Area (cm²) | Non-Dense Area (cm²) |
| | n | β-Estimate¹ (95% CI) | P² | n | β-Estimate¹ (95% CI) | P² | n | β-Estimate¹ (95% CI) | P² |
| Age at menarche, years | 59 | 0.02 (-0.24, 0.29) | 0.86 | 55 | 0.08 (-0.23, 0.40) | 0.59 | 55 | -0.02 (-0.48, 0.44) | 0.93 |
| Parity | | | | | | | | | |
| Nulliparous | 13 | ref | 11 | ref | 11 | ref |
| Parous | 46 | -0.50 (-1.34, 0.33) | 0.23 | 44 | -0.65 (-1.66, 0.37) | 0.21 | 44 | 0.41 (-1.09, 1.91) | 0.58 |
| Total live births³ | 46 | -0.32 (-0.84, 0.21) | 0.23 | 44 | -0.30 (-0.84, 0.24) | 0.27 | 44 | 0.55 (-0.34, 1.44) | 0.22 |
| Breastfeeding³ | | | | | | | | | |
| Never | 10 | ref | 10 | ref | 10 | ref |
| Ever | 36 | 0.06 (-1.02, 1.14) | 0.91 | 34 | -0.51 (-1.62, 0.61) | 0.37 | 34 | -1.07 (-2.9, 0.75) | 0.24 |
| Breastfeeding duration, months³ | 46 | 0.02 (-0.03, 0.07) | 0.44 | 44 | 0.02 (-0.03, 0.08) | 0.42 | 44 | -0.04 (-0.13, 0.05) | 0.35 |
| OC use | | | | | | | | | |
| Never | 5 | ref | 4 | ref | 4 | ref |
| Ever | 53 | 0.78 (-0.44, 2.01) | 0.20 | 50 | 0.47 (-1.13, 2.07) | 0.56 | 50 | -0.90 (-3.19, 1.40) | 0.44 |
| Duration of OC use, years | 53 | -0.01 (-0.06, 0.04) | 0.60 | 50 | 0.01 (-0.05, 0.08) | 0.70 | 50 | 0.04 (-0.05, 0.13) | 0.43 |
| HRT use | | | | | | | | | |
| Former | 43 | ref | 39 | ref | 39 | ref |
| Current | 7 | 0.27 (-0.93, 1.47) | 0.66 | 7 | 0.34 (-1.04, 1.72) | 0.62 | 7 | -0.32 (-2.29, 1.65) | 0.75 |

¹β-Estimates and 95% confidence intervals are from analyses using untransformed mammographic density measures.
²P-values are from analyses using square root-transformed mammographic density measures.
³Among parous women only.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The parity (total live births) and breastfeeding models were additionally adjusted for age at first birth (continuous). OC, oral contraceptives. HRT, hormone replacement therapy.
Table 5.2D. Adjusted mean mammographic density measures according to reproductive and hormonal exposures among postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Postmenopausal women (n = 59)</th>
<th>Non-Dense Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent Density (%)</td>
<td>Dense Area (cm²)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean¹ PD</td>
</tr>
<tr>
<td>Age at menarche</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;13 years</td>
<td>21</td>
<td>12.3</td>
</tr>
<tr>
<td>≥13 years</td>
<td>38</td>
<td>11.7</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>13</td>
<td>14.8</td>
</tr>
<tr>
<td>Parous</td>
<td>46</td>
<td>11.1</td>
</tr>
<tr>
<td>Total live births³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>18.8</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>10.3</td>
</tr>
<tr>
<td>≥3</td>
<td>18</td>
<td>10.0</td>
</tr>
<tr>
<td>Breastfeeding³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10</td>
<td>10.8</td>
</tr>
<tr>
<td>Ever</td>
<td>35</td>
<td>11.2</td>
</tr>
<tr>
<td>Breastfeeding duration³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11 months</td>
<td>26</td>
<td>10.0</td>
</tr>
<tr>
<td>≥11 months</td>
<td>20</td>
<td>12.7</td>
</tr>
<tr>
<td>OC use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>5</td>
<td>7.4</td>
</tr>
<tr>
<td>Ever</td>
<td>53</td>
<td>12.2</td>
</tr>
<tr>
<td>Duration of OC use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;9 years</td>
<td>26</td>
<td>13.3</td>
</tr>
<tr>
<td>≥9 years</td>
<td>27</td>
<td>11.6</td>
</tr>
<tr>
<td>HRT use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>43</td>
<td>11.6</td>
</tr>
<tr>
<td>Former</td>
<td>9</td>
<td>12.3</td>
</tr>
<tr>
<td>Current</td>
<td>7</td>
<td>13.5</td>
</tr>
</tbody>
</table>

¹Adjusted least-square means and 95% confidence intervals are from analyses using untransformed mammographic density measures.
²P-values are from analyses using square root-transformed mammographic density measures.
³Among parous women only.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The parity (total live births) and breastfeeding models were additionally adjusted for age at first birth (continuous). OC, oral contraceptives. HRT, hormone replacement therapy.
Table 5.3A. Difference in mammographic density measures according to anthropometric and lifestyle factors among premenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal women ($n = 97$)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$\beta$-Estimate ($95%\ CI$)</td>
<td>$P^2$</td>
<td>$n$</td>
<td>$\beta$-Estimate ($95%\ CI$)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>97</td>
<td>-0.05 (-0.08, -0.03)</td>
<td>&lt;0.0001</td>
<td>88</td>
<td>-0.01 (-0.04, 0.01)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>97</td>
<td>0.05 (0.00, 0.10)</td>
<td>0.06</td>
<td>88</td>
<td>0.02 (-0.04, 0.09)</td>
</tr>
<tr>
<td>TWA (MET-hrs/week)</td>
<td>97</td>
<td>0.00 (-0.01, 0.01)</td>
<td>0.98</td>
<td>88</td>
<td>0.00 (-0.01, 0.02)</td>
</tr>
<tr>
<td>MVPA (MET-hrs/week)</td>
<td>97</td>
<td>0.00 (-0.02, 0.01)</td>
<td>0.80</td>
<td>88</td>
<td>0.00 (-0.02, 0.02)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>64</td>
<td>ref</td>
<td>ref</td>
<td>58</td>
<td>ref</td>
</tr>
<tr>
<td>Former</td>
<td>26</td>
<td>0.03 (-0.79, 0.84)</td>
<td>0.95</td>
<td>23</td>
<td>0.24 (-0.82, 1.30)</td>
</tr>
<tr>
<td>Current</td>
<td>7</td>
<td>-1.20 (-2.66, 0.26)</td>
<td>0.11</td>
<td>7</td>
<td>-1.43 (-3.27, 0.42)</td>
</tr>
<tr>
<td>Age at first use</td>
<td>33</td>
<td>0.17 (0.00, 0.33)</td>
<td>0.05</td>
<td>30</td>
<td>0.32 (0.15, 0.49)</td>
</tr>
<tr>
<td>Packs smoked per week</td>
<td>33</td>
<td>-0.21 (-0.50, 0.09)</td>
<td>0.16</td>
<td>30</td>
<td>-0.40 (-0.77, -0.03)</td>
</tr>
<tr>
<td>Duration of use (years)</td>
<td>33</td>
<td>-0.08 (-0.13, -0.02)</td>
<td>0.01</td>
<td>30</td>
<td>-0.13 (-0.19, -0.07)</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>10</td>
<td>ref</td>
<td>ref</td>
<td>10</td>
<td>ref</td>
</tr>
<tr>
<td>Former</td>
<td>15</td>
<td>-0.07 (-1.49, 1.35)</td>
<td>0.92</td>
<td>15</td>
<td>-0.51 (-2.33, 1.32)</td>
</tr>
<tr>
<td>Current</td>
<td>72</td>
<td>-0.26 (-1.43, 0.91)</td>
<td>0.66</td>
<td>72</td>
<td>-0.36 (-1.91, 1.20)</td>
</tr>
<tr>
<td>Age at first use</td>
<td>87</td>
<td>0.15 (0.01, 0.28)</td>
<td>0.03</td>
<td>79</td>
<td>0.20 (0.04, 0.36)</td>
</tr>
<tr>
<td>Drinks per week</td>
<td>87</td>
<td>0.02 (-0.10, 0.14)</td>
<td>0.56</td>
<td>79</td>
<td>0.02 (-0.12, 0.16)</td>
</tr>
</tbody>
</table>

$^1$-$\beta$-Estimates and 95% confidence intervals are from analyses using untransformed mammographic density measures.

$^2$-$P$-values are from analyses using square root-transformed mammographic density measures.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The height and weight models were mutually adjusted for each other (continuous) instead of BMI. The physical activity models were additionally adjusted for smoking status (never, former, current). The smoking models were additionally adjusted for the number of alcoholic drinks consumed per week (continuous). The alcohol models were additionally adjusted for smoking status (never/ever). TWA, total weekly activity. METs, metabolic task equivalent. MVPA, moderate to vigorous physical activity.
Table 5.3B. Adjusted mean mammographic density measures according to anthropometric and lifestyle factors among premenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal women (n = 97)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent Density (%)</td>
<td>Dense Area (cm²)</td>
<td>Non-Dense Area (cm²)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Mean¹ PD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;77.0 kg</td>
<td>71</td>
<td>28.4</td>
<td>24.0-33.1</td>
</tr>
<tr>
<td>≥77.0 kg</td>
<td>26</td>
<td>13.9</td>
<td>9.0-19.8</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;170 cm</td>
<td>69</td>
<td>22.2</td>
<td>18.4-26.3</td>
</tr>
<tr>
<td>≥170 cm</td>
<td>28</td>
<td>28.7</td>
<td>21.9-36.3</td>
</tr>
<tr>
<td>TWA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;43.0 MET-hrs/week</td>
<td>69</td>
<td>21.8</td>
<td>16.7-27.5</td>
</tr>
<tr>
<td>≥43.0 MET-hrs/week</td>
<td>28</td>
<td>20.6</td>
<td>14.3-28.1</td>
</tr>
<tr>
<td>MVPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;27.0 METs/week</td>
<td>72</td>
<td>21.9</td>
<td>17.1-27.3</td>
</tr>
<tr>
<td>≥27.0 METs/week</td>
<td>25</td>
<td>19.5</td>
<td>12.9-27.5</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Never</td>
<td>64</td>
<td>24.8</td>
<td>20.7-29.3</td>
</tr>
<tr>
<td>Former</td>
<td>26</td>
<td>25.0</td>
<td>18.7-32.2</td>
</tr>
<tr>
<td>Current smoker</td>
<td>7</td>
<td>14.3</td>
<td>5.8-26.5</td>
</tr>
<tr>
<td>Age at first use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;16 years</td>
<td>10</td>
<td>17.1</td>
<td>9.7-26.5</td>
</tr>
<tr>
<td>≥16 years</td>
<td>23</td>
<td>26.3</td>
<td>20.0-33.4</td>
</tr>
<tr>
<td>Packs smoked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 packs/week</td>
<td>20</td>
<td>27.6</td>
<td>20.7-35.5</td>
</tr>
<tr>
<td>≥3 packs/week</td>
<td>13</td>
<td>17.4</td>
<td>10.7-25.6</td>
</tr>
<tr>
<td>Duration of use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;14 years</td>
<td>16</td>
<td>32.9</td>
<td>24.6-42.4</td>
</tr>
<tr>
<td>≥14 years</td>
<td>17</td>
<td>15.8</td>
<td>10.4-22.3</td>
</tr>
</tbody>
</table>
Table 5.3B continued

<table>
<thead>
<tr>
<th>Alcohol use</th>
<th>Percent Density (%)</th>
<th>Dense Area (cm²)</th>
<th>Non-Dense Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean PD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Never</td>
<td>10</td>
<td>25.8</td>
<td>15.8-38.2</td>
</tr>
<tr>
<td>Former</td>
<td>15</td>
<td>25.1</td>
<td>16.8-35.1</td>
</tr>
<tr>
<td>Current user</td>
<td>72</td>
<td>23.2</td>
<td>19.3-27.5</td>
</tr>
</tbody>
</table>

| Age at first use    |                  |                  |                  |
|<18 years           | 29  | 20.1    | 14.7-26.3    |      | 27  | 25.4    | 18.1-34.0 |      | 27  | 124.5    | 101.5-149.9 | 0.77 |
|≥18 years           | 58  | 26.0    | 21.4-31.0   | 0.13 | 52  | 30.5    | 24.3-37.4 | 0.33 | 52  | 95.6     | 80.3-112.1 | 0.04 |

| Drinks consumed     |                  |                  |                  |
|<2 drinks/week       | 60  | 23.0    | 18.5-27.9   |     | 57  | 28.3    | 22.3-35.0 |     | 57  | 103.1    | 86.9-120.8 |     |
|≥2 drinks/week       | 27  | 25.5    | 19.1-32.9   | 0.55 | 22  | 29.4    | 20.6-39.8 | 0.85 | 22  | 110.5    | 85.9-138.1 | 0.64 |

1 Adjusted least-square means and 95% confidence intervals are from analyses using untransformed mammographic density measures.
2 P-values are from analyses using square root-transformed mammographic density measures.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The height and weight models were mutually adjusted for each other (continuous) instead of BMI. The physical activity models were additionally adjusted for smoking status (never, former, current). The smoking models were additionally adjusted for the number of alcoholic drinks consumed per week (continuous). The alcohol models were additionally adjusted for smoking status (never/ever). TWA, total weekly activity. MVPA, moderate-to-vigorous physical activity. METs, Metabolic Equivalent of Task.
Table 5.3C. Difference in mammographic density measures according to anthropometric and lifestyle factors among postmenopausal women

<table>
<thead>
<tr>
<th>Percent Density (%)</th>
<th>Dense Area (cm²)</th>
<th>Non-Dense Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>β-Estimate¹ (95% CI)</td>
<td>P²</td>
</tr>
<tr>
<td>------</td>
<td>-------------------</td>
<td>-----</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59</td>
<td>-0.04 (-0.07, -0.02)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>59</td>
<td>0.00 (-0.06, 0.06)</td>
</tr>
<tr>
<td>TWA (MET-hrs/week)</td>
<td>59</td>
<td>0.00 (-0.01, 0.01)</td>
</tr>
<tr>
<td>MVPA (MET-hrs/week)</td>
<td>59</td>
<td>0.00 (-0.01, 0.01)</td>
</tr>
</tbody>
</table>

