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A causal investigation of soy isoflavone intake for primary prevention of post-menopausal breast cancer among Asian women

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Abstract

The incidence of breast cancer is increasing at an alarming rate across Asia, by up to 6% annually, compared to near stable incidence rates in many Western countries. While selective oestrogen receptor modulators and aromatase inhibitors are actively being studied as chemoprevention among high-risk Caucasian women, the risks may outweigh the benefits among Asian women with lower population risk of breast cancer. Modifiable lifestyle targets for primary prevention have long been identified, such as post-menopausal obesity, alcohol intake, and hormone replacement therapy use, but these risk factors are less prevalent among women in Asian countries. There remains an urgent need to find primary prevention strategies that are low risk, acceptable, and effective for Asian women.

Epidemiological evidence in Asian women suggests that high soy intake is associated with lower risk of breast cancer, but these findings were not observed in epidemiological studies of Caucasian women nor in clinical trials of soy isoflavone supplements. To date, there are no clinical trials that examine the effect of soy isoflavone intake from diet nor supplement on breast cancer risk among Asian women. In this thesis, I present the research studies undertaken to investigate if soy isoflavone intake is causally and inversely associated with post-menopausal breast cancer risk among Asian women.

The objective of the first research study was to identify mammographic density measures that are suitable biomarkers of breast cancer risk in the target population (**Chapter 3**). In this study, volume-based mammographic density measures and breast cancer risk factors were compared between 1,501 Malaysian women and 4,501 age- and BMI-matched Swedish women with no personal history of cancer. The analysis demonstrated that absolute dense volume, rather than percent density, may be a better biomarker of breast cancer risk among post-menopausal Asian women.

Based on the above findings, the second research study sought to determine if mammographic density mediates the association between soy intake and breast cancer risk in the target population (**Chapter 4**). A cross-sectional analysis of 3,277 healthy Malaysian women showed that mammographic density was lower among women with frequent soy intake compared to non-consumers, by up to 2.5cm^3 dense volume or 2.0cm^2 dense area, but this was not statistically significant. Intriguingly, regular soy intake was associated with lower mammographic density among overweight or obese women, but for leaner women,

regular soy intake was associated with higher mammographic density. This interaction was statistically significant among pre-menopausal women ($p_{interaction}$ = 0.029).

Prior to designing a robust clinical trial to test the causal association between soy intake and mammographic density as a biomarker of breast cancer risk, the feasibility of a dietary soy intervention was assessed in a small sample of the target population (n=10, **Chapter 5**). Overall, women in the study were able to maintain a diet of 70-90mg/day of soy isoflavones for 2 months, but the target of 100mg/day was not feasible and may have led to some adverse events. Thematic analysis of semi-structured interviews revealed that women participated in the study for altruistic reasons and due to emotional attachments to the cause, and that adherence was largely influenced by the practicability of the new diet or routine.

Building from the results of the previous three chapters, the primary objective of the last research study was to test the effect of daily soy isoflavone intake for 1 year on breast cancer risk among Asian women, using mammographic density as a biomarker of risk (**Chapter 6**). In this clinical trial, 57 healthy post-menopausal Malaysian women were randomized into the Supplement arm (100mg/day isoflavones, with >90% daidzein), the Dietary Soy arm (50mg/day isoflavones), or the Control arm. After 1 year of intervention, women in the Supplement arm experienced 4.1cm^2 lower dense area and 2.4% lower area-based percent density compared to women in the Control arm, but these associations were not statistically significant. The associations were weaker for women in the Dietary Soy arm and for volume-based mammographic density measures. Interestingly, stronger associations were observed when the analysis was limited to women with high BMI or low dietary fat intake, but the sample size was too small for robust analyses.

In conclusion, the data presented in this thesis suggest a causal association between soy isoflavone intake and lower post-menopausal breast cancer risk among Asian women. However, due to the small sample size, the analysis was underpowered to show statistically significant effects and will require confirmation in a larger trial. Nonetheless, the research undertaken here adds to existing evidence that the soy isoflavone daidzein may be responsible for the protective effect of soy. Furthermore, it proposes new hypotheses in understanding the association between soy intake and breast cancer risk across populations, including possible effect modification by BMI or dietary fat intake.

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Abbreviations

95% CI	95% confidence interval
AE	Adverse event
ASR	Age-standardized incidence rate
BMI	Body mass index
Erβ	Oestrogen receptor beta
FDA	Food and Drug Administration
FFDM	Full-field digital mammography
FFQ	Food frequency questionnaire
FGV	Fibro-glandular volume
HRT	Hormone replacement therapy
IQR	Interquartile range
ISF	Isoflavones
MANS	Malaysian Adult Nutrition Survey
MD	Mammographic density
MHLQ	Medical History and Lifestyle Questionnaire
MLO	Mediolateral oblique
MyMammo	The Malaysian Mammography study
NAF	Nipple aspirate fluid
OC	Oral contraceptive
OR	Odds ratio
PD	Percent mammographic density
RCT	Randomized clinical trials
RM	Malaysian Ringgit
RR	Relative risk
SD	Standard deviation
SERM	Selective oestrogen receptor modulator
SHBG	Sex hormone binding globulin
SJMC	Subang Jaya Medical Centre
UK	United Kingdom
UMMC	University Malaya Medical Centre
USA	The United States of America
Vs	Versus
WHR	Waist-to-hip ratio

List of peer-reviewed manuscripts

- Rajaram N, Mariapun S, Eriksson M, Tapia J, Kwan PY, Ho WK, et al (2017). Differences in mammographic density between Asian and Caucasian populations: a comparative analysis. Breast Cancer Research and Treatment; 161: 353–62.

List of conference abstracts

- Rajaram N, Mariapun S, Eriksson M, Tapia J, Kwan PY, Ho WK, et al. Differences in mammographic density between Asian and Caucasian populations: a comparative analysis. The 2016 American Association for Cancer Research (AACR) Annual Meeting, 16-20 April 2016, New Orleans, United States of America. *Recipient of the Susan G. Komen Scholar-in-Training Award.*

- Rajaram N, Ho WK, Mitra SM, Khor GL, Teo SH. Increasing Dietary Soy for Breast Cancer Prevention: A Feasibility Study. The 11th National Conference for Clinical Research, 27-29 September 2017, Putrajaya, Malaysia.

- Rajaram N, Ho WK, Mitra SM, Khor GL, Teo SH. Increasing Dietary Soy for Breast Cancer Prevention. The National Cancer Research Initiatives Annual Cancer Conference, 4-6 November 2018, Glasgow, United Kingdom.

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Chapter 1 : General introduction

1.1. Breast cancer risk in Asia

The burden of breast cancer is large and inequitably distributed across the world. In 2020, 2.26 million new breast cancer cases were diagnosed globally, with more than 680,000 deaths (1). Breast cancer incidence is high in developed countries, with age-standardized incidence rates (ASR) of up to 254 per 100,000 women, compared to 82-138 cases per 100,000 women in Asian countries (2). However, due to the large population sizes in Asia, women in this region account for 45.5% of the global number of breast cancer diagnosed in 2020, and 50.5% of breast cancer deaths (1). Furthermore, Asia has observed the largest increases in breast cancer incidence in the past two decades (2). For example, the average annual increase in breast cancer incidence was between 5.8-6.1% for South Korea, 3.2-5.0% for Japan, and 1.9-4.0% for Thailand, compared to the less than 1% increase seen in the United Kingdom (UK) or the decrease observed in the United States (USA) (2).

In Malaysia, breast cancer is the most common cancer and accounts for 19% of all cancer diagnoses (3). The ASR for breast cancer over a 5-year period (2012-2016) was 34.1 cases per 100,000 women, and approximately 43% of women presented at later stages of disease (stage 3 and 4) (3). There are ethnic differences in breast cancer incidence in Malaysia, where Chinese women have the highest ASR (40.7 cases per 100,000 women), followed by Indian women (38.1 cases per 100,000 women) and Malay women (31.5 cases per 100,000 women) (3). The incidence of breast cancer among women was highest for women aged 50-64 years old, with ASRs greater than 100 cases per 100,000 women between 2012-2016 (3). Alarmingly, breast cancer incidence in Malaysia is projected to increase by 64% over the next 20 years for all women, and by up to 85% among women over 50 years old (1).

Changes in reproductive and lifestyle factors have been proposed as major determinants of the rising breast cancer incidence in Asian countries (4,5). This includes having fewer children or having children later in life, reduced breast-feeding, higher prevalence of obesity coupled with low physical activity levels, and changing dietary patterns (4,5). Studies have shown that Asian migrant women in the USA or the UK with greater degrees of acculturation often have higher breast cancer incidence, compared to Asian women living in Asia. This further supports the argument that risk changes as women change the way they live (6).

With the growing number of breast cancer cases and the higher burden of breast cancer deaths in Asian countries, there is an urgent need to explore effective, practical, and culturally-tailored strategies to reduce breast cancer incidence in the region. The preventability of breast cancer is explored in the following sections, particularly in the context of post-menopausal breast cancers in Asia. This chapter discusses the primary prevention strategies that are currently explored for use in developed countries and why these strategies may not be suitable for developing Asian countries. Furthermore, it presents an argument for the use of mammographic density as a possible biomarker and/or target for breast cancer prevention in Asia. Lastly, it will describe the work undertaken in this thesis, which seeks to investigate the causal effect of soy isoflavone intake on breast cancer risk among post-menopausal Asian women, using suitable biomarkers of breast cancer risk.

1.2. Prevention of breast cancer

With the right strategy in place, primary prevention could significantly reduce the number of women who are diagnosed with breast cancer (7). At present, there is great interest in using selective oestrogen receptor modulators (SERMs) to prevent breast cancer among high-risk women. Tamoxifen, for example, was shown to reduce the number of breast cancer cases by 7 per 1,000 women over 5 years, with effects lasting 8 years after discontinuation (8). However, side effects from tamoxifen were serious, including an increased risk of thromboembolic events (relative risk (RR) = 1.93, 95% confidence interval (CI) = [1.33, 2.68]) and endometrial cancer (RR = 2.25, 95% CI = [1.17, 4.41]) (8). The overall prevalence of these events were low, approximately 0.9 and 0.6 per 1,000 women for thromboembolic events and endometrial cancers, respectively (8). Raloxifene, on the other hand, proved as effective as tamoxifen and resulted in fewer side effects, but there was still an increased risk for thromboembolic events (RR = 1.56, 95% CI = [1.11, 2.60]) (8). Apart from SERMs, the use of aromatase inhibitors also led to reductions in the number of women diagnosed with breast cancer, by up to 16 per 1,000 women (8). Aromatase inhibitors do not appear to increase the risk of thromboembolic events nor endometrial cancer, but vasomotor and musculoskeletal side effects were reported (8).

Beyond the use of SERMs and aromatase inhibitors, researchers have looked to repurposing commonly used drugs for breast cancer prevention. In a recent meta-analysis of 38 studies, aspirin use was associated with an 8% relative reduction in breast cancer risk, compared to non-users (9). The effect of aspirin was also stronger among post-menopausal women (RR = 0.89, 95% CI= [0.83, 0.96]) and with frequent use (RR = 0.88, 95% CI = [0.82,

0.96]) (9). Metformin, a common anti-diabetic drug, also shows some promise in reducing breast cancer risk attributable to post-menopausal obesity (10,11). At present, however, there is insufficient evidence for the widespread use of these repurposed drugs to reduce breast cancer incidence (11,12).

Several modifiable lifestyle factors have been conclusively shown to increase the risk of breast cancer, such as obesity, frequent alcohol intake (13), smoking (14), and hormone replacement therapy (HRT) use (15). Interestingly, body fatness was protective of pre-menopausal breast cancer, while it was strongly associated with increased breast cancer risk among post-menopausal women (13). Among post-menopausal women, there is strong evidence that high physical activity levels could reduce breast cancer risk, possibly through its' effect on post-menopausal obesity and adult weight gain (13).

The evidence is sparse for the role of diet in reducing incidence of breast cancer. Studies of non-starchy vegetables, carotenoids, and calcium have all yielded no conclusive evidence (13). In an overview of reviews or meta-analyses, or an umbrella review, the authors suggest that consumption of red or processed meat was associated with a higher risk of breast cancer, while low-fat dairy intake appeared to be protective (16). The umbrella review also showed that the association between soy intake and breast cancer risk is consistent across studies and appears to be protective, particularly for Asian post-menopausal women (16). The components of interest in soy are isoflavones, which are structurally similar to 17-B-estradiol, and its' effect on breast cancer risk may be similar to that of the SERMs discussed in this section (17,18), with potentially fewer side effects.

Unfortunately, many of the primary prevention strategies or targets discussed in this section may be unsuitable for use in developing Asian countries. For instance, while SERMs, aromatase inhibitors, and repurposed drugs are promising for women at highest risk of breast cancer, the risk of side effects may outweigh the benefits in populations with lower breast cancer incidence (19). Furthermore, many of the lifestyle risk factors discussed are less prevalent in Asian countries (20), and are therefore less effective targets for primary prevention. Soy, on the other hand, is commonly available and affordable in many Asian countries, making it a possible candidate for breast cancer prevention in this region. However, there is much debate about the effect of soy on breast cancer risk, as will be discussed in **Chapter 2**.

1.3. Mammographic density as a biomarker of breast cancer risk in epidemiological studies

Mammographic density is the radio-dense area on a mammogram image that represents the connective and epithelial tissue of the breast. Conversely, fat in the breast is radiologically translucent and appears black on a mammogram. In the first reported characterization of breast parenchymal patterns by Wolfe et al., women with more prominent ductal patterns on film mammograms were more likely to be diagnosed with breast cancer (21). He also provided a method of classifying women based on their breast parenchyma, namely the Wolfe classification method (21). Later, the Tabar method was developed using a 3-dimentional technique to assess mammographic patterns (22). The Tabar method classified women into 5 groups and was shown to have greater correlation to breast cancer risk and risk factors, such as parity (22). Currently, the most commonly used method of classifying breast parenchymal patterns for breast cancer risk management is the BI-RADS method by the American College of Radiology (23). According to the 5th edition of BI-RADS, mammographic parenchymal patterns can be categorized into four groups, from entirely fatty breasts (A) to very dense breasts (D), as seen in **Figure 1-1** (23).

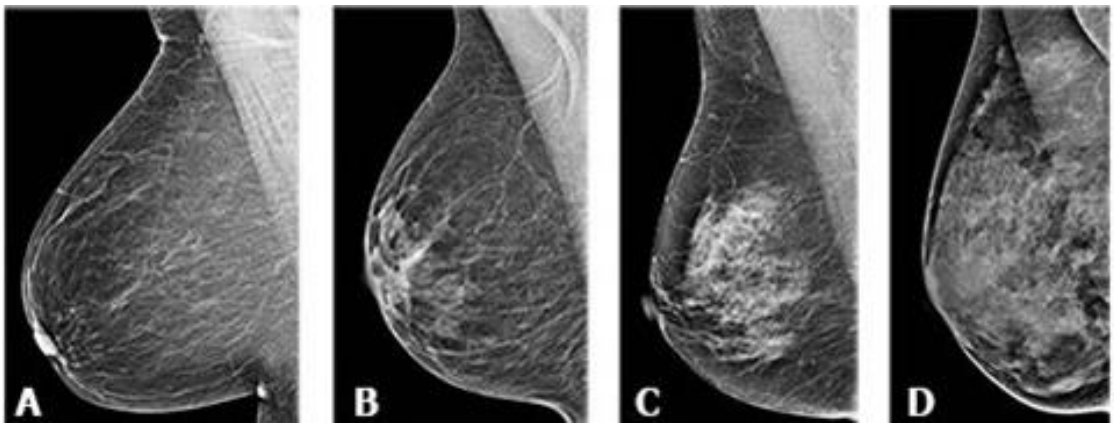


Figure 1-1: BI-RADS classification of mammographic density[1].

Qualitative categorization of mammographic parenchymal patterns, however, are subject to reader variation and bias, and is highly dependent on the quality of the film

[1]Image taken from the American College of Radiology (www.acr.org, date retrieved: 24 October 2020).

mammogram available (24,25). Categorizing women into 4-5 groups based on visual inspection of a mammogram may also result in breast cancer risk misclassification (26). In 1995, Boyd and colleagues developed a computer-aided technique to quantify the absolute area of dense tissues and non-dense tissues (27). Since then, various computer-aided or fully-automated methods have been developed to quantify mammographic density from digitized film mammogram images and more recently, digital mammogram images (28).

Consistently, there is a strong and significant association between mammographic density measures and breast cancer risk. In 1995, Boyd et al. showed that women with extensive mammographic density had 4 times higher risk of breast cancer compared to women with the least dense breasts (27). This finding is replicated in a landmark meta-analysis of mammographic density and breast cancer risk by McCormack et al. in 2006, where women with highest breast density (>75% percent density) had 4-6 times greater risk of developing breast cancer, compared to women with the lowest breast density (<10% percent density) (29). High mammographic density is also associated with factors known to increase breast cancer risk, such as lower parity, older age at first child birth, HRT use and obesity (30–33).

Paradoxically, as women age and undergo menopause, breast cancer risk increases but mammographic density declines (34). As women age, lobular involution occurs when the glandular elements of the breast is progressively replaced by collagen, and subsequently fat (35). Greater degree of involution leads to less dense area and increased non-dense area, and is associated to less breast cancer risk (36,37). However, the rate of age-related mammographic density decline is not the same for all women (38). Therefore, in every age bracket, higher mammographic density is consistently associated with higher risk of breast cancer (39).

In the few reports that compare absolute measures of mammographic density across ethnicities, mammographic density appears to be lower among Asian women compared to Caucasian women (40,41), an effect that is consistent with population differences in breast cancer risk. Conversely, percent density (the area of density relative to the total area of the breast) is consistently higher among Asian women, compared to Caucasian women who have higher population risk (41–43). Percent density is a commonly reported mammographic density measure. However, it is heavily influenced by breast size and body mass index (BMI), which can vary greatly across ethnic groups (40,42,44). Negative confounding by BMI has

been reported as a concern in studies reporting percent density and can lead to an underestimation of the true effect (45).

Promisingly, recent data shows that if all Caucasian women with very dense breast were shifted to the least dense category, up to 36% of pre-menopausal breast cancer and 26% of post-menopausal breast cancer could be avoided (46). This effect is similar to that for obesity, which accounts for up to 23% of post-menopausal breast cancer (46). Among Asian women, on the other hand, reducing mammographic density could prevent almost 50% of all breast cancer cases. Shifting overweight and obese women to the normal BMI range accounts for only 16% of breast cancer cases (47). Therefore, mammographic density may be a more important target for primary prevention of breast cancer in Asia.

1.4. Scope of research

In this thesis, I hypothesize that there is an inverse causal association between soy isoflavone intake and breast cancer risk among post-menopausal Asian women. The following chapters seek to test this hypothesis, using suitable mammographic density measures as a biomarker of breast cancer risk.

Chapter 2 provides a thorough literature review of the factors that are considered in this research. It will describe the association between soy intake and breast cancer risk across populations, as reported by numerous meta-analysis and systematic reviews. It will also describe the use of mammographic density in chemoprevention trials of soy and breast cancer risk. This chapter seeks to identify the gaps in the existing body of evidence for the causal relationship between soy and breast cancer risk, and highlights the importance of addressing some of these gaps through research among Asian women living in Asia.

Chapter 3 examines the utility of fully-automated volume-based mammographic density measures as a biomarker of breast cancer risk in an Asian population. In this study, the distribution of volume-based mammographic density among healthy Asian women is compared to that of Swedish women with similar age and BMI distributions. The study also assesses if the differences in mammographic density between the two cohorts can be explained by population differences in established breast cancer risk factors.

In **Chapter 4,** a cross-sectional analysis investigates if mammographic density is a suitable biomarker to study the effect of soy on breast cancer risk among healthy Asian women. Two fully-automated methods of mammographic density estimation are used in this

analysis. Additionally, this analysis seeks to explore if the association between soy intake and mammographic density may be restricted to certain subgroups, such as by menopausal status or BMI category.

Chapter 5 describes the feasibility of conducting a dietary soy intervention study among post-menopausal healthy Asian women, prior to planning a robust randomized controlled trial of soy intake on breast cancer risk. This chapter seeks to identify the motivators and barriers to participating in a dietary intervention study, as well as the factors that promote adherence to the intervention. Furthermore, this research sheds light on the acceptable dose of soy intake for long-term dietary intervention in the target population.

Building on the research done in the preceding chapters, **Chapter 6** seeks to test the causal association between soy intake and breast cancer risk, using suitable mammographic density measures, among healthy post-menopausal Asian women. In this trial, women were randomized into the soy supplement arm (100mg/day isoflavones), the dietary soy arm (50mg/day isoflavones), or the control arm, and followed over 12 months. This chapter reports on the effect of the soy interventions on mammographic density at 12 months and explores for possible effect modifiers of this association.

Chapter 7 summarizes the overarching conclusions from the research presented in this thesis, and draws inferences about the causal association between soy and breast cancer risk. Finally, **Chapter 8** highlights the remaining gaps needed to establish causal evidence for the association between soy isoflavone intake and breast cancer risk, and propose avenues for future research.

1.5. Selection of patient population

Malaysian cohort

The study population under research in the following chapters were largely selected from the Malaysian Mammography (MyMammo) study. In the MyMammo study, Malaysian women were recruited between 2011 and 2015 by Cancer Research Malaysia at Subang Jaya Medical Centre (SJMC) and University Malaya Medical Centre (UMMC) (20). In total, 4,014 women were recruited through the MyMammo study from it's' inception up to 1st January 2017 (**Figure 1-2**).

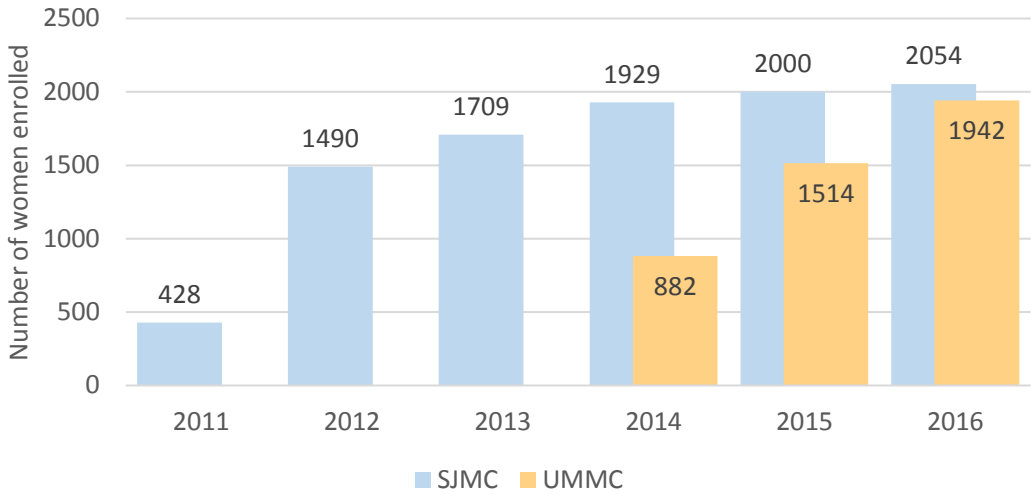


Figure 1-2 Cumulative number of women recruited into the MyMammo programme between 2011-2016.

Women were eligible to participate in the MyMammo study if they were healthy (no personal history of breast cancer) and were between 40-74 years old. Women were recruited through various forms of media and via physician referrals. All women provided written informed consent, completed a baseline questionnaire, provided blood samples, and had their mammogram. The study was approved by the Independent Ethics Committee of Ramsay Sime Darby Health Care and the Medical Ethics Committee of University Malaya Medical Centre.

This large hospital-based cohort provides a unique opportunity to study the lifestyle factors that are associated with mammographic density among Malaysian women (48,49). The observational studies presented in **Chapter 3** and **Chapter 4** have drawn samples for analysis from this cohort. Furthermore, it forms the target population for the interventional aspects of this research, as presented in **Chapter 5** and **Chapter 6**.

Swedish cohort

Through collaboration between Cancer Research Malaysia and the Karolinska Institutet in Sweden, data was obtained for healthy Swedish women who participated in the Karma study. The Karma study is a population-based prospective cohort study in Sweden of over 70,000 women attending the national screening programme since 2011 (50). The study collected breast cancer risk factor data, blood samples, and digital mammograms from healthy Swedish women. This cohort will be used in **Chapter 3** in a comparative analysis with the Malaysian cohort described above.

1.6. Mammographic density estimation

Many of the studies on mammographic density and breast cancer risk have used either qualitative assessments or computer-aided quantitative methods to measure mammographic density, which could introduce reader-dependent variation and biases (25). Fully-automated methods, on the other hand, remove this variability and increase the efficiency and reliability of measuring mammographic density in large batches (25,51,52). In this thesis, mammographic density is measured using two fully-automated, high-throughput software. Volpara™ is a commonly used software which produces volume-based mammographic density estimates. Volpara™ has been tested across populations and has been shown to be stable across mammography systems (53). These estimates are strongly correlated with breast cancer risk in Caucasian populations, where every 10cm^3 increase in dense volume is associated to a 3% increase in relative risk (54). STRATUS is a recently developed software which measures area-based mammographic density. It has shown good consistency across various mammography systems (55,56), and has been calibrated for use in the target population.

Chapter 2 : Literature review

2.1. Objectives

Identifying safe and effective strategies for primary prevention among Asian women with low-to-moderate breast cancer risk remain a key area of research. The objective of this chapter is to summarize the existing evidence for the association between soy isoflavone intake and breast cancer risk across populations as well as the gaps that exist in establishing causality for this association.

2.2. Biological plausibility

Soy foods are rich in isoflavones. The isoflavones in soy include genistein and daidzein, and to a less degree, glycitein (17). These isoflavones are found almost exclusively in soy, and are the subject of study in breast cancer research (17).

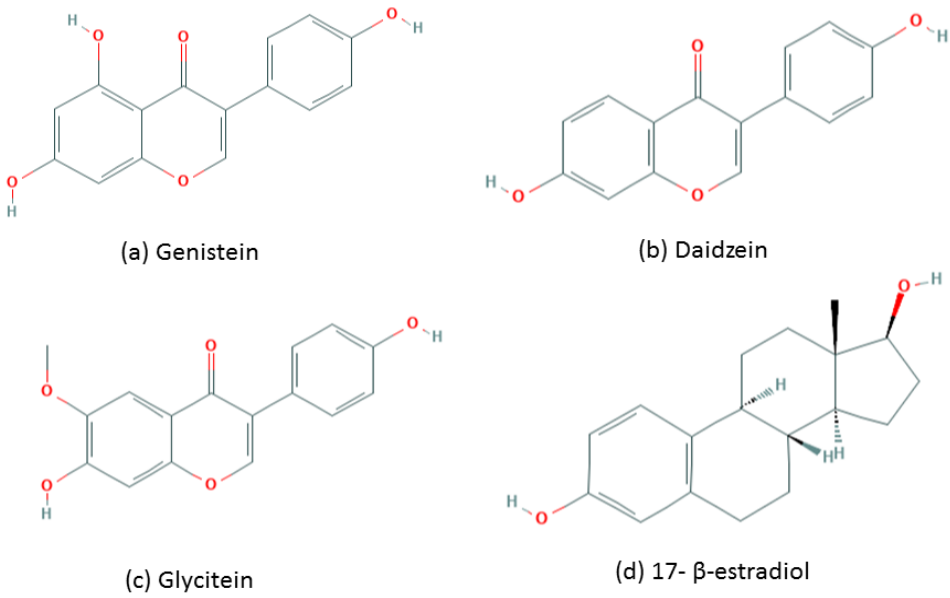


Figure 2-1: Chemical structure of soy isoflavones and 17- β-oestradiol[2].