Smoking

<p>| | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>30</td>
<td>ref</td>
<td>ref</td>
<td>29</td>
<td>ref</td>
<td>ref</td>
<td>29</td>
<td>ref</td>
</tr>
<tr>
<td>Former</td>
<td>22</td>
<td>1.13 (0.41, 1.84)</td>
<td>0.003</td>
<td>20</td>
<td>1.12 (0.24, 1.99)</td>
<td>0.01</td>
<td>20</td>
<td>-1.35 (-2.66, -0.03)</td>
</tr>
<tr>
<td>Current</td>
<td>7</td>
<td>-0.06 (-1.10, 0.98)</td>
<td>0.91</td>
<td>6</td>
<td>-0.42 (-1.70, 0.86)</td>
<td>0.51</td>
<td>6</td>
<td>-0.10 (-2.02, 1.82)</td>
</tr>
<tr>
<td>Age at first use</td>
<td>29</td>
<td>0.14 (-0.11, 0.39)</td>
<td>0.25</td>
<td>26</td>
<td>0.17 (-0.14, 0.48)</td>
<td>0.28</td>
<td>26</td>
<td>0.00 (-0.46, 0.46)</td>
</tr>
<tr>
<td>Packs smoked per week</td>
<td>29</td>
<td>0.11 (-0.18, 0.40)</td>
<td>0.44</td>
<td>26</td>
<td>0.02 (-0.37, 0.42)</td>
<td>0.90</td>
<td>26</td>
<td>-0.21 (-0.77, 0.35)</td>
</tr>
<tr>
<td>Duration of use (years)</td>
<td>29</td>
<td>0.01 (-0.03, 0.05)</td>
<td>0.55</td>
<td>26</td>
<td>0.01 (-0.04, 0.06)</td>
<td>0.77</td>
<td>26</td>
<td>-0.05 (-0.12, 0.02)</td>
</tr>
</tbody>
</table>

Alcohol

<p>| | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>5</td>
<td>ref</td>
<td>ref</td>
<td>4</td>
<td>ref</td>
<td>ref</td>
<td>4</td>
<td>ref</td>
</tr>
<tr>
<td>Former</td>
<td>8</td>
<td>-0.03 (-1.61, 1.54)</td>
<td>0.97</td>
<td>8</td>
<td>-0.12 (-2.02, 1.79)</td>
<td>0.90</td>
<td>8</td>
<td>0.37 (-2.37, 3.10)</td>
</tr>
<tr>
<td>Current</td>
<td>46</td>
<td>-0.28 (-1.51, 0.95)</td>
<td>0.65</td>
<td>43</td>
<td>-0.24 (-1.85, 1.36)</td>
<td>0.76</td>
<td>43</td>
<td>1.16 (-1.15, 3.46)</td>
</tr>
<tr>
<td>Age at first use</td>
<td>54</td>
<td>0.12 (0.04, 0.19)</td>
<td>0.003</td>
<td>51</td>
<td>0.17 (0.08, 0.25)</td>
<td>0.0002</td>
<td>51</td>
<td>-0.08 (-0.22, 0.06)</td>
</tr>
<tr>
<td>Drinks per week</td>
<td>54</td>
<td>0.01 (-0.06, 0.09)</td>
<td>0.72</td>
<td>51</td>
<td>0.04 (-0.05, 0.13)</td>
<td>0.37</td>
<td>51</td>
<td>0.06 (-0.07, 0.19)</td>
</tr>
</tbody>
</table>

¹β-Estimates and 95% confidence intervals are from analyses using untransformed mammographic density measures.
²P-values are from analyses using square root-transformed mammographic density measures.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The height and weight models were mutually adjusted for each other (continuous) instead of BMI. The physical activity models were additionally adjusted for smoking status (never, former, current). The smoking models were additionally adjusted for the number of alcoholic drinks consumed per week (continuous). The alcohol models were additionally adjusted for smoking status (never/ever). TWA, total weekly activity. MVPA, moderate-to-vigorous physical activity. METs, Metabolic Equivalent of Task.
Table 5.3D. Adjusted mean mammographic density measures according to anthropometric and lifestyle factors among postmenopausal women

<table>
<thead>
<tr>
<th>Weight</th>
<th>n</th>
<th>Mean(^1) PD</th>
<th>95% CI</th>
<th>P</th>
<th>n</th>
<th>Mean(^1) DA</th>
<th>95% CI</th>
<th>P</th>
<th>n</th>
<th>Mean(^1) NDA</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;77.0 kg</td>
<td>43</td>
<td>13.6</td>
<td>10.6-16.8</td>
<td>0.04</td>
<td>40</td>
<td>16.2</td>
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<td>13.3-20.7</td>
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<td>45</td>
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<td>11.4-19.2</td>
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<td>116.7-150.4</td>
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<td>10.3-17.9</td>
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<td>0.19</td>
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<td>20.0</td>
<td>13.3-28.1</td>
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<td>20</td>
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<td>17.0-29.5</td>
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<td>20</td>
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<td>4.2-15.3</td>
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<td>6</td>
<td>10.5</td>
<td>4.4-19.3</td>
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<td>6</td>
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<td>15.3</td>
<td>7.3-26.1</td>
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<td>92.1-172.3</td>
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<td>0.27</td>
<td>14</td>
<td>22.1</td>
<td>13.0-33.6</td>
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<tr>
<td>&lt;3 packs per week</td>
<td>8</td>
<td>10.0</td>
<td>4.3-18.0</td>
<td>ref</td>
<td>7</td>
<td>16.8</td>
<td>7.2-30.4</td>
<td>ref</td>
<td>7</td>
<td>162.6</td>
<td>116.2-216.7</td>
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<td>11.0-21.5</td>
<td>0.20</td>
<td>19</td>
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<td>12.7-27.8</td>
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<td>19</td>
<td>127.8</td>
<td>102.4-156.0</td>
<td>0.21</td>
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<td>Duration of use</td>
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<tr>
<td>&lt;14 years</td>
<td>13</td>
<td>13.4</td>
<td>7.7-20.7</td>
<td>0.78</td>
<td>11</td>
<td>18.2</td>
<td>9.7-29.3</td>
<td>0.78</td>
<td>11</td>
<td>156.1</td>
<td>118.9-198.4</td>
<td>0.21</td>
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<td>≥14 years</td>
<td>16</td>
<td>14.6</td>
<td>9.2-21.3</td>
<td>0.78</td>
<td>15</td>
<td>19.3</td>
<td>11.7-28.8</td>
<td>0.78</td>
<td>15</td>
<td>123.4</td>
<td>95.2-155.2</td>
<td>0.19</td>
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Table 5.3D continued

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<th>Alcohol use</th>
<th>Percent Density (%)</th>
<th>Dense Area (cm²)</th>
<th>Non-Dense Area (cm²)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean¹ PD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Never</td>
<td>5</td>
<td>13.6</td>
<td>6.3-23.5</td>
</tr>
<tr>
<td>Former</td>
<td>8</td>
<td>13.3</td>
<td>6.9-21.8</td>
</tr>
<tr>
<td>Current user</td>
<td>46</td>
<td>11.5</td>
<td>9.1-14.3</td>
</tr>
<tr>
<td>&lt;18 years</td>
<td>20</td>
<td>9.9</td>
<td>6.4-14.0</td>
</tr>
<tr>
<td>≥18 years</td>
<td>34</td>
<td>12.5</td>
<td>9.5-16.0</td>
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<tr>
<td>Drinks consumed</td>
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<td>&lt;2 drinks per week</td>
<td>35</td>
<td>11.0</td>
<td>8.2-14.3</td>
</tr>
<tr>
<td>≥2 drinks per week</td>
<td>19</td>
<td>12.4</td>
<td>8.3-17.3</td>
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</table>

¹Adjusted least-square means and 95% confidence intervals are from analyses using untransformed mammographic density measures.
²P-values are from analyses using square root-transformed mammographic density measures.

All models were adjusted for age (continuous) and BMI (continuous) at the time of mammogram, and parity (continuous). The height and weight models were mutually adjusted for each other (continuous) instead of BMI. The physical activity models were additionally adjusted for smoking status (never, former, current). The smoking models were additionally adjusted for the number of alcoholic drinks consumed per week (continuous). The alcohol models were additionally adjusted for smoking status (never/ever). TWA, total weekly activity. MVPA, moderate-to-vigorous physical activity. METs, Metabolic Equivalent of Task.
Chapter 6
Discussion

6
6.1 Discussion

It is of interest to evaluate the relationships between breast cancer risk factors and mammographic density among women at an elevated familial risk, based on prior studies conducted in the general population suggesting that mammographic density may play an important intermediary role between these risk factors and their influence on breast cancer risk. While the relationship between mammographic density and breast cancer risk has been evaluated among other familial breast cancer cohorts [5, 169, 172, 237, 238], the relationships between breast cancer risk factors and mammographic density have not been evaluated in these familial studies. Therefore, the current study represents the first investigation of the relationships between breast cancer risk factors and mammographic density among a cohort of high-risk women.

In this cross-sectional analysis of breast cancer risk factors and mammographic density among 97 premenopausal and 59 postmenopausal high-risk women, an increasing number of live births and body weight were associated with significantly lower mammographic density, while height was inversely associated with non-dense area. Former smoking, but not current smoking, was positively associated with percent density and dense area, and inversely associated with non-dense area in postmenopausal women. A longer duration of smoking and an increasing number of packs of cigarettes smoked per week were inversely associated with percent density and dense area in premenopausal women. Increasing age at initiation of smoking and alcohol were associated with higher percent density and dense area among both premenopausal and postmenopausal women.

Parity has a protective effect on breast cancer and is inversely associated with mammographic density in the general population. In the current study, increasing live births was associated with significantly lower percent density ($P = 0.05$) among parous premenopausal women. Among
parous postmenopausal women, women in the highest category of parity had significantly lower adjusted mean percent density (10.0% vs. 18.8%; \( P = 0.06 \)) and dense area (14.3 cm\(^2\) vs. 24.3 cm\(^2\); \( P = 0.08 \)), and higher adjusted mean non-dense area (146.9 cm\(^2\) vs. 95.3 cm\(^2\); \( P = 0.03 \)), compared to women in the lowest parity category. Pregnancy may lower breast cancer risk by reducing the number of mammary stem cells in the breast that are potentially at-risk for genetic change, which may explain why parous women have lower mammographic density [42]. The cessation of breastfeeding is thought to stimulate epithelial cell remodelling into adipose tissue [42], thus reducing the amount of dense tissue and increasing the amount of non-dense tissue. Although not significant, in our study we found that parous premenopausal women who breastfed had lower dense area and greater non-dense area than women who did not breastfeed, but this comparison was limited by the small number of women who did not breastfeed (n = 4).

We observed an inverse association between body weight and percent density and a positive association between body weight and non-dense area. An increasing body weight was associated with significantly lower percent density and greater non-dense area in both premenopausal women (\( P < 0.0001 \)) and postmenopausal women (\( P \leq 0.002 \)). The relationship between body size (measured either by body weight or BMI) and breast cancer risk varies by menopausal status, with a protective effect in premenopausal women and an increase in risk in postmenopausal women. Our findings are consistent with studies among the general population reporting similar associations [57, 60, 61, 129, 194, 195]. Body size is inversely associated with percent mammographic density due to a positive association with non-dense area in both premenopausal and postmenopausal women [57]. While the biological role of adipose tissue in breast cancer carcinogenesis is not well understood, adipose tissue is an important source of endogenous estrogens, especially among postmenopausal women [57, 61]. Higher levels of estrogen in the breast can stimulate the proliferation of dense epithelial tissue [61]. In addition, adipose tissue can stimulate the release of adipokines that may be associated with breast cancer risk, such as leptin, and lower levels of adipokines that may prevent cancer, such as adiponectin [61]. Obesity in premenopausal women may be protective of breast cancer risk due to modifications in the function of the ovaries [239]. Given that body weight was found to be inversely associated with percent density, mammographic density may mediate the association between body weight and breast cancer in premenopausal women, but it likely does not mediate this association among postmenopausal women.
In our study, an increasing height was associated with significantly higher percent density ($P = 0.06$) and significantly lower non-dense area ($P = 0.005$) in premenopausal women. Among postmenopausal women, women with greater height had a lower adjusted mean of non-dense area of 106.3 cm$^2$ compared to 144.6 cm$^2$ among women with lower height ($P = 0.02$). A higher adult attained height is thought to be positively associated with percent mammographic density as a result of endocrine hormones and circulating growth factors [195]. Several mitogens that are responsible for growth, most notably IGF-1 and prolactin, also play a role in the development of the mammary epithelium and stromal tissue [195]. Further, positive associations of circulating levels of IGF-1 and prolactin and percent mammographic density have been reported among premenopausal and postmenopausal women, respectively [57, 84, 195]. Similar to our findings of a positive association between height and percent density only among premenopausal women, a study of young women by Dorgan et al. found height was significantly and positively associated with percent dense breast volume [194]. This aligns with the fact that premenopausal women tend to have denser breasts and are likely to have less body fat compared to postmenopausal women [3].

Among premenopausal women, an increasing duration of smoking was associated with significantly lower percent density ($P = 0.01$), and an increasing age at initiation of smoking was associated with greater percent density ($P = 0.05$) and greater dense area ($P = 0.001$). Women who smoked at least three packs of cigarettes per week had significantly lower adjusted mean percent density (17.4% vs. 27.6%; $P = 0.07$) and dense area (16.5 cm$^2$ vs. 37.3 cm$^2$; $P = 0.01$) compared to women who smoked less packs per week. Overall, our results suggest an inverse association between smoking and mammographic density, which agree with most [197, 220, 240-243], but not all [221, 244], of the existing literature. While our findings of a positive association between age at initiation and percent density and dense area conflict with what is known about early exposure to smoking and breast cancer risk, other studies have also reported the same positive association between age at initiation and percent density, in both premenopausal and postmenopausal cohorts [220, 240]. For example, Jacobsen et al. found that initiation of smoking prior to age 16 was significantly associated with a lower odds of having mixed/dense breasts (OR = 0.79, 95% CI 0.64, 0.96) [220]. Furthermore, other studies have also found inverse associations between the number of cigarettes smoked per day and percent density [220, 240, 241]. A cross-sectional study of Spanish women found women who smoked
(currently or accumulated over the lifetime) 15 or more cigarettes per day were 32\% less likely to be in high mammographic density categories based on Boyd’s semi-quantitative scale [241]. Interestingly, we found that postmenopausal former smokers, but not current smokers, had higher adjusted mean percent density (17.3\% vs. 9.2\%; \( P = 0.003 \)) and dense area (22.8 cm\(^2\) vs. 13.4 cm\(^2\); \( P = 0.01 \)), and significantly lower non-dense area (116.8 cm\(^2\) vs. 147.7 cm\(^2\); \( P = 0.05 \)) compared to never smokers. In line with this, two studies have also observed higher mammographic density levels among both premenopausal and postmenopausal women who quit smoking compared to non-smokers and current smokers [220, 221]. Whether the changes in mammographic density that are associated with the cessation of smoking increase breast cancer risk is unknown. Overall, our findings suggest that smoking exerts an anti-estrogenic effect on the dense tissue in the breast, and is therefore associated with lower dense area and lower percent density [240]. Given that our findings of a positive association between age at initiation of smoking and percent density and dense area conflict with the known increased risk of breast cancer associated with early exposure to tobacco, the influence of active smoking on breast cancer risk may not be mediated by mammographic density [220]. However, our findings of higher percent density and dense area and lower non-dense area among postmenopausal former smokers suggest that the cessation of long-term smoking may have an important impact on mammographic density.

The majority of the literature investigating the influence of alcohol on mammographic density reports positive associations [69, 219, 221, 241], with the strongest effects emerging among women consuming seven or more servings per week (e.g., 10 grams per day) compared to non-drinkers [245, 246]. These observations conflict with the findings of a cross-sectional study evaluating the effect of alcohol intake during early adulthood (age 20 to 29 years), which reported an inverse association between seven or more servings per week and percent density [247]. In the current study, our study sample had low levels of alcohol intake, where only two drinks per week were the average among ever users, and only 30\% of ever users reported consuming seven or more servings per week. An increasing age at initiation of alcohol was associated with significantly greater percent density and dense area among both premenopausal (\( P \leq 0.03 \)) and postmenopausal (\( P \leq 0.003 \)) women. Premenopausal women who started consuming alcohol after age 18 had a significantly lower adjusted mean non-dense area of 95.6 cm\(^2\) compared to 124.5 cm\(^2\) among women who started consuming alcohol earlier (\( P = 0.04 \)).
Another study found that an earlier age at initiation of alcohol use was associated with higher percent density [248], suggesting that alcohol-related changes in percent mammographic density may be due to an increase in dense area and/or a reduction in non-dense area, at least for premenopausal women. Other studies have proposed that the time interval between menarche and first full-term pregnancy may be important when assessing the effect of alcohol consumption on breast cancer risk. For example, a prospective analysis within the Nurses’ Health Study found that among women who consumed 10 grams of alcohol per day, those whose time interval between menarche and first full-term pregnancy was 15 years or longer, had an increased relative risk of breast cancer of 1.25 (95% CI 1.06, 1.48) [249]. Breast tissue undergoes the highest rate of proliferation between menarche and first full-term pregnancy, and this time period is associated with the highest breast cancer risk accumulation until the breast undergoes pregnancy-related tissue differentiation [19, 249]. In addition, alcohol consumption is thought to be associated with higher levels of circulating estrogens and IGF-1, which may also stimulate the proliferation of dense epithelial tissue [219, 249]. Therefore, alcohol consumption during the time between menarche and first full-term pregnancy may have a greater impact on mammographic density and breast cancer risk than alcohol consumption after first full-term pregnancy. Overall, our findings suggest an inverse association between alcohol consumption and mammographic density, suggesting that mammographic density likely does not mediate the association between alcohol and breast cancer risk in this cohort.

We observed that each unit increase in levels of total weekly physical activity was associated with significantly higher non-dense area in postmenopausal women (β = 0.02; 95% CI 0.00 0.03; P = 0.05). Given that total weekly activity was also positively associated with percent density and dense area, although non-significantly, this is likely a spurious result and may reflect the limitations of questionnaire-based physical activity assessment.

Lastly, there were eight pairs of relatives included in this study. Five of the pairs were sisters, two pairs were an aunt and a niece, and one pair was comprised of maternal first-cousins. Of these eight pairs, only three of the five sister-pairs were in the same menopausal status category (one pair was premenopausal and two pairs were postmenopausal). As previously covered in Chapter 2, Section 2.2.2.3.1, mammographic density is highly correlated between sisters [4], and it has previously been suggested that lifestyle factors such as body weight, physical activity and
sedentary behaviours, and smoking and alcohol consumption are highly correlated between close relatives [250-252]. Therefore, it was important to determine if including related individuals into the analyses would significantly influence the results. This is especially important given that linear regression was used to conduct the analyses, which depends on the assumption that all observations are independent. We conducted sensitivity analyses by excluding one woman from each of the three sister-pairs with the same menopausal status. Overall, these analyses did not substantially change the study findings.

6.2 Strengths and Limitations

Other prospective cohort studies of women with a family history of breast cancer have been established, such as the Breast Cancer Prospective Family Study Cohort [253], the Minnesota Breast Cancer Family Study [254, 255], and the Sisters Study [256]. However, the family history criteria of these cohorts vary by the number of affected relatives required, the relationship of these relatives to the study participant (e.g., first-, second- or third-degree relative), and their age(s) of diagnosis. Other studies of high-risk women have included women who do not carry a BRCA mutation but belong to BRCA mutation-positive families [172]. Even though these women do not carry a BRCA mutation, the known BRCA mutation in their family may cause these women to have a different familial risk profile than women from BRCA mutation-negative families.

Based on this, the primary strength of the current study is the novelty of the study population. Our prospective cohort study based at Women’s College Hospital differs from other familial breast cancer studies given our strict family history criteria, which considers both the relationship of the affected relatives to the study participant and the age(s) at diagnosis: two breast cancer cases between first-degree relatives under the age of 50, or three breast cancer cases at any age on one side of the family [9]. In addition, all families in our cohort have been genetically tested and are confirmed to not carry a predisposing BRCA mutation using full genome sequencing, which is the current gold standard for genetic testing [26].

In order to ensure that a representative sample of the ‘Negative Study’ cohort was captured in the current study, we evaluated whether there were significant differences in age, parity, total live births, and BMI between the 157 women who were included in the study and the 87 women who were not, stratified by menopausal status. Among premenopausal women, those who were not
included in the study were younger (32.5 years vs. 43.8 years; \( P < 0.0001 \)) and were more likely to be nulliparous (29\% vs. 73\%; \( P < 0.0001 \)) compared to the women who were included. Given the young average age of the women who were not included, it is reasonable that these women have not started regular mammography screening or have had children. Although not statistically significant, the women who were not included had a lower mean BMI compared to the women who were included (25.8 kg/m\(^2\) vs. 24.2 kg/m\(^2\); \( P = 0.07 \)), which may also be a reflection of their younger age. Among parous women, the total number of live births were not significantly different between the two groups (\( P = 0.46 \)).

Among postmenopausal women, women who were included in the study did not significantly differ from the women who were not included by age, parity, or by BMI (\( P \geq 0.54 \)). However, parous women who were included in the study had a greater mean number of live births compared to parous women who were not included (2.3 births vs. 1.6 births; \( P = 0.004 \)). Overall, these findings suggest a reasonable internal validity of the study sample for both premenopausal and postmenopausal women.

Another strength of this study involves the use of the Cumulus software to assess mammographic density. This semi-automated, quantitative method has been shown to perform well against qualitative and fully-automated methods [100]. In our study, Reader 2 (O.M.) underwent thorough training consisting of reading batches of practice images belonging to an unrelated study and comparing the percent density values with those obtained from an experienced reader. These practice modules were completed several times before the images belonging to the current study were read. All mammographic images were de-identified and randomized for each read, and 15\% of the images were randomly chosen to be repeated. The reliability across all three batches was high (correlation coefficients range: 0.77 to 0.98) [257]. In addition, a high correlation coefficient of 0.86 was observed among the subset of study images that were read by both Reader 1 and Reader 2, indicating that both readers were well-trained.

The distributions of the mammographic density measures were graphed and were visually assessed for deviations from normal distribution prior to conducting the linear regression models. An extremely high percent density value in a postmenopausal woman was observed, and she was removed from the analyses. This participant was nulliparous, reported a diagnosis of fibroadenoma (a form of benign breast disease), and a low BMI of 18.5 kg/m\(^2\), which are all
factors that likely contributed to her extensive mammographic density. Missing observations in the data set were imputed using the median value of the exposure in order to maintain the sample size. Covariates included in the statistical models were chosen based on backward selection procedures, in order to ensure that the chosen covariates best suited the data.

A primary limitation of this study is the small sample size, which included only 97 premenopausal women and 59 postmenopausal women after exclusions. The limited sample size is likely a consequence of the strict eligibility criteria of the ‘Negative Study,’ which requires participants to have a strong family history of breast cancer as well as an affected relative test negative for BRCA mutations. Further limiting the sample size, we stratified our statistical analyses by menopausal status, however, this is essential when working with mammographic density. In general, the small sample size may have limited the statistical power of our analyses, and therefore increased the likelihood of Type II error. As a result, we may have failed to observe a statistically significant association between the exposures of interest and the mammographic density measures. In addition, it is also important to note that multiple comparisons were made, therefore there is the possibility that our significant findings were due to chance. However, our findings coincide with the relationships between the exposures of interest and mammographic density established within the general population, as well as with the postulated biological mechanisms that may be responsible for these relationships. Based on this, we feel that there is reasonable external validity to support our findings.

The size of the study population limited our ability to test for effect modification by BMI and hormone replacement therapy use, which have been shown to be important when evaluating the associations between alcohol and mammographic density [221]. In our sample, only 16 postmenopausal women reported ever using hormone replacement therapy, and very few women reported never consuming alcohol (n = 15). Due to the low number of smokers and nulliparous women in the sample, we could not evaluate the effects of smoking and alcohol before first full-term pregnancy on mammographic density, which may be a more important risk factor than the age at initiation [240, 249]. Moreover, only six women reported ever using chemopreventive drugs, and only three have taken tamoxifen, therefore the relationship between tamoxifen use and mammographic density could not be evaluated in this sample.
The current study utilized a cross-sectional approach to carry out the study objectives. While longitudinal prospective cohort studies are the ideal design for epidemiological studies, this strategy is unrealistic when the endpoint of the study is a disease that occurs at a slow rate, such as cancer. Moreover, the ‘Negative Study’ cohort is young, with a mean age at enrollment of 43.7 years (range: 18.2 to 64.9 years), and to date, there are only four incident breast cancers. Based on this, a cross-sectional design was chosen to evaluate an intermediate biomarker of breast cancer risk (i.e., mammographic density) as the outcome instead of breast cancer risk. However, a primary limitation associated with cross-sectional approaches is that the exposure and the outcome are ascertained generally at the same point in time, thus, a causal relationship between the exposure and the outcome cannot be established [258].

There were several barriers to obtaining mammographic images. The most frequent limitation was that the hospital or imaging centre indicated by the participant could not locate their records. While most diagnostic imaging centres utilize digital mammography, other sites used films that we were able to scan, but were often of poorer quality than the digital images. One imaging centre refused to provide a copy of the requested mammographic images. There was also wide variation in the storage and format of the mammographic images across the different imaging centres. For example, eleven mammographic images that were obtained were not compatible with Cumulus because they were in a compressed format, and consequently, could not be measured for mammographic density. In addition, 13% of the images read by Reader 1 were screen-film mammograms (n = 13). Of these, nine were digitized by the respective imaging centres. This is a limitation because the pixel size spacing and the digitization methods that were used are unknown, which can affect the measurements of the dense and non-dense areas. Moreover, film mammograms systematically produce higher percent mammographic density readings compared to digital mammograms [75]. Despite being aware of this issue, we did not adjust for the types of mammograms in the current study because determining the number of digital images read by Reader 2 that were once films is ongoing. Given that only 13% of the images read by Reader 1 were of this nature, it is likely to be a small number and may contribute minimal measurement bias to our mammographic density readings.

For all participants, the mammogram that was closest in time to their baseline research questionnaire was requested, but this mammogram was not always available. For some participants, the mammogram used was performed prior to their enrollment into the study (n =
90), and for others, after enrollment (n = 67). The gap in time between the mammogram and research questionnaire is reflected in the baseline characteristics of the sample: among premenopausal women, the time between the mammogram and the questionnaire used was significantly longer than among postmenopausal women (0.9 years vs. 0.6 years; P = 0.01) (Table 5.1). However, given that all analyses were adjusted for age at mammogram, this likely has a minimal effect, if any, on the results.

While the Cumulus software has been considered the gold standard for measuring mammographic density for several years, a primary limitation associated with area-based methods include the inability to consider the three-dimensional depth (i.e., volume) of the breast [75]. As reported previously in Chapter 2, Section 2.2.1, volumetric methods may be better able to capture the overlapping fibroglandular tissue than area-based methods. In addition, volumetric methods utilize more sophisticated methods to distinguish dense tissue from non-dense tissue in comparison to area-based methods [100]. Lastly, all of the currently available volumetric methods are fully automated and therefore do not require a human reader. Consequently, the possibility of inherent bias that is associated with the Cumulus method is eliminated among these fully-automated methods [75]. As these volumetric methods become more widely used, it is possible that they will replace Cumulus as the gold standard of mammographic density assessment.