Isoflavones are structurally similar to 17-β-oestradiol (**Figure 2-1**). They are able to bind to oestrogen receptors in the body, and have a preference for oestrogen receptor beta (ERβ) (17). When bound to oestrogen receptors, these phytoestrogens exert a weaker effect

[2] Chemical structure was taken from PubChem, under the Creative Commons Attribution License (https://pubchem.ncbi.nlm.nih.gov/, date retrieved: 18 October 2020)

compared to the activity by endogenous oestrogen (17). Isoflavones may also have anti-proliferative, anti-angiogenic, anti-oxidative, and anti-inflammatory qualities, either independently or due to lower endogenous oestrogen exposure (17,18). Interestingly, in high oestrogen environments, isoflavones exert anti-estrogenic effects while estrogenic effects are observed in low-oestrogen environment (57). This has led researchers to suggest that soy may not benefit all women equally, and its' effect on breast cancer may be dependent on menopausal status (17).

Thus far, animal studies and *in vitro* studies have shown mixed results (58). Several animal studies have reported that high doses of soy isoflavones increased cell proliferation (59,60), serum IGFBP-3 (61), and tumour aggression (62). It is important to consider *in vitro* and *in vivo* studies carefully, as the dosage of soy isoflavones used in these studies are often far greater than what is clinically relevant for humans (63). For example, a systematic review of rodent studies showed a great variation in isoflavone doses, even as high as 200mg of isoflavones per kilogram body weight (58), compared to typical human doses of between 25-50mg/day in Asian countries (64). Furthermore, there is some argument that the metabolism of soy isoflavones is different for rodents and humans (63). In the former, isoflavones circulate in its' unconjugated, biologically active aglycone form, particularly if the isoflavones were injected subcutaneously and has bypassed important digestive phases (63). Humans, on the other hand, are able to efficiently conjugate isoflavones in circulation while actively deconjugating isoflavones in the intestines for maximal absorption (63,65). Therefore, the observations from rodent studies may not be directly applicable to humans (63).

2.3. Evidence from epidemiological studies

The epidemiological research into the impact of soy intake on breast cancer risk is extensive. These studies show that high soy intake is associated with 14-41% lower relative risk of breast cancer compared to women with low soy intake, but the effects appear to be limited to Asian women (66–69). For instance, a large meta-analysis of 35 studies reported a strong association between soy intake and breast cancer risk among Asian women, with similar effects among post-menopausal (odds ratio (OR) = 0.59, 95% CI = [0.44, 0.74]) and pre-menopausal women (OR = 0.59, 95% CI = [0.48, 0.69]) (69). Similarly, high soy intake (>20mg/day isoflavones) was associated with a 29% decrease in breast cancer risk among Asian women (OR = 0.71, 95% CI = [0.60, 0.85]) (67). Among Caucasian women with habitually low soy intake, the association between soy intake and breast cancer risk was often null (66,69).

Given the interaction between soy isoflavones and endogenous oestrogen, it is possible that the effect of soy on breast cancer risk is different by menopausal status (17). Trock et al. showed that the association was marginally stronger for pre-menopausal women (OR = 0.70, 95% CI = [0.58, 0.85]) compared to post-menopausal women (OR = 0.77, 95% CI = [0.60, 0.98]) (66). In the large meta-analysis, the effect of soy on breast cancer risk was similar for both pre-menopausal and post-menopausal Asian women (69). At present, there is insufficient evidence to conclude whether the association between soy and breast cancer risk differs by menopausal status.

Also, there is no consensus about the effective dosing range for soy isoflavones in the context of breast health. In a dose-response meta-analysis, habitual consumption of 10mg/day isoflavones was associated with a 3-16% reduction in breast cancer risk (74). More recently, up to 10% decrease in relative risk of breast cancer was observed for every 10g/day of tofu (approximately 2.6mg/day isoflavones) consumed (68,71). However, a prospective observational study among 300,000 Chinese women showed that there was no association between soy intake and breast cancer risk among women with an average isoflavone intake of 19mg/day (70). Instead, doses as high as 40mg/day may be required to show a protective effect (70). Furthermore, it is possible that the dose-response effect of soy on breast cancer risk is not linear. In fact, Heaney et al. proposed that the physiological response to nutrient intake follows a sigmoid curve, where the dose-response association is only observed when the nutrient intake meets a certain threshold (72). Below this threshold, there is likely no clinically meaningful effect, while intake beyond the effective dosing range may increase the risk of adverse effects (72).

The type and processing of soy foods may affect its' association with breast cancer risk (73). An analysis of 16 prospective studies showed that high amounts of soy foods, rather than soy isoflavone intake, was associated with 13% lower relative risk of breast cancer (95% CI = [0.76, 1.00]) (74). Among Korean women, fermented soybean curd intake was associated with lower relative risk of breast cancer (RR = 0.47, 95% CI = [0.34, 0.66]), compared to overall soy intake (RR = 0.61, 95% CI = [0.38, 0.99]) (75). The ratio of isoflavone components (genistein, daidzein, or glycitein) or isoflavone forms (as glycosides or aglycones) varies greatly across soy foods and isoflavone supplements, and may affect its' efficacy, bioavailability, and/or absorption efficiency (63,76). Unfortunately, there is insufficient evidence to determine the type of soy food or soy isoflavone that is more strongly associated with breast cancer risk.

2.4. Randomized controlled trials of soy isoflavones on biomarkers of breast cancer risk

To date, there are no intervention studies of soy isoflavones with breast cancer occurrence as the primary endpoint because it requires large cohorts followed over long periods of time, making such studies expensive and difficult to implement (77). Instead, randomized controlled trials (RCTs) studying the effect of soy isoflavones have focused on modifiable biomarkers of breast cancer risk (78)**.** These studies were conducted in predominantly Caucasian populations.

RCTs of soy isoflavones have investigated a wide range of biomarkers for breast cancer risk, which are summarized in **Table 2-1**. Studies of serum samples have focused on adipocytokines (79), IGF-1 (80), and circulating oestrogen (81–83), which do not appear to be modulated by soy isoflavone intake. Other researchers have hypothesized that soy isoflavones, acting as phytoestrogens, may directly impact the breast tissue and its' environment (84,85), and have suggested the use of more localized biomarkers of breast cancer. For example, oestrogen levels in nipple aspirate fluid (NAF) may be a better measure of breast cancer risk compared to circulating oestrogen (84). Khan et al used the Ki-67 labelling index as a measure for breast epithelial cell proliferation (86). Interventions of soy food or high doses of soy isoflavone supplements did not modify these biomarkers of risk.

Mammographic density is the most commonly used biomarker of breast cancer risk in RCTs of soy isoflavones (**Table 2-2**). Overall, there were no significant effect of soy isoflavones on mammographic density measures (87–92). In a meta-analysis of these RCTs, Hooper et al. showed that women on the isoflavone intervention have less percent mammographic density compared to women in the placebo arms (by -1.1%, 95% CI = [-3.22, 1.03]), but this was not statistically significant (78). Notably, a significant increase in percent density was observed among pre-menopausal women on the soy isoflavone intervention, by 1.8% (95% CI = [0.25, 3.40]) (78).

Table 2-1 Summary of RCTs of soy on biomarkers of breast cancer risk

Author (year)	Population*	Sample size†	(1) Exposure (2) Control	Duration (months)	Outcome measure	Main findings
Nadadur, et al (2015) (79)	Post-menopausal women (USA)	57	(1) 50mg ISF (tofu) (2) Low-fat diet	2	Adipocytokines level in serum	No significant difference in circulating adipocytokines
Khan, et al (2012) (86)	Women at risk for breast cancer (USA)	126 (98)	(1) 235mg ISF (tablets) (2) Placebo	6	Breast epithelial cell proliferation	No significant difference in biomarker level except for Ki-67 labelling index among pre-menopausal women
Maskarinec, et al (2011) (84)	Pre-menopausal women (USA)	96 (82)	(1) 50mg ISF (food) (2) Regular diet	6	Oestrogen levels in NAF	No significant difference in Oestrogen levels in NAF or serum
Teas, et al (2010) (80)	Post-menopausal women (USA)	30	(1) 2mg/kg body weight ISF (powder) (2) Placebo	3.5	Serum IGF-1	Significant increase in serum IGF-1 concentrations compared to placebo
Nettleton, et al (2006) (81)	Post-menopausal with and without breast cancer (USA)	(40)	(1) 0.64mg body weight ISF (powder) (2) Milk protein	1.4	Circulating oestrogen	No significant difference in oestrogen levels in cancer patients or healthy women
Wu, et al (2005) (83)	Post-menopausal women (USA)	57	(1) 50mg ISF (tofu) (2) Low-fat diet	2	Estradiol, SHBG, insulin, leptin	No significant difference in hormone levels
Maskarinec, et al (2004) (82)	Pre-menopausal women, excluding soy consumers (USA)	220 (189)	(1) 50mg ISF (food) (2) Regular diet	24	Oestrogen levels in serum	No significant difference in oestrogen levels in serum

Annotations: *Population of study (country of study), †number of participants randomized (number of participants analysed, if different). Abbreviations: NAF = nipple aspirate fluid; IGF = insulin-like growth factor, SHBG = sex hormone binding globulin.

Table 2-2: Summary of RCTs of soy on mammographic density as a biomarker of breast cancer risk

Author (year)	Population*	Sample size†	(1) Exposure (2) Control	Duration (months)	Outcome measure‡	Main findings
Wu, et al (2015) (91)	Women with and without breast cancer (USA)	112 (85)	(1) 50mg ISF (tablet) (2) Placebo	12	Area-based MD, digital images, by computer assisted method (Madena)	No difference in dense area, PD and percent FGV by intervention group, for pre- and post-menopausal women
Delmanto et al (2013) (92)	Post-menopausal women (Brazil)	98 (80)	(1) 100mg ISF (tablet) (2) Placebo	10	Qualitative MD (BIRADS)	No difference in mammographic density
Maskarinec, et al (2009) (88)	Post-menopausal women (USA)	406 (358)	(1) 80/120mg ISF (2) Placebo	24	Area-based MD, from digitized films, by computer assisted method (Cumulus)	No difference in dense area, PD and non-dense area, no differences by age or BMI
Verheus, et al (2008) (87)	Post-menopausal women (Netherlands)	175 (126)	(1) 99mg ISF (powder) (2) Placebo	12	Area-based MD, from digitized films, by computer assisted method (Cumulus)	No difference in dense area and PD
Maskarinec, et al (2004) (90)	Pre-menopausal women, excluding regular soy consumers (USA)	220 (189)	(1) 50mg ISF (food) (2) Regular diet	24	Area-based MD, from digitized films, by computer assisted method (Cumulus)	No difference in dense area and PD, across all ethnicities. Lifetime soy intake was associated with PD.
Maskarinec, et al (2003) (89)	Pre-menopausal women (USA)	30	(1) 100mg ISF (tablets) (2) Placebo	12	Area-based MD, from digitized films, by computer assisted method (Cumulus)	No difference in dense area and PD.

Annotations: *Population of study (country of study), †number of participants randomized (number of participants analysed, if different), †Type of mammographic density measure (software). Abbreviations: ISF = isoflavones, MD = mammographic density, PD = percent density, FGV = fibro-glandular volume.

Notably, the RCTs reported thus far have estimated mammographic density using qualitative methods or computer-aided quantitative methods, where mammographic density was measured from digitized images of mammogram films (87–90,92). The process of digitization could affect the contrast and visualization of the mammogram image, thus altering mammographic density (93). This, coupled with the use of computer-aided methods which may be subject to human error, reader variability, and inter-batch variations, could have led to the lack of a measurable impact on breast cancer risk. This is substantiated in the meta-analysis discussed above (78), where a stronger protective effect was observed in the studies that have used fully-automated measures, such as Standard Mammogram Form (94,95) (mean difference in percent density between soy intervention and control group = -0.22, 95% CI = [-1.57, 1.13]), compared to Cumulus and similar computer-aided methods (87–90,96) or visual inspection (97), where mean percent density was higher by 0.67-0.70 among the soy intervention group.

2.5. The potential effect of soy on breast cancer risk in Asian women in Asian women

To date, there are no reports of RCTs that investigates the effect of soy isoflavone intake among Asian women living in Asia. Several theories have been put forth to explain the null associations from RCTs in Caucasian women, some of which could be addressed in an RCT among Asian women living in Asia. These theories are discussed in the following paragraphs.

Firstly, it is possible that the lack of association in previous RCTs is due to the form in which soy isoflavones were consumed (98). As shown in **Table 2-1** and **Table 2-2**, a majority of these RCTs have used an intervention of soy isoflavone supplements or isolated soy protein, rather than foods made from whole soybeans, which are more commonly consumed in Asian populations (98). Using a supplement in a clinical trial is ideal because it is easily implemented, provides a consistently measured dose of the intervention, and allows for a placebo-controlled trial, thereby improving the internal validity of the study. However, foods made from whole soybeans contain other nutritional components that may interact with soy isoflavones to reduce breast cancer risk (90,91). Furthermore, Khan et al. hypothesized that habitual dietary soy intake typically involves consuming small doses of soy throughout the day and leads to sustained exposure, as compared to a once daily supplement (86). A dietary soy intervention may be more readily implemented in an Asian population, where soy foods are commonly available, affordable, and can be easily incorporated into meals.

Secondly, the effect of soy isoflavones on breast cancer risk may be dependent on early life or lifelong exposure to soy (90,91,99–102). While the exact mechanism for the association between early life soy intake and adult breast cancer risk is not known, it is postulated that exposure to these phytoestrogens can lead to earlier mammary gland development (101) and may change breast cells in a way that reduces the risk of carcinogenesis (103). The effect of early exposure of soy has been likened to that of early pregnancy, which is protective against breast cancer development (103). However, data from the Shanghai Women's Study suggests that lifelong soy intake, and not just intake in early life, conferred the strongest protective effect against breast cancer (104). Taken together, this suggests that a soy isoflavone intervention later in life may only benefit women who have been habitually consuming soy since puberty. Studies of Malaysian women show that soy foods are a regular part of diet among young adults and women in their reproductive years (105,106).

Thirdly, there may be population differences in the ability to metabolize soy isoflavones into more effective and biologically available forms. For example, research suggests that Asian women can more efficiently metabolize the isoflavone daidzein into (S)-equol (107,108). Compared to daidzein, (S)-equol has been shown to have greater affinity to oestrogen receptors in *in vitro* studies, and therefore, may be more potent in preventing carcinogenesis (109). However, this is unconfirmed, with more recent studies suggesting that higher serum concentrations of daidzein, rather than (S)-equol, may be associated to lower breast cancer risk (110). This theory requires robust investigation in a Asian population, where the prevalence of (S)-equol producers is often greater that 50%, compared to less than 30% among Caucasians (111,112).

Lastly, it has been suggested that the effect of soy isoflavone intake on breast cancer risk may not be mediated through mammographic density (99). In studies of predominantly Caucasian participants, there were no significant associations between soy isoflavone intake and mammographic density measures (78,107,113–118). However, cross-sectional studies of Asian women with average soy isoflavone intake of 10-18mg/day showed significant inverse associations between soy intake and mammographic density (108,119–121). Only one Japanese study of predominantly pre-menopausal women showed no significant association between an average soy intake of 57mg/day with mammographic density (122). It is possible that the inverse association between soy isoflavone intake and mammographic density may be stronger among post-menopausal Asian women (120).

2.6. Conclusion

In summary, there is convincing biological and epidemiological evidence that high soy intake may reduce population incidence of breast cancer, possibly through direct or indirect effects of soy isoflavones on oestrogen receptors in the breast. This inverse association appears to be limited to Asian women, and may be stronger for post-menopausal women.

Well-designed RCTs of soy isoflavone supplements among Caucasian women have shown no effect on biomarkers of breast cancer risk, including mammographic density. It is not known whether these null effects are attributed to the lack of early life intake or differences in the ability to metabolize soy isoflavones between populations. Furthermore, evidence suggests that soy isoflavones obtained through whole soy foods may be required to show a clinically meaningful effect. However, little is known about the effective, acceptable dosing range for dietary soy isoflavones. Also, none of the RCTs for soy have used mammographic density measured by high-throughput fully-automated software, which are less subject to inter-reader and inter-batch variability.

Most importantly, there are no reports of a soy isoflavone RCT among Asian women living in Asia, despite the numerous observational studies suggesting that soy isoflavone intake may reduce breast cancer incidence among Asian women.

Chapter 3 : Mammographic density as a biomarker of breast cancer risk among Asian women

3.1. Rationale and objectives

Studies of Asian women with lower population incidence of breast cancer often report higher relative mammographic density measures compared to Caucasian women, by approximately 2-4% (29,40,42,123–126). Research on absolute density, on the other hand, have shown that Asian women have less dense area ($5cm^2$) or dense volume ($5cm^3$) compared to Caucasian women, but these differences were often not statistically significant after adjustment of age and BMI (40,42,127,128). Overall, these data do not reflect population differences in breast cancer risk and remains a paradox in mammographic density research.

It is important to note that studies comparing mammographic density across population groups typically include small proportion of Asian women who were at least first-generation immigrants in Caucasian-dominant countries. It is possible that their risk for breast cancer may already be changing in parallel to acculturation as women adapt to their new environment (129). Studies of mammographic density and breast cancer risk in Asian countries are limited and vary greatly, and most studies have reported on percent density measures (130). Less is known about the use of absolute mammographic density as a biomarker of breast cancer risk among Asian women living in Asia.

The aim of this chapter is to understand the utility of absolute and relative mammographic density measures as biomarkers for breast cancer risk in an Asian population. To do so, the specific objectives of this chapter are (1) to compare the distribution of absolute and relative mammographic density measures between Asian and Caucasian populations, (2) to understand the factors associated with mammographic density for both populations, and (3) to evaluate breast cancer risk factors that account for population differences in mammographic density. This analysis will shed light onto the type of mammographic density measures that are suitable for use in an Asian population, and the breast cancer risk factors that should be considered in future analyses of Asian mammographic density.

The findings from this chapter has been published in the Breast Cancer Treatment and Research journal (131).

3.2. Methodology

3.2.1. Study Participants

Women enrolled in the MyMammo programme (as described in **Chapter 1**) between year 2011-2015 were included in the analysis if raw digital mammogram images were available for them (n=1,575). Women were excluded from analysis if they reported a prior cancer diagnosis or was diagnosed post-screening (n=18). Further exclusions were made for women who were not between 40-75 years old at enrolment (n=10), if they were missing age, BMI, or menopause information (n=30), had incomplete questionnaires (n=4), or if they were non-Malaysians (n=10). Two duplicate records were removed. A total of 1,501 Malaysian women were included in the analysis.

Through collaboration between Cancer Research Malaysia and the Karolinska Institutet in Sweden, data was obtained for healthy Swedish women who participated in the Karma study, as described in **Chapter 1**. A total of 4,501 Swedish women with no personal history of breast cancer were exact-matched to the Malaysian women by age and BMI at a ratio of 3:1.

3.2.2. Breast cancer risk factors

Both Malaysian and Swedish women completed baseline questionnaires at their screening visit. Most of the data collected were comparable between the two questionnaires. However, education level, menopause status, alcohol intake and physical activity were collected differently and were standardized for better comparability. The definitions for all variables are presented in **Table 3-1**. There were substantial missing data for several variables. Age at menopause was missing for 13.2% of Swedish women, and was imputed as previously reported mean age at menopause in this population (49.9 years old) (132,133). Education level was also missing for 13.8% of Swedish women, who were assumed to have completed tertiary education (132).

3.2.3. Mammographic density measurements

Both studies collected raw and processed mammograms using full-field digital mammography (FFDM) systems. There were a variety of mammogram machines used across all the study sites, as per **Table 3-2**.

Table 3-1 Definition of variables used in this thesis (in alphabetical order)

Variable	Definition
Age	Calculated from year of birth to year of consent
Age at first full-term pregnancy	Calculated as year of birth to year of first child's birth
Age at last full-term pregnancy	Calculated as year of birth to year of last child's birth
Age at menarche	Self-reported age at first menstrual period
Age at menopause	Calculated from date of birth to date of last period
BMI	Calculated from measured height and weight (kg/m^2)
Breast cancer family history	Self-report family history of breast cancer among first degree relatives among mother, sisters, or daughters
Body shape change in youth	Using the Stunkard figure rating scale, reported shape at 18 years old – reported shape at 7 years old
Body shape change since youth	Using the Stunkard figure rating scale, reported shape at study visit – reported shape at 18 years old
Education	Self-reported last attained education level, categorized as primary education or less, secondary education, or tertiary education
Ethnicity	Self-reported ethnicity, categorized as Malay, Chinese, Indian or others
Ever smoked	Self-reported, at least I year of use at any point in life
Full-term pregnancies	Self-reported number of full-term pregnancies
Gynaecological surgery	Self-reported history of hysterectomy, oophorectomy, and sterilization
Height	Measured or self-reported, in cm
HRT ever use	Self-reported use of any type of HRT for at least one year at any point in life
HRT current use	Self-reported current use of any type of HRT
Monthly household income	Self-reported household income per month, categorized as <RM5,000,, RM5-10,000, or ≥RM10,000.
Nulliparity	Self-reported no live births
OC ever use	Self-reported use of any type of OC for at least three months at any point in life
Parity	Self-reported number of first full-term pregnancies
Post-menopause	At least one year prior to the screening date, or had a hysterectomy or oophorectomy at least one year prior to the screening date, or older than 55 years old
Regular alcohol intake	Consumption of any alcohol at least once a month
Regular coffee intake	At least 1 cup per day
Regular physical activity	Self-reported physical activity at least 2 hours/week, including recreational and household activity
Regular physical activity (MET-hours/week)	Calculated from self-reported physical activity levels. MET score (7 for 1 hour of strenuous activity, 4 for moderate activity, and 3 for gentle activity) are multiplied by number of hours spent in each activity. Categorized as low (<10.0 MET-hours/week), moderate (10.0 – 20.0 MET-hours/week), and high (≥ 20.0 MET-hours/week) physical activity
Weight	Measured or self-reported, in kg

Volpara™ (134) mammographic measures were used in this analysis as it was available for both cohorts, and will be referred to as volume-based measures. Absolute dense volume was estimated as the sum of 3-dimensional dense pixels observed on digital mammograms. Non-dense volume was estimated by subtracting dense volume from total breast volume. Percent density was calculated as the dense volume relative to the total breast volume. Volume-based measures were assessed from mediolateral oblique (MLO) view mammograms, and the average across the left and right breast measurement was used in this study.

Table 3-2: Distribution of mammogram machine used in the MyMammo study (n=1,501) and Karma study (n=4,501)

Type of mammogram machine	**MyMammo (n=1,501)**	**Karma (n=4,501)**
	n (%)	n (%)
Hologic Selenia Dimensions	1,089 (72.6)	0 (0.0)
General Electric Company Senographe Essential	249 (16.6)	1,817 (40.4)
Siemens MAMMOMAT Novation	160 (10.7)	0 (0.0)
Sectra Mammography System	0 (0.0)	2537 (56.4)
Philips Mammography System	0 (0.0)	100 (2.2)
Siemens MAMMOMAT Inspiration	0 (0.0)	6 (0.1)

Annotations: n = number; % = proportion over total in the cohort.

3.2.4. Statistical analysis

Mean and standard deviation (SD) was used to describe the distribution of continuous variables that were normally distributed, while median and interquartile ranges (IQR, 75th percentile – 25th percentile) were used to describe distributions that were not normally distributed. Number and percentages were used for categorical and ordinal variables. Student's t-test and Pearson chi-square tests were used to describe the variables of interest. The volume-based measures were not normally distributed, and were $\log_{10}$-transformed to enable the use of parametric tests and models for analysis.

Log-linear regression models were used to assess the univariate and multivariable association between mammographic density and breast cancer risk factors, for each cohort and by menopausal status. Risk factors that may be associated with mammographic density (using a conservative p value < 0.25) in univariate analyses were included in multivariable models. This included age (in years), BMI (kg/m2), highest attained education, height, changes in body shape in youth and since youth, age at menarche, parity factors (nulliparity,

number of births, age at last birth), use of OC, family history of breast cancer, history of smoking, current physical activity and coffee intake, and among post-menopausal women, use of HRT. In the analysis of Malaysian women, ethnicity was also included in the multivariable model. All multivariable models were also adjusted for differences in mammography systems. Variance inflation factor of <3 was used as the threshold for low multicollinearity. Linear regression assumptions, such as linearity and normality of the outcome, were assessed visually using residual plots. To ease the interpretation of results, the model coefficients and 95% CIs were back-transformed and mean-centred with the formula, $\Delta = (\exp(\beta) \times w) - w$, where Δ is the mean-centred difference in mammographic density for every unit increase in the exposure, β is the regression coefficient, and w is the mean mammographic density in each stratum of analysis.

The Baron and Kenny's four steps approach for mediation analysis (135) was conducted to determine the risk factor(s) which account for the variation in mammographic density between Malaysian and Swedish women. In this analysis, the cohort effect is tested as a predictor variable and mammographic density as the response variable in a linear regression model. Risk factors that cause significant attenuation in the cohort effect are considered as important mediators of population differences in mammographic density. The Preacher and Hayes bootstrap method (136) was used to determine the statistical significance of the mediation effect.

For all analyses, *p* value < 0.05 were considered statistically significant. All analysis was carried out using the R statistical environment, version 3.2.0.

3.3. Results

3.3.1. Differences in demographics, reproductive and other breast cancer risk factors between the two study populations

As shown in **Table 3-3**, age and BMI were similar across the two cohorts, and is a consequence of exact-matching. The overall mean age was 54 years old and average BMI was 25.6kg/m^2. Despite BMI-matching, Swedish women were taller (by 10cm, *p* value < 0.001) and weighed more (by 9kg, *p* value < 0.001) on average compared to Malaysian women. Among Malaysian women, the predominant ethnicity was Chinese (52.9%), followed by Malay (21.6%) and Indian (19.2%), while the Swedish cohort consisted of Caucasian women.

Table 3-3: Distribution of breast cancer risk factors for Malaysian (n=1,501) and Swedish women (n=4,501)

Risk factors	Malaysian women (n=1,501)		Swedish women (n=4,501)		
	x̄ ± SD	n (%)	x̄ ± SD	n (%)	*p* value
Age, in years	54.0 ± 8.5		54.0 ± 8.5		0.879
Ethnicity					
Chinese		794 (52.9)			
Malay		324 (21.6)			
Indian		288 (19.2)			
Education					
Primary or less		130 (8.7)		45 (1.0)	<0.001**
Secondary		748 (49.8)		442 (9.8)	
Tertiary		592 (39.4)		4014 (89.2)	
Age at menarche	12.9 ± 1.4		13.0 ± 1.5		0.002*
Age at first full-term pregnancy†	27.1 ± 4.8		27.2 ± 5.2		0.480
Full-term pregnancies†	2.8 ± 1.2		2.2 ± 0.8		<0.001**
Nulliparity		239 (15.9)		557 (12.4)	<0.001**
Post-menopause		805 (53.6)		2493 (55.4)	0.045*
Age at menopause‡	49.3 ± 4.7		48.8 ± 5.8		0.019*
Breast cancer family history		205 (13.7)		576 (12.8)	0.431
OC ever use		378 (25.2)		3180 (70.7)	<0.001**
HRT ever use‡		156 (19.4)		872 (35.0)	<0.001**
HRT current use‡		21 (2.6)		148 (5.9)	<0.001**
Gynaecological surgery‡		273 (33.9)		774 (31.0)	0.183
Height (cm)	156.1 ± 5.7		166.6 ± 6.0		<0.001**
Weight (kg)	62.4 ± 11.8		71.2 ± 13.8		<0.001**
BMI (kg/m^2)	25.6 ± 4.8		25.6 ± 4.8		0.926
Body shape change					
In youth (units)	0.8 ± 0.9		0.6 ± 1.1		<0.001**
Since youth (units)	1.6 ± 1.2		1.5 ± 1.2		0.001*
Regular alcohol intake		68 (4.5)		3476 (77.2)	<0.001**
Ever smoked		65 (4.3)		2380 (52.9)	<0.001**
Regular physical activity		260 (17.3)		2049 (45.5)	<0.001**
Regular coffee intake		845 (56.3)		3591 (79.8)	<0.001**

Annotations: ***p* value < 0.001, **p* value < 0.05, †among parous women, ‡among post-menopausal women, x̄ = mean, SD = standard deviation, n = number, % = column proportion.