The epidemiological data used for this cross-sectional study originates from a longitudinal, prospective cohort study, which is an ideal study design for observational studies. However, the use of self-reported questionnaires represents an important limitation of observational studies. At baseline and every two years thereafter, participants in the ‘Negative Study’ are mailed a follow-up questionnaire, where they report changes in their reproductive, hormonal, and medical histories, as well as lifestyle habits such as physical activity, smoking, and alcohol use [9]. Given the self-reported nature of the research questionnaires, the presence of non-differential misclassification is a possibility, and as a result, may have underestimated the true association between the exposures of interest and the mammographic density measures. Misclassification bias is often a problem when assessing physical activity, anthropometric variables such as height and weight, and the use of hormone replacement therapy and oral contraceptives. To minimize this, we incorporated a validated physical activity assessment tool [225] into our research questionnaires, which have been used by our research group for over 20 years. In addition, self-
reported height, weight, and BMI have been shown to be highly correlated with standardized measurements [259]. Furthermore, good agreement between self-reported hormone replacement therapy and physician records have previously been reported [260]. While a previous study within the Nurses’ Health Study showed acceptable validity for self-reported oral contraceptive history [261], our participants had particular difficulty recollecting the brand and/or dose of oral contraceptive(s) they used in the past. As a result, analyses stratifying by different types of oral contraceptives and assessing their independent associations with mammographic density could not be conducted.

Extracting data on alcohol consumption from the DHQs completed by the ‘Negative Study’ participants was originally planned, but they were not analyzed in time for inclusion in these analyses. Using this data would have been helpful since the DHQ calculates the approximate amount of alcohol consumed in grams per day, which is how the majority of epidemiological studies assess alcohol consumption, compared to our research questionnaire which captured the number of drinks consumed per week. The DHQ also considers the seasonality of certain alcoholic drinks, such as beer, which our research questionnaire is not able to consider. Despite these differences, the DHQ is also based on the number of self-reported drinks consumed per week, and therefore may be subjected to the same biases as our research questionnaire. In addition, other epidemiological studies of alcohol and mammographic density have found variations in the associations by type of alcohol. For example, red wine has been found to be inversely associated with mammographic density, while beer and white wine may be positively associated with mammographic density [69].

6.3 Implications and Future Directions

The primary findings of the current study suggest that parity, body weight, height, smoking, and alcohol are associated with mammographic density among women at an increased risk for familial breast cancer. Apart from active smoking, alcohol consumption, and postmenopausal body weight, it is plausible that mammographic density may be acting as an intermediate factor in the pathway between these risk factors and their influence on breast cancer risk. Therefore, the implications of these findings include the development of evidence-based recommendations regarding the management of mammographic density, and subsequently breast cancer risk, among this unique study population. Targeting mammographic density, one of the few
modifiable risk factors for breast cancer, may be of particular relevance for this study population since the breast cancer prevention options available (e.g., prophylactic surgery, chemoprevention) were initially established for BRCA mutation carriers, whom inherently represent a different risk profile.

These findings may also have important implications for the utility of mammographic density in breast cancer risk prediction models, which are essential tools used by physicians and genetic counsellors to stratify women by risk, in order to determine who is eligible for high-risk breast cancer screening and to identify who will benefit from primary prevention strategies. These risk prediction models incorporate several breast cancer risk factors that are associated with mammographic density, such as body weight, height, parity and exogenous hormone use [71, 73, 150, 151]. Given that mammographic density is a stronger risk factor for breast cancer compared to these other risk factors, the inclusion of mammographic density in these risk assessment tools is imperative. The predictive value of mammographic density has only been evaluated among some risk prediction models with promising improvement [148, 158, 165], and is currently only included in the latest version of the IBIS model for clinical use [72]. In this version of IBIS, there are three different ways to include mammographic density in the risk assessment: 1) by imputing one of the four BI-RADS categories; 2) imputing a percentage of mammographic density derived from visual assessment (note: this includes Cumulus and other non-automated, area-based techniques); and 3) imputing a percentage of mammographic density derived from Volpara, a volumetric method [72]. In addition to IBIS, mammographic density should be included in other widely used risk prediction tools, such as the BOADICEA model, which is often used in conjunction with the IBIS model to determine eligibility for the Ontario Breast Screening Program [70]. The most feasible approach to achieve this would include incorporating the BI-RADS classification system into these risk prediction models since it is the most widely used method of mammographic density assessment for all women, and therefore, would not entail extra work from radiologists [32]. While using BI-RADS is an attractive option, quantitative measures of percent mammographic density, which can be derived from methods such as Cumulus, are more strongly associated with breast cancer risk and tend to improve prediction models more than BI-RADS [148, 158, 165]. However, as previously mentioned in Chapter 2, Section 2.2.1.1.2, Cumulus requires extensive training before use, which may limit its implementation into clinical practice [100]. While volumetric methodologies such as Volpara
are fully automated and do not require training, many of these techniques must be used at the time the mammogram is taken, which is a limitation for widespread use [75]. Ultimately, before mammographic density can be implemented into more risk prediction models, a standardized technique of measuring mammographic density must first be established.

Given its prospective design, the ‘Negative Study’ currently only has four incident breast cancer cases. Because of this, mammographic density was used as an intermediate marker of breast cancer risk in the current study. Future studies including a larger sample size and a longer follow-up period are needed to determine the joint effects of breast cancer risk factors and mammographic density on breast cancer risk as the primary endpoint. A longitudinal follow-up on these women will also allow for multiple mammographic images to be assessed in order to measure changes in the relationships between breast cancer risk factors and mammographic density over time. Since mammographic density is also a strong risk factor for breast cancer among BRCA mutation carriers [170-172], whether these other breast cancer risk factors are similarly associated with mammographic density among this high-risk population should also be evaluated.

The influence of early life exposures, such as during childhood or adolescence, may be more important predictors of mammographic density rather than exposures during adulthood. This is especially important since breast tissue proliferates at the highest rate after menarche and until the first full-term pregnancy, and the breast is the most susceptible to genetic change during this window of time [249, 262]. Based on this, evaluation of the relationships between childhood and adolescent growth patterns, body size, and physical activity and mammographic density and breast cancer risk may be of interest [262].

Approximately 18% of the SNPs associated with breast cancer risk are also associated with mammographic density [175]. In the current study, participants were not genotyped for such genetic variants. This may be of interest for future studies, given the likely shared genetic basis between breast cancer risk, mammographic density, and a family history of the disease. In addition, some studies have reported a significant predictive value of including these SNPs together with mammographic density into breast cancer risk prediction models [263], suggesting that these genetic variants may also be useful to improve the identification of high-risk women.
The biological mechanisms underlying the association between mammographic density and breast cancer risk have not yet been elucidated, but likely involve the cumulative exposure of the breast epithelium to exogenous and endogenous hormones and growth factors [84]. Briefly, the receptor activator of nuclear factor κB (RANK)/RANK ligand (RANKL) signalling pathway may also be of importance here, given its emerging role in mammary epithelial cell proliferation and in breast cancer development [264]. We recently evaluated the relationship between serum osteoprotegerin (OPG), the endogenous antagonist of RANK/RANKL signalling, and mammographic density among a subset of the ‘Negative Study’ population (Under review). Among postmenopausal women, those with low serum OPG levels had significantly higher adjusted mean percent mammographic density of 21% compared to 14% among those with high serum OPG levels ($P = 0.04$), suggesting an alternate mechanism by which mammographic density may influence breast cancer risk (Under review). These findings should be replicated in other high-risk populations, such as among BRCA mutation carriers. Our group previously reported that BRCA mutation carriers with high levels of plasma OPG had a significantly lower risk of breast cancer compared to those with low plasma OPG levels (HR = 0.25 95% CI 0.08, 0.78; $P = 0.02$) [233]. Collectively, these findings suggest that inhibiting the RANK/RANKL/OPG signalling pathway in these high-risk populations, especially among women with high mammographic density, may reduce breast cancer incidence, thereby identifying a novel target for chemoprevention [265].

### 6.4 Summary of Thesis

In conclusion, this thesis evaluated the relationships between important reproductive, hormonal, anthropometric, and lifestyle risk factors for breast cancer and measures of mammographic density among a cohort of women at an increased risk of familial breast cancer. It was found that an increasing number of live births and body weight were significantly associated with lower mammographic density while increasing height was associated with higher mammographic density. Smoking was associated with lower mammographic density in premenopausal women and higher mammographic density among former postmenopausal smokers. A later age of initiation of smoking and alcohol were associated with higher mammographic density. These findings suggest that mammographic density may mediate the influence of parity, body weight among premenopausal women, and height on breast cancer risk among this high-risk cohort. Mammographic density does not seem to mediate the relationships between postmenopausal
body weight, active smoking, alcohol consumption, and breast cancer risk. The relationship between mammographic density and former smoking must be evaluated further. Overall, mammographic density is an important risk factor in this unique population.

The public health significance of these findings includes the incorporation of mammographic density into breast cancer risk prediction models in order to accurately identify women at an increased risk for disease. It is crucial that a woman’s risk of breast cancer is accurately evaluated in order to determine which women are eligible for high-risk screening, and who will benefit from primary prevention (i.e., chemoprevention, prophylactic surgery). In addition, establishing personalized breast cancer screening regimens will improve health care spending and reduce the number of unnecessary biopsies, while ensuring that women who are high risk are receiving the intensive surveillance they need to identify breasts cancers while they are small and easier to treat.
References


Appendices

1. Invitation Letter for Proband
2. Invitation Letter for Relative
3. Study Package Letter
4. Consent Form
5. Authorization of Medical Release Form
6. Diet History Questionnaire: due to its large size, the actual document has not been included but may be accessed at https://epi.grants.cancer.gov/DHQ/.
8. Follow-Up Questionnaire for Risk Factor Analysis for Familial Breast Cancer
Appendix 1: Invitation Letter to the Proband

Monday, September 25, 2017

Dear Jane Doe,

The Familial Breast Cancer Unit at Women’s College Hospital recently reported that women with a strong family history of breast cancer, but no BRCA1 or BRCA2 mutation, are two to four times more likely to develop breast cancer than women without a family history. Despite this elevated risk, no clinical guidelines have been developed for the care of these women. We are currently conducting a study to evaluate factors which may influence cancer risk in women with a strong family history of breast cancer without a BRCA mutation.

We are currently looking for women in families with a strong family history of breast cancer who have not been diagnosed with breast cancer. We understand that you have visited the centre in the past, and although you are not eligible to participate because of your breast cancer, we would like to invite your family members to participate in our current research initiative. The information obtained from the pedigree you provided, indicates that your (eligible relatives) may be eligible to participate. We are not able to contact them directly and thus we require your help. There are three ways you can help us contact your relatives in order to receive a study package: 1) you can provide us with their contact information, 2) you can ask them to contact us, or 3) we can send you a study package directly in which you can then pass on to them.

Participation entails completing a consent form, medical release form, and two research questionnaires that inquire about diet, lifestyle and other health-related factors. Similar questionnaires will be completed every two years, over a 10 year period. In addition, there is an optional fasting blood and urine sample that can be taken during a one-time visit to the Familial Breast Cancer Research Unit at Women’s College Research Institute.

Please read the enclosed study information. I encourage you and/or your relatives to contact us with any questions or comments that you may have, in addition to contacting us for the study participation package to be mailed. You may reach our research associate by email, negative.study@wchospital.ca with the subject heading “Negative Study” or 416-351-3800 ext. 2875. For more detailed information regarding the study, we invite you to visit our website: www.womensresearch.ca/noncarriersonline/. We will contact you in two weeks to ensure you have received this letter and answer any questions.

We greatly appreciate your help in making this research possible.

Sincerely,

Steven Narod, MD, FRCPC

Joanne Kotsopoulos, PhD
Appendix 2: Invitation Letter to the Relative

Monday, September 25, 2017

Dear Jane Doe,

The Familial Breast Cancer Unit at Women’s College Hospital recently reported that women with a strong family history of breast cancer, but no BRCA1 or BRCA2 mutation, are two to four times more likely to develop breast cancer than women without a family history. Despite this elevated risk, no clinical guidelines have been developed for the care of these women. We are currently conducting a study to evaluate factors which may influence cancer risk in women with a strong family history of breast cancer without a BRCA mutation.

We are currently looking for women in families with a strong family history of breast cancer who have not been diagnosed with breast cancer. We understand that you have visited the unit in the past, and we would like to invite you to participate in our current research initiative.

Participation entails completing a consent form, medical release form, a collection of toenail clippings, and two research questionnaires that ask about diet, lifestyle and other health-related factors as well as family history of cancer. A similar, but shorter, questionnaire will be completed every two years, over a 10 year period. In addition, there is an optional fasting blood and urine sample that can be taken during a one-time visit to the Familial Breast Cancer Research Unit at Women’s College Research Institute.

Please read the enclosed study information. We encourage you and/or your relatives to contact the research associate with any questions or comments that you may have, in addition to contacting us for study participation package to be mailed. You may reach the research associate by email, negative.study@wchospital.ca with the subject heading “Negative Study” or 416-351-3732 ext 2875. For more detailed information regarding the study, we invite you to visit the study’s website: www.womensresearch.ca/noncarrierstudy/.

We greatly appreciate your help in making this research possible.

Sincerely,

Steven Narod, MD, FRCPC

Joanne Kotsopoulus, PhD
Appendix 3: Study Package Letter

Monday, September 25, 2017

Dear Jane Doe,

Thank you for agreeing to take part in our ‘Risk Factor Analysis for Familial Breast Cancer’ study. We recently reported that women with a strong family history of breast cancer, but no BRCA1 or BRCA2 mutation, are two to four times more likely to develop breast cancer than women without a family history. Despite this elevated risk, no clinical guidelines have been developed for the care of these women. The purpose of this study is to evaluate factors which may influence their risk.

As you are aware, participation in this study includes the completion of a questionnaire, every two years that collects information about your health and lifestyle, a medical release form and collection of toenail clippings. We would also appreciate if you would take some time to provide an optional fasting blood and urine sample at the Women’s College Research Institute (76 Grenville Street, Toronto). Having access to blood and urine samples, along with your information collected from a questionnaire, could help us investigate more risk factors for breast cancer. Our research associate will contact you to discuss this appointment after you return your study package. Please note, if you are currently pregnant or breastfeeding, your appointment should be scheduled after you have stopped breastfeeding.

The package includes the following:
1. **Informed Consent Form** for “Risk Factor Analysis for Familial Breast Cancer”. This will confirm that you understand the study and your role if you choose to participate. Please read carefully, sign, date and return.
2. **Authorization for Medical Release Form.** By signing this document, you give us permission to access your medical records in relation to any cancer screening or diagnosis. Please read carefully, sign, date and return.
3. **Research Questionnaire.** Please take your time in order to complete the whole questionnaire, and answer each question as accurately as possible. Please complete and return.
4. **Toenail Clippings.** Please clip clean, unpolished toenails and place in the envelope provided.
5. **Postage-paid Return Envelope and Return Checklist.**

*You will receive a Diet History Questionnaire aimed to assess regular dietary intake after returning your completed study package.

If you have any questions regarding the enclosed information or about our research in general, please feel free to contact our research associate, Olivia Moran, at negative.study@wchospital.ca or 416-351-3732 ext. 2875 to address your questions, comments or concerns. You may also visit our website www.womensresearch.ca/noncarrierstudy for additional information. We appreciate your commitment to helping us better understand how to prevent familial breast cancer.

Sincerely,

Steven Narod, MD, FRCPC

Joanne Kotsopoulos, PhD
Appendix 4: Consent Form

INFORMED CONSENT FORM
RISK FACTOR ANALYSIS FOR FAMILIAL BREAST CANCER

PRINCIPAL INVESTIGATORS: Dr. Joanne Kotsopoulos & Dr. Steven Narod
SITE: Women’s College Research Institute
SPONSOR: Canadian Breast Cancer Foundation

Background
Approximately 15% of women with a diagnosis of breast cancer have a family history of breast cancer. Within this 15%, approximately one out of four carry a mutation in one of the two known breast cancer genes, BRCA1 and BRCA2. This means that the majority of women that qualify for genetic testing receive uninformative results, despite their elevated significant family history of breast cancer. There is currently little information available to inform these women and their physicians about screening and prevention practices. Our goal is to better understand the influence of family history on the risk of developing breast cancer.

You are being invited to participate in this study because you and/or your family has been in contact with the Familial Breast Cancer Clinic and you and/or your family member has received a genetic test that showed no mutation in either of the two breast cancer genes (i.e. negative for BRCA1 and BRCA2 mutations). In addition, your family is deemed as having a strong family history of breast cancer, which is defined here as either two female relatives with breast cancer under 50 or three (or more) female relatives with breast cancer diagnosed at any age.

Your participation is voluntary and you may decide to withdraw at any time. Your clinical care will be unaffected by your decision to participate or not participate in this study.

Purpose
Assessing risk factors that may influence breast cancer in high-risk women may lead to improving prevention strategies, counselling and treatment provided for women from families like yours. This information will aid in identifying preventive measures that are successful in reducing the risk of breast cancer development in these high-risk women, which may lead to future clinical guidelines for the management of high-risk women.

Who can participate in this study?
- Women who fully understand the study and give informed consent to participate as demonstrated by signing this consent form.
- Women who are 25-65 years of age with no personal history of cancer.
- Have 2 or more female relatives with breast cancer ≤ 50 years OR 3 breast cancers at any age from only ONE side of the family (mother or father, not both).
- Have at least one first-degree relative with breast cancer (i.e., mother, sister, daughter).
- Have at least one relative with breast cancer receive a negative result for a BRCA1 or BRCA2 mutation.
Who cannot participate in this study?
- Women who do not meet the criteria above.
- Women who have received a positive test for either of the BRCA1/2 mutations.
- Women who have a first or second-degree relative that has received a positive test for either of the BRCA1/2 mutations.
- Individuals who are unable to give voluntary, informed consent.

Study Procedures
Participation entails completing this consent form as well as a medical release form, a research questionnaire that asks about lifestyle and other health-related factors as well as family history of cancer, and a diet history questionnaire that gathers information about dietary factors. A similar, but shorter, questionnaire will be completed every two years, over a 10 year period. We estimate that the questionnaires should take about 30 minutes to complete. If you prefer, the questionnaire may be completed over the telephone with a research assistant.

To update our files, we request results from any breast MRIs, mammograms and breast biopsies that you have had performed. Any results that are received will be marked as confidential and will only be handled and interpreted by study personnel. We will only request this information from other health centres if you give us permission to do so, by completing the enclosed Medical Release Form that we can copy and provide to these centres.

This study also requires you to provide a blood and a urine sample, as well as toenail clippings. Toenail clippings can be sent to us using the enclosed envelope labelled ‘Toenail Clippings’ and will be used to assess levels of various dietary factors. Blood and urine samples will be collected at the Familial Breast Cancer Research Unit during a short visit. After a nightly fast, a phlebotomist (someone who is trained in taking blood) will draw 20 cubic centimetres of blood (about four tablespoons) by venipuncture. Blood samples will be used to assess levels of various nutrients, hormones and to obtain DNA so we can evaluate other genetic factors that might influence breast cancer risk. At this time, we will also request a urine sample to assess hormone levels. To respect your privacy, no identifying data will accompany these samples. Please note that if you do not reside near Toronto, the study coordinator will help you make an appointment at an outside laboratory closer to your home to have blood and urine collected.

If you are not familiar with your family history in sufficient detail, we will ask you to identify a second person in the family who may be able to provide additional details. We will not contact this individual directly, but we will ask you to provide them with the research assistant’s contact information. If this individual proceeds to contact a member of the study team and agrees to provide additional information, he/she will be sent a copy of this consent form for completion.

Confidentiality
Your confidentiality will be respected to the extent permitted by applicable laws and regulations and your medical and study records will not be publicly available. No information that discloses your identity will be released or published without your specific consent. Your identity will not be used in any reports about the study; you will only be identified by a study code. The only people who will have access to the study information will be the research team. All information associated with this study will be kept behind locked doors or in secure computer files.

Research records and medical records identifying you may be inspected in the presence of the Investigator and the WCH Research Ethics Board for the purpose of monitoring the research. However, no records that identify you will be allowed to leave the centre. These organizations have policies of strict confidentiality and the individuals inspecting your records must sign a confidentiality form.
The information gathered from this study, with your identifying information removed, will be used to determine the risk of developing cancer in women with a strong family history of breast cancer but have uninformative genetic testing results. The information will be shared with the sponsor of the study, the governmental regulatory agencies that oversee such research, the investigators who have conducted this study, genetic counsellors in Canada, non-profit organizations involved in breast cancer care, and other doctors and researchers throughout the world through the publication of the results of this study.

Your rights to privacy are protected and guaranteed by the “Personal Health Information Act.” This act lays down safeguards respecting your privacy, and also gives you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about this act are available on request. If you have any concerns about your treatment or rights as a research participant, you may telephone Diana Raymond-Watts, Manager of Research Ethics Board at (416) 351-2535.

Benefits & Risks
As we have taken steps to ensure confidentiality, we do not anticipate any risks related to your involvement in this research. However, due to the rare risk associated with providing blood samples, if you become ill or are physically injured as a result of participation in this study, medical treatment will be provided. In no way does signing this consent form waive your legal rights nor does it relieve the investigators, sponsors or involved institutions from their legal and professional responsibilities.

Participation in this research does not provide any direct benefit to you. However, the information collected from this study will be used to establish valuable cancer risk information for women who have a strong family history of breast cancer but have uninformative genetic results.

Contact
Your participation in this study is entirely voluntary. Please feel free to ask questions at any time. You can reach the study’s coordinator, Olivia Moran at 416-351-3732 ext. 2875.

Consent
You may decide not to participate in this research. Whether or not you participate in the project will not affect your health care in any way. If you do participate, you will have the right to ask questions and/or withdraw at any time. If you choose to withdraw, information that you have provided to us will be destroyed.
CONFIRMATION OF PARTICIPATION:
I confirm that the purpose of the research, the study procedures that I will undergo and the possible discomforts, as well as benefits that I may experience, have been provided to me in sufficient detail.

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care. I understand that my confidentiality will be respected to the fullest extent of the law.

I give permission to Dr. Kotsopoulos, Dr. Narod and the study team to contact me by telephone if additional information is needed.

YES  _____  NO  _____  Telephone ______________________

Please check the appropriate box:
☐ I do not consent to participate in this study.
☐ I consent to participate in this study. By checking this box, your signature below indicates that you have read and understood this consent form and agree to participate in this study.

I give permission to Dr. Kotsopoulos, Dr. Narod and the study team to use my blood, urine and toenail samples for analysis in future studies on cancer susceptibility.  YES  _____  NO  _____

If YES: Please contact me in the future if my sample is being used for future studies on cancer susceptibility.

YES  _____  NO  _____

__________________________________________________________________________
Participant’s Signature  Date  ___________

__________________________________________________________________________
Witness’ Signature  Date  ___________

I have provided ______________________ with information that outlines the purpose of this research, the procedures required and the possible risks and benefits of the study.

__________________________________________________________________________
Investigator’s Signature  Date  ___________
Appendix 5: Authorization of Medical Release Form

Consent Form for Release of Medical Records

I, (Print name) _____________________________, Date of Birth: ____________________ hereby authorize the release of information from physicians and from hospitals pertaining to my screening, diagnosis, and treatment of breast cancer (i.e. MRI or Mammograms) at:

(Full Address of Clinic)

____________________________________________________________________________

Released to:

Principle Investigator: Dr. Joanne Kotsopoulos
Co-Investigator: Dr. Steven Narod
Familial Breast Cancer Research Unit
Women’s College Research Institute
76 Grenville Street, 6th Floor
Toronto, ON M5S 1B2
Fax: 416-351-3767

Signature:___________________________ Date:_______________________
Witness:____________________________ Date:_______________________

I give permission to Dr. Joanne Kotsopoulos and Dr. Steven Narod and the study team to contact me by telephone if additional information is needed.

YES______________________TELEPHONE#:________________________

NO__________________________
Appendix 6: Diet History Questionnaire

Due to the large size of this questionnaire, the actual document has not been included but may be accessed at https://epi.grants.cancer.gov/DHQ/.
Appendix 7: Research Questionnaire for the Risk Factor Analysis for Familial Breast Cancer Study

RISK FACTOR ANALYSIS FOR FAMILIAL BREAST CANCER

This questionnaire is part of a research study to improve our understanding of the prevention and treatment of hereditary breast cancer. Please complete to the best of your ability and contact us (416-351-3800 ext. 2875) if you have any questions.

Name: ____________________________

Date of Birth: ____/____/______  Age: ___

Date Completed: ____/____/______

mm  dd  yyyy
1. Have you ever been pregnant?
   □ No    → Go to question 6.
   □ Yes

2. Please consider all pregnancies, in order, from first to last. Give year of pregnancy. Place an ‘X’ in the appropriate column for the outcome of each pregnancy. For live-born children try to recall for how long you breast-fed each child and indicate months of breast-feeding in the right column (if not breast-fed, enter ‘0’).

   **PREGNANCY OUTCOMES**

<table>
<thead>
<tr>
<th>#</th>
<th>Year of Pregnancy</th>
<th>Miscarriage</th>
<th>Therapeutic Abortion</th>
<th>Still Born</th>
<th>Live Born (Month/Year of Birth)</th>
<th>Length of Pregnancy (In Weeks)</th>
<th>Birth Weight (If known)</th>
<th>Months of Breast Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>9</td>
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</tr>
</tbody>
</table>

   *(for twins, enter ‘T’ at the corresponding cell)*

3. How many of these pregnancies were caesarean sections? ________

   Circle which pregnancies (1-9 in chart above):
   
   1 2 3 4 5 6 7 8 9

4. Did you ever have difficulty breast-feeding?
   □ No
   □ Yes    → Reason: __ poor milk production    __ pain
               __ premature infant    __ mastitis (breast inflammation)
               __ other (please specify): __________________

   Circle which child (1-9 in chart above) you experienced this with:
   
   1 2 3 4 5 6 7 8 9
5. Have you ever taken medication to stop milk production?
   □ No
   □ Yes → Name of medication (if known): _________________________
   Method: ___ injections or ___ pills

   Circle with which child (1-9 in preceding chart) you took this medication.
   1  2  3  4  5  6  7  8  9

6. How old were you when you had your first menstrual period? _____ YEARS OLD

7. How would you describe your menstrual cycle? By regular we mean that the start of your period was predictable within 5 days. (Please check one)
   □ My periods are/were **always regular.**
   □ My periods are/were **usually regular.**
   □ My periods are/were **never regular.**

8. How many days apart are your menstrual periods? That is, from the start of one period to the start of the next period. For example, many women have cycles of 28-32 days. (Please check one)
   __ 28 days __ 29 days __ 30 days __ 31 days __ 32 days Other→____ days

9. Do you currently have menstrual periods? That is, have you had a menstrual period within the last year? Please answer “Yes” if you are currently pregnant or breast-feeding.
   □ No
   □ Yes

10. Have your periods stopped completely?
    □ No → Go to question 13.
    □ Yes

11. How old were you when your periods stopped completely? ______ YEARS OLD

12. What was the reason your periods stopped? (Select one only)
    □ **Natural Menopause** (change of life)
    □ **Hysterectomy** (uterus removed/ovaries not removed)
    □ **Uterus and ovaries removed**
    □ **Oophorectomy** (ovaries removed/uterus not removed)
    □ **Medication / Chemotherapy**
    □ **Other** (please specify): ___________________________________________
13. Have you ever seen a doctor for a problem of difficulty in getting pregnant or in carrying a pregnancy, such as several miscarriages?
   □ No → Go to question 16.
   □ Yes → What reason did the doctor give to explain why you had trouble getting or staying pregnant? (Please check all that apply.)
   ___ no problem was found ___ problem with cervix
   ___ problem with ovaries ___ partner has fertility problem
   ___ problem with fallopian tubes ___ endometriosis
   ___ other (please specify): ______________________

14. Have you ever taken medication to increase your chances of becoming pregnant?
   □ No
   □ Yes → Name of medication(s): ______________________________
   For how many months did you take this medication? _____ months
   What years did you take this medication? _______, ______

15. Have you ever received fertility treatment such as in vitro fertilization/Embryo Transfer (IVF/ET) to help you get pregnant?
   □ No
   □ Yes → What type of treatment did you receive? _______________________ 

16a. Have you ever used birth control pills, Norplant (implants), Depo-Provera (injections), or an IUD (i.e., Mirena, copper) to prevent pregnancy or for any other reason?
   □ No → Go to question 17.
   □ Yes → Can you describe the times?