Among parous women, the age at first birth was similar across the two cohorts, approximately 27 years old. Malaysian women reported more children compared to Swedish women (2.8 versus (vs) 2.2 children per woman on average, *p* value < 0.001). However, up to 16% of Malaysian women reported nulliparity, compared to only 12% among Swedish women (*p* value < 0.001). Compared to Swedish women, Malaysian women reported an earlier age at menarche (12.9 vs 13.0 years old, *p* value = 0.002) and a later age at menopause (49.3 vs 48.8 years old, *p* value = 0.019), suggesting a longer reproductive period in this cohort of Malaysian women.

In terms of lifestyle risk factors, Malaysian women were less likely to report high risk activities such as hormone replacement therapy use (19.4% vs 35.0% among Swedish women, *p* value < 0.001), oral contraceptive use (25.2% vs 70.7%, *p* value < 0.001), smoking (4.3% vs 52.9%, *p* value < 0.001), and regular alcohol intake (4.5% vs 77.2%, *p* value < 0.001). On the other hand, Malaysian women were less likely to report regular physical activity (17.0% vs 45.5% among Swedish women, *p* value < 0.001), and were marginally more likely to gain weight over time (by 0.8 vs 0.6 units in youth and 1.6 vs 1.5 units since youth). Interestingly, the proportion of women reporting a first degree relative with breast cancer is similar for both cohorts (13.7% among Malaysian women and 12.8% among Swedish women, *p* value = 0.431).

3.3.2. Distribution of mammographic density for Malaysian and Swedish women

The distribution of mammographic density in this age- and BMI-matched analysis is shown in **Figure 3-1**. Consistent with published literature, Malaysian women presented with higher percent density compared to Swedish women (by 2%, *p* value < 0.001). The difference appears largest among pre-menopausal women, where Malaysian women had an average percent density of 12.1% compared to 9.3% among Swedish women (*p* value < 0.001). Among post-menopausal women, the difference was less apparent, by 1.3%, but this was still statistically significant (*p* value < 0.001).

Non-dense volume was significantly lower among Malaysian women (531.5cm^3) compared to Swedish women (722.2cm^3), and the differences were similar for both pre-menopausal women (by -133cm^3, *p* value < 0.001) and post-menopausal women (by -152.2cm^3, *p* value < 0.001).

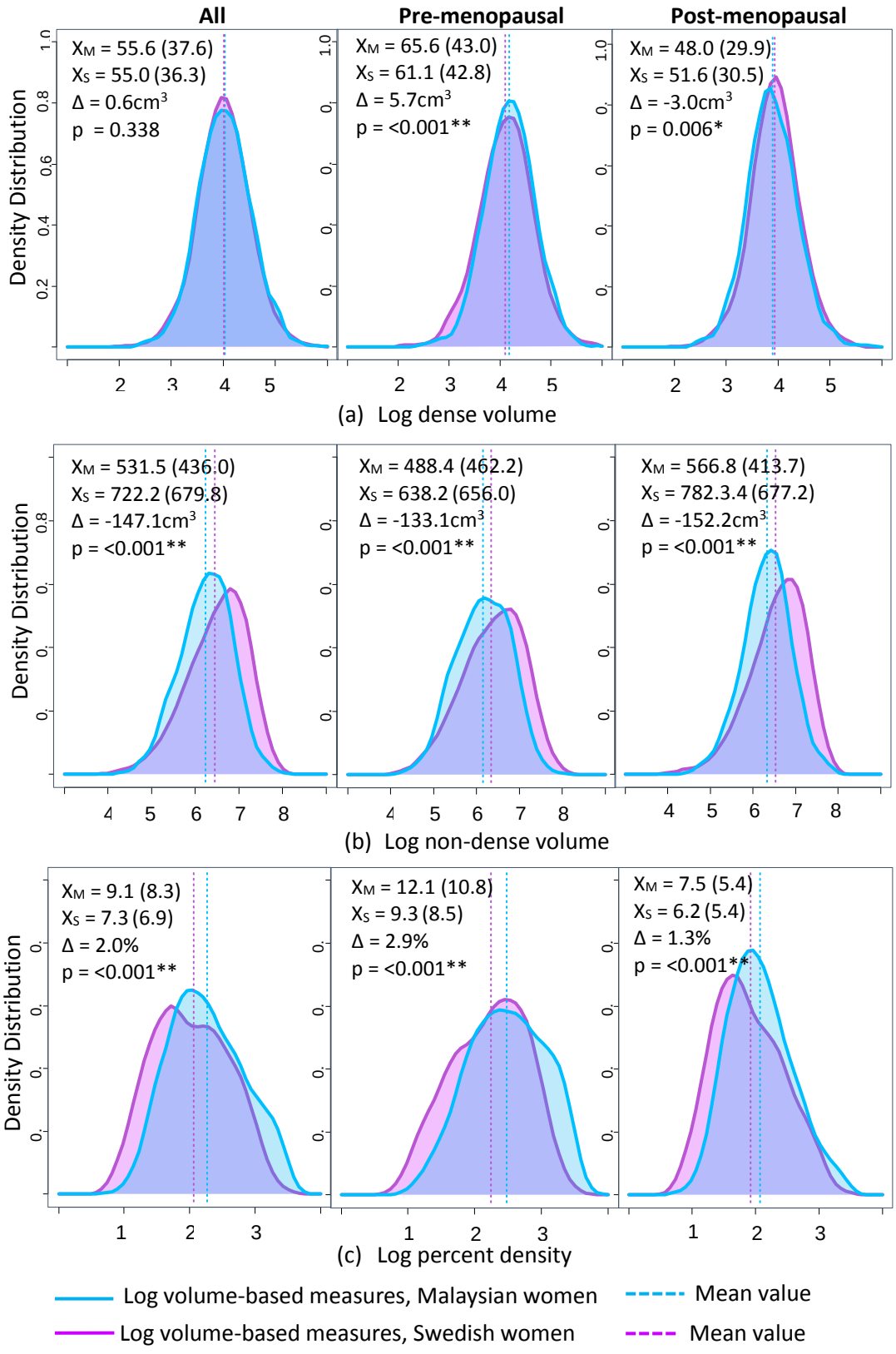


Figure 3-1: Distribution of age- and BMI-matched mammographic density for both cohorts. *Annotations*: ***p* value < 0.001, **p* value < 0.05, X_M = Median (IQR) volume-based measures among Malaysian women, X_S = Median (IQR) volume-based measures among Swedish women in original scale (cm^3 or %), Δ = Exponentiated mean difference and p = *p* value for comparison of log-transformed volume-based measures between Malaysian and Swedish women.

Post-menopausal Malaysian women had significantly lower absolute dense volume compared to Swedish women (by -3.0cm^3, *p* value = 0.009). The inverse was true for premenopausal women, where Malaysian women had on average 5.7cm^3 higher dense volume compared to Swedish women (*p* value < 0.001). The above analysis shows that differences in absolute dense volume, rather than percent density, better reflects population differences in breast cancer risk, particularly for post-menopausal women. Further analyses will be conducted with absolute dense volume only.

3.3.3. Factors attributed to within-cohort variation in mammographic density

Multivariable linear regression models were used to test for independent associations between mammographic density measures and breast cancer risk factors for each population and by menopausal status. As shown in **Figure 3-2**, the assumptions for linearity and normality of residuals for the analysis of $\log_{10}$ volume-based mammographic density measures were met.

Overall, multivariable log-linear regression models show that the associations between dense volume and breast cancer risk factors were in the same direction for both cohorts, with small differences in the magnitude of association. Among pre-menopausal women (**Table 3-4**), dense volume was lower by 1.2cm^3 (95% CI = [-1.9, -0.5]) among Malaysian women and by 0.6cm^3 (95% CI = [-0.9, -0.2]) among Swedish women for every year increase in age. The effect was weaker among post-menopausal women (**Table 3-5**), where dense volume was lower by 0.5cm^3 (95% CI = [-0.8, -0.2]) among Malaysian women and 0.3cm^3 (95% CI = [-0.5, -0.2]) among Swedish women as age increased. The association between dense volume and BMI were similar across cohorts and menopausal status, where dense volume was higher by 1.2-1.5cm^3 for every unit increase in BMI (*p* value < 0.001).

There were strong, significant associations between reproductive factors and mammographic density across cohorts. Nulliparity was associated with 6.3-7.7cm^3 higher dense volume compared to parous women. Consistent with this, dense volume decreased with increasing number of births. Among Malaysian women, this effect was stronger and statistically significant among pre-menopausal women (by -3.8cm^3, 95% CI = [-6.7, -0.7]) compared to post-menopausal women (by -1.0cm^3, 95% CI = [-2.8, 0.9]). Among Swedish women, dense volume was lower by 2.8cm^3 for every birth, and the effect was similar for both pre-menopausal and post-menopausal women (*p* value < 0.001).

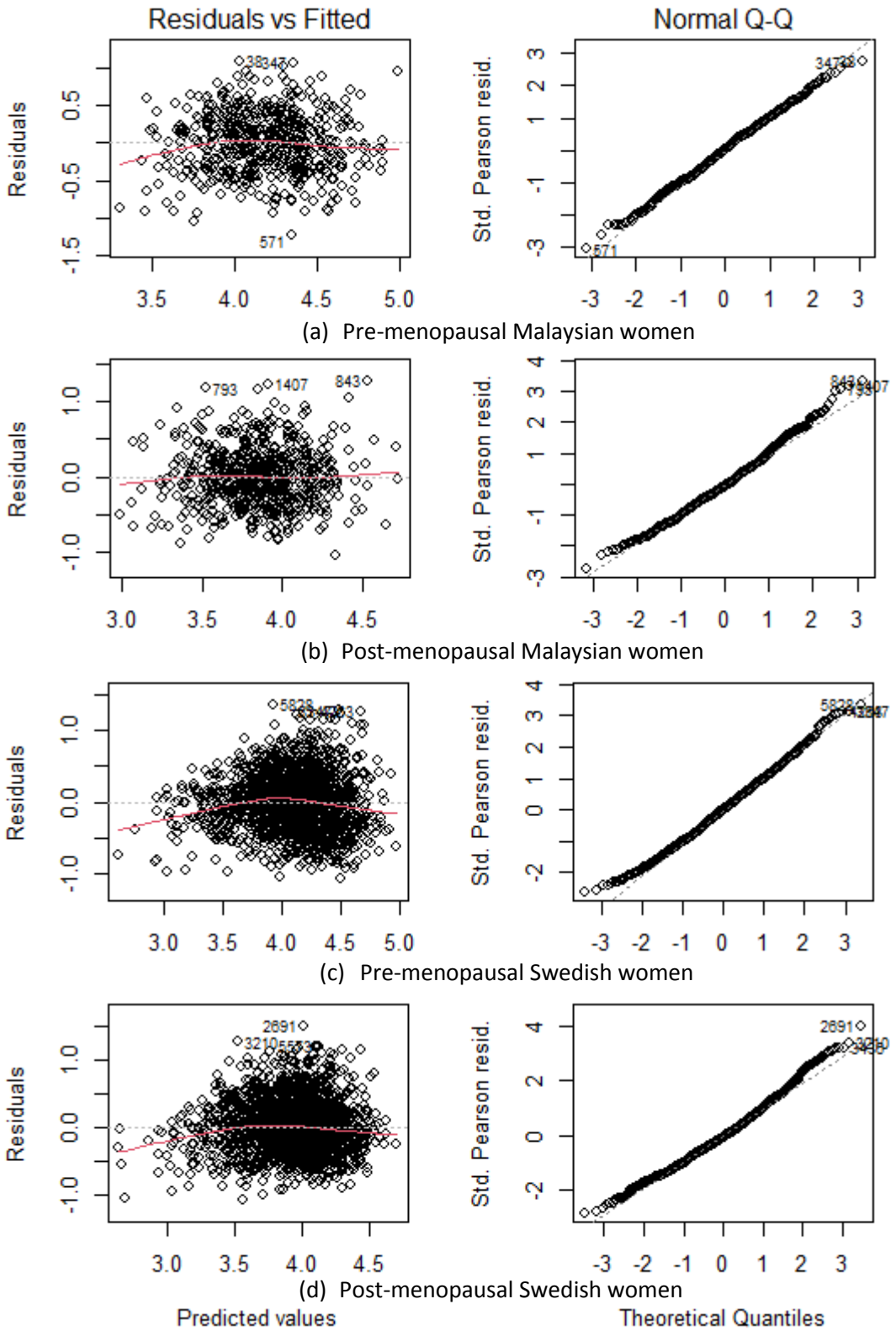


Figure 3-2 Assessment of linearity and normality of residuals in multivariable linear regression analyses for factors associated with dense volume among (a) pre-menopausal and (b) post-menopausal Malaysian women, and (c) pre-menopausal and (d) post-menopausal Swedish women. The assumption for linearity is met if the red line of the "Residuals vs Fitted" plot is approximately horizontal. The assumption for normality of residuals is met if there are no large deviations from the diagonal line in the "Normal Q-Q" plot.

Table 3-4 Multivariable log-linear regression analysis of dense volume and breast cancer risk factors, among pre-menopausal Malaysian (n=696) and Swedish (n=2,008) women

Model variables	Multivariable associations with dense volume (cm^3)			
	Malaysian (n=696)		Swedish (n=2,008)	
	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Age	-1.2 [-1.9, -0.5]	<0.001**	-0.6 [-0.9, -0.2]	0.002*
BMI	1.2 [0.4, 2.0]	0.002*	1.5 [1.1, 1.9]	<0.001**
Ethnicity				
Malay	Ref			
Chinese	11.3 [3.1, 20.4]	0.006*		
Indian	8.1 [-1.4, 19.0]	*0.099*		
Education				
Secondary	Ref		Ref	
Primary or less	-1.7 [-13.3,12.5]	0.799	NA	
Tertiary	-3.1 [-8.5, 2.7]	0.287	6.8 [-1.8, 16.7]	0.129
Height	0.0 [-0.5, 0.6]	0.874	0.5 [0.2, 0.7]	<0.001**
Body shape change in youth	1.5 [-1.5, 4.6]	0.328	2.3 [0.7, 3.9]	0.004*
Body shape change since youth	2.1 [-0.6, 4.9]	0.139	3.2 [1.5, 5.1]	<0.001**
Age at menarche	0.5 [-1.7, 2.8]	0.656	0.5 [-0.6, 1.6]	0.365
Nulliparous	7.7 [-0.1, 16.4]	*0.055*	6.3 [1.2, 11.8]	0.015*
OC ever use	-4.0 [-10.0, 2.7]	0.233	0.8 [-2.7, 4.5]	0.675
Breast cancer family history	-4.8 [-12.3, 3.8]	0.265	3.5 [-1.3, 8.7]	0.153
Ever smoked	10.7 [-2.4, 26.5]	0.117	-1.2 [-4.2, 2.1]	0.471
Physical activity	3.0 [-4.1, 10.9]	0.428	-1.6 [-4.6, 1.7]	0.337
Regular coffee intake	2.4 [-3.2, 8.4]	0.419	-1.1 [-4.9, 3.0]	0.593
Number of births†	-3.8 [-6.7, -0.7]	0.018*	-2.8 [-4.9, -0.6]	0.012*
Age at last birth†	0.1 [-0.8, 0.9]	0.797	0.1 [-0.3, 0.4]	0.775

All variables listed in the table were included in multivariable analysis (except for ethnicity in the Swedish analysis). The models were also adjusted for type of mammography system." Variables were not included if they were not significant in univariate analysis at *p* value > 0.05 or were in high collinearity with other variables (age at first birth, weight). *Annotations:* ***p* value < 0.001, **p* value < 0.05, †among parous women, Δ = Mean-centered difference in dense volume, Ref = Reference category.

Interestingly, height and changes in body shape over time was significantly associated with dense volume among Swedish women, but not Malaysian women. Every 1cm increase in height was associated with 0.4-0.5cm^3 higher dense volume among Swedish women (p-value<0.001). Similarly, increases in body size in youth was associated with 2.3-2.5cm^3 higher dense volume (p<0.001). Higher dense volume was also associated to

increasing body size from youth to adulthood, by 3.2cm^3 (95% CI: [1.5, 5.1]) among pre-menopausal women and by 2.7cm^3 (95% CI: [1.6, 3.8]) among post-menopausal women.

Table 3-5 Multivariable log-linear regression analysis of dense volume and breast cancer risk factors, among post-menopausal Malaysian (n=805) and Swedish (n=2,493) women

Model variables	Multivariable associations with dense volume (cm^3)			
	Malaysian (n=805)		Swedish (n=2,493)	
	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Age, in years	-0.5 [-0.8, -0.2]	<0.001**	-0.3 [-0.5, -0.2]	<0.001**
BMI	1.5 [0.9, 2.1]	<0.001*	1.3 [1.0, 1.6]	<0.001**
Ethnicity				
Malay	Ref			
Chinese	3.1 [-2.1, 8.9]	0.259		
Indian	10.2 [3.7, 17.4]	0.001*		
Education				
Secondary	Ref		Ref	
Primary or less	-1.1 [-6.6, 5.2]	0.726	0.3 [-7.4, 9.4]	0.946
Tertiary	2.7 [-1.3, 7.0]	0.197	-0.8 [-3.8, 2.3]	0.595
Height	0.0 [-0.3, 0.3]	0.994	0.4 [0.3, 0.6]	<0.001**
Body shape change in youth	0.3 [-1.6, 2.3]	0.782	2.5 [1.5, 3.5]	<0.001**
Body shape change since youth	0.0 [-1.7, 1.8]	0.985	2.7 [1.6, 3.8]	<0.001**
Age at menarche	0.9 [-0.4, 2.2]	0.177	0.3 [-0.4, 1.0]	0.422
Nulliparous	6.6 [1.1, 12.8]	0.018*	9.0 [5.3, 12.9]	<0.001**
OC ever use	-1.9 [-5.6, 2.3]	0.366	-1.2 [-3.3, 1.0]	0.291
HRT ever use	0.0 [-4.8, 5.4]	0.994	-0.3 [-2.6, 2.1]	0.798
HRT current use	-12.2 [-20.3, -1.7]	0.026*	-0.2 [-4.5, 4.5]	0.924
Gynaecological surgery	-2.9 [-6.5, 1.0]	0.146	2.9 [0.6, 5.2]	0.012*
Breast cancer family history	3.4 [-1.5, 8.9]	0.183	4.3 [1.2, 7.5]	0.005*
Ever smoked	5.1 [-7.1, 20.7]	0.448	-0.4 [-2.4, 1.7]	0.682
Physical activity	2.8 [-2.1, 8.2]	0.269	2.2 [0.1, 4.4]	0.041*
Regular coffee intake	2.9 [-0.7, 6.9]	0.122	0.3 [-2.6, 3.4]	0.851
Number of births†	-1.0 [-2.8, 0.9]	0.300	-2.8 [-4.1, -1.4]	<0.001**
Age at last birth†	0.2 [-0.2, 0.7]	0.356	0.1 [-0.1, 0.3]	0.392

All variables listed in the table were included in multivariable analysis (except for ethnicity in the Swedish analysis). The models were also adjusted for type of mammography system." Variables were not included if they were not significant in univariate analysis at *p* value > 0.05 or were in high collinearity with other variables (age at first birth, weight). *Annotations:* ***p* value < 0.001, **p* value < 0.05, †among parous women, Δ = Mean-centered difference in dense volume, Ref = Reference category.

There were two variables that had associations with mammographic density that were not in parallel to breast cancer risk. Firstly, current use of HRT was strongly associated to lower dense volume among post-menopausal Malaysian women (by -12.2cm^3, 95% CI = [20.3, -1.7]), while no association was observed among Swedish women. Secondly, physical activity was associated with higher dense volume among post-menopausal Swedish (by 2.2cm^3, 95% CI = [0.1, 4.4]) and Malaysian women (by 2.8cm^3, 95% CI = [-2.1, 8.2]). There were no significant associations among pre-menopausal women.

Table 3-6 Mediation analysis among pre- and post-menopausal women

	Linear regression associations				**Mediation analysis**
	Variable effect		**Cohort effect**		
Model variables	β	*p* value	β_c	*p* value	$p_{mediation}$
(a) Pre-menopausal women					
No variables			**0.08**	**<0.001****	
Height	0.01	0.002*	0.14	<0.001**	
Weight	0.01	<0.001**	0.16	<0.001**	
Breast Volume	0.44	<0.001**	-0.17	<0.001**	
Body shape change in youth	0.04	<0.001**	0.08	0.001*	
Body shape change since youth	0.09	<0.001**	0.05	0.030*	<0.001**
Nulliparity	0.11	<0.001**	0.08	<0.001**	
Number of births†	-0.04	<0.001**	0.11	<0.001**	
Physical activity	-0.07	0.002*	0.06	0.008*	0.090
(b) Post-menopausal women					
No variables			**-0.05**	**0.008***	
Height	0.00	0.004*	-0.01	0.771	<0.001**
Weight	0.01	<0.001**	0.05	0.015*	<0.001**
Breast Volume	0.42	<0.001**	-0.05	0.007*	
Body shape change in youth	0.04	<0.001**	-0.07	<0.001**	
Body shape change since youth	0.08	<0.001**	-0.06	0.001*	
Nulliparity	0.13	<0.001**	-0.05	0.005*	
Number of births†	-0.03	<0.001**	-0.03	0.160	<0.001**
Physical activity	-0.03	0.125	-0.06	0.003*	

Mediation analysis is only conducted when there is an attenuation of the cohort effect. *Annotations:* ***p* value < 0.001, **p* value < 0.05, †among parous women, β = regression coefficient for the variable entered, β_c = regression coefficient for cohort effect comparing Malaysian to Swedish cohort (referent group, $p_{mediation}$ = *p* value from mediation analyses.

Among Malaysian women, the highest dense volume was observed among pre-menopausal Chinese women (by 11.3cm^3 compared to Malay women, 95% CI = [3.1, 20.4]). Among post-menopausal women, however, the difference was smaller and not statistically significant (by 3.1cm^3, 95% CI = [-2.1, 8.9]). Instead, Indian women observed the highest

dense volume post-menopause (by 10.2cm^3 compared to Malay women, 95% CI = [3.7, 17.4]).

3.3.4. Factors attributed to between-cohort variation in mammographic density

Mediation analyses identified several factors that account for between-cohort differences in dense volume (**Table 3-6**). Mediation occurs when the addition of a model variable leads to an attenuation of the cohort effect, indicating that the model variable may explain some of the variation in dense volume between the two cohorts. For post-menopausal women, significant attenuation in the cohort effect was observed when body size variables (i.e. height and weight) and number of births were added to model. When height was added the model, the cohort effect (β_c) attenuated from -0.05 to -0.01 (*p* value < 0.001), but it changed to 0.05 when weight was included in the model (*p* value < 0.001). With the addition of number of children, the cohort effect attenuated from -0.05 to -0.03 (*p* value < 0.001). These effects were not observed among pre-menopausal women. The only significant attenuation observed among pre-menopausal women was with the introduction of changes in body shape since youth (change in β_c from 0.08 to 0.05, *p* value < 0.001).

Interestingly, despite large differences in non-dense volume, breast size did not explain the differences in dense volume between Asian and Caucasian women. Other breast cancer risk factors that were differentially distributed between the two cohorts, such as body changes in youth, nulliparity and physical activity, did not explain the differences in mammographic density between the two cohorts.

3.4. Discussion

3.4.1. Summary of main findings

This comparative analysis between age- and BMI-matched Asian and Caucasian women supports the hypothesis that absolute density may more accurately reflect population differences in breast cancer risk, compared to percent density, particularly for post-menopausal women. Among post-menopausal women, specifically, absolute dense volume was significantly lower among Asian women compared to age- and BMI-matched Caucasian women, and this may correlate to population differences in breast cancer risk. Population differences in absolute dense volume between post-menopausal Asian and Caucasian women can be explained by important breast cancer risk factors, such as height,

weight, and parity. This further substantiates the utility of absolute dense volume as a biomarker of breast cancer risk for Asian women.

3.4.2. Mammographic density as a biomarker of breast cancer risk across populations

One of the most notable findings from this analysis is that absolute density measures, rather than relative or percent density measures, was significantly lower among post-menopausal Asian women compared to age- and BMI-matched Caucasian women, and this may correlate to population differences in breast cancer risk. Researchers have postulated that associations with percent density, taken as a ratio of fibro-glandular tissue to the whole breast volume, are more strongly confounded by breast size and BMI, and may underestimate the amount of dense tissue in the breast (137). Absolute dense measures, therefore, may more accurately represent the amount of breast tissue at risk for cancer development (44). The findings in this study is consistent with a previous meta-analysis which suggests that absolute dense measures are more strongly correlated to breast cancer risk compared to percent density for Asian women (130). In previous comparative studies, Asian women living in the UK or USA had up to 5 units lower absolute dense area or volume compared to Caucasian women, but the differences were not significant after adjustments for age and BMI (29,40,42,123–126).

This study also suggests that the mammographic density measures studied here may not accurately reflect population risk to breast cancer among pre-menopausal Asian women. The findings here are in line with prior studies of East Asian women which show mammographic density predicts breast cancer well in post-menopausal women, but not pre-menopausal women (126,138). Herein lies the paradox, where younger Asian women with presumably lower breast cancer risk observed higher absolute dense volume, compared to their Caucasian counterparts. However, there were important associations between absolute dense volume and breast cancer risk factors among pre-menopausal Asian women in this study, including for age, BMI, and parity. This suggests that volume-based absolute density may still be useful, albeit imperfect, as a biomarker of risk among pre-menopausal Asian women.

Thus far, the distribution of Asian mammographic density have been reported in relatively homogenous populations, such as in Korea and Japan (130). Studies of Singaporean women describe mammographic density in a predominantly Chinese cohort (139). There are limited reports on the variation in mammographic density across Asian ethnicities. In this

study, younger Chinese women observe the highest absolute dense volume, compared to Malay and Indian women. The associations observed for Chinese women are in contrast to a previous report of mammographic density in the MyMammo cohort, where computer-aided methods show that Chinese women had the lowest dense area, both pre- and post-menopause (48). Compared to the previous report, however, the analysis in this chapter contains a much larger sample size of Chinese women (n=794 vs n=205) and had an older cohort overall (average age 54 vs 50 years old), which may in part drive the differences observed between the two studies. Notably, the distributions observed here are more concordant with ethnic differences in breast cancer incidence, where the incidence of breast cancer is highest among Chinese women (3). This suggests that mammographic density measured with fully-automated software likely provide reliable estimates of breast cancer risk across different Asian ethnicities.