<table>
<thead>
<tr>
<th>Name of Medication</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
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<td>4</td>
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</tr>
</tbody>
</table>
   □ Pills □ Implants □ IUD □ Injections

16b. Are you currently using birth control pills, Depo-Provera, Norplant, or an IUD?
   □ No
   □ Yes
17a. Have you ever taken hormone replacement therapy for menopause (i.e. estrogen, progesterone)?

☐ No → Go to question 18.

☐ Yes → Complete table below:

<table>
<thead>
<tr>
<th>Name of Hormone</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used (Years)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>3</td>
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<tr>
<td>4</td>
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</tbody>
</table>

☐ Pills ☐ Skin Patches ☐ Vaginal Suppositories

17b. Are you currently taking hormone replacement therapy?

☐ No

☐ Yes → Name of hormone: ___________________ dose? __________

SECTION II – Breast Cancer Screening/Prevention

18. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?

☐ No

☐ I'm not sure

☐ Yes → Name of Drug:  
  ____ Tamoxifen (Nolvadex)  
  ____ Raloxifene (Evista)  
  ____ Aromasin (Exemestane)  
  ____ Femara (Letrozole)  
  ____ Arimidex (Anastrozole)  
  ____ Other (please name) ____________________________

Number of pills per day: _______________

Date started: __ __ / __ __ __ __ (mm / yyyy) to __ __ / __ __ __ __ (mm / yyyy)
19. Have you ever participated in a clinical trial for the prevention of breast cancer?
   □ No
   □ Yes → Which one?  ____ Tamoxifen Trial (Tamoxifen vs. placebo)
   □ STAR Trial (Tamoxifen vs. Raloxifene)
   □ Other: __________________________
   □ Unknown
   Do you know which drug you took? □ No  □ Yes
   If yes, which one? __________________________

20. Have you ever had breast implants or breast reconstruction?
   □ No
   □ Yes → Type: ___ Saline  ___ Silicone  ___ TRAM-flap
   □ Other __________________________
   Year of surgery: ___ ___ ___

21. Have you ever had a breast reduction?
   □ No
   □ Yes
   Year of surgery: ___ ___ ___

22. Have you undergone surgery at any time in order to prevent breast cancer (e.g. preventive removal of the breasts)?  NOTE: The surgery is only preventive if you’ve never previously been diagnosed with cancer in that breast.
   □ No
   □ Yes → What year? _________
   Which breast? ___ Left  ___ Right  ___ Both
   Procedure: ___ Total mastectomy (nipple and areola removed)
   □ Subcutaneous mastectomy (nipple and areola preserved)

23. Have you ever had a breast biopsy?
   □ No
   □ Yes → Number of biopsies: _________
   Month/Year of last biopsy: ______/___________
   What was the result of the biopsy? (Check all that apply)
   ___ normal  ___ DCIS  ___ atypical hyperplasia
   ___ cancer  ___ LCIS  ___ fibroadenoma
   ___ other (please specify): __________________________
24. Approximately how many mammograms have you had in your lifetime?
   I have had _____ mammograms. If you do not recall the exact number, please provide a range.
   Age at first mammogram? ______ years old.
   Age at last mammogram? ______ years old.

25. Have you ever had any abnormalities detected by mammogram?
   ☐ No
   ☐ Yes → Please describe the abnormality: _________________________________
   Month/Year of abnormality: ______/___________

26. Have you ever had MRI screening of your breasts?
   ☐ No
   ☐ Yes → Age at first MRI: ______
   How many MRIs have you had? __________________
   Where were they done: __________________________
   What year was your last MRI done? __ __ __ __
   Were there any abnormalities detected by MRI? ☐ No ☐ Yes
   If yes, please describe: _______________________________
   Year of abnormality: __ __ __ __

SECTION III – Medical History

27. Are you taking or have you taken any prescription medication (i.e., Metformin, Lipitor) and/or over-the-counter medications (i.e., baby aspirin, Advil, Benadryl) on a regular basis?
   Note: Please do not include birth control pills or hormone replacement therapy.
   ☐ No
   ☐ Yes → Complete:

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Dosage</th>
<th>Date Started</th>
<th>Date Ended</th>
<th>Reason</th>
</tr>
</thead>
</table>

28. Have you ever been diagnosed with any cancer other than breast or ovarian?
   ☐ No
   ☐ Yes → What type? _______________ Year of Diagnosis? ________
29. Please describe briefly any medical problems that you have had in the past, especially those that may have required hospitalization.

_____________________________________________________________________
_____________________________________________________________________

30. Have you received your genetic test results?

☐ No
☐ Yes → Date you received your results: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

31. What do you think your risk of developing breast cancer over your lifetime is? Example: If you think you have an 80% risk of developing breast cancer, place a mark at 80.

[100] 0 20 40 60 80 100

I don’t know _____________

32. What do you think your risk of developing ovarian cancer over your lifetime is? Example: If you think you have an 80% risk of developing ovarian cancer, place a mark at 80.

[100] 0 20 40 60 80 100

I don’t know _____________

SECTION IV — Reproductive/Abdominal Surgeries

33. Have you had surgery performed on your reproductive organs: including ovaries, fallopian tubes, or uterus? (e.g. preventative measures, fibroids, cyst, scar tissue, or pain)

☐ No
☐ Yes → Year of surgery: __________

Type of surgery (please check one):

☐ Hysterectomy ☐ Cervix removed
☐ Myomectomy ☐ D & C
☐ Oophorectomy:

Number of ovaries removed: ___ One ___ Two ___ Unsure
Fallopian tubes removed: ___ One ___ Two ___ Unsure
Reason for the surgery: __________________________
34. Have you ever had a tubal ligation (fallopian tubes tied)?
   □ No
   □ Yes → Year: _________

35. Have you ever had another operation on your abdomen? (e.g. gall bladder, appendix, laparoscopy, hernia, etc)
   □ No
   □ Yes → 1. Type of surgery: ____________________________ Year: _________
               2. Type of surgery: ____________________________ Year: _________
               3. Type of surgery: ____________________________ Year: _________

SECTION V – Personal Information

37. Place of Birth: ____________________________________________
               City Province/State

38. Current Residence: __________________________________________
               City Province/State

39. Ethnic Background: _________________________________________

40a. What is the major ancestry of your father (paternal)? (Please circle one option.)
   African/Caribbean/African American  Irish
   (country of origin: _________)  Italian
   Ashkenazi Jewish  Native American (Amer. Indian)
   Asian/Pacific Islander  Polish/Slavic/Eastern
   (country of origin: _________)  Russian
   Dutch  Scandinavian (Swedish/Finnish/ Norwegian/Dane)
   English  Scot-Irish or Scottish
   European Bloc countries  Sephardic Jewish
   French Canadian  Other (specify: _____________)
   German  Unknown
   Hispanic  (country of origin: _____________)
40b. What is the major ancestry of your mother (maternal)? (*Please circle one option.*)

- African/Caribbean/African American
- (country of origin: __________)
- Ashkenazi Jewish
- (country of origin: __________)
- Asian/Pacific Islander
- (country of origin: __________)
- Dutch
- (country of origin: __________)
- English
- European Bloc countries
- Scandavian (Swedish/Finnish/ Norwegian/Dane)
- French Canadian
- German
- Hispanic
- (country of origin: _____________)
- Italian
- Native American (Amer. Indian)
- Polish/Slavic/Eastern
- Russian
- Asian/Pacific Islander
- Polish/Slavic/Eastern
- Russian
- Dutch
- Scandavian (Swedish/Finnish/ Norwegian/Dane)
- French Canadian
- German
- Hispanic
- (country of origin: _____________)
- Italian
- Native American (Amer. Indian)
- Polish/Slavic/Eastern
- Russian
- Dutch
- Scandavian (Swedish/Finnish/ Norwegian/Dane)
- French Canadian
- German
- Hispanic
- (country of origin: _____________)

40c. Please indicate if your grandparents were:

- Maternal Grandmother:  
  - Ashkenazi Jewish  
  - Sephardic Jewish  
  - Other
- Maternal Grandfather:  
  - Ashkenazi Jewish  
  - Sephardic Jewish  
  - Other
- Paternal Grandmother:  
  - Ashkenazi Jewish  
  - Sephardic Jewish  
  - Other
- Paternal Grandfather:  
  - Ashkenazi Jewish  
  - Sephardic Jewish  
  - Other

41. What is your highest level of education? (*please check one*)

- ___ Attended elementary school
- ___ Graduated from elementary school
- ___ Attended high school
- ___ Graduated from high school
- ___ Attended college/university
- ___ Graduated from college/university
- ___ Attended graduate school
- ___ Graduated from graduate school

42. What is your current occupation? ________________________

43. In the past, have you worked permanent nights or in a rotating shift system that has included at least three nights per month in addition to days and evenings in that month?

- □ No
- □ Yes

44. How long altogether have you been working night shifts? _____ Years _____ Months

45. What is your:  
- current weight? _______ pounds
- current height? _______ feet _____ inches

46. Think back to when you were 18 years old, about the time you graduated from high school.

   How much did you weigh then? _______ pounds
   at age 30? _______ pounds
   at age 40? _______ pounds

   What is the most you have ever weighed (exclude pregnancy)? _______ pounds

   How old were you when you weighed the most? _______ years old
47. Do you know how much you weighed when you were born?
   □ No
   □ Yes → ______ pounds ______ ounces  OR  ______ grams

48. What is your mother’s year of birth? ______________

49. What is your birth order (i.e. first-born, second-born, third-born etc.)? ______________

50. Were you part of a multiple birth (i.e. twin, triplet)?
   □ No
   □ Yes → (Please check one)
   ___ twin → Are you an identical twin?  □ No  □ Yes
   ___ triplet       ___ other (please specify): __________

SECTION VI – Lifestyle

51. Have you ever smoked cigarettes regularly?
   □ No
   □ Yes → From: __________ (age first started) to __________ (age last used)
   On average, how many packs do/did you smoke per week? ______________
   Do you still smoke?  □ No  □ Yes

52. Do you or did you ever drink coffee regularly?
   □ No
   □ Yes → From: __________ (age first started), Until: __________ (age last used)

<table>
<thead>
<tr>
<th>Caffeinated</th>
<th>□ Yes</th>
<th>□ No</th>
<th>Decaffeinated</th>
<th>□ Yes</th>
<th>□ No</th>
</tr>
</thead>
<tbody>
<tr>
<td>If you answered yes, please check the best answer:</td>
<td>If you answered yes, please check the best answer:</td>
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<td></td>
</tr>
<tr>
<td>1 – 2 cups/day</td>
<td>□</td>
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<td>1 – 2 cups/day</td>
<td>□</td>
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<td>3 – 4 cups/day</td>
<td>□</td>
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<td>3 – 4 cups/day</td>
<td>□</td>
<td></td>
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<tr>
<td>5 or more cups/day</td>
<td>□</td>
<td></td>
<td>5 or more cups/day</td>
<td>□</td>
<td></td>
</tr>
</tbody>
</table>

   If you currently drink coffee, please indicate the last time you drank coffee:
   □ Yes, Today
   □ Yes, Daily during the last week
   □ Yes, Occasionally during the last week
   □ Yes, Other. Please specify ____________________________________________
53. Do you or did you ever drink tea regularly?

☐ No

☐ Yes → From: __________ (age first started), Until: __________ (age last used)

<table>
<thead>
<tr>
<th>Black Tea ☐ No ☐ Yes</th>
<th>Green Tea ☐ No ☐ Yes</th>
<th>Herbal Tea ☐ No ☐ Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>If you answered yes, please check the best answer:</strong></td>
<td><strong>If you answered yes, please check the best answer:</strong></td>
<td><strong>If you answered yes, please check the best answer:</strong></td>
</tr>
<tr>
<td>☐ 1 – 2 cups/day</td>
<td>☐ 1 – 2 cups/day</td>
<td>☐ 1 – 2 cups/day</td>
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<td>☐ 3 – 4 cups/day</td>
<td>☐ 3 – 4 cups/day</td>
<td>☐ 3 – 4 cups/day</td>
</tr>
<tr>
<td>☐ 5 or more cups/day</td>
<td>☐ 5 or more cups/day</td>
<td>☐ 5 or more cups/day</td>
</tr>
</tbody>
</table>

If you currently drink tea, please indicate the last time you drank tea:

☐ Today

☐ Daily during the last week

☐ Occasionally during the last week

☐ Other. Please specify_________________________________________

54. Do you or have you ever had alcoholic beverages?

☐ No

☐ Yes → From: __________ (age first started), Until: __________ (age last used)

On average, how many alcoholic drinks do/did you have per week? *(Check one)*

☐ 0-3  ☐ 4-9  ☐ 10-20  ☐ 20 or more

What type of alcoholic beverages do/did you drink? *(Check all that apply.)*

☐ Beer  ☐ Wine  ☐ Hard liquor

Please indicate when was the last time you drank alcoholic beverages?

☐ Today

☐ Daily during the last week

☐ Occasionally during the last week

☐ Other. Please specify_________________________________________
### SECTION VII – PHYSICAL ACTIVITY

55. The following are questions about your physical activity at various times in your life and at various intensity levels. For each age range below, please estimate the average amount of time that you spend in these activities. We recognize that this is a difficult task, but we ask that you average your activity over seasons and years during the given age categories.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Average hours per WEEK</th>
<th>Grades 7-8</th>
<th>Grades 9-12</th>
<th>Ages 18-22</th>
<th>Ages 23-29</th>
<th>Ages 30-34</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Walking to and from school or work</td>
<td>None</td>
<td>0.5</td>
<td>1-2</td>
<td>3-4</td>
<td>5-6</td>
<td>7-10</td>
</tr>
<tr>
<td>b) TV watching</td>
<td>None</td>
<td>1</td>
<td>2-5</td>
<td>6-10</td>
<td>11-20</td>
<td>21-40</td>
</tr>
<tr>
<td>c) Strenuous Recreational Activity causing increased breathing, heart-rate or sweating (e.g., running, aerobics, lap swimming)</td>
<td>None</td>
<td>0.5</td>
<td>1-2</td>
<td>3-4</td>
<td>5-6</td>
<td>7-10</td>
</tr>
<tr>
<td>d) Moderate Recreational Activity e.g., hiking, walking for exercise, casual cycling, yard work (do not count activities already reported)</td>
<td>None</td>
<td>0.5</td>
<td>1-2</td>
<td>3-4</td>
<td>5-6</td>
<td>7-10</td>
</tr>
</tbody>
</table>
56. **DURING THE PAST YEAR**, what was your average time **PER WEEK** spent at each of the following recreational activities?