3.4.3. Breast cancer risk factors that account for population differences in mammographic density

In a study of British women, differences in BMI largely explained the differences in mammographic density between ethnic groups (41). BMI is strongly associated to mammographic density, and has been shown to negatively confound associations with mammographic density (34). An important strength of this study is the ability to match Asian and Caucasian women on BMI, thereby reducing its' confounding effect. It has also allowed the independent evaluation of height and weight, which are established post-menopausal breast cancer risk factors (140). In this study, Swedish women were 10cm taller and 9kg heavier on average when compared to Malaysian women, despite having the same BMI, and these differences accounted for population differences in absolute dense volume. This study also highlights the need for caution when using BMI as a standard measure of body fatness across populations, which is common practice.

The association between breast cancer risk and parity is observable from global trend of rising breast cancer incidence in countries that are experiencing rapid declines in number of births per woman (2). In Asia, the total fertility rate has reduced from 5.4 births per woman in 1970 to 2.0 births per woman in 2020 (141). Beyond global trends, there is extensive research that parity factors, including the number of children, age at first birth, and breast-feeding habits, are strongly associated to breast cancer risk later in life (142). It is noteworthy that this strong breast cancer risk factor could account for population differences in mammographic density in this study, as it gives added confidence that this

measure of mammographic density is a suitable biomarker of breast cancer risk among Asian post-menopausal women.

Interestingly, the differences in HRT use between the Caucasian and Asian women in this study did not explain the differences in absolute dense volume between the two cohorts. HRT use is a widely known risk factor for post-menopausal breast cancer (143), and has been shown to be associated with mammographic density in Caucasian populations (144). However, there is evidence that HRT use may only modify mammographic density during exposure (145). Also, the proportion of women currently on HRT in this study was less than 6% and may represent a very select group of women, which may have led to the spurious inverse association between HRT use and absolute dense volume observed among Asian women. Thus far, there is no published description of the characteristics and risk profiles of Malaysian HRT users. Therefore, the associations observed for HRT use in this study should be interpreted with caution.

There was a significant positive association between absolute dense volume and physical activity among post-menopausal Swedish women, suggesting that physical activity may increase breast cancer risk in this population. Again, this observation is in contrast to the strong, protective association between physical activity and breast cancer risk observed across populations (13). Largely, there have been no reported association between physical activity and mammographic density, even for studies conducted in the MyMammo (49) and Karma (133) cohorts. Therefore, the positive association observed here is unexpected.

3.4.4. Strengths

This is the first study that robustly investigates if differences in mammographic density can be attributed to variation in breast cancer risk factors across two geographically distinct populations. Many of the variables of interest were collected in a comparable manner for the two cohorts, and mammographic density was estimated using the same software. Matching of participants from the two cohorts enabled an assessment of breast cancer risk factors that was independent of age and BMI. It is also the one of few reports of mammographic density in a multi-ethnic Asian population, and the only study to do so using fully-automated, volume-based mammographic density measures.

3.4.5. Limitations

Firstly, the study samples may not be representative of their respective populations. The Malaysian cohort was a convenient sample of women attending a subsidized screening programme in an urban, private hospital. The proportion of women who reported a first degree relative with breast cancer was similar for Malaysian and Swedish women, suggesting that this cohort of Malaysian women may experience higher breast cancer risk, compared to the general population. Swedish women, on the other hand, were matched by age and BMI to the selected Malaysian women, and therefore may not be representative of the overall distribution of Swedish women.

Secondly, the majority of Asian women studied were of Chinese ethnicity. Therefore, the associations observed for might be more applicable to Chinese women, compared to women of other Asian ethnicities. The sample size of Malay and Indian women in this study were too small for stratified analysis. Future studies of the distribution of mammographic density in these understudied Asian ethnicities are warranted.

Thirdly, there were substantial missing data for several risk factors. For risk factors that were homogenous, such as age at menopause or education status, imputations were necessary to include the variable in analysis. Some factors, however, could not be included in the analysis, such as breast-feeding habits or soy intake. If available, this study could have provided unique insight into the population differences in breast-feeding habits and its' effect on mammographic density. Also, studying the association between population differences in soy intake and mammographic density could have provided important evidence for the causal relationship between soy intake and breast cancer risk.

3.5. Conclusion

Compared to relative measures of mammographic density, absolute dense volume may be a better biomarker of post-menopausal breast cancer risk for Asian women. More robust, area-based mammographic density measures may be required for pre-menopausal Asian women. To improve sensitivity in risk estimation, future studies comparing breast cancer risk across and within populations should take into account the interaction between ethnicity, breast size, and BMI when selecting mammographic density measures as a biomarker of risk.

Chapter 4 : Cross-sectional association between soy intake and mammographic density among multi-ethnic Asian women

4.1. Rationale and objectives

Increasingly, mammographic density has been used as a biomarker of breast cancer risk in observational studies and in randomized control trials. For example, mammographic density is correlated to changes in breast cancer risk in response to tamoxifen use among breast cancer patients (146) and among high-risk women (147,148). Mammographic density has also been used to study lifestyle and dietary interventions for breast cancer prevention, including green tea intake (149), vitamin D exposure (150) and physical activity (151), but these have typically reported null findings. Similarly, RCTs of soy supplements on mammographic density among Caucasian women have reported no significant association (78), leading some authors to question if mammographic density can mediate the effect of soy intake on breast cancer risk (91,99).

Observational studies investigating the association between soy intake and mammographic density among Caucasian women have reported mixed results (107,113–118). These inconsistencies have been largely attributed to the low prevalence of soy isoflavone intake as well as low amount of intake even among soy consumers in Caucasian populations (117). Soy intake is expected to be higher and more frequent in Asian populations, and in the few studies conducted in Asian populations, a majority have shown inverse associations between soy intake and mammographic density (108,119–121). The associations appear to be limited to subsets of the population, such as among post-menopausal women or non-green tea drinkers (91), or for women who are able to metabolize daidzein to (S)-equol efficiently (108).

Furthermore, research in Chinese and Singaporean women suggests that the inverse association between soy intake and breast cancer risk may be stronger for women with higher BMI, compared to lean women (102,152). Given that BMI is an important determinant of mammographic density, its' role in the association between soy intake and mammographic density requires investigation. Thus far, observational studies for soy and mammographic density have included BMI as a confounder, but have not explored its' role as an effect modifier.

This chapter evaluates the utility of mammographic density as a biomarker in studying the association between soy intake and breast cancer risk among Asian women. Specifically, the primary objective of this chapter is to determine the association between soy intake and mammographic density in a cross-sectional analysis of pre-menopausal and post-menopausal Asian women. Additionally, this chapter seeks to (1) to understand the factors that are associated with frequent soy intake among Asian women, and (2) to evaluate the interaction between soy intake and BMI on mammographic density.

4.2. Methodology

4.2.1. Study population

The analysis was conducted with data collected from the MyMammo programme at SJMC and UMMC, as described in **Chapter 1**. Among 4,014 women recruited between 2011 and 2016, 737 (18.4%) women were excluded from the analysis (**Figure 4-1**). Women were excluded if they were diagnosed with breast cancer prior- or post-recruitment (n=47), there was missing information on age (n=18) and BMI (n=25), if breast implants were seen on mammogram (n=5), and if there was missing data on soy intake (n=166).

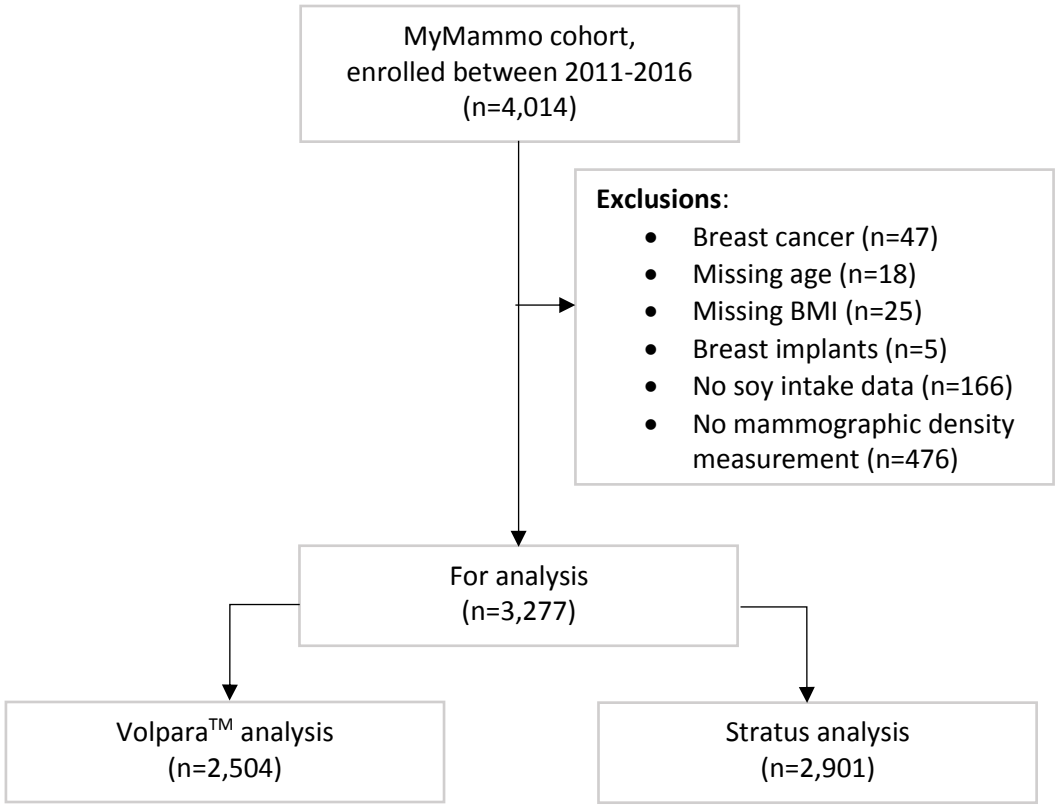


Figure 4-1 Participant selection for volume-based and area-based analysis.

Further exclusions were made for participants who did not have mammographic density measurements, due to missing mammogram images or errors in mammographic density measurement (n=476). A total of 3,277 women were included in the final analysis.

4.2.2. Frequency of soy intake

At enrolment, women were asked how frequently they currently consumed soymilk or soy foods. The frequency of consumption was categorized as more than once a week ("Frequent"), at least once a month ("Regular"), or less than once a month or rarely ("Non-consumer"). Frequency of total soy intake was derived from the above two variables (**Table 4-1**). In this composite variable, participants who reported frequent consumption of soy food and/or soymilk were categorized as frequent soy consumers. Among participants who did not report frequent intake, those who reported regular soy food intake and/or soymilk intake were categorized as regular soy consumers. Remaining participants were considered as non-consumers.

Table 4-1 Matrix for developing the "Frequency of Total Soy Intake†" variable (n=3,277)

		Frequency of soy food intake				
		Non-consumers	**Regular**	**Frequent**	**Missing**	**Total (%) for soymilk**
Frequency of soymilk intake	**Non-consumers**	241	728	72	40	1,081 (33.0)
	Regular	180	820	132	8	1,140 (34.8)
	Frequent	25	129	56	2	212 (6.5)
	Missing	452	56	35	0	844 (25.8)
Total (%) for soy food		898 (27.4)	2,034 (62.1)	295 (9.0)	50 (1.5)	3,277

Annotations: †Cells in green were categorized as frequent consumers, cells in blue were regular consumers and cells in pink were non-consumers in the "Frequency of Total Soy Intake" variable.

4.2.3. Possible confounding variables

At screening, participants responded to a cross-sectional questionnaire about their demographic, lifestyle, and reproductive factors. Age was calculated from date of birth to date of screening, in years. Height and weight were measured at screening, and used to calculate BMI (kg/m^2).

All other factors were self-reported, and is defined in **Table 3-1**. Reproductive factors include age at menarche, age at first full-term pregnancy, number of full-term pregnancies, nulliparity, and menopausal status. OC and HRT use were also assessed. Socio-demographic and lifestyle factors include ethnicity, monthly household income, and highest attained education. Women were asked to report some their body size in youth and since youth using Stunkard pictograms (153). Current physical activity frequency and duration was converted into MET-hours/week, as previously published (49) and described in **Table 3-1**.

4.2.4. Mammographic density measures

Volpara™ (version 1.5.4.0) was used to determine volume-based measures of mammographic density, including dense volume and percent density, and has been described in **Chapter 3**. Of the 3,277 women in this analysis, 2,504 women (76.4%) had volume-based mammographic density measurements (**Figure 4-1**).

Stratus (version 1.7.0) was used to determine area-based measures of mammographic density. Stratus builds upon an FDA (Food and Drug Administration) approved density tool (iCAD) and uses machine-learning to conduct high-throughput mammographic density measurement (56). The software estimates the dense area (cm^2) and total breast area (cm^2), which is then used to calculate percent density (dense area ÷ total breast area X 100%). Of the 3,277 women in this analysis, 2,901 women (88.5%) had Stratus mammographic density measurements (**Figure 4-1**). A total of 2,128 (64.9%) participants had both volume-based and area-based measures available.

Mammographic density was taken as the average from left and right breast in MLO view. The Pearson correlation between left and right mammographic density for volume-based mammographic density was 0.81 for dense volume and 0.90 for percent density, and for area-based mammographic density it was 0.88 for dense area and 0.89 for percent density. A majority of the mammograms were taken on the Hologic Selenia systems (87.7%), followed by the General Electric Company Senographe Essential system (7.3%), and the Siemens MAMMOMAT Novation system (4.8%).

4.2.5. Statistical analysis

Standard descriptive statistics were used to describe frequency of soy intake as well as demographic, reproductive, and lifestyle factors. Mean and SD was used to describe the distribution of continuous variables that were normally distributed, while median and IQR

(75^{th} percentile – 25^{th} percentile) were used to describe distributions that were not normally distributed. Number and percentages were used for categorical and ordinal variables.

Chi-square tests of independence (for categorical variables) and one-way ANOVA tests (for continuous variables) were used to test for variables that were univariately associated with frequency of soy intake (non-consumer, regular and frequent intake), under the null hypothesis that there were no association between frequency of soy intake and the variable tested. Variables that were associated with frequency of soy intake at a lenient threshold of *p* value < 0.100 were then included in a multivariable ordinal logistic regression model. The variables in the model were assessed for multicollinearity by the variance inflation factor analysis, using a cutoff of <3 to indicate low collinearity. An important assumption of the ordinal logistic regression model is the proportional odds assumption, which assumes that the slopes of the regression is parallel for each pair of outcomes. The assumption was tested by visual assessment of the slopes for each variable (154).

Linear regression models were used to test for variables that were associated with mammographic density. Volume-based mammographic density measures were $\log_{10}$-transformed and area-based mammographic density measures were square-root transformed to meet the assumption for normality of residuals. Each variable was tested in univariate models, under the null hypothesis that there were no association between mammographic density and the variable tested. Variables that were associated with mammographic density at a lenient threshold of *p* value < 0.100 were then included in a multivariable model. Variance inflation factor of <3 was used as the threshold for low multicollinearity. Linear regression assumptions, such as linearity and normality of the outcome, were assessed visually using residual plot.

The causal diagram in **Figure 4-2** describes the analytical plan for the primary analysis. Linear regression models were used to test for the association between mammographic density and frequency of soy intake. Fully adjusted models include variables that were independently associated with frequency of soy intake and mammographic density (ethnicity and parity), previously established determinants of mammographic density among Asian women (age, BMI, and menopausal status) and recruitment factors (type of mammography system and recruitment site). Linear regression assumptions, such as linearity and normality of the outcome, were assessed visually using residual plot. The analysis was further stratified by menopausal status.

The interaction between frequency of soy intake and BMI was assessed by adding the interaction term in linear regression models, under the null hypothesis that there is no significant interaction between frequency of soy intake and BMI on mammographic density. To further illustrate significant interactions, predicted values of mammographic density from the multivariable models were plotted against BMI and stratified by frequency of soy intake.

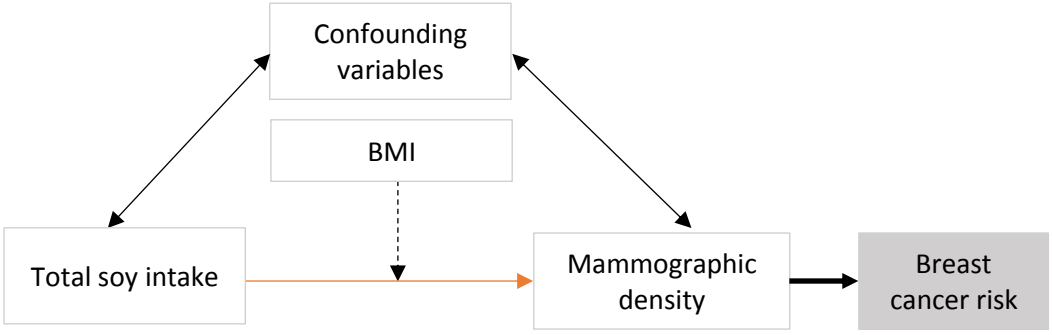


Figure 4-2 Causal diagram for the analysis of soy intake and mammographic density.

For ease of interpretation, all coefficients and 95% CIs from linear regression models were transformed back to original scale and centered to median value of mammographic density, $\Delta = (\exp(\beta) \times w) - w$ for volume-based measures and $\Delta = 2\beta(\sqrt{w}) + \beta^2$, where Δ is the difference in mammographic density compared to the referent category, β is the regression coefficient from linear regression models and w is the median mammographic density.

All statistical analyses were conducted using the R Statistical Environment (v4.0.3). All hypothesis testing was two-sided, and *p* value < 0.05 was considered as significant.

4.3. Results

4.3.1. Distribution and determinants of frequent soy intake among Asian women

The distribution and determinants of soy intake among Asian women is presented in **Table 4-2**. Of the study population, 13.8% were categorized as frequent consumers (consuming soy food or soymilk more than once a week) while most women (63.9%) were regular consumers (consuming soy food or soymilk at least once a month). Only 22.4% of the study population were categorized as non-consumers.

Table 4-2 The association between total soy intake and demographic, reproductive, and lifestyle risk factors (n=3,277)

		Distribution by frequency of soy intake				
	Overall (n=3,277)	**Non-consumers (n=733)**	**Regular (n=2093)**	**Frequent (n=451)**	**Multivariable association**	
	x̄ ± SD n (%)	x̄ ± SD n (%)	x̄ ± SD n (%)	x̄ ± SD n (%)	β [95%CI]	*p* value
Age	53.1 ± 8.3	54.9 ± 8.5	52.6 ± 8.2	52.6 ± 7.9	0.0 [0.0, 0.0]	0.508
Ethnicity[r%]						
Malay	646 (19.7)	183 (28.3)	394 (61.0)	69 (10.7)	Ref	
Chinese	1,834 (56.0)	383 (20.9)	1178 (64.2)	273 (14.9)	0.2 [0.0, 0.5]	*0.056*
Indian	672 (20.5)	136 (20.2)	445 (66.2)	91 (13.5)	0.4 [0.1, 0.6]	0.005*
Other	125 (3.8)	31 (24.8)	76 (60.8)	18 (14.4)	0.1 [-0.3, 0.6]	0.519
Income [r%]						
<RM5,000	1,798 (54.9)	457 (26.4)	1121 (62.3)	220 (12.2)	Ref	
RM5-10,000	870 (26.5)	165 (19.0)	578 (66.4)	127 (14.6)	0.0 [-0.2, 0.2]	0.730
>RM10,000	524 (16.0)	90 (17.2)	339 (64.7)	95 (18.1)	0.0 [-0.3, 0.2]	0.899
Education [r%]						
Secondary	1,646 (50.2)	361 (21.9)	1085 (65.9)	200 (12.2)	Ref	
Tertiary	1,201 (36.6)	233 (19.4)	765 (63.7)	203 (16.9)	0.2 [0.0, 0.3]	0.105
Primary/less	260 (7.9)	77 (29.6)	148 (56.9)	35 (13.5)	-0.2 [-0.5, 0.1]	0.254
Recruiting hospital [r%]						
UMMC	1,349 (41.2)	452 (33.5)	768 (56.9)	129 (9.6)	Ref	
SJMC	1,928 (58.8)	281 (14.6)	1325 (68.7)	322 (16.7)	0.8 [0.6, 1.0]	<0.001**
Height (cm)	156.4 ± 5.7	155.8 ± 5.9	156.6 ± 5.7	156.8 ± 5.5	0.0 [0.0, 0.0]	0.829
Weight (kg)	61.9 ± 11.6	61.7 ± 11.7	62.1 ± 11.4	61.3 ± 11.9		
BMI (kg/m^2)	25.3 ± 4.6	25.4 ± 4.8	25.3 ± 4.6	24.9 ± 4.8	0.0 [0.0, 0.0]	0.795
Lean, < 25.0 kg/m^2	1,727 (52.7)	386 (52.7)	1,086 (51.9)	255 (56.5)		
Overweight, 25.0-30.0 kg/m^2	1,065 (32.5)	225 (30.7)	701 (33.5)	139 (30.8)		
Obese, ≥ 30.0 kg/m^2	485 (14.8)	122 (16.6)	306 (14.6)	57 (12.6)		

Multivariable model excludes variables not associated with frequency of soy intake in univariate analysis at *p* value > 0.100 (height, change in body size since youth, OC use, family history of breast cancer, age at menarche, and number of full-term pregnancies). *Annotations:* ***p* value < 0.001, **p* value < 0.05, [†]among parous women, [‡]among post-menopausal women, x̄ = mean, SD = standard deviation, n = number; % = column proportions, r% = row proportions, β = regression coefficient, Ref = Reference category.

Table 4.2 (continued): The association between total soy intake and demographic, reproductive, and lifestyle risk factors (n=3,277)

	Overall (n=3,277)	Distribution by frequency of soy intake			Multivariable association	
		Non-consumers (n=733)	Regular (n=2093)	Frequent (n=451)		
	x̄ ± SD n (%)	x̄ ± SD n (%)	x̄ ± SD n (%)	x̄ ± SD n (%)	β [95%CI]	*p* value
Body shape change in youth	0.7 ± 1.0	0.6 ± 0.9	0.8 ± 1.0	0.8 ± 1.1	0.1 [0.0, 0.2]	0.014*
Body shape change since youth	1.6 ± 1.4	1.6 ± 1.4	1.7 ± 1.4	1.5 ± 1.5		
Physical Activity [r%]						
Low	1,219 (37.2)	326 (26.7)	770 (63.2)	123 (10.1)	Ref	
Moderate	1270 (38.8)	259 (20.4)	818 (64.4)	193 (15.2)	0.4 [0.2, 0.5]	<0.001**
High	756 (23.1)	139 (18.4)	488 (64.6)	129 (17.1)	0.5 [0.2, 0.7]	<0.001**
Ever smoked	194 (5.9)	29 (4.0)	122 (5.8)	43 (9.5)	0.3 [0.0, 0.6]	*0.091*
Regular alcohol intake	501 (15.3)	74 (10.1)	343 (16.4)	84 (18.6)	0.1 [-0.1, 0.4]	0.265
HRT use [‡, r%]						
Never	859 (44.3)	263 (30.6)	511 (59.5)	85 (9.9)	Ref	
Ever	361 (18.6)	103 (28.5)	210 (58.2)	48 (13.3)	0.0 [-0.4, 0.3]	0.772
Current	70 (3.6)	22 (31.4)	33 (47.1)	15 (21.4)	0.2 [-0.4, 0.8]	0.459
OC ever use	969 (29.6)	202 (27.6)	635 (30.3)	132 (29.3)		
Breast cancer family history	418 (12.8)	99 (13.5)	275 (13.1)	44 (9.8)		
Age at menarche	12.9 ± 1.4	13.0 ± 1.4	12.9 ± 1.4	12.8 ± 1.5		
Full-term pregnancies [r%]						
None	383 (11.7)	101 (26.4)	225 (58.7)	57 (14.9)		
1-2	935 (28.5)	203 (21.7)	606 (64.8)	126 (13.5)		
≥ 3	1764 (53.8)	372 (50.8)	1,144 (54.7)	248 (55.0)		
Nulliparous	428 (13.1)	110 (15.0)	253 (12.1)	65 (14.4)	-0.2 [-0.4, 0.0]	*0.071*
Age at first pregnancy [†]	27.1 ± 4.7	26.9 ± 5.0	27.1 ± 4.6	27.5 ± 4.5	0.0 [0.0, 0.0]	0.997
Menopause status [r%]						
Pre-menopause	1,338 (40.8)	242 (18.1)	906 (67.7)	190 (14.2)	Ref	
Post-menopause	1,937 (59.1)	490 (25.3)	1186 (61.2)	261 (13.5)	0.0 [-0.2, 0.2]	0.906
Age at menopause‡	49.3 ± 4.6	48.9 ± 4.5	49.3 ± 4.6	49.7 ± 4.6	0.0 [0.0, 0.1]	0.160

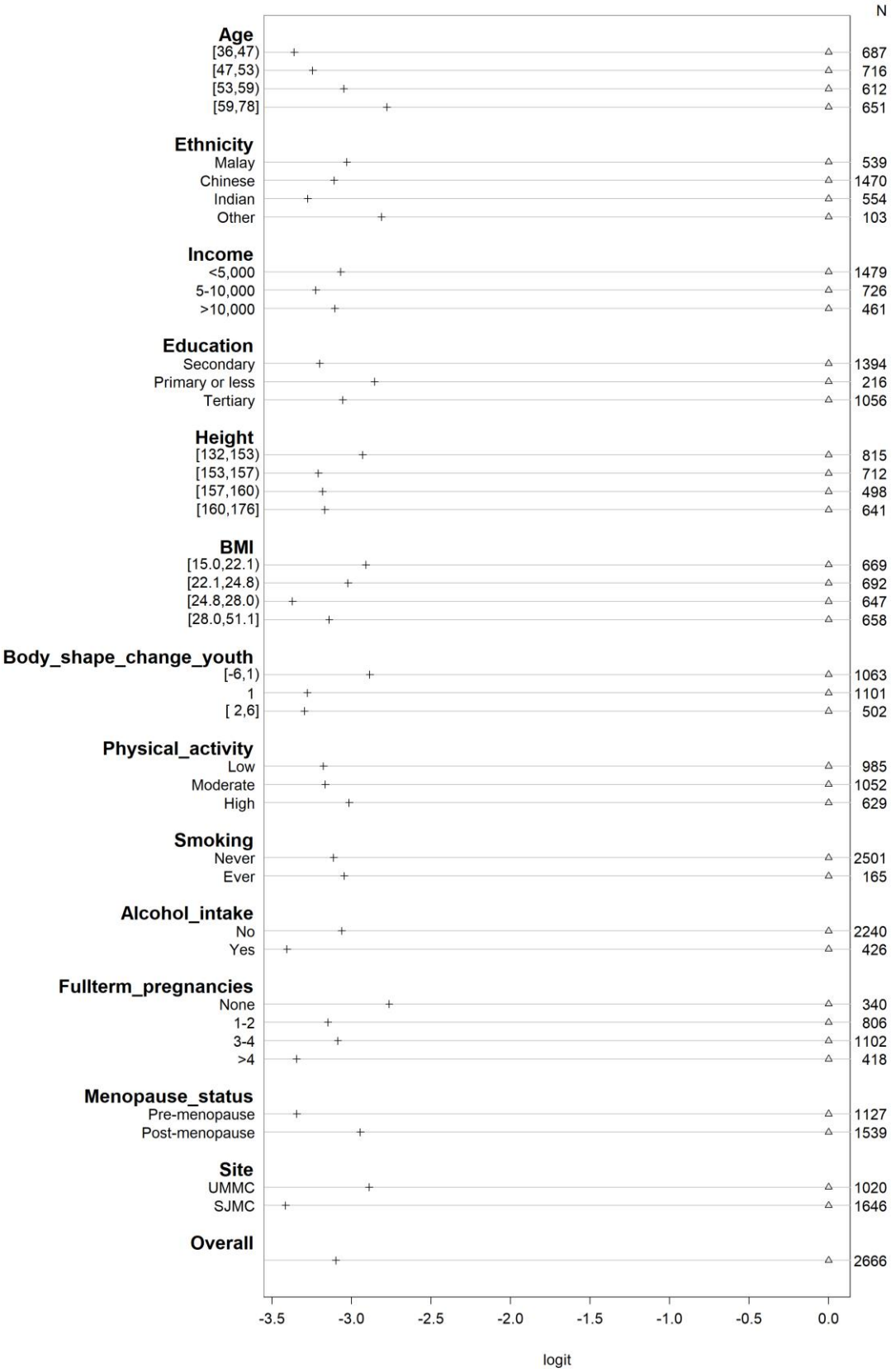


Figure 4-3 Plot of proportional odds assumption for the ordinal logistic regression model with frequency of soy intake as the outcome variable. The "Δ" sign refers to coefficient comparing non-consumers and regular/frequent consumers, and is normalized to zero. The "+" sign refers to the coefficient comparing non-/regular consumers vs frequent consumers. Equal distances between "+" and "Δ" indicates proportional odds.

The proportional odds assumption for the ordinal regression model was assessed in **Figure 4-3**. The difference in predicted values between models using varying thresholds of the outcome variable (i.e., non-consumers vs regular/frequent consumers and non-/regular consumers vs frequent consumers) were not very different across the categories of each variable for inclusion in the multivariable model, indicating that the proportional odds or parallel slopes assumption likely holds for these variables.

Multivariable ordinal logistic regression models showed that there were several independent predictors of frequent soy intake (**Table 4-2**). Indian women were more likely to have regular or frequent soy intake, compared to Chinese and Malay women (*p* value = 0.005). Interestingly, women who experienced an increase in body size between 7 and 18 years old were more likely to consume soy regularly as an adult (*p* value = 0.014). The analysis also showed that women with moderate or high levels of physical activity were more likely to consume soy regularly, compared to women with low physical activity (*p* value < 0.001, respectively).

Despite adjustment for various demographic and breast cancer risk factors in the regression model, there was still a strong significant association between frequency of soy intake and recruiting hospital. Women who were recruited from the SJMC site were more likely to report regular or frequent soy intake, compared to women recruited from the UMMC site (*p* value = 0.002).

There were no significant associations between the remaining variables with frequency of soy intake. While socio-economic variables, such as income and education, were significantly associated with frequency of soy intake in univariate analysis, the associations attenuated in multivariable analysis (*p* value > 0.05). Previously established determinants of mammographic density, such as age, BMI, parity factors, and menopausal status, were not independently associated with frequency of soy intake.

4.3.2. Distribution and determinants of volume-based mammographic density

Figure 4-4 shows the distribution of volume-based mammographic density measures in the study cohort. The median dense volume for this cohort was 56.9cm^3 (IQR = 37.1cm^3) and the median percent density was 8.8% (IQR = 7.9%).

Multivariable linear regression models were used to test for independent associations between mammographic density measures and variables of interest. As shown

in **Figure 4-5**, the assumptions for linearity and normality of residuals for the analysis of $\log_{10}$ volume-based mammographic density measures were met.

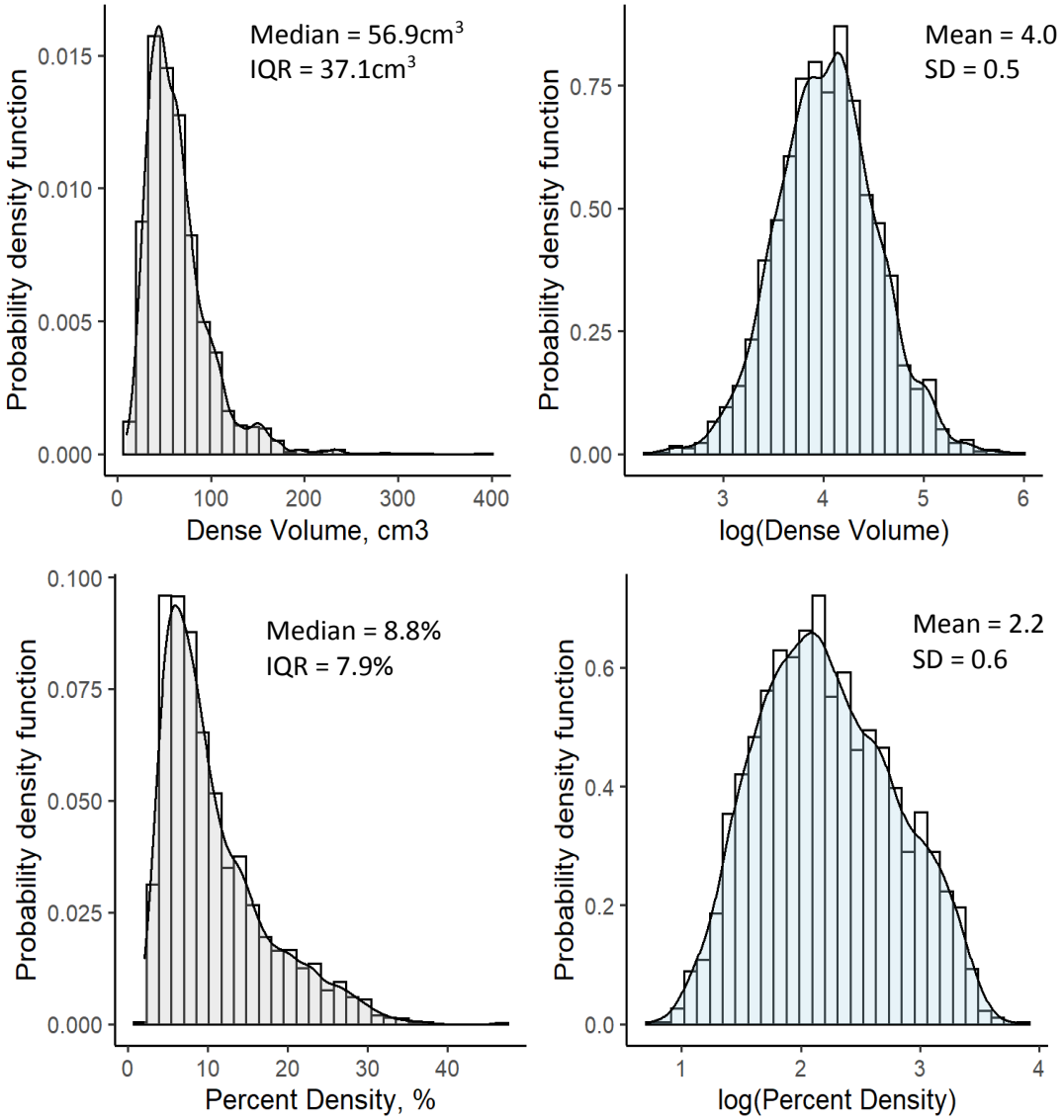
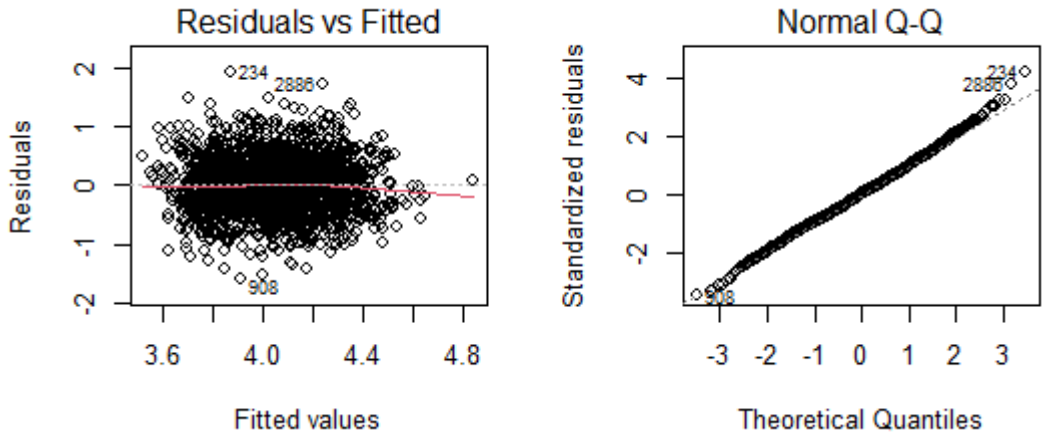


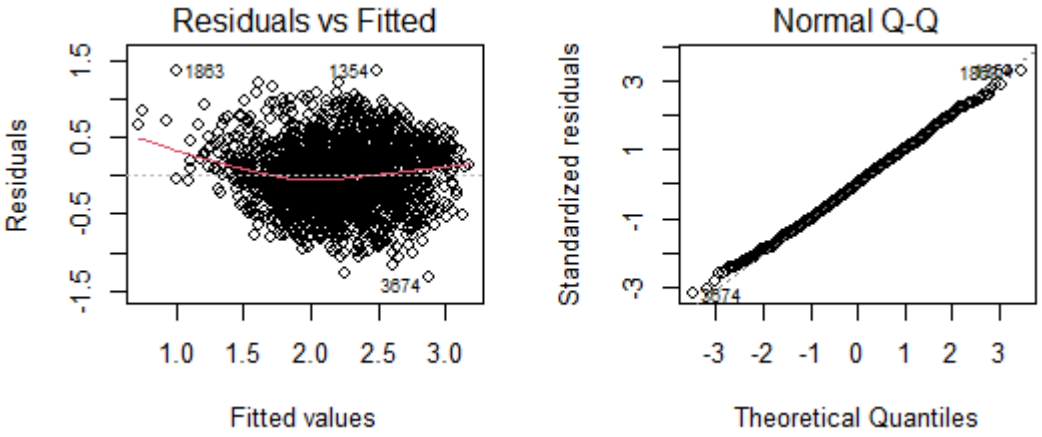
Figure 4-4 Distribution of volume-based mammographic density, in original and $\log_{10}$ scale.

In this study, volume-based mammographic density was independently associated with age, menopausal status, parity, and BMI (**Table 4-3**). Mammographic density was inversely associated with age, where dense volume was lower by 0.7cm^3 (95% CI = [-0.9, -0.5]) and percent density was lower by 0.1% (95% CI = [-0.2, -0.1]) for every unit increase in age. As expected, post-menopausal women had significantly lower dense volume and percent density compared to pre-menopausal women (by -7.5cm^3, 95% CI = [-10.5, -4.4] and -1.7%, 95% CI = [-2.0, -1.3], respectively). For every unit increase in BMI, dense volume was higher by 1.3cm^3 (95% CI = [0.9, 1.6]) while percent density was lower by 0.5% (95% CI = [-

0.5, -0.4]). Nulliparous women had 6.6cm^3 higher (95% CI = [3.0, 10.4]) dense volume and 0.8% higher (95% CI = [0.3, 1.3]) percent density compared to parous women. Older age at first full term pregnancy was also associated to higher mammographic density, by 0.4cm^3 (95% CI = [0.1, 0.7]) and 0.1% (95%CI = [0.0, 0.1]) for every year older.



(a) Dense volume



(b) Volume-based percent density

Figure 4-5 Assessment of linearity and normality of residuals in multivariable linear regression analysis for factors associated with (a) dense volume or (b) volume-based percent density. The assumption for linearity is met if the red line of the "Residuals vs Fitted" plot is approximately horizontal. The assumption for normality of residuals is met if there are no large deviations from the diagonal line in the "Normal Q-Q" plot.

There were also significant ethnic differences in mammographic density in this Asian cohort. Indian women had highest dense volume compared to Chinese and Malay women, by 6.2cm^3 (95% CI = [2.3, 10.4]). However, Chinese women had the highest percent density, by 1.6% (95% CI = [1.1, 2.2]), compared to Malay and Indian women.

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Table 4-3 The multivariable association between volume-based mammographic density and demographic, reproductive, and lifestyle risk factors (n=2,504)

Model variables	Multivariable associations with mammographic density			
	Dense volume (cm^3)		Percent density (%)	
	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Age	-0.7 [-0.9, -0.5]	<0.001**	-0.1 [-0.2, -0.1]	<0.001**
Ethnicity (Ref: Malay)				
Chinese	1.5 [-1.8, 5.1]	0.378	1.6 [1.1, 2.2]	<0.001**
Indian	6.2 [2.3, 10.4]	0.001*	0.0 [-0.5, 0.5]	0.863
Other	1.2 [-4.9, 8.0]	0.718	0.1 [-0.7, 1.1]	0.795
Income (Ref: <RM5,000)				
RM5-10,000	-1.0 [-3.9, 2.0]	0.487	0.2 [-0.2, 0.7]	0.275
>RM10,000	-3.1 [-6.6, 0.6]	*0.099*	-0.3 [-0.8, 0.2]	0.243
Education (Ref: Secondary)				
Primary/less	-4.3 [-8.1, -0.3]	0.035*	-0.2 [-0.7, 0.4]	0.599
Tertiary	1.4 [-1.4, 4.4]	0.323	0.4 [0.0, 0.8]	*0.081*
Height (per 10 cm)	0.5 [-1.7, 2.8]	0.672	-0.3 [-0.6, 0.0]	0.046*
BMI (kg/m2)	1.3 [0.9, 1.6]	<0.001**	-0.5 [-0.5, -0.4]	<0.001**
Body shape change in youth	0.2 [-1.0, 1.4]	0.793	-0.1 [-0.2, 0.1]	0.322
Body shape change since youth	0.5 [-0.5, 1.5]	0.321	0.0 [-0.1, 0.2]	0.718
Physical Activity (Ref: Low)				
Moderate	-0.6 [-3.1, 2.1]	0.670	-0.1 [-0.4, 0.3]	0.786
High	-0.6 [-3.6, 2.6]	0.721	0.0 [-0.5, 0.4]	0.875
Ever smoked	2.2 [-3.2, 8.3]	0.435	-0.3 [-1.0, 0.5]	0.404
Regular alcohol intake	2.4 [-1.1, 6.2]	0.186	0.1 [-0.4, 0.6]	0.664
Nulliparity	6.6 [3.0, 10.4]	<0.001**	0.8 [0.3, 1.3]	0.001*
Age at first pregnancy†	0.4 [0.1, 0.7]	0.010	0.1 [0.0, 0.1]	0.012*
Oral contraceptive use	-0.2 [-2.7, 2.5]	0.879	0.3 [-0.1, 0.7	0.150
Post-menopause (Ref: Pre-menopause)	-7.5 [-10.5, -4.4]	<0.001**	-1.7 [-2.0, -1.3]	<0.001**
Age at menopause‡	0.1 [-0.3, 0.5]	0.650	0.0 [0.0, 0.1]	0.435
HRT use‡ (Ref: Never)				
Ever	-1.1 [-5.2, 3.4]	0.630	-0.3 [-0.9, 0.3]	0.349
Current	1.8 [-5.9, 10.5]	0.667	0.4 [-0.7, 1.7]	0.514
SJMC (Ref: UMMC)	-1.0 [-3.5, 1.7]	0.465	0.2 [-0.1, 0.6]	0.233

All variables listed in the table were included in multivariable analysis. The models were also adjusted for type of mammography system. Variables that were not included: (1) not significant in univariate analysis at *p* value > 0.100 (age at menarche, family history of breast cancer), (2) high collinearity with variables in the model (number of pregnancies, weight). *Annotations:* ***p* value < 0.001, **p* value < 0.05, †among parous women, ‡among post-menopausal women, n = number; % = proportion over total in group, Δ = Mean-centered difference in mammographic density, Ref = Reference category.

There were interesting observations for height and education level. An inverse association was observed between height and percent density, but the association was small and only marginally significant (p value = 0.046). Also, women who did not complete formal schooling had significantly lower dense volume, by 4.3cm3 (95%CI = [-8.1, -0.3]), compared to women who completed at least secondary education

4.3.3. Association between volume-based mammographic density and frequency of soy intake

Absolute dense volume was inversely association with soy intake, but these associations were not statistically significant in multivariable models and in stratified analysis by menopausal status (**Table 4-4**). Women with frequent soy intake had 2.1cm^3 lower (95% CI = [-5.5, 1.6]) dense volume compared to non-consumers.

This association appears to be marginally stronger among post-menopausal women, where the difference in dense volume was 2.5cm3 (95% CI = [-6.5, 2.0]) for frequent consumers compared to non-consumers. A significant positive association was observed between percent density and total soy intake in the unadjusted model, but the association attenuated in the multivariable model. Also, there were no significant interactions between BMI and frequency of soy intake on volume-based mammographic density measures.

Table 4-4 Multivariable analysis for volume-b*a*sed mammographic density and soy intake (n=2,504)

Frequency of soy intake	n	Multivariable associations with mammographic density					
		Dense volume (cm^3)			Percent density (%)		
		Δ [95% CI]	*p* value	p_{BMI}	Δ [95% CI]	*p* value	p_{BMI}
(a) Unadjusted model							
Non-consumer	619	Ref			Ref		
Regular	1,572	1.7 [-0.9, 4.4]	0.200		0.7 [0.3, 1.2]	0.002*	
Frequent	313	-1.0 [-4.6, 2.8]	0.601		1.0 [0.3, 1.8]	0.003*	
(b) Full model†							
Non-consumer	572	Ref		Ref	Ref		Ref
Regular	1,482	-0.5 [-2.9, 2.1]	0.715	0.697	0.0 [-0.4, 0.3]	0.846	0.537
Frequent	296	-2.1 [-5.5, 1.6]	0.263	0.732	0.1 [-0.4, 0.6]	0.842	0.833
(c) Pre-menopause‡							
Non-consumer	171	Ref		Ref	Ref		Ref
Regular	622	1.2 [-3.4, 6.2]	0.629	0.418	0.1 [-0.5, 0.8]	0.722	0.741
Frequent	118	-1.4 [-7.4, 5.2]	0.660	0.383	-0.2 [-1.0, 0.7]	0.678	0.376
(d) Post-menopause‡							
Non-consumer	401	Ref		Ref	Ref		Ref
Regular	860	-1.2 [-4.1, 1.8]	0.415	0.692	-0.1 [-0.5, 0.3]	0.563	0.938
Frequent	178	-2.5 [-6.5, 2.0]	0.268	0.552	0.2 [-0.5, 0.8]	0.625	0.844

Annotations: ***p* value < 0.001, **p* value < 0.05, n = number, Δ = Mean-centered difference in mammographic density, Ref = Reference category, †linear regression models adjusted for age, BMI, ethnicity, menopausal status, nulliparity, type of mammogram machine and recruitment site; ‡linear regression models adjusted for all variables included in full model, except menopausal status, p_{BMI} = *p* value for the interaction term between frequency of soy intake and BMI.

4.3.4. Distribution and determinants of area-based mammographic density

The distribution of area-based mammographic density measures is shown in **Figure 4-6**. The median dense area was 20.4cm^2 (IQR = 26.8cm^2), while the median area-based percent density was 17.4% (IQR = 25.4%).

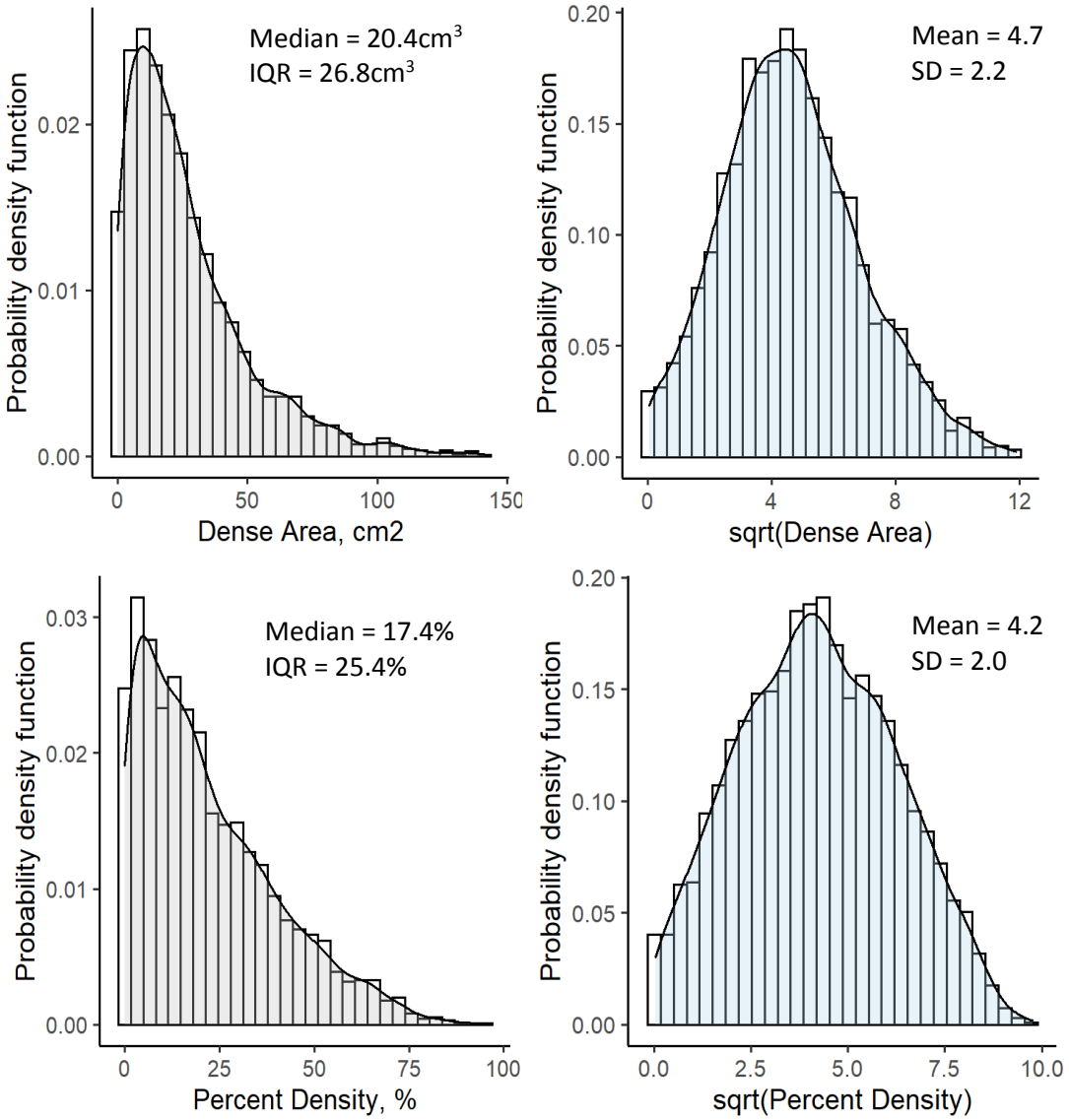
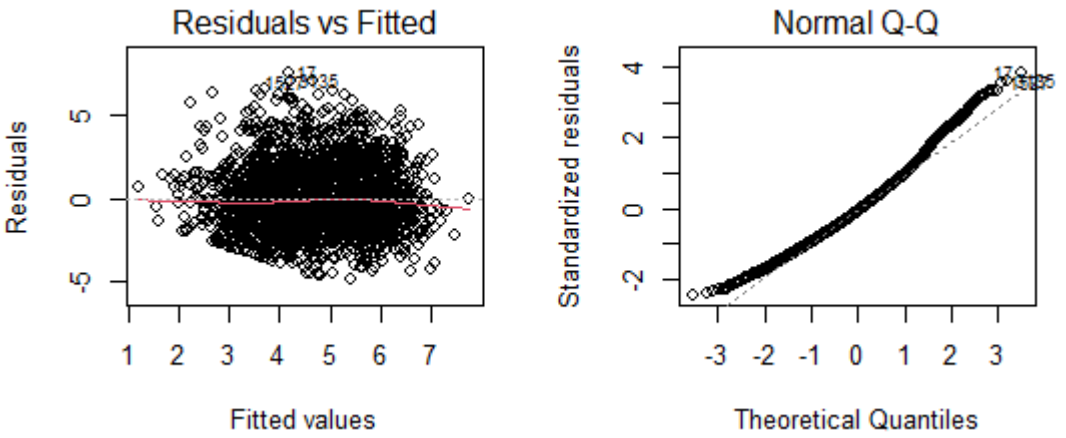
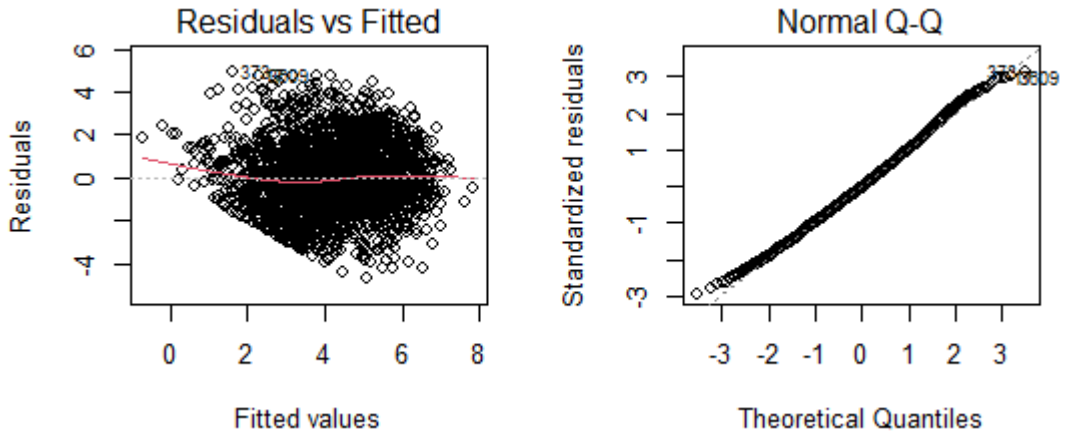


Figure 4-6 Distribution of area-based mammographic density, in original and square-root (sqrt) scale.

Multivariable linear regression models were used to test for independent associations between mammographic density measures and variables of interest. As shown in **Figure 4-7**, the assumptions for linearity and normality of residuals for the analysis of square-rooted area-based mammographic density measures were met.



(a) Dense area



(b) Area-based percent density

Figure 4-7 Assessment of linearity and normality of residuals in multivariable linear regression analysis for factors associated with (a) dense area or (b) area-based percent density. The assumption for linearity is met if the red line of the "Residuals vs Fitted" plot is approximately horizontal. The assumption for normality of residuals is met if there are no large deviations from the diagonal line in the "Normal Q-Q" plot.

Area-based mammographic density measures were associated with age, menopausal status, BMI, and parity, with similar associations for absolute and relative measures of mammographic density (**Table 4-5**). Dense area was lower by 0.6cm^2 (95% CI = [-0.8, -0.5]) and percent density was lower by 0.6% (95% CI = [-0.7, -0.5]) for every year increase in age. Correspondingly, women who were post-menopause at the time of mammography had on average 6.3cm^2 lower dense area (95% CI = [-8.1, -4.4]) and 5.5% lower percent density (95% CI = [-6.9, -4.2]) compared to women who were pre-menopausal. BMI was inversely associated with both dense area (-1.1 cm^2 (95% CI = [-1.3, -0.9]) and percent density (-1.6% (95% CI = [-1.7, -1.4]).