<table>
<thead>
<tr>
<th>Activity</th>
<th>TIME PER WEEK</th>
<th>Zero</th>
<th>1-4 min</th>
<th>5-19 min</th>
<th>20-59 min</th>
<th>One hour</th>
<th>1-1.5 hrs</th>
<th>2-3 hrs</th>
<th>4-6 hrs</th>
<th>7-10 hrs</th>
<th>11+ hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking for exercise or walking to work</td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
<td>☐</td>
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<td>☐</td>
</tr>
<tr>
<td>Jogging (slower than 6 minutes/km)</td>
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<td>☐</td>
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<tr>
<td>Running (6 minutes/km or faster)</td>
<td></td>
<td>☐</td>
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</tr>
<tr>
<td>Bicycling (include stationary machine)</td>
<td></td>
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</tr>
<tr>
<td>Tennis, squash, racquetball</td>
<td></td>
<td>☐</td>
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<tr>
<td>Lap swimming</td>
<td></td>
<td>☐</td>
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<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other aerobic exercise (aerobic, dance, ski or stair machine, etc.)</td>
<td></td>
<td>☐</td>
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<tr>
<td>Lower intensity exercise (yoga, stretching, toning)</td>
<td></td>
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<tr>
<td>Other vigorous activities (e.g., lawn mowing)</td>
<td></td>
<td>☐</td>
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<td>☐</td>
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<tr>
<td>Weight training or resistance exercises (include free weights or machines)</td>
<td></td>
<td>☐</td>
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</tr>
</tbody>
</table>

57. **DURING THE PAST YEAR**, on average, how many hours **PER WEEK** did you spend:

<table>
<thead>
<tr>
<th>Activity</th>
<th>TIME PER WEEK</th>
<th>None</th>
<th>1-2.5</th>
<th>2.6-5</th>
<th>5.1-10</th>
<th>10.1-20</th>
<th>20.1-40</th>
<th>40.1-60</th>
<th>60.1-90</th>
<th>90.1-110</th>
<th>110+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing or walking around work or away from home</td>
<td></td>
<td>☐</td>
<td>☐</td>
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<tr>
<td>Standing or walking around home</td>
<td></td>
<td>☐</td>
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<tr>
<td>Sitting at work or away from home or while driving</td>
<td></td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
<td>☐</td>
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<td>☐</td>
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<tr>
<td>Sitting at home while watching TV</td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
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</tr>
<tr>
<td>Other sitting at home (eg, reading, meal times, at desk)</td>
<td></td>
<td>☐</td>
<td>☐</td>
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</tbody>
</table>

### SECTION VIII – Current Vitamin and Supplement Use

58. Are you currently taking **Vitamins or Multivitamins or Supplements**? *(For example: folic acid, selenium, diindolylmethane (DIM), vitamin D).*

- [ ] No
- [ ] Yes →If yes, please specify in table below

<table>
<thead>
<tr>
<th>Vitamin or Multivitamin or Supplement</th>
<th>Name</th>
<th>Pills/Week</th>
<th>Dosage</th>
<th>Duration of Use</th>
<th>Age started</th>
<th>Age last used</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>
59. Think back to the time you were pregnant. In the **12 months before pregnancy**, did you take any dietary supplements (i.e., folic acid, vitamin B6, vitamin B12, multivitamin, prenatal vitamin)? Please indicate the type, brand name, frequency of intake and the month when intake was started/stopped for prior to each pregnancy.

<table>
<thead>
<tr>
<th>Pregnancy (i.e., 1st, 2nd)</th>
<th>Vitamin (i.e., folate, B12)</th>
<th>Brand</th>
<th>Dose (i.e., pills per day)</th>
<th>Month Started</th>
<th>Month Stopped</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

60. When you found out you were pregnant, did you take any dietary supplements **during pregnancy**? Please indicate the type, brand name, frequency of intake and the month when intake was started for each pregnancy.

<table>
<thead>
<tr>
<th>Pregnancy (i.e., 1st, 2nd)</th>
<th>Vitamin (i.e., folate, B12)</th>
<th>Brand</th>
<th>Dose (i.e., pills per day)</th>
<th>Month Started</th>
<th>Month Stopped</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

**Thank you for taking the time to complete this questionnaire.**

Please provide your contact information for Follow-Up:

Contact Number: __________________________
Email: __________________________
Address:  

In the case we cannot reach you, please provide an alternate contact:

Name: __________________________ Relationship: __________________________
Contact Number: __________________________
Email: __________________________
Address:  

Please call us if you are changing contact information – 416-351-3800 ext. 2875

**FOR OFFICE USE**

Date questionnaire received: __________________________
Month – Day – Year
As a participant in the study ‘Risk Factor Analysis for Familial Breast Cancer’, we request that you complete this follow-up questionnaire. Please complete to the best of your ability and contact us (416-351-3800 ext. 2875) if you have any questions.

Date Completed: __________________________(dd / mm / yyyy)

Name: ________________________________________________

Date of Birth: ________________________________(dd / mm / yyyy)

Fam/Study ID#: ________________________________

Telephone #: ________________________________

Date of Previous Questionnaire: ______________________(dd / mm / yyyy)
SECTION I - REPRODUCTIVE UPDATE

1. Have you had any pregnancies since __ __ / __ __ __ __ (mm / yyyy) (including still born, miscarriage, abortion)?
   - No
   - Yes → Year of pregnancy: __ __ __ __ Date of birth: __ __ / __ __ / __ __ __ __ (dd/mm/yyyy)
   
   Pregnancy outcome (please check one):
   - ___ Liveborn
   - ___ Stillborn
   - ___ Abortion
   - ___ Miscarriage
   - ___ Other: ____________________

2. Have you ever had any difficulty breastfeeding?
   - I have never breastfed
   - No
   - Yes → Reason: _____________________________

3. Are you still having menstrual periods? That is, have you had a menstrual period within the last year? Please answer “Yes” if you are currently pregnant, breast-feeding, or taking hormones which temporarily stop your menses (i.e., Mirena IUD or birth control pills.)
   - Yes
   - No → At what age did they stop? ______________
   
   Reason they stopped? (Please check one)
   - ___ Ovaries removed (uterus remained)
   - ___ Medication/Chemotherapy
   - ___ Uterus removed (ovaries remained)
   - ___ Natural Menopause
   - ___ Both uterus and ovaries removed
   - ___ Other: ____________________

4. Have you ever taken birth control medication?
   - No
   - Yes → (i) From: __ __ __ __ to __ __ __ __ (year)
   
   Length of time used: __________ years __________ months
   
   Name of medication: __________________________
   
   Method (please check one):
   - ___ Pill
   - ___ Injection
   - ___ IUD
   - ___ Implant
   - ___ Other: ____________________

   (ii) From: __ __ __ __ to __ __ __ __ (year)
   
   Length of time used: __________ years __________ months
   
   Name of medication: __________________________
   
   Method (please check one):
   - ___ Pill
   - ___ Injection
   - ___ IUD
   - ___ Implant
   - ___ Other: ____________________

   Are you still using birth control medication?  □ No  □ Yes
5. Have you ever taken hormone replacement therapy (HRT)?
   □ No
   □ Yes → (i) From: __ __ __ __ to __ __ __ __ (year)
   Length of time used: __________ years __________ months
   Name of medication: _____________________________
   Dose (mg/day): _____________________________
   Method (please check one): ___ Pills   ___ Cream   ___ Gel    ___ Patch
   ___ Vaginal Suppositories   ___ Other: _______

   (ii) From: __ __ __ __ to __ __ __ __ (year)
   Length of time used: __________ years __________ months
   Name of medication: _____________________________
   Dose (mg/day): _____________________________
   Method (please check one): ___ Pills   ___ Cream   ___ Gel   ___ Patch
   ___ Vaginal Suppositories   ___ Other: _______

   Are you still using HRT? □ No   □ Yes

SECTION II – BREAST CANCER SCREENING/PREVENTION

6. Have you ever had a mammogram?
   □ No
   □ Yes → Age at first mammogram: __________
   How many mammograms have you had? __________
   Where was your most recent mammogram done (name of center): __________
   Were there any abnormalities detected by mammogram? □ No   □ Yes
   If yes, please describe: _____________________________
   Year of abnormality: __ __ __ __
   What year was your last mammogram done? __ __ __ __

7. Have you ever had MRI screening of your breasts?
   □ No
   □ Yes → Year of first MRI: __________
   How many MRIs have you had? __________
   Where was your most recent MRI done (name of center): __________
   What year was your last MRI done? __ __ __ __
   Were there any abnormalities detected by MRI? □ No   □ Yes
   If yes, please describe: _____________________________
   Year of abnormality: __ __ __ __
8. Have you ever had a breast biopsy (this includes needle and core biopsies)?

☐ No

☐ Yes  →  Number of biopsies: ____________________________

Date of first biopsy: __ __ / __ __ / __ __ __ __ (dd / mm / yyyy)
Date of last biopsy: __ __ / __ __ / __ __ __ __ (dd / mm / yyyy)
Result of biopsies: ____________________________

9. Have you undergone preventive removal of your breasts?

☐ No

☐ Yes  →  Date: __ __ / __ __ / __ __ __ __ (dd / mm / yyyy)

Which breast was removed?  ____ Left  ____ Right  ____ Both

Procedure:  ____ Subcutaneous Mastectomy (nipple and areola preserved)

____ Total Mastectomy (nipple and areola removed)

____ Other: ____________________________

10. Have you ever had breast implants or breast reconstruction?

☐ No

☐ Yes  →  Type:  ____ Saline  ____ Silicone  ____ TRAM-flap

____ Other: ____________________________

Year of surgery: __ __ __ __

11. Have you ever had breast reduction?

☐ No

☐ Yes

Year of surgery: __ __ __ __

12. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?

☐ No

☐ Yes  →  Name of Drug:  ____ Tamoxifen (Nolvadex)  ____ Raloxifene (Evista)

____ Aromasin (Exemestane)  ____ Femara (Letrozole)

____ Arimidex (Anastrozole)  ____ Other (please name): ______

Dosage (mg/day): __________________

Date started: __ __ / __ __ / __ __ __ __ (dd/mm/yyyy)  to  __ __ / __ __ / __ __ __ __ (dd/mm/yyyy)

13. Have you ever participated in a clinical trial for the prevention of breast cancer?

☐ No

☐ Yes  →  Which one?  ____ Tamoxifen Trial (Tamoxifen vs. placebo)

____ STAR Trial (Tamoxifen vs. Raloxifene)

____ Other: ____________________________

____ Unknown

Do you know which drug you took?  ☐ No  ☐ Yes

If yes, which one? ____________________________
14. Have you been diagnosed with breast cancer since date of previous questionnaire?

☐ No  ➔ Go to Question 16 (Section IV).
☐ Yes  ➔ Date of diagnosis: ___ / ___ / ___ ___ (dd / mm / yyyy)
   Which breast? ___ Left  ___ Right  ___ Both

**Type of Cancer (check all that apply):**

☐ It was an invasive cancer.
☐ It was a non-invasive cancer (early stage breast cancer).
   ➔ Was it Ductal Carcinoma In-situ (DCIS)?  ☐ No  ☐ Yes
   ➔ Was it Lobular Carcinoma In-situ (LCIS)?  ☐ No  ☐ Yes
☐ I’m not sure.

**Treatment:** (i) Surgery?  ☐ No  ☐ Yes

  **If Yes:** ____ Mastectomy (whole breast removed)
   Which breast? ___ Left  ___ Right  ___ Both
   Date of mastectomy: ___ / ___ / ___ ___ (dd / mm / yyyy)

  ____ Lumpectomy (part of breast removed)
   Which breast? ___ Left  ___ Right  ___ Both
   Date of lumpectomy: ___ / ___ / ___ ___ (dd / mm / yyyy)

  ____ Axillary Node Dissection (lymph nodes removed)
   Number removed: ______
   Were any found to have cancer?  ☐ No  ☐ Yes
   How many? ______

(ii) Chemotherapy?  ☐ No  ☐ Yes

  **If yes,** name of drug: __________________

(iii) Radiation therapy?  ☐ No  ☐ Yes

(iv) Other treatment drugs (e.g. Tamoxifen, Femara, etc.)?  ☐ No  ☐ Yes

  **If yes,** name of drug: ___________________ Dosage (mg/day): ______
   Date started: ___ / ___ ___ to ___ / ___ ___ (mm / yyyy)

Name of hospital: ___________________________ City: _______________________
How was the cancer first detected?