Table 4-5 The association between area-based mammographic density and demographic, reproductive, and lifestyle risk factors (n=2,901)

Model variables	Multivariable associations with mammographic density			
	Dense area (cm^2)		Percent density (%)	
	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Age	-0.6 [-0.8, -0.5]	<0.001**	-0.6 [-0.7, -0.5]	<0.001**
Ethnicity (Ref: Malay)				
Chinese	2.4 [0.1, 4.8]	0.038*	4.0 [2.2, 5.8]	<0.001**
Indian	1.9 [-0.6, 4.5]	0.139	0.3 [-1.5, 2.2]	0.723
Other	-0.3 [-4.3, 4.0]	0.869	-0.3 [-3.2, 2.9]	0.872
Income (Ref: <RM5,000)				
RM5-10,000	-1.1 [-2.9, 0.7]	0.227	-0.5 [-1.8, 0.9]	0.489
>RM10,000	-1.6 [-3.8, 0.6]	0.148	-1.2 [-2.8, 0.4]	0.148
Education (Ref: Secondary)				
Primary/less	-0.8 [-3.5, 2.1]	0.563	-0.3 [-2.3, 1.9]	0.805
Tertiary	2.0 [0.3, 3.9]	0.025*	1.6 [0.2, 2.9]	0.020*
Height (per 10cm)	-1.5 [-2.8, -0.2]	0.027*	-3.2 [-4.8, -1.4]	0.001*
BMI (kg/m2)	-1.1 [-1.3, -0.9]	<0.001**	-1.6 [-1.7, -1.4]	<0.001**
Body shape change in youth	-0.2 [-1.0, 0.5]	0.514	-0.5 [-1.0, 0.0]	*0.064*
Body shape change since youth	0.2 [-0.5, 0.8]	0.621	0.0 [-0.5, 0.4]	0.923
Physical Activity (Ref: Low)				
Moderate	-0.5 [-2.2, 1.2]	0.551	-0.5 [-1.7, 0.8]	0.466
High	-1.5 [-3.4, 0.4]	0.115	-0.8 [-2.2, 0.6]	0.262
Ever smoked	1.2 [-1.8, 4.5]	0.426	-0.2 [-2.3, 2.1]	0.877
Regular alcohol intake	1.3 [-0.7, 3.5]	0.207	1.2 [-0.3, 2.8]	0.122
Nulliparity	3.2 [1.0, 5.6]	0.005*	2.9 [1.2, 4.6]	0.001*
Age at first pregnancy†	0.2 [0.0, 0.4]	0.019*	0.2 [0.1, 0.3]	0.003*
Oral contraceptive use	-0.7 [-2.2, 1.0]	0.420	-0.1 [-1.3, 1.1]	0.892
Post-menopause (Ref: Pre-menopause)	-6.3 [-8.1, -4.4]	<0.001**	-5.6 [-6.9, -4.2]	<0.001**
Age at menopause‡	0.0 [-0.3, 0.3]	0.958	0.0 [-0.2, 0.2]	0.957
HRT use‡ (Ref: Never)				
Ever	-1.6 [-4.4, 1.4]	0.286	-1.4 [-3.6, 0.8]	0.207
Current	-1.9 [-6.5, 3.4]	0.459	-2.1 [-5.6, 1.9]	0.284
SJMC (Ref: UMMC)	-4.5 [-6.0, -2.9]	<0.001**	-3.6 [-4.7, -2.4]	<0.001**

All variables listed in the table were included in multivariable analysis. The models were also adjusted for type of mammography system. Variables that were not included: (1) not significant in univariate analysis (p>0.100) = age at menarche, family history of breast cancer, (2) high collinearity with variables in the model = number of pregnancies, weight. *Annotations*: ***p* value < 0.001, **p* value < 0.05, †among parous women, ‡among post-menopausal women, n = number; % = proportion over total in group, Δ = Mean-centered difference in mammographic density, Ref = Reference category.

Women who were nulliparous had higher dense area (by 3.2cm^2, 95% CI = [1.0, 5.6]) and percent density (by 2.9%, 95% CI = [1.2, 4.6]) compared to parous women, and mammographic density increased with increasing age at first full term pregnancy (by 0.2cm^2, 95% CI = [0.0, 0.4] and by 0.2%, 95% CI = [0.1, 0.3]). Also, Chinese women were observed to have the highest dense area (by 2.4cm^2, 95% CI = [0.1, 4.8]) and percent density (by 4.0%, 95% CI = [2.2, 5.8]) compared to the other Asian ethnicities. Interestingly, women with tertiary education had significantly higher dense area and percent density, compared to women with secondary education or lower, by 2.0cm^2 (95% CI = [0.3, 3.9]) and 1.6% (95% CI = [0.2, 2.9]), respectively.

Similar to volume-based analysis, there was a statistically significant difference in distribution of area-based mammographic density measures by recruiting hospital (*p* value < 0.001 respectively), despite adjustment for various socio-demographic factors. Also, height was inversely associated with area-based mammographic density, where for every 10cm increase in height, dense area decreased by 1.5cm^2 (95% CI = [-2.8, -0.2]) while percent density decreased by 3.2% (95% CI = [-4.8, -1.4]).

4.3.5. Association between area-based mammographic density and frequency of soy intake

As shown in **Table 4-6**, there were no significant associations between area-based mammographic density measures and frequency of soy intake. In fully adjusted models, women with frequent soy intake had lower dense area compared to regular and non-consumers, by 0.5cm^2 (95% CI = [-2.7, 1.9]). There were no associations for area-based percent density.

The inverse association between area-based mammographic density and frequency of soy intake was more strongly observed among pre-menopausal women, where frequent consumers observed 2.0cm^2 lower dense area (95% CI = [-5.4, 1.9]) and 1.5% lower percent density (95% CI = [-3.9, 1.2]), compared to non-consumers. Among post-menopausal women, there were small, non-significant positive associations observed for both dense area and area-based percent density.

Table 4-6 The association between area-based mammographic density and total soy intake (n=2,901)

Frequency of soy intake	n	Multivariable associations with mammographic density					
		Dense area (cm^2)			Percent density (%)		
		Δ [95% CI]	*p* value	p_{BMI}	Δ [95% CI]	*p* value	p_{BMI}
(e) Unadjusted model							
Non-consumer	595	Ref			Ref		
Regular	1,896	1.2 [-0.6, 3.1]	0.202		1.3 (-0.2, 2.9]	*0.092*	
Frequent	410	-0.1 [-2.5, 2.4]	0.918		0.9 (-1.2, 3.0]	0.417	
(f) Full model†							
Non-consumer	545	Ref		Ref	Ref		Ref
Regular	1,793	0.2 [-1.5, 2.0]	0.800	0.103	0.1 (-1.1. 1.4]	0.826	0.378
Frequent	392	-0.5 [-2.7, 1.9]	0.676	0.423	-0.1 (-1.7, 1.7]	0.943	0.934
(g) Pre-menopause‡							
Non-consumer	186	Ref		Ref	Ref		Ref
Regular	800	0.0 [-2.8, 3.1]	0.982	0.029*	0.2 (-1.9, 2.3]	0.882	0.201
Frequent	170	-2.0 [-5.4, 1.9]	0.305	0.810	-1.5 (-3.9, 1.2]	0.269	0.455
(h) Post-menopause‡							
Non-consumer	359	Ref		Ref	Ref		Ref
Regular	993	0.2 [-1.9, 2.4]	0.834	0.743	0.0 (-1.5, 1.7]	0.975	0.941
Frequent	222	0.6 [-2.3, 3.7]	0.698	0.230	1.0 (-1.2, 3.3]	0.398	0.351

Annotations: ***p* value < 0.001, **p* value < 0.05, n = number, Δ = Mean-centered difference in mammographic density, Ref = Reference category, †linear regression models adjusted for age, BMI, ethnicity, menopausal status, nulliparity, type of mammogram machine and recruitment site; ‡linear regression models adjusted for all variables included in full model, except menopausal status, p_{BMI} = *p* value for the interaction term between frequency of soy intake and BMI.

Of note is the significant interaction between regular soy intake and BMI on dense area among pre-menopausal women (*p* value = 0.029), which is illustrated in **Figure 4-8**. Among lean pre-menopausal women, regular soy intake was associated with higher dense area. Among overweight or obese women, however, regular soy intake was associated with lower dense area. Importantly, premenopausal women with frequent soy intake had consistently lower dense area, compared to non-consumers, across the BMI scale. From **Figure 4-8**, it appears that there was an interaction between frequent soy intake and BMI on dense area among post-menopausal women, but the effect was small and not statistically significant

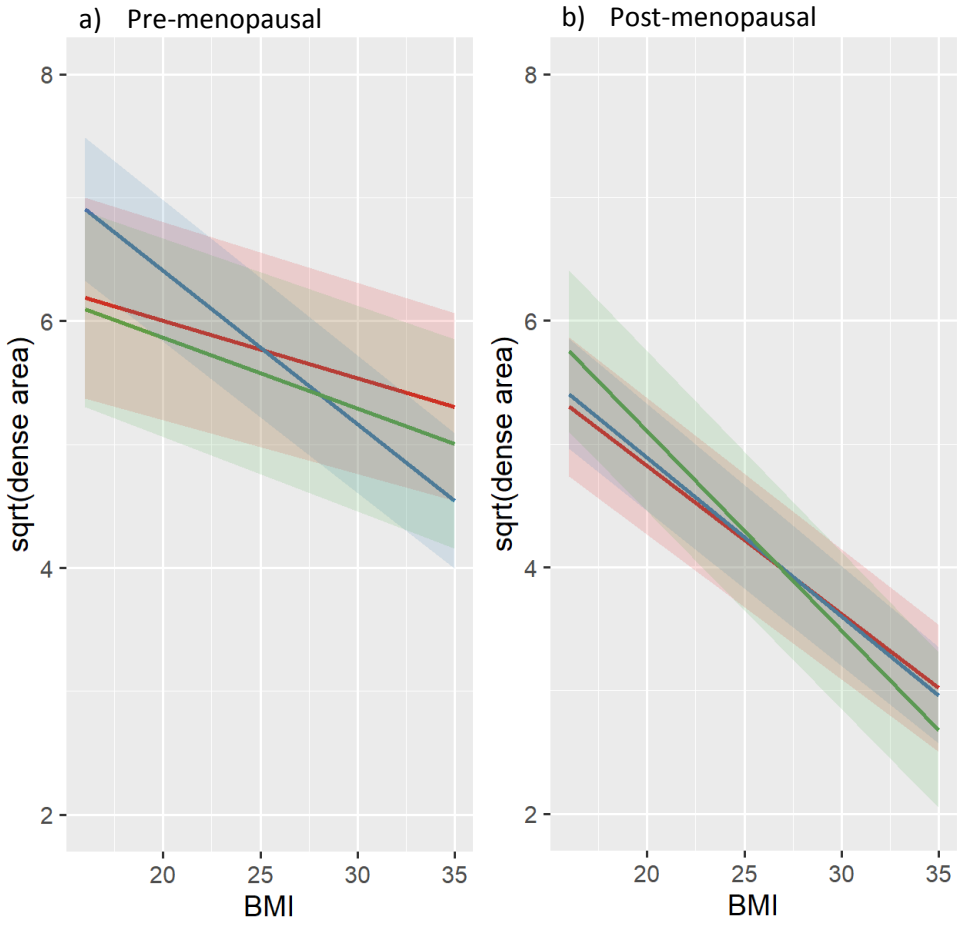


Figure 4-8 Interaction between total soy intake and BMI on area-based mammographic density measures among (a) pre-menopausal women and (b) post-menopausal women. Annotations: red line = non-consumer, blue line = regular consumers, and green line = frequent consumer.

Table 4-7 The multivariable association between mammographic density and demographic, reproductive, and lifestyle risk factors for the subset where both volume-based and area-based measures are available (n=2,504)

	Multivariable associations with mammographic density							
	Volume-based				**Area-based**			
	Dense volume (cm^3)		**Percent density (%)**		**Dense area (cm^2)**		**Percent density (%)**	
Model variables	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Age	-0.7 [-0.9, -0.5]	<0.001**	-0.1 [-0.2, -0.1]	<0.001**	-0.7 [-0.8, -0.5]	<0.001**	-0.6 [-0.7, -0.5]	<0.001**
Ethnicity (Ref: Malay)	Ref		Ref		Ref		Ref	
Chinese	2.3 [-1.2, 6.0]	0.204	1.7 [1.1, 2.3]	<0.001**	2.8 [0.3, 5.6]	0.029*	4.3 [2.4, 6.4]	<0.001**
Indian	5.2 [1.2, 9.4]	0.011*	-0.1 [-0.7, 0.4]	0.631	1.9 [-0.9, 4.8]	0.186	0.6 [-1.4, 2.6]	0.582
Other	0.6 [-5.7, 7.6]	0.859	-0.2 [-0.8, 1.2]	0.770	-1.1 [-5.4, 3.8]	0.644	-0.7 [-3.9, 2.8]	0.678
Income (Ref: <RM5,000)	Ref		Ref		Ref		Ref	
RM5-10,000	-1.3 [-4.2, 1.7]	0.385	0.3 [-0.2, 0.7]	0.269	-0.9 [-2.9, 1.3]	0.409	-0.1 [-1.6, 1.5]	0.902
>RM10,000	-2.6 [-6.1, 1.1]	0.164	-0.2 [-0.8, 0.3]	0.393	-1.4 [-3.9, 1.4]	0.320	-0.7 [-2.5, 1.3]	0.510
Education (Ref: Secondary)	Ref		Ref		Ref		Ref	
Primary/less	-3.8 [-7.7, 0.4]	*0.078*	0.0 [-0.7, 0.6]	0.885	-2.1 [-4.9, 1.0]	0.181	-1.1 [-3.3, 1.1]	0.316
Tertiary	1.2 [-1.6, 4.2]	0.402	0.3 [-0.1, 0.8]	0.142	1.5 [-0.6, 3.6]	0.171	1.1 [-0.4, 2.7]	0.138
Height (per 10 cm)	0.4 [-1.8, 2.8]	0.709	-0.3 [-0.6, 0.0]	0.045*	-1.2 [-2.8, 0.4]	0.136	-2.7 [-4.5, -0.5]	0.018*
BMI (kg/m2)	1.2 [0.9, 1.6]	<0.001**	-0.5 [-0.5, -0.5]	<0.001**	-1.1 [-1.3, -0.9]	<0.001**	-1.5 [-1.7, -1.4]	<0.001**
Body shape change in youth	0.4 [-0.8, 1.7]	0.508	-0.1 [-0.2, 0.1]	0.566	0.2 [-0.6, 1.1]	0.613	-0.1 [-0.7, 0.6]	0.807
Body shape change since youth	0.9 [-0.1, 1.9]	*0.094*	0.0 [-0.4, 0.5]	0.380	0.2 [-0.5, 0.9]	0.597	0.1 [-0.4, 0.7]	0.592

All variables listed in the table were included in multivariable analysis. The models were also adjusted for type of mammography system. Variables that were not included: (1) not significant in univariate analysis at *p* value > 0.100 (age at menarche, family history of breast cancer), (2) high collinearity with variables in the model (number of pregnancies, weight). *Annotations:* ***p* value < 0.001, **p* value < 0.05, †among parous women, ‡among post-menopausal women, n = number; % = proportion over total in group, Δ = Mean-centered difference in mammographic density, Ref = Reference category.

Table 4-7 (cont'd) The multivariable association between mammographic density and demographic, reproductive, and lifestyle risk factors for the subset where both volume-based and area-based measures are available (n=2,504)

	Multivariable associations with mammographic density							
	Volume-based				**Area-based**			
	Dense volume (cm^3)		**Percent density (%)**		**Dense area (cm^2)**		**Percent density (%)**	
Model variables	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value	Δ [95% CI]	*p* value
Physical Activity (Ref: Low)	Ref		Ref		Ref		Ref	
Moderate	-1.0 [-3.6, 1.7]	0.451	-0.1 [-0.5, 0.3]	0.504	-0.5 [-2.4, 1.4]	0.592	-0.5 [-1.8, 0.9]	0.510
High	-0.2 [-3.3, 3.0]	0.899	0.0 [-0.4, 0.5]	0.964	-1.5 [-3.6, 0.8]	0.191	-0.9 [-2.5, 0.7]	0.277
Ever smoked	3.5 [-2.0, 9.4]	0.221	-0.4 [-1.1, 0.4]	0.319	1.5 [-2.3, 5.6]	0.466	-0.6 [-3.3, 2.2]	0.656
Regular alcohol intake	2.3 [-1.2, 6.1]	0.197	0.1 [-0.4, 0.6]	0.679	1.4 [-1.1, 4.1]	0.269	1.1 [-0.7, 3.1]	0.236
Nulliparity	7.2 [3.5, 11.1]	<0.001**	0.9 [0.3, 1.4]	0.001*	3.5 [1.0, 6.2]	0.006*	3.2 [1.3, 5.1]	0.001*
Age at first pregnancy[†]	0.4 [0.1, 0.7]	0.006*	0.0 [0.0, 0.1]	0.026*	0.3 [0.1, 0.5]	0.004*	0.3 [0.1, 0.4]	0.001*
Oral contraceptive use	-0.7 [-3.2, 2.0]	0.621	0.3 [-0.1, 0.7]	0.185	0.0 [-1.9, 2.0]	0.993	0.5 [-0.9, 1.9]	0.525
Post-menopause (Ref: Pre-menopause)	-6.3 [-9.4, -3.0]	<0.001**	-1.7 [-2.1, -1.3]	<0.001**	-5.9 [-8.0, -3.7]	<0.001**	-5.1 [-6.6, -3.4]	<0.001**
Age at menopause[‡]	0.1 [-0.3, 0.5]	0.679	0.0 [0.0, 0.1]	0.443	0.0 [-0.3, 0.3]	0.883	0.0 [-0.2, 0.2]	0.952
HRT use[‡] (Ref: Never)	Ref		Ref		Ref		Ref	
Ever	-0.3 [-4.7, 4.4]	0.882	-0.2 [-0.9, 0.5]	0.487	-1.7 [-4.8, 1.6]	0.293	-1.6 [-3.9, 0.9]	0.196
Current	0.5 [-7.1, 9.5]	0.896	0.0 [-1.2, 1.3]	0.965	-0.1 [-5.6, 6.3]	0.980	-0.6 [-4.7, 4.2]	0.793
SJMC (Ref: UMMC)	1.8 [-0.9, 4.6]	0.203	0.5 [0.1, 0.9]	0.018*	-5.4 [-7.0, -3.7]	<0.001**	-4.3 [-5.5, -3.0]	<0.001**

Table 4-8 Multivariable analysis for mammographic density and soy intake for the subset where both volume-based and area-based measures are available (n=2,504)

Frequency of soy intake	n	Multivariable associations with mammographic density							
		Volume-based				Area-based			
		Dense volume (cm^3)		Percent density (%)		Dense area (cm^2)		Percent density (%)	
		Δ [95% CI]	p_{arm}	Δ [95% CI]	p_{arm}	Δ [95% CI]	p_{arm}	Δ [95% CI]	p_{arm}
a) *Unadjusted model*									
Non-consumer	481	Ref		Ref		Ref		Ref	
Regular	1375	4.2 [1.4, 7.1]	0.003*	1.1 [0.6, 1.7]	<0.001**	2.0 [-0.1, 4.2]	*0.058*	2.1 [0.4, 3.9]	0.014*
Frequent	272	2.0 [-1.8, 6.0]	0.311	1.3 [0.5, 2.1]	0.001*	0.3 [-2.5, 3.3]	0.852	1.2 [-1.1, 3.7]	0.312
b) *Full model*†									
Non-consumer	444	Ref		Ref		Ref		Ref	
Regular	1302	1.5 [-1.0, 4.2]	0.247	0.2 [-0.2, 0.5]	0.413	0.2 [-1.7, 2.2]	0.828	0.2 [-1.1, 1.6]	0.793
Frequent	257	0.3 [-3.2, 4.1]	0.867	0.3 [-0.2, 0.9]	0.294	-0.4 [-3.0, 2.4]	0.782	0.1 [-1.8, 2.0]	0.944
c) *Pre-menopause*‡									
Non-consumer	132	Ref		Ref		Ref		Ref	
Regular	556	3.1 [-1.7, 8.5]	0.210	0.3 [-0.4, 1.0]	0.370	0.4 [-3.0, 4.0]	0.830	0.1 [-2.1, 2.5]	0.914
Frequent	103	-0.7 [-6.6, 6.1]	0.837	-0.2 [-1.0, 0.7]	0.696	-1.4 [-5.6, 3.4]	0.551	-1.0 [-3.9, 2.1]	0.507
d) *Post-menopause*‡									
Non-consumer	312	Ref		Ref		Ref		Ref	
Regular	746	0.7 [-2.3, 3.8]	0.654	0.0 [-0.4, 0.5]	0.835	-0.1 [-2.3, 2.2]	0.913	0.0 [-1.6, 1.7]	0.966
Frequent	154	0.9 [-3.3, 5.5]	0.689	0.7 [-0.1, 1.3]	0.102	0.2 [-3.0, 3.7]	0.887	0.7 [-1.7, 3.2]	0.589

Annotations: **p value < 0.001, *p value < 0.05, n = number, Δ = Mean-centered difference in mammographic density, Ref = Reference category, †linear regression models adjusted for age, BMI, ethnicity, menopausal status, nulliparity, type of mammogram machine and recruitment site; ‡linear regression models adjusted for all variables included in full model, except menopausal status.

4.3.6. Comparison of results between volume-based and area-based mammographic density measures, using a subset of the sample where both measures are available

In Tables 4-3 to 4-6, there were some observed differences between volume-based and area-based analyses, which could be attributed to the differences in the samples analysed or to the use of different algorithms to measure mammographic density. Therefore, multivariable linear regression analyses were performed on a subset of the sample where both volume-based and area-based mammographic density measures were available (n=2,128).

In Table 4-7, the associations between mammographic density with demographic, reproductive, and lifestyle factors were similar for volume-based and area-based measures. The only exception to this was for BMI, where increased BMI is associated with significantly higher dense volume but significantly lower dense area. When studying the association between mammographic density and soy intake (Table 4-8), similar associations were observed for volume-based and area-based mammographic density measures.

4.4. Discussion

4.4.1. Summary of main findings

In this cross-sectional study of multi-ethnic Asian women, 63.9% of women reported regular soy intake (defined as consuming soymilk or soy food more than once a month) while 13.8% of women reported frequent soy consumption (defined as consuming soymilk or soy food more than once a week). Here, non-significant inverse associations were observed between mammographic density measures and frequency of soy intake. The associations observed for volume-based and area-based measures were of similar magnitude and direction. Notably, among pre-menopausal women, a statistically significant interaction was observed for regular soy intake and BMI on absolute dense area. No significant interaction was observed for post-menopausal women nor for volume-based mammographic density measures.

4.4.2. Interaction between soy and BMI on area-based mammographic density

In this study, regular soy intake was associated with higher mammographic density among lean women, but among overweight or obese women, an inverse association was observed. This was observed for all women, but the effect was larger and statistically

significant among younger women. Previous studies reporting the association between mammographic density measures and soy intake have consistently incorporated BMI into the analytical model as an important confounding variable (108,119–121), but there has not been an investigation into the effect of BMI on the association between mammographic density and soy intake. The findings in this chapter are consistent with previous reports from population-based studies in China and Singapore, where the protective effect of soy on breast cancer risk was limited to overweight or obese women (102,152). Obesity is correlated to higher adiposity, and adipose tissues have been shown to serve as a reservoir for endogenous oestrogen (155). Isoflavones, which are structurally and functionally similarly to endogenous oestrogen, which directly and indirectly influence adipose tissue distribution (156). However, more research is required to understand the biological mechanism of soy isoflavone intake on adiposity in post-menopausal women, and its' effect on breast cancer risk.

This analysis also suggests that a soy intervention may not benefit all women, and may even increase breast cancer risk among younger, leaner women. This is in contrast to the findings from the China Kadoori Bank, which reported a significant inverse association between soy intake and breast cancer risk even among women with BMI less than 24kg/m^2 (70). Currently, there is insufficient evidence to draw a conclusion about the effect of soy on breast cancer risk among young, lean women.

4.4.3. Non-significant inverse associations between soy intake and MD

In this study, frequent soy consumers had lower absolute mammographic density measures, but these associations were not statistically significant. The associations with percent density measures were weaker or null. In many of the studies of East Asian women, consistent inverse associations were reported (119,120), where high soy intake was associated to 4-5% lower percent density compared to low consumers, an effect the authors likened to menopause (121). Only one study of predominantly pre-menopausal Japanese women show no significant association between percent density and soy intake, even though the average soy isoflavone intake in this cohort was high, between 42-57mg/day (122).

There is some suggestion that the inverse association between soy intake and mammographic density may be limited to post-menopausal Asian women (120). In this chapter, stronger inverse associations were noted for post-menopausal women when using

volume-based mammographic density measures. However, strong inverse associations were observed for pre-menopausal women in area-based analysis. It is possible that volume-based measures are not suitable biomarkers of breast cancer risk among pre-menopausal Asian women, as demonstrated in **Chapter 3.** The findings here underscore the importance of assessing the ability of biomarkers to accurately capture risk in the target population prior to use in research or in clinical settings.

4.4.4. Association between mammographic density and other variables

Two interesting observations were noted in this study. Firstly, the data suggests that taller Asian women have lower mammographic density, and the association was statistically significant for both volume-based and area-based analysis. There is strong evidence that increased height is correlated to higher breast cancer risk (13). Correspondingly, height has been shown to be positively, albeit weakly, correlated to mammographic density in Caucasian and Asian populations (157–159). Only one other study of mammographic density among a small sample of women with a strong family history of breast cancer showed an inverse association between height and mammographic density measures (31). Therefore, it is possible that the findings observed here are entirely due to chance. However, given the consistency across two methods of mammographic density measures, the association between height and mammographic density in Asian populations warrants further investigation.

The second interesting observation was the significant positive association between mammographic density and education. The findings were consistent for both volume-based and area-based analysis, and was also observed in **Chapter 3**. By contrast, a study of Korean women showed an inverse association between education level and mammographic density, particularly for non-dense measures (160). On the other hand, a study of American women showed that early life socioeconomic factors, including education, had no effect on mammographic density later in life (161). One possible explanation for the positive association observed in this chapter is that higher education levels likely reflects the "Westernization" of habits and a greater accumulation of risk factors (162). Importantly, this suggests that there are key risk factors that are unmeasured in this analysis. Identifying these risk factors remain a priority for understanding the association between mammographic density and breast cancer risk among Asian women.

4.4.5. Strengths

This is the first study to investigate the effect of BMI on the association between soy intake and mammographic density as a biomarker of risk. This relatively large study enabled stratified analysis by menopausal status. Furthermore, the inclusion of two types of mammographic density measurements provided a measure of reproducibility of results. The inverse associations observed here, though not statistically significant, support the hypothesis that regular soy intake may be associated with lower breast cancer risk among pre-menopausal and post-menopausal Asian women.

4.4.6. Limitations

The most important limitation in this chapter is the lack of data on the quantity of soy consumed by participants. While frequency of intake may be a reasonable indicator of soy consumption, there can be great differences in how much soy is consumed at every intake. Furthermore, the concentration of soy isoflavones also differ by type and degree of processing of soy foods (75). Furthermore, there was substantial data missing from soymilk intake, up to 26%. These issues may have led to the misclassification of soy intake, and has likely biased the findings towards the null.

Even with a sample size of 3,277 women, there were small sample sizes in some strata of analysis. Therefore, the findings from the stratified analyses have to be interpreted with caution. Furthermore, as discussed in **Chapter 3,** the analysis was conducted on a hospital-based cohort recruited from private and government hospitals, and is not representative of the general Malaysian population. However, this population may form a higher risk Malaysian cohort for whom prevention research is most needed.

There were several questions that could not be addressed in this analysis. For example, previous studies have reported that the inverse association between mammographic density and soy intake may be limited to non-green tea drinkers (120). In this study, however, the role of green tea intake was not evaluated due to the large number of missing data points. Furthermore, this study only collected data on a few dietary variables, namely soy, coffee, tea, and alcohol intake. The lack of information for other dietary variables limited the analysis, including the ability to adjust for total energy intake or potential confounding dietary variables. The effect of (S)-equol concentrations on the association between soy intake and breast cancer risk was another key point of interest in

both Asian and Caucasian studies (107,108,115,118). Unfortunately, (S)-equol producing status is not available for this study.

4.5. Chapter conclusion

Higher frequency of soy intake may be associated to lower breast cancer risk among Asian women, but this warrants confirmation in a larger study with more robust measurement of soy isoflavone intake. This study hypothesizes that not all women may benefit equally from a soy isoflavone intervention, and the benefits may be greatest among women who are overweight or obese.

Chapter 5 : Feasibility of a dietary soy intervention among healthy post-menopausal Asian women: a mixed methods analysis

5.1. Rationale and objectives

While esteemed as the gold standard in clinical research, RCTs are often subject to poor recruitment and high attrition rates. A review of 115 trials in the UK showed that less than a third of trials met their sample size requirements (163), and many trials are forced to close due to poor participation (164). Poor recruitment and high drop-out rates lead to small sample sizes for analysis and an increased chance for selection bias, thus disrupting the external validity of an RCT (165,166).

Dietary intervention studies are especially challenging to implement. Apart from well-recognized problems with recruitment and retention, adherence to the dietary intervention remains a major struggle, even in short term trials [138,139]. This is because dietary intervention studies typically require high and consistent commitment to complex behaviour change [140].

A successful dietary RCT can be achieved with a study design that takes into account the population-specific drivers and barriers to recruitment and adherence to the dietary change (167,168). Increasingly, feasibility studies are recognized as important for the successful implementation of clinical trials (169,170). The information collected in a feasibility study can be used to identify study designs that will maximize recruitment and retention rates (171).

Therefore, as a primer to an RCT to determine the effect of dietary soy on breast cancer risk, a feasibility study was conducted to determine the motivators and barriers to participation in a dietary soy intervention study among healthy post-menopausal Malaysian women. The specific objectives of this study are (1) to measure adherence to a dietary soy intervention (100mg/day of soy isoflavones) over 2 months, (2) to assess for other dietary changes during the intervention period, and (3) to determine the motivators and barriers to participation in a dietary soy intervention study, as well as intent to participate in long-term intervention studies for cancer prevention.

5.2. Methodology

5.2.1. Study participants

Women were selected from the SJMC recruitment site of the MyMammo cohort (described in **Chapter 1**). Of the 2,022 women in the cohort, 1,752 women were excluded as they did not meet the study criteria (**Figure 5-1**). After applying the exclusions, 270 women were eligible for participation in this study. From this list, a total of 63 women were randomly selected for invitation. During the phone call, women were informed about study requirements and were scheduled for an appointment at the study site (Breast Care Clinic, SJMC) if they were interested to learn more about the programme.

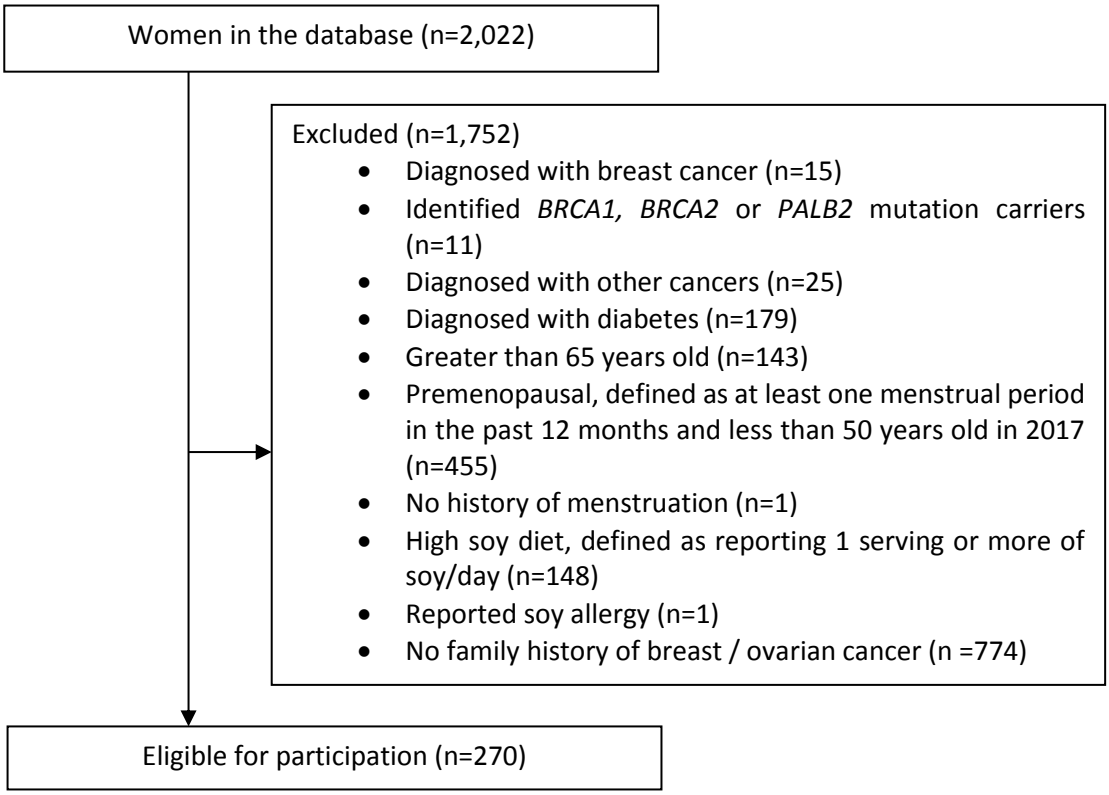


Figure 5-1: Cohort selection from MyMammo.

Women who were interested to participate signed the Informed Consent Form prior to any study assessments. The study protocol and all study-related forms were approved by the Independent Ethics Committee of Ramsay Sime Darby Health Care in January 2017 (Reference #: 201612.3) and the University of Nottingham Malaysia Science and Engineering Research Ethics Committee.

5.2.2. Dietary soy intervention

All participants in the study were required to adhere to a dietary soy intervention, defined as 100mg/day of soy isoflavones through easily available, minimally processed soy foods and drinks. This dosage represents the upper limit of typical soy isoflavone intake in Asian countries with high soy intake (64) and is the commonly used dosage in previous RCTs of soy isoflavone supplements (78). Each participant received a food guide and a cash subsidy of RM100/month to offset the cost of purchasing soy products. The subsidy was estimated based on the price of 1 litre of soymilk per day (>100mg isoflavones), estimated at Malaysian Ringgit (RM) 3.50 per box. The food guide included a daily soy diary for participants to record their adherence to the intervention.

5.2.3. Data collection & follow up

Participants visited the study site 3 times over the study period. The study assessments conducted per visit are described in **Table 5-1**.

Table 5-1: Study assessments

Assessment	Study assessments by visit		
	Enrolment	**1-month**	**2-month**
Baseline questionnaire	√		
Food frequency questionnaire on soy	√		
Food frequency questionnaire on protein/dairy	√		√
Semi-structured interview		√	
Feasibility survey			√
Anthropometric measurement	√	√	√
Urine sample collection	√	√	√
Daily food diary collection		√	√

The questionnaire administered at enrolment assessed participants' medical and family history, as well as breast cancer risk factors, such as physical activity, smoking, and alcohol consumption. Semi-quantitative food frequency questionnaires were administered to measure soy and protein intake pre- and post-intervention. Participants were asked about the frequency of consuming each food item and the size of each serving. The soy products listed in the questionnaire was derived from a questionnaire previously validated among Malaysian women (105). Height and weight were measured at the study site using a floor model that includes a manual scale for height measurement and a digital weighing scale.

Adherence to the study intervention was defined as reporting at least, on average, 80mg/day of isoflavones consumed through soy foods and drinks. Soy consumption at enrolment were derived from semi-quantitative food frequency questionnaires. The average intake of soy foods during the intervention period was derived from daily self-reported intake.

Semi-structured interview questions were administered to participants 1-month post-enrolment. The duration for each interview was between 30-45 minutes per participant. Participants were asked for their perceptions about being in a dietary soy intervention study, and the motivators and barriers to participation. All interviews were recorded.

A structured feasibility survey was administered to participants who completed the study, 2-months post-intervention. The survey collected information on participants' experience during the intervention period and measured the likelihood of participating in future intervention studies for cancer prevention.

5.2.4. Sample size calculation

The desired sample size of this feasibility study was a trade-off between statistical sample size estimation and what was achievable in the time frame. Assuming that 85% of participants will adhere to intervention, the study requires at least 12 participants to describe this proportion with a 20% margin of error. Assuming a participation rate of 20%, approximately 63 women would have to be contacted to achieve the desired sample size. Sample size calculation for one sample proportions were conducted in the R statistical environment software (version 3.4.2).

5.2.5. Statistical Analysis

Standard descriptive analyses were used in the study. Proportions were used to describe response rates, participation rates, the reasons for non-participation, and participant characteristics. Responses from 5-point Likert scales were described using mean and 95% CIs.

The average daily isoflavone intake was calculated as the sum of the daily intake of each soy food item (in grams) times the estimated total isoflavone content for the item (in mg per gram of soy food). The estimated total isoflavone content in commonly consumed

soy food are described in **Table 5-2**, and was summarized from a study of isoflavone content of soy food in Singapore and Hawaii (172). Median and IQR were used to describe the distribution of isoflavone intake (mg/day).

Table 5-2 Conversion of soy food intake to soy isoflavone intake per serving

Food item	Serving size	Isoflavone intake per 100g food (mg)	Isoflavone intake per serving (mg)
Soymilk	1 cup	12.5	31.3
Tofu	100 grams	25.9	25.9
Taufoofah	1 bowl	34.7	106.5
Soy beans	100 grams	128.2	128.2
Tofu puffs	1 piece	16.2	2.49
Tempeh	100 grams	72.8	72.8
Foochuk	1 piece	55.4	14.5
Edamame	100 grams	19.3	19.3
Miso soup	1 cup	23.0	29.1

Daily energy intake (kCal/day) from protein was calculated using the Malaysian Food Composition Database 1997 (173). Within-women changes in protein intake over the study period was assessed using Wilcox-sign-rank tests for non-parametric data.

All descriptive statistics and statistical tests were performed using the R statistical environment software (version 3.4.2). All tests were two-sided and was considered statistically significant if the *p* value < 0.05.

5.2.6. Thematic Analysis

Semi-structured interview recordings (administered 1-month post-intervention) were transcribed by an external service provider. Transcripts were read and coded in random order, using the NViVo software (version 11). Statements that describe perception, motivators or barriers were coded as nodes. Similar nodes were then classified into themes. The analysis achieved thematic saturation after 8 of the interviews were coded. The themes were further organized into domains, using the Theoretical Domain Framework (174). This framework is an amalgamation of 33 theories on behaviour and behaviour change, and is commonly used in implementation science (175). The framework is used here to describe and discuss the results of the thematic analysis.

5.3. Results

5.3.1. Response & non-participation

Of the 63 women who were selected from the database, only 48 (76%) were contactable. Of this, 13 women (27%) agreed to attend the baseline visit (**Table 5-3**). Interestingly, half of the Malay women who were contacted agreed to participate, but only 32% of Chinese women and 11% of Indian women agreed.

Table 5-3: Participation rate (n=63)

	Participation rate							
	Chinese		Indian		Malay		Overall	
	n	%	n	%	n	%	n	%
Total selected	28		23		12		63	
Number contactable	22	(79%)	18	(78%)	8	(67%)	48	(76%)
Participation rate	7	(32%)	2	(11%)	4	(50%)	13	(27%)

Annotations: n = number; % = column proportion.

The primary reasons for non-participation are recorded in **Table 5-4**. The most common reason for non-participation was lack of interest in the study (n = 15, 43%) and logistical difficulties (n = 8, 23%). Six women (18%), respectively, said that they were reluctant to consume a high soy diet due to soy intolerance or concern about side effects. Four women (11%) reported that the study would be time consuming. Women were also concerned that participation in the study would interfere with their current diet (n = 1, 3%) or that they would not be able to comply with the protocol (n = 1, 3%).

Table 5-4: Primary reasons for non-participation (n=35)

Reasons for non-participation	n (%)
Not interested	15 (43%)
Lives too far away/no transport	8 (23%)
Reluctant to consume a high diet	6 (18%)
Too time consuming	4 (11%)
Worried about compliance to the diet	1 (3%)
Worried about changes to diet	1 (3%)

Annotations: n = number; % = column proportion.

5.3.2. Characteristics of study participants

Of the 13 women who visited the study site, two women were not eligible for participation (pre-menopausal at enrolment) and 1 woman declined participation. A total of 10 women signed the Informed Consent Form, and are henceforth referred to as study participants. Overall, there were no significant differences in demographic and risk factors between the women who were contacted and those who subsequently enrolled into the study (**Table 5-5**).

Table 5-5: Characteristics of participants in the study (n=10) compared to women who were contacted (n=48)

Characteristics	Comparison of participant characteristics		
	Contacted	Enrolled (n=10)	
	n (%)	n (%)	p-value
Age			
≤ 55	25 (52%)	5 (50%)	0.749
56 – 60	13 (27%)	3 (30%)	
> 60	10 (21%)	2 (20%)	
Ethnicity			
Chinese	22 (46%)	4 (40%)	0.226
Malay	8 (17%)	4 (40%)	
Indian	18 (38%)	2 (20%)	
Highest education attainment			
At least secondary education	28 (58%)	4 (40%)	0.469
Some tertiary education	19 (40%)	6 (60%)	
Monthly household income			
< RM 5,000	26 (54%)	4 (40%)	0.712
RM 5,000-10,000	17 (35%)	5 (50%)	
≥ RM 10,000	5 (10%)	1 (10%)	
Breast cancer family history	20 (42%)	5 (50%)	0.999
Employed			
Full time		5 (50%)	
Part time		2 (20%)	
Unemployed/Retired		3 (30%)	
BMI (kg/m^2)			
< 23		4 (40%)	
23-28		5 (50%)	
≥ 28		1 (10%)	
Year since last mammogram			
≤1 year		3 (30%)	
1-5 years		4 (40%)	
≥ 5 years		3 (30%)	

Annotations: n = number; % = column proportion

Al participants were between 50 and 65 years old. Chinese and Malay women made up the majority of the study population (40% respectively), while 20% of participants were Indian. Most women reported tertiary education (60%) and a monthly household income of at least RM5,000. This indicates that the cohort includes well-educated women from at least middle-income families. Most women were working full time at the time of the study (50%). Only 3 women (30%) had a mammogram within the last year.

Having family history of breast or ovarian cancer (including first, second, or third degree) may be an important motivator to participate in prevention studies and therefore was a criterion for inclusion in this feasibility study. Almost half of the participants reported having at least 1 first degree family member diagnosed with breast cancer (42%). This is comparable to the women in the database (50%). One woman did not meet the inclusion criteria (she did not have any family history of cancer), but she was included in this feasibility study as she was very motivated to participate.

5.3.3. Follow up

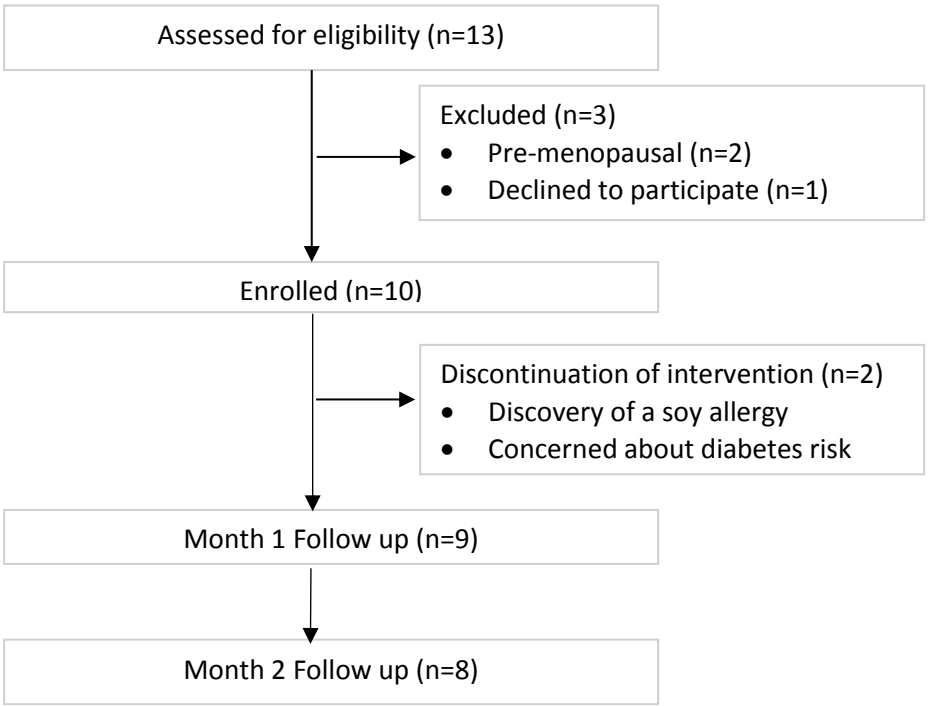


Figure 5-2: Participant follow up during the intervention period.

There was a total of 75 person-weeks of follow up for 10 participants. The median number of weeks of follow up per person was 8 weeks (IQR = 1.8). All women completed 1-

month follow up (**Figure 5-2**). Two participants discontinued the intervention within the first month (20%). One participant discovered an underlying soy intolerance which led to nausea, diarrhoea, and other allergy symptoms. The second participant reported an increase in fasting blood glucose level post-enrolment. Eight women completed the study.

5.3.4. Baseline intake of soy among post-menopausal Malaysian women

The median soy isoflavone intake at enrolment was 11.4mg/day (IQR = 7.2, **Table 5-6**). The most common source of soy isoflavones among participants in this study were tofu (median = 2.8 mg/day, IQR = 3.3) and soybean curd (median = 2.7 mg/day, IQR = 3.4). Soymilk contributed 1.7 mg/day (IQR = 1.1) to overall soy isoflavone intake. Interestingly, consumption of soybean curd skin or *foojuk* was relatively common, contributing 2.2 mg/day (IQR = 2.2) to total isoflavone intake, while tofu puffs contributed only 0.3 mg/day (IQR = 0.6). Participants in this study rarely consumed whole mature soybeans, whole young soybeans (edamame), or tempeh.

Table 5-6: Average daily intake of isoflavone from soy foods at baseline and during the study period (n=10)

Soy food	Distribution of soy isoflavone intake (mg/day)			
	Enrolment (n=10)	**1-month (n=10)**	**2-month (n=8)**	**Overall**
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Total	11.4 (7.2)	98.3 (24.7)	76.1 (41.8)	87.0 (31.1)
By soy food:				
Soymilk	1.7 (1.1)	67.8 (16.8)	57.7 (32.1)	63.1 (19.1)
Tofu	2.8 (3.3)	15.2 (10.2)	7.1 (9.4)	11.6 (7.2)
Soybean curd	2.7 (3.4)	12.2 (12.5)	8.0 (18.1)	9.6 (8.8)
Tempeh	0 (0.6)	0 (0.2)	0 (0.0)	0.1 (0.1)
Whole soybeans	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tofu puffs	0.3 (0.6)	0 (0.0)	0.3 (0.6)	0 (0.2)
Foojuk	2.2 (2.2)	0 (0.2)	0.3 (0.7)	0.3 (0.6)
Edamame	0 (0.0)	0 (0.0)	0 (2.2)	0.5 (1.2)

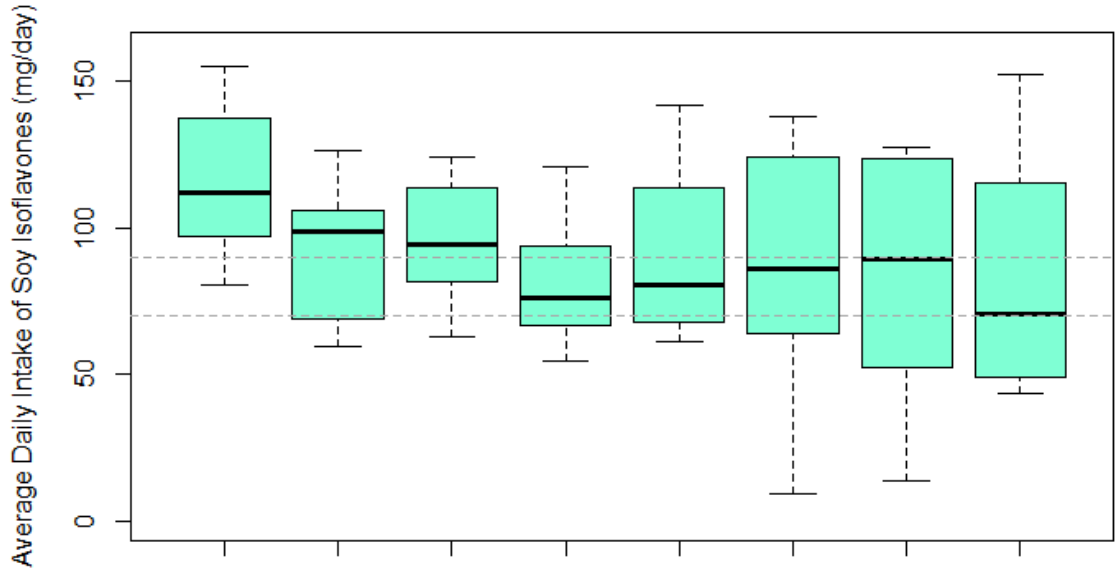
5.3.5. Adherence to the intervention

Participants were able to maintain isoflavone intake at an average of 87.0 mg/day (IQR = 31.1) during their participation in the study (**Table 5-6**). Of this, the majority of isoflavones were consumed through soymilk (median = 63.1 mg/day, IQR = 19.1). This

translated to approximately 2 cups of soy milk per day. Tofu was the next most common source of soy isoflavones (median = 11.6 mg/day, IQR = 7.2), followed by soybean curd (median = 9.6 mg/day, IQR = 8.8). Other soy food, such as tempeh, *foojuk*, edamame and tofu puffs were consumed in smaller amounts.

In the first month, the median isoflavones consumed was on average 98.3 mg/day (IQR = 24.7). Four women (40%) consumed more than 100mg/day isoflavones in this month, while one woman consumed up to 260mg/day (*data not shown*). In the second month, the median consumption declined to 76.1 mg/day (IQR = 41.8).

Figure 5-3 shows the weekly changes in adherence over the study period, as measured from study diaries. Adherence to the soy intervention was highest in the first three weeks of the study period, where in Week 1 the median intake of soy isoflavones was 111.9 mg/day (IQR = 36.9). Adherence reduced and stabilized by Week 4-5, and was between 70-90 mg isoflavones/day on average.



Week	1	2	3	4	5	6	7	8
n	10	10	10	9	9	8	8	7
Median (mg/day)	111.9	98.7	94.2	76.3	80.6	85.9	89.4	70.7
IQR (mg/day)	36.9	34.7	31.9	27.2	30.9	49.1	71.3	66.1

Figure 5-3: Average daily soy isoflavone intake over the study period. Grey dotted lines represent average soy isoflavone intake over time (70-90mg/day).

5.3.6. Changes to protein intake over the study period

There were no significant differences in intake of other sources of protein, including dairy, seafood and meat, over the intervention period (**Table 5-7**). There was a decrease in dairy and egg intake, and a slight increase in pork intake over the study period, but these were not statistically significant.

Table 5-7: Changes of protein and dairy intake over the study period (n=8)

Protein/dairy intake	Energy intake, kCal/day (n=8)		*p* value
	Enrolment	**At 2-months**	
	Median (IQR)	Median (IQR)	
Eggs	16.5 (14.3)	9.5 (10.0)	0.203
Fish	6.8 (5.1)	6.8 (4.1)	0.599
Shellfish/prawns	1.1 (3.6)	0.4 (2.1)	0.599
Beef	0.3 (1.5)	0 (0.8)	0.371
Chicken	12.1 (41.5)	10.5 (8.5)	0.204
Pork	0.5 (12.7)	4.0 (12.7)	0.999
Mutton/lamb	0.7 (0.7)	0.3 (0.9)	0.999
Dairy	20.0 (89.7)	17.5 (88.5)	0.999

5.3.7. Motivators and barriers

Eleven common themes were identified from transcripts of interviews conducted at the end of the first month. Of this, 7 themes were classified as motivators and 4 were classified as barriers. Further grouping of themes using the Theoretical Domain Framework resulted in 6 domains (**Table 5-8**).

In this study, social influence presents both as a motivator and barrier. A majority of participants (80%) felt that support from their family and friends was a powerful motivator for them to participate in the study and meet the daily soy requirements (Quote 1 and 2, **Table 5-9**). Beyond this, support from the research staff were important reinforcements of adherence to the intervention (Quote 3). However, 80% of participants also reported reading that soy is not good for them or have heard so from the people in their social circles. This created a sense of doubt about their participation in this study (Quote 4).

Participants in this study reported that they participated for altruistic reasons (70%). Participants were also intrigued by the study and wanted to learn about their risk for breast

cancer and how to protect themselves (Quote 5 and 6). Up to 80% of women mentioned in the interview that they considered participation because they were emotionally connected to the cause or they knew someone with cancer. Interestingly, this was not limited to family history, and included peers and other members of their social circle (Quote 7 and 8).

Table 5-8: Common patient motivators and barriers arising from thematic analysis (n=10)

Category	Theme	Percentage, %
Social Influence	Support from family/friends	80
	Public misconceptions about soy	80
Identity/emotional connection	Knows someone diagnosed with cancer	80
Behavioural regulation	Difficulty in changing habits	80
	Difficulty in obtaining soy products	80
Intention	Altruism	70
	Self-awareness/education	70
Belief about consequences	Prior knowledge that soy is beneficial	70
	Perceived side effects	60
Reinforcement	Support from the research team	60
	Commitment to the study	60

Despite negative feedback from their peers, most participants believed soy was beneficial to their health. Many did not know of the correlation between soy and breast cancer risk prior to the study, but believed that soy was good for skin, in menopause management, as a calcium supplement, as well as improving pregnancy outcomes.

The main barriers in this study were in the behavioural regulation domain. Many participants (80%) faced challenges due to the changes in their diet or routine during the study. They also felt that the intervention was too much and was not sustainable over time (Quote 9). Another difficulty faced by participants is the lack of availability of soy products at restaurants or the short self-life of soy foods. This partly explains why soymilk was the most common form of soy consumed in this study, as it is easily accessible and can be purchased fresh or packaged for longer storage.