If a lump was felt, was a mammogram done to confirm the cancer?  ☐ No  ☐ Yes

15. Have you had a breast cancer recurrence?

☐ No

☐ Yes  ➔ Date: ___ / ___ / ___ ___ (dd / mm / yyyy)
   Site of recurrence: __________________Treatment: ___________________
### SECTION IV – OTHER CANCERS

16. Have you been diagnosed with any cancer other than breast cancer?

- [ ] No
- [ ] Yes  →  Date of diagnosis: ___/___/____ (dd/mm/yyyy)
  Type: ______________________________________
  Treatment: ___________________________________

### SECTION V – MEDICAL HISTORY

17. Have you ever had any of the following medical conditions? *Check ALL that apply.*

<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Age(s) at onset</th>
<th>Medications (including non-prescription drugs or dietary) and/or treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>___ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Eczema</td>
<td>___ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Hay fever</td>
<td>___ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Date(s) ended:</td>
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<tr>
<td></td>
<td></td>
<td>[ ] No treatments</td>
</tr>
<tr>
<td><strong>Arthritis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>___ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>___ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Other: ______________</td>
<td>___ years</td>
<td>Date(s) started:</td>
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<tr>
<td></td>
<td></td>
<td>Date(s) ended:</td>
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<tr>
<td></td>
<td></td>
<td>[ ] No treatments</td>
</tr>
<tr>
<td><strong>Bone health</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteopenia</td>
<td>___ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>___ years</td>
<td>Dosage(s):</td>
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<tr>
<td></td>
<td></td>
<td>Date(s) started:</td>
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<tr>
<td></td>
<td></td>
<td>Date(s) ended:</td>
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<tr>
<td></td>
<td></td>
<td>[ ] No treatments</td>
</tr>
<tr>
<td><strong>Thyroid problems</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hyperthyroid (overactive)</td>
<td>___ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Hypothyroid (underactive)</td>
<td>___ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Other: ______________</td>
<td>___ years</td>
<td>Date(s) started:</td>
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<tr>
<td></td>
<td></td>
<td>Date(s) ended:</td>
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<tr>
<td></td>
<td></td>
<td>[ ] No treatments</td>
</tr>
<tr>
<td><strong>Heart health</strong></td>
<td></td>
<td></td>
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<tr>
<td>High cholesterol</td>
<td>___ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>___ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Stroke</td>
<td>___ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Blood clot</td>
<td>___ years</td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[ ] No treatments</td>
</tr>
</tbody>
</table>

*Where was the clot? ______________*
<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Age(s) at onset</th>
<th>Medications (including non-prescription drugs or dietary) and /or treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
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<tr>
<td>Coronary heart disease</td>
<td></td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Cardiomyopathy (heart muscle weak</td>
<td></td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Angina</td>
<td></td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Heart attack (myocardial infarcti</td>
<td></td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td>Other: __________________________</td>
<td>________ years</td>
<td>No treatments</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I diabetes (autoimmune)</td>
<td>________ years</td>
<td>Insulin ➔ Brand: _______________</td>
</tr>
<tr>
<td>Type II diabetes</td>
<td>________ years</td>
<td>Dosage: __________</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>________ years</td>
<td>Gliclazide (Diamicron) Dosage: ________</td>
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<td></td>
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<td>Metformin (Glucophage) Dosage: ________</td>
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<td></td>
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<td>Rosiglitazone (Avandia) Dosage: ________</td>
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<td></td>
<td>Pioglitazone (Actos) Dosage: ________</td>
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<tr>
<td></td>
<td></td>
<td>Glyburide (Diabeta) Dosage: ________</td>
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<td></td>
<td></td>
<td>Other: __________________________ Dosage: ________</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No treatments</td>
</tr>
<tr>
<td>Autoimmune Diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus (SLE)</td>
<td>________ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>________ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>________ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Other: __________________________</td>
<td>________ years</td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td>Eye conditions</td>
<td></td>
<td>No treatments</td>
</tr>
<tr>
<td>Cataracts</td>
<td>________ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>________ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Detached retina</td>
<td>________ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Other: __________________________</td>
<td>________ years</td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td>Gastrointestinal (GI) problems</td>
<td></td>
<td>No treatments</td>
</tr>
<tr>
<td>Gall stones</td>
<td>________ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Lactose intolerance</td>
<td>________ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>________ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>________ years</td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td>Other: __________________________</td>
<td>________ years</td>
<td>No treatments</td>
</tr>
<tr>
<td>Urinary and genital problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic ovary syndrome (PCOS)</td>
<td>________ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Cystocele (fallen bladder)</td>
<td>________ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td>Urinary tract infection (UTI)</td>
<td>________ years</td>
<td>Date(s) started:</td>
</tr>
<tr>
<td>Fibroids</td>
<td>________ years</td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>________ years</td>
<td>No treatments</td>
</tr>
<tr>
<td>Other: __________________________</td>
<td>________ years</td>
<td></td>
</tr>
<tr>
<td>Mental Health</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>________ years</td>
<td>Drug name(s):</td>
</tr>
<tr>
<td>Anxiety</td>
<td>________ years</td>
<td>Dosage(s):</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Date(s) started:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Date(s) ended:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No treatments</td>
</tr>
</tbody>
</table>
18. Have you had any surgery since _____ / ________ (mm / yyyy) not previously mentioned?  
☐ No  ☐ Yes  (Complete table below)

<table>
<thead>
<tr>
<th>Date of Surgery</th>
<th>Type of Surgery</th>
<th>Reason for Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

19. Have you taken any other prescription medications and/or over-the-counter medications regularly (i.e., Tylenol, baby aspirin) since _____ / ________ (mm / yyyy) not previously mentioned?  
Note: Please do not include birth control pills or hormone replacement therapy.  
☐ No  ☐ Yes  (Complete table below)

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Drug 1</th>
<th>Drug 2</th>
<th>Drug 3</th>
<th>Drug 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

20. Please describe briefly any other medical problems that you have had in the past, especially those that may have required hospitalization.

__________________________________________________________________________________
__________________________________________________________________________________
__________________________________________________________________________________
__________________________________________________________________________________
__________________________________________________________________________________

SECTION VI – PERSONAL INFORMATION

21. What is your:  
- Current weight? _______ Pounds
- Current height? _______ Feet, _____ Inches
22. What is your highest level of education? _(please check one)_
   ___ No Schooling
   ___ Attended elementary school __ Graduated from elementary school
   ___ Attended high school __ Graduated from high school
   ___ Attended college/university __ Graduated from college/university
   ___ Attended graduate school __ Graduated from graduate school

23. What is your current occupation? ____________________________

24. In the past, have you worked permanent nights or in a rotating shift system that has included at least three nights per month in addition to days and evenings in that month?
   □ Yes    □ No

   How long altogether have you been working night shifts? ____ Years ____ Months

25. Have you ever smoked cigarettes regularly?
   □ No
   □ Yes → From: __________ (age first started), Until: __________ (age last used)
     On average, how many packs do/did you smoke per week? __________ Packs
     Do you still smoke? □ Yes    □ No

   **When was the last time you smoked?**
   □ Yesterday
   □ Daily during last week
   □ Occasionally during last week
   □ Other. Please specify___________________________________________________

26. Do you or did you ever drink coffee regularly?
   □ No    □ Yes → From: __________ (age first started), Until: __________ (age last used)

<table>
<thead>
<tr>
<th>Caffeinated</th>
<th>□ No</th>
<th>□ Yes</th>
<th>Decaffeinated</th>
<th>□ No</th>
<th>□ Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If you answered yes, please check the best answer:</td>
<td>If you answered yes, please check the best answer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – 2 cups/day</td>
<td>□</td>
<td>1 – 2 cups/day</td>
<td></td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>3 – 4 cups/day</td>
<td>□</td>
<td>3 – 4 cups/day</td>
<td></td>
<td>□</td>
<td></td>
</tr>
<tr>
<td>5 or more cups/day</td>
<td>□</td>
<td>5 or more cups/day</td>
<td></td>
<td>□</td>
<td></td>
</tr>
</tbody>
</table>

   **If you currently drink coffee, please indicate the last time you drank coffee:**
   □ Today
   □ Daily during the last week
   □ Occasionally during the last week
   □ Other. Please specify___________________________________________________
27. Do you drink tea regularly?

☐ No  ☐ Yes → From: __________ (age first started), Until: __________ (age last used)

<table>
<thead>
<tr>
<th>Black Tea ☐ No ☐ Yes</th>
<th>Green Tea ☐ No ☐ Yes</th>
<th>Herbal Tea ☐ No ☐ Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If you answered yes, please check the best answer:</td>
<td>If you answered yes, please check the best answer:</td>
<td>If you answered yes, please check the best answer:</td>
</tr>
<tr>
<td>1 – 2 cups/day ☐</td>
<td>1 – 2 cups/day ☐</td>
<td>1 – 2 cups/day ☐</td>
</tr>
<tr>
<td>3 – 4 cups/day ☐</td>
<td>3 – 4 cups/day ☐</td>
<td>3 – 4 cups/day ☐</td>
</tr>
<tr>
<td>5 or more cups/day ☐</td>
<td>5 or more cups/day ☐</td>
<td>5 or more cups/day ☐</td>
</tr>
</tbody>
</table>

If you currently drink tea, please indicate the last time you drank tea:

☐ Today
☐ Daily during the last week
☐ Occasionally during the last week
☐ Other. Please specify________________________________________

28. Do you or have you ever had alcoholic beverages?

☐ No
☐ Yes → From: __________ (age first started), Until: __________ (age last used)

On average, how many alcoholic drinks do/did you have per week? (Please check one.)

☐ 0-3 ☐ 4-9 ☐ 10-20 ☐ 20 or more

What type of alcoholic beverages do/did you drink? (Check all that apply.)

☐ Beer ☐ Wine ☐ Hard liquor

Please indicate when was the last time you drank alcoholic beverages?

☐ Today
☐ Daily during the last week
☐ Occasionally during the last week
☐ Other. Please specify________________________________________
**SECTION VIII – Physical Activity**

The following are questions about your physical activity at various intensity levels. For each exercise listed below, please estimate the *average* amount of time that you spend in these activities. We recognize that this is a difficult task, but we ask that you *average* your activity over the seasons.

### 29. DURING THE PAST YEAR, what was your average time PER WEEK spent at each of the following recreational activities?

<table>
<thead>
<tr>
<th>Activity</th>
<th>TIME PER WEEK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero</td>
</tr>
<tr>
<td></td>
<td>1-4 min</td>
</tr>
<tr>
<td></td>
<td>5-19 min</td>
</tr>
<tr>
<td></td>
<td>20-59 min</td>
</tr>
<tr>
<td></td>
<td>One hour</td>
</tr>
<tr>
<td></td>
<td>1-1.5 hrs</td>
</tr>
<tr>
<td></td>
<td>2-3 hrs</td>
</tr>
<tr>
<td></td>
<td>4-6 hrs</td>
</tr>
<tr>
<td></td>
<td>7-10 hrs</td>
</tr>
<tr>
<td></td>
<td>11+ hrs</td>
</tr>
<tr>
<td>Walking for exercise or walking to work</td>
<td></td>
</tr>
<tr>
<td>Jogging (slower than 6 minutes/km)</td>
<td></td>
</tr>
<tr>
<td>Running (6 minutes/km or faster)</td>
<td></td>
</tr>
<tr>
<td>Bicycling (include stationary machine)</td>
<td></td>
</tr>
<tr>
<td>Tennis, squash, racquetball</td>
<td></td>
</tr>
<tr>
<td>Lap swimming</td>
<td></td>
</tr>
<tr>
<td>Other aerobic exercise (aerobic, dance, ski or stair machine, etc.)</td>
<td></td>
</tr>
<tr>
<td>Lower intensity exercise (yoga, stretching, toning)</td>
<td></td>
</tr>
<tr>
<td>Other vigorous activities (e.g., lawn mowing)</td>
<td></td>
</tr>
<tr>
<td>Weight training or resistance exercises (include free weights or machines)</td>
<td>Arms [Legs]</td>
</tr>
</tbody>
</table>

### 30. DURING THE PAST YEAR, on average, how many hours PER WEEK did you spend:

<table>
<thead>
<tr>
<th>Activity</th>
<th>TIME PER WEEK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2-5</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
</tr>
<tr>
<td></td>
<td>11-20</td>
</tr>
<tr>
<td></td>
<td>21-40</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
</tr>
<tr>
<td></td>
<td>61-90</td>
</tr>
<tr>
<td></td>
<td>91+</td>
</tr>
<tr>
<td>Standing or walking around work or away from home</td>
<td></td>
</tr>
<tr>
<td>Standing or walking around at home</td>
<td></td>
</tr>
<tr>
<td>Sitting at work or away from home or while driving</td>
<td></td>
</tr>
<tr>
<td>Sitting at home while watching TV</td>
<td></td>
</tr>
<tr>
<td>Other sitting at home (e.g, reading, meal times, at desk)</td>
<td></td>
</tr>
</tbody>
</table>
31. Are you currently taking **Vitamins or Multivitamins or Supplements**? *(For example: folic acid, selenium, diindolylmethane (DIM), vitamin D).*

- [ ] No
- [ ] Yes → If yes, please specify in table below

<table>
<thead>
<tr>
<th>Vitamin or Multivitamin or Supplement</th>
<th>Name</th>
<th>Pills/Week</th>
<th>Dosage</th>
<th>Duration of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age started</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Age last used</td>
</tr>
</tbody>
</table>

32. Think back to the time you were pregnant. In the **12 months before pregnancy**, did you take any dietary supplements (i.e., folic acid, vitamin B6, vitamin B12, multivitamin, prenatal vitamin)? Please indicate the type, brand name, frequency of intake and the month when intake was started/stopped for prior to each pregnancy.

*(If you have never had children, please skip this section).*

<table>
<thead>
<tr>
<th>Pregnancy (i.e., 1st, 2nd)</th>
<th>Vitamin (i.e., folate, B12)</th>
<th>Brand</th>
<th>Dose (i.e., pills per day)</th>
<th>Month Started</th>
<th>Month Stopped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

33. When you found out you were pregnant, did you take any dietary supplements **during pregnancy**? Please indicate the type, brand name, frequency of intake and the month when intake was started for each pregnancy.

<table>
<thead>
<tr>
<th>Pregnancy (i.e., 1st, 2nd)</th>
<th>Vitamin (i.e., folate, B12)</th>
<th>Brand</th>
<th>Dose (i.e., pills per day)</th>
<th>Month Started</th>
<th>Month Stopped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thank you very much for taking the time to complete this questionnaire. Should the need arise, may we contact you again? ☐ No ☐ Yes

<table>
<thead>
<tr>
<th>Telephone number:</th>
<th>Alternate contact:</th>
</tr>
</thead>
<tbody>
<tr>
<td>________________</td>
<td>________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E-mail address:</th>
</tr>
</thead>
<tbody>
<tr>
<td>________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mailing address:</th>
</tr>
</thead>
<tbody>
<tr>
<td>______________________________</td>
</tr>
</tbody>
</table>

City: ________________ Province: ________________ Postal Code: ________________

Please call us if you are changing contact information – 416-351-3800 ext. 2875

**FOR OFFICE USE**

Date questionnaire received: ________________

Day – Month – Year
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