Importantly, up to 60% of participants reported side effects from the soy intervention. This includes gastrointestinal issues, increased urinary frequency, and bloating. This could have affected participants' motivation to meet study requirements.

Table 5-9: Participant quotes from semi-structured interviews (n=10)

Quote Number	Quote
1	*"The first time you called me, I was a bit lazy. I was thinking, no, I don't want to take part. Then I was talking about it with my family and then they said, no, you should [participate]."*
2	*"I am busy I sort of forgot [that] I am on this soya diet kind of thing and my husband will remind [me], have you taken your soya today?"*
3	*"I think you guys have done you level best you give me support intermittently, that I think is a good reminder that that actually keep one focus as well."*
4	*"…then that lady (food vendor) was telling me, you have been consuming quite a lot [of soy], you know it's not good for you."*
5	*"And I thought about it but I said if it helps others, it is fine…"*
6	*"That sort of increase my awareness and of course wanting to contribute mainly because […] it's not just breast cancer or any cancer, it's like we know not much about it, so it's good to contribute."*
7	*"I have a very good friend from South Africa who also died of breast cancer within 2 [to] 3 months of finding out she had cancer."*
8	*"…his (son's) ex-girlfriend died of cancer […] in a way I am also just like doing it for her plus if you remember my maternal side has a lot cases […] of cancer..."*
9	*"So, when you incorporate a lot of tofu in your diet it's like you eat tofu but there is a part of the body that also wants to eat chicken..."*

5.3.8. Participant experiences and intent to participate in future cancer prevention studies

Participants were asked to rate their experience in their study after 2 months of intervention (**Table 5-10**). Most women rated that they understood the purpose of the study (mean = 4.6, 95% CI = [4.2, 5.0]) and were motivated to participate (mean = 4.4, 95% CI = [3.9, 4.8]). They agreed that the subsidy provided was sufficient (mean = 4.3, 95% CI = [3.3, 5.0]). Participants appear to be less agreeable for questions pertaining to amount of soy (mean = 4.0, 95% CI = [3.4, 4.6]) and incorporation of soy into routine (mean = 3.9, 95% CI = [3.1, 4.7]). This is consistent with the results of the thematic analysis in this chapter.

Furthermore, participants were asked to rate their likelihood of participation in future trials (**Table 5-11**). Participants reported that they were more likely to participate in a soy intervention study with half the dose (mean = 3.5, 95% CI = [2.5, 4.5]), compared to a trial of 100mg/day soy isoflavones (mean = 2.6, 95% CI = [1.7, 3.5]). Participants were also willing to participate in a study of green tea (mean = 3.4, 95% CI = [2.3, 4.5]) but not a study of high intensity physical activity (mean = 2.6, 95%CI = [1.6, 3.6]).

Table 5-10: Participant experience in the study (n=8)

Participant experiences	Mean score†	[95% CI]
Understand the purpose of the study	4.6	[4.2-5.0]
Motivated to participate	4.4	[3.9-4.8]
Easily meeting daily soy requirement	4.0	[3.4-4.6]
Easily incorporate soy in their diets	3.9	[3.1-4.7]
Subsidy provided was enough	4.3	[3.3-5.0]

Annotations: †Mean score from 5-point Likert scale from 1 (completely disagree) to 5 (completely agree).

Table 5-11: Perception towards future participation (n=8)

Choice of intervention	Mean score	[95% CI]
Soy, same amount as in pilot study	2.6	[1.7-3.5]
Soy, half the amount	3.5	[2.5-4.5]
Green tea, 2 cups once daily	3.4	[2.3-4.5]
High intensity physical activity, 3 times a week	2.6	[1.6-3.6]

Annotations: †Mean score from 5-point Likert scale from 1 (very unlikely) to 5 (very likely).

5.4. Discussion

5.4.1. Summary of main findings

In this feasibility study of 10 post-menopausal Malaysian women, there was good adherence to the dietary intervention. On average, women were able to consume 70-90 mg/day of isoflavones via dietary soy intake over two months. The main barriers to adherence were difficulties in obtaining the soy foods regularly and incorporating large amounts of soy into their diet. Familial support, emotional investment and altruism were common motivators to participation and compliance among Malaysian women. Furthermore, there was intent to participate in future trials of dietary soy, particularly if the dose was reduced.

5.4.2. Recruitment and retention in a dietary soy intervention study

The recruitment rate in this study was 27%. The reasons for non-participation include lack of interested (43%), logistical issues (23%) and reluctance to incorporate soy into their diet (18%). The finding of this study mirror that of Crowder et al., who reported a recruitment rate of 25% among Caucasian head and neck survivors (176). The main reasons for non-participation in the Crowder et al. study were distance, lack of interest, or lack of time (176). Expectedly, research has shown that recruitment rates decline as complexity and

duration of the study increase (177,178), especially when the purpose of the study is not well understood (179). Therefore, a crucial first step in any dietary or lifestyle intervention trial should involve developing tools that can effectively communicate study goals and requirements to potential participants.

Most dietary or lifestyle interventions have investigated the motivators and barriers to participation amongst people with disease or at high risk for disease. For example, a majority of these studies have targeted individuals seeking weight loss or better control of their cardiovascular risk factors, and this risk awareness has been shown to be an important predictor of participation and compliance in dietary intervention studies (180). Among breast cancer survivors, the motivations to participate in lifestyle intervention studies are likely different because of the desire to regain of control over their life and health (167,181). Dietary intervention studies are particularly challenging when the study population consists of healthy women, for whom the intervention will likely not result in a direct, observable benefit (165,182).

Another factor that can significantly affect the external validity of the study is attrition or drop-out during the study. Attrition is a particularly important problem in dietary intervention studies as participants may be less willing to incorporate or sustain the new diet or lifestyle in the long term (183). In this study, however, women dropped out solely due to adverse events, at a rate of 20% over the two months. The rate of attrition is comparable to a Cochrane review of 38 dietary intervention studies, where the attrition rate was between 20-30% (184). The findings in this chapter suggest that proper consideration of the isoflavone dosage may be a key strategy to prevent attrition in a dietary soy intervention trial among Asian women.

5.4.3. Adherence to a dietary soy intervention

To ensure robust study results, high and consistent adherence to the study intervention is required. In this study, the overall adherence to the dietary soy intervention was good, between 70-90 mg/day isoflavones out of the prescribed 100mg/day dose. The compliance reported here is comparable, if not higher, compared to many dietary intervention studies among healthy women. For example, in the Herbal Alternatives Trial, only 40% of women in the soy intervention group adhered to 2 servings/day of dietary soy over 1 year (185). In a meta-analysis of weight loss intervention studies, adherence was approximately 60.5% on average (186). In intervention studies amongst cancer survivors,

short-term adherence was often greater than 70% (187–189). It is important to note that self-reported adherence to diet is likely subject to bias. Alternatively, biomarkers have become increasingly popular in intervention studies as an objective measure of dietary intake, but it should be used concurrently with validated dietary assessment tools (183,190).

Furthermore, adherence in this study declined over time, a phenomenon that is common in dietary intervention studies that require rigorous self-monitoring and self-efficacy (191,192). According to Peters et al., the first 4 months of the intervention is critical for the "adoption" of the dietary intervention (193). Therefore, incorporating motivational components, particularly in the first part of the participant's journey, is important to reinforce the behavioural change required to meet adherence to the intervention.

5.4.4. Motivators to adherence

In this study, social support was a major motivator for participation and adherence. This behaviour is aligned to the social cognitive theory, which describes self-efficacy as a product of social support, motivation and self-regulation (194). Previous studies have also reported that support from family and friends were important facilitators to behaviour change, even more so when the support person is involved in the study (167,168,186). Women have reported feeling demotivated when there was a lack of support and understanding from their family (167). Furthermore, women may be more inclined to prioritize the dietary needs of the family rather than her requirements as a study participant (167,181). Therefore, integrating design components that focus on building relationships with family members or peers could increase compliance to the intervention and reduce attrition in dietary intervention studies.

Besides social support, a good relationship with the study personnel was also reported as a motivator of adherence in this study. Trust, encouragement, clarity of information, and regular contact were positive influences on behaviour change (167,168,176,195,196). A review of dietary intervention trials showed that frequent and varied support by the research staff was key to successful intervention (186). Lemstra et al. showed that close supervision and monitoring of participants improved adherence by 65%, increased accountability, and the increased the likelihood of achieving set goals, compared to self-monitoring (186). Nevertheless, relationship building and close monitoring requires significant human and financial resources, and remain a challenge for dietary or lifestyle intervention studies.

This study demonstrates that healthy Asian women participate in lifestyle intervention studies for altruistic reasons, which is further strengthened by having a personal relationship to a cancer survivor. Altruism is the most common motivator to participate in biomedical research in low- and middle-income countries (197). For example, Poore et al. found that up to 83% of women participated for altruistic reasons, compared to 48% with self-rewarding reasons (182). They also found that women who participated with altruistic reasons were more motivated and were more likely to achieve desired outcomes (182). Incorporating culturally-tailored altruistic messages in recruitment and study materials may improve recruitment rates and adherence to the study intervention.

5.4.5. Difficulties faced by participants

Self-regulation and self-monitoring are crucial elements in dietary intervention trial of free-living participants. Similar to previous studies, the lack of choice and availability of soy products in this study limited women's ability to meet study requirements (165–167). Furthermore, high participant burden and the lack of adequate guidance in fulfilling study requirements have been shown to be a major deterrent in lifestyle intervention studies (167,168,178,197) Better compliance could be achieved by easing the burden felt by participants, such as by increasing access to the intervention, better guidance in estimating serving sizes, and by the use of technology for monitoring (196,198). For example, Turner et al. suggested that the use of a points-based reward system or gamification could improve self-monitoring habits (191).

Another deterrent to adherence in this study was the onset of side effects. In this study, the average isoflavone intake at enrolment was low, up to 12mg/day. A drastic increased in soy isoflavone intake may have led to some side effects, such as weight gain, more frequent bowel movements, diarrhoea or constipation, urinary frequency, and bloating, similar to previous reports (78). Concern for safety is a major reason for non-participation in research, particularly among healthy women in prevention studies (197,199). Unfortunately, there is not much is not known about the safe and effective dosing range for soy isoflavones for reducing breast cancer risk. Previous RCTs have tested the effects of 80-120mg/day soy isoflavone supplements (78), while epidemiological studies suggest that soy isoflavone intake of 40mg/day may be sufficient to show a clinically meaningful effect (70). In a safety study, doses of 60mg/day of isoflavones were reported to be safe for breast and endometrial tissues (200). Taken together with the difficulties in adherence, 100mg/day of

soy isoflavones obtained through diet may not be feasible among post-menopausal Malaysian women.

5.4.6. Strengths

This is the first study to look at the feasibility of a dietary intervention for breast cancer prevention among Asian women. It enables an understanding of the motivators and barriers to recruitment and adherence to a dietary soy intervention prior to a large-scale trial within the same population. Despite common misperceptions or ambiguity about the benefits of soy, this study shows that Malaysian women were willing and motivated to participate in a dietary soy intervention study. Importantly, in the absence of an established dosing range for soy isoflavone intake, this study sheds some light on the acceptable dose of dietary soy isoflavones for long-term intervention in this population.

5.4.7. Limitations

Firstly, the study only recruited 10 of the anticipated 12 people required to measure 85% adherence with a relatively large margin of error (20%). This sample size calculation also did not consider the analysis of other study objectives, such as the change in protein intake over the study period. The participation rate in this study was low, about 27%, and was the main reason for the small sample size. Nevertheless, the sample size was sufficient for the thematic analysis, which provides crucial information for the design of the larger trial.

Secondly, women recruited in this study are likely not representative of the women in the MyMammo programme or the general population of Malaysian women. The women in this study were also more likely to be well-educated, from at least middle-income families, and were more likely to report healthier habits, such as frequent physical activity. Many women in the study had extensive family history of cancer, which was not limited to breast cancer. Hence, this group may represent highly motivated women living in urban areas who are more likely to adopt healthy habits. The conclusions from the study, therefore, should be extrapolated with caution.

A third important limitation is the measurement of soy isoflavones from reported soy intake. In this study, participants were asked to purchase soy foods on their own, based on a food guide. Many participants reported being uncertain about the amount of soy they consumed on a daily basis and frequently reported estimates. This could have led to the over-reporting or under-reporting of soy intake, the extent to which is not measurable in this

study. Better methods of controlling serving size, such as providing study participants with standardized servings of soy foods are required to increase adherence as well as ensure uniformity in dosing. This will enable a more robust examination of the impact of increased dietary soy on breast cancer risk in the larger prevention trial.

5.5. Conclusion

A dietary soy intervention may be feasible among healthy post-menopausal Asian women, but an intervention of 100mg/day isoflavone is not achievable for a long-term clinical trial of dietary soy intake on breast cancer risk. Strategies to reduce participant burden and increase rapport are necessary to improve the internal validity for a dietary intervention trial. Furthermore, developing culturally-tailored communication tools that incorporate altruistic messages may lead to higher recruitment rates in this population.

Chapter 6 : The effect of soy isoflavone intake on mammographic density as a biomarker of breast cancer risk: a randomized, three-arm, non-placebo-controlled clinical trial

6.1. Rationale and objectives

In observational studies of Asian women, typical soy intake of between 25-50mg/day isoflavones has been shown to be protective against breast cancer, particularly for post-menopausal women (66–69). However, RCTs using 50-120mg/day isoflavone supplements for 1-2 years among Caucasian women have yielded null findings on biomarkers of risk, including mammographic density (87–92). It is possible that soy isoflavones obtained through food made from whole soybeans are more potent in its' effect on breast cancer risk, compared to isoflavone or soy protein isolate supplements (98). Furthermore, there is some debate as to whether Asian women may benefit more from a dietary soy intervention, perhaps due to lifelong exposure to soy isoflavones or differences in the ability to metabolize soy, as described in **Chapter 2** (105–108). To date, there has not been an RCT of soy isoflavone intake among Asian women.

The research studies in the preceding chapters have laid the foundation for an RCT of soy isoflavone intake among Asian women. The findings from **Chapter 3** and **Chapter 4** suggests that mammographic density is a suitable biomarker of breast cancer risk among post-menopausal Asian women, and may be used to understand the association between soy intake and breast cancer risk. **Chapter 5** demonstrates that a dietary soy intervention is feasible among post-menopausal Asian women, but a dose of 100mg/day of isoflavones through diet is not sustainable over time.

The primary objective of this chapter is to evaluate if consuming 100mg/day of soy isoflavones through supplements or 50mg/day of soy isoflavones through diet for 12 months would modify mammographic density among healthy peri- and post-menopausal Asian women in a randomized, three-armed, non-placebo-controlled trial (The Malaysian Soy and Mammographic Density Study or MiSo Study). Additionally, this chapter explores for possible effect modifiers of the relationship between isoflavone intake and mammographic density.

6.2. Methodology

6.2.1. Ethical approval

The study protocol was approved by the Ramsay Sime Darby Healthcare Independent Ethics Committee (Reference number: 201805.1), the University of Nottingham, Malaysian Campus Science and Engineering Research Ethics Committee, and is registered with the National Medical Research Register (NMRR-18-287-40385) and ClinicalTrials.gov (NCT03686098).

Prior to participation in the study, participants were informed about all procedures they would undertake in the study, the risks and benefits of participation, their right to withdraw from the study, as well as the measures taken to protect their data and confidentiality. Participants signed an Informed Consent Form before any study procedures were performed.

6.2.2. Study population & recruitment

Women were invited from the SJMC recruitment site of the MyMammo cohort and from the general population between November 2018 and August 2019. Recruitment material was sent via the Whatsapp mobile app (for women in the MyMammo study) and placed at various clinics at SJMC. Women who were interested to participate were invited to the Breast Care Clinic at SJMC for screening and enrolment.

6.2.3. Enrolment & Randomization

Women were eligible for participation if they were between 45-65 years old and if they responded "No" to the criteria listed in **Table 6-1**. The final criteria (Score of 2-5 using ACR BIRADS) was assessed based on participant's mammogram report at enrolment.

Eligible women were enrolled and randomized into three study arms (100mg/day Soy Supplement, 50mg/day Dietary Soy, Control). A stratified, block randomization approach was used to account for potential differences in distribution by ethnicity (Chinese, Indian, and Malay) and menopausal status (peri-menopausal and post-menopausal)[3].

[3] Randomization list per stratum was generated using https://www.sealedenvelope.com/simple-randomiser/v1/lists), dated 26 October 2018, with seed 199098584295839.

Table 6-1 List of exclusion criteria in the study

Exclusion criteria
• A menstrual period in the past 3 months • Diagnosis of cancer, stroke, and other serious health conditions • Diagnosis of benign breast disease • Diagnosis of gout, hyperuricemia and associated conditions • Diagnosis of diabetes • Diagnosis of hypothyroidism • Diagnosis of gastrointestinal conditions (i.e., irritable bowel syndrome) • Allergy or intolerance to soy and soy products • Recent use of hormone replacement therapy drugs, including alternative and traditional therapies for menopause symptom management (within the last 6 months) • Recent smoking (within the last 6 months) • Recent high soy diet, defined as daily consumption of soy products or soy-based supplements (within the last 6 months) • A mammogram in the past 12 months • Breast augmentation • BI-RADS Score of 2-5 at screening mammography with additional tests (i.e., ultrasound) or upon the advice of a Consultant Radiologist

6.2.4. Intervention & Compliance

In the Soy Supplement arm, participants received 100mg/day of soy isoflavones through a locally-produced, commercially available supplement. Each soy isoflavone tablet contained 125mg of soybean (Glycine max) standardized extract, which delivered 50mg of soy isoflavones (approximately 46mg of daidzein and 4mg of genistein, in aglycone weight). The supplier provided a certificate of analysis for high performance liquid chromatography tests, which confirmed the concentration and purity of the isoflavone supplement. Participants were asked to consume 2 tablets per day, after meals and at a consistent time every day. Furthermore, participants were asked to limit their dietary soy intake to less than 3 times/week for the duration of the study. Participants were defined as compliant if they consumed at least 80% of intended intake, which was assessed by counting remaining tablets in supplement bottles.

In the Dietary Soy arm, participants were asked to consume 50mg soy isoflavones daily via soy food. Participants in this arm received a Study Diary consisting of a portion guide and a food diary. The portion guide was developed based on the isoflavone content in

commonly consumed soy foods in Singapore and Hawaii (172), and is illustrated in **Figure 6-1**. Participants were provided RM65/month to subsidize the cost of purchasing the soy food throughout the study period. This is estimated based on the average price of 2 servings of soymilk per day. Participants were defined as compliant if they consumed at least 80% of the intended soy servings (≥40mg/day of soy isoflavones), which was based from self-reported intake in the Study Diary.

In the Control arm, participants were asked to continue their regular diet, and to ensure that their dietary soy intake was less than 3 times/week for the duration of the study. Compliance in the Control arm was defined as intake of less than 40mg/day of soy isoflavones through diet.

Figure 6-1 Instructions in the study diary for women in the Dietary soy arm.

6.2.5. Data collection

Study assessments were conducted as per **Table 6-2**. Height and weight were measured at the study site using a single floor model that includes a manual scale for height measurement and a digital weighing scale. BMI was calculated as the weight (in kilograms) divided by height squared (in metres). Waist and hip circumference were measured in centimetres using a measuring tape. Waist circumference was measured at the midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line, approximately at the belly button (201). Hip circumference was measured at the

largest circumference of the buttocks (201). Waist-to-hip ratio (WHR) was calculated as the waist circumference divided by hip circumference. Waist-to-hip ratio is a simple and effective measure of abdominal obesity, which is an important risk factor for post-menopausal breast cancer (202).

Table 6-2 Study assessments

Study Phases:	**Screening**	**Enrolment**	**Intervention**			
Study Site Visits:	1	1	2	3	4	5
Months from enrolment:		0	3	6	9	12
Eligibility screening	√					
Anthropometrics	√		√	√	√	√
Mammography	√					√
Ultrasound	√					√
Medical History & Lifestyle Questionnaire		√	√	√	√	√
Urine collection		√		√		√
Compliance			√	√	√	√

The Medical History and Lifestyle Questionnaire (MHLQ) collected data on past and present medical history, medication use, family history of cancer, and demographic, reproductive and lifestyle factors. Participants were also asked to provide mid-stream, spot urine samples at the clinic. Boric acid was added to urine samples within 2 hours from collected, and were stored as 1.5ml aliquots in the -80°C freezer at the study site.

6.2.6. Calorie, macronutrient and isoflavone intake

Habitual dietary intake over the preceding 3 months was recorded using a 171-item food frequency questionnaire (FFQ) in the MHLQ. The FFQ was adapted from a questionnaire validated for use in the general Malaysian population (the Malaysian Adult Nutrition Survey, MANS) (203). For each food item, data collected in the FFQ was converted into standard portions per day, which was subsequently converted into calorie (kCal/day) and macronutrient intake (g/day). The conversions were based on data compiled from publicly available food composition databases, namely the Malaysian Food Composition Database 1997 (173) and the Singapore Health Promotion Board's Energy and Nutrient Composition of Food (204). Macronutrient intake assessed here include carbohydrate, protein, and fat.

The average daily isoflavone intake was calculated from intake of all soy food reported in the FFQ. Isoflavone content in soy food (in mg/day) was estimated based on a

study of isoflavone content in Singapore (172), as described in **Table 5-2**. The MHLQ was administered at every visit for participants in the intervention arms, but only at Month 0, 6, and 12 for women in the Control arm who had fewer study visits as per protocol. Therefore, this data is presented for Month 0, 6, and 12 only, and each point of data collection reflects intake in the preceding 3 months.

6.2.7. Mammogram variables

Mammography was conducted at enrolment and at 12-month follow up. All mammograms were conducted at SJMC, using the Hologic Selenia Mammography System. All women attending mammography were seen by a consulting general physician prior and post-mammogram, as per hospital procedures for screening mammography.

Digital copies of the mammogram were retrieved from the main hospital server and were de-identified. Mammographic density was estimated from raw mammogram images using fully-automated, high-throughput methods, namely Volpara™ (volume-based) and STRATUS (area-based), as described in **Chapter 1.** Absolute and relative mammographic density measures were estimated as the average mammographic density measured from left and right MLO images. The Pearson correlation between left and right absolute density and percent density were 0.89 and 0.91 for volume-based mammographic density and 0.83, respectively, for area-based mammographic density.

Mammogram acquisition parameters, namely compression force (N), compression pressure (kPa), paddle tile (mm), and recorded breast thickness (mm) were exported from the DICOM tags from the mammogram image. The average of left and right mammogram acquisition parameters was used in the analysis.

6.2.8. Adverse events

Throughout the study period, participants were monitored for potential adverse events through regular online surveys and at in-person visits. An adverse event (AE) was defined as any untoward medical occurrence in a participant who has received the intervention and does not necessarily have a causal relationship with the intervention, as per the Malaysian Guidelines for Good Clinical Practice (205).

6.2.9. Statistical Analysis

Standard descriptive statistics were used to describe participant flow, as well as demographic, reproductive, and lifestyle risk factors. Mean and SD were used to describe the distribution of normally-distributed continuous variables. Student's T-tests were used to test for differences in distribution across arms, under the null hypothesis that there were no differences in distribution. Number and percentages were used for categorical and ordinal variables, and Fisher's Exact tests were used to compare the distributions of these variables across intervention arms.

Time-dependent variables (including anthropometry, physical activity, and dietary intake variables) and mammographic acquisition variables were not normally distributed. For these variables, the distributions were described by median and IQR (75^{th} percentile – 25^{th} percentile). The Kruskal-Wallis test was used to test for differences in distribution of these variables, under the null hypothesis that there were no differences in distribution across the intervention arms. The Wilcoxon-rank-sum test were used to test for within-woman differences across the intervention period.

Linear regression models were used to test for difference in mammographic density at 12 months among the study arms, whilst accounting for mammographic density at baseline and within-woman differences in compression force. Volume-based mammographic density measures were $\log_{10}$-transformed and area-based mammographic density measures were square-root transformed to meet the assumption for normality of residuals. Linear regression assumptions, such as linearity and normality of the outcome, were assessed visually using residual plot. For ease of interpretation, the coefficients from the models were transformed back to original scale and centered to the median value of mammographic density in the Control arm using the formula, $\Delta = (\exp(\beta) \times w) - w$ for volume-based measures and $\Delta = 2 \beta \sqrt{w} + \beta^2$, where Δ is the difference in 12-month mammographic density compared to the Control arm, β is the regression coefficient and w is the median mammographic density of the Control arm.

Intention-to-treat analysis included all participants who were randomized and for whom outcome data was available, regardless of compliance to treatment. Restricted analyses were subsequently carried out to test for internal validity of the analysis. This includes per protocol analysis (excluding women who discontinued treatment during the study period) and modified per-protocol analysis (further restricting the analysis to women

who were compliant to the study intervention, which was defined as consuming at least 80% of the supplements or dietary soy requirements in the intervention arms, or consuming less than 40mg/day isoflavones in the Control arm over the study period).

In exploratory analysis was, variables of interest (body measurement and dietary variables that appear to change over time, using a lenient threshold of *p* value < 0.100) were added to multivariable linear regressions to assess for interaction or effect modification of the primary effect, which is the differences in mammographic density at 12 months by study arm.

All statistical analyses were conducted using the R Statistical Environment (v4.0.3). All hypothesis testing was two-sided, and *p* value < 0.05 was considered as statistically significant.

6.2.10. Sample size calculation

In *apriori* sample size calculation, a total of 270 women (90 women per arm) was required to detect a change in percent density by 3.5% in the intervention arms at 80% power, compared to an expected 1% change in the control arm over 1 year (206). The effect size was estimated based on the largest effect observed in an RCT of green tea on annual mammographic density change (149). This sample size calculation takes into consideration a 5.0% standard deviation of percent density change, the probability of type 1 error (α=0.05), two-sided testing, and Bonferroni correction for multiple comparisons. The estimated sample size also accounts for a potential 15% drop out rate (99,149).

Post-hoc power calculations were also conducted to determine statistical power in the analysis, based on the actual analytical plan, sample size and observed distributions of volume-based and area-based analysis. This analysis was conducted using the G*Power software (version 3.1.9.7), using one-way ANOVA omnibus F test to compare mean 12-month mammographic density across 3 groups, with standard probability of type 1 error (α=0.05).

6.3. Results

6.3.1. Participation and follow up

Between November 2018 and August 2019, 128 women were screened for eligibility (**Figure 6-2**). Of this, 47 (36.7%) women did not meet the criteria for eligibility. Eighty-one women were enrolled into the study and were randomized into the three study arms.

Twenty-four women who were randomized did not complete the study (29.6%, **Table 6-3**). While the the Supplement arm observed the highest rate of lost to follow up (39.3% compared to 25.9% in the Dietary Soy arm and 23.1% in the Control arm), this difference was not statistically significant (*p* value=0.402). The most common reason for loss to follow up was the onset of adverse events and the Covid-19 pandemic (n=10, 41.7%, respectively), followed by lack of interest (n=4, 16.6%). The onset of adverse events was most commonly reported in the Supplement arm (54.5%), whereas in the Dietary arm, lack of interest was also a common reason (42.9% respectively). In the Control arm, participants were lost to follow up mainly because they were not willing to attend 12-month assessments during the Covid-19 pandemic (66.6%).

Among the 57 women who completed the study, 6 women in the Supplement arm (23%) and 2 women in the Dietary Soy arm (8%) discontinued the intervention during the study period (**Figure 6-2**). The 2 women in the Dietary Soy arm accepted the option to switch to a low dose supplement intervention (50mg/day).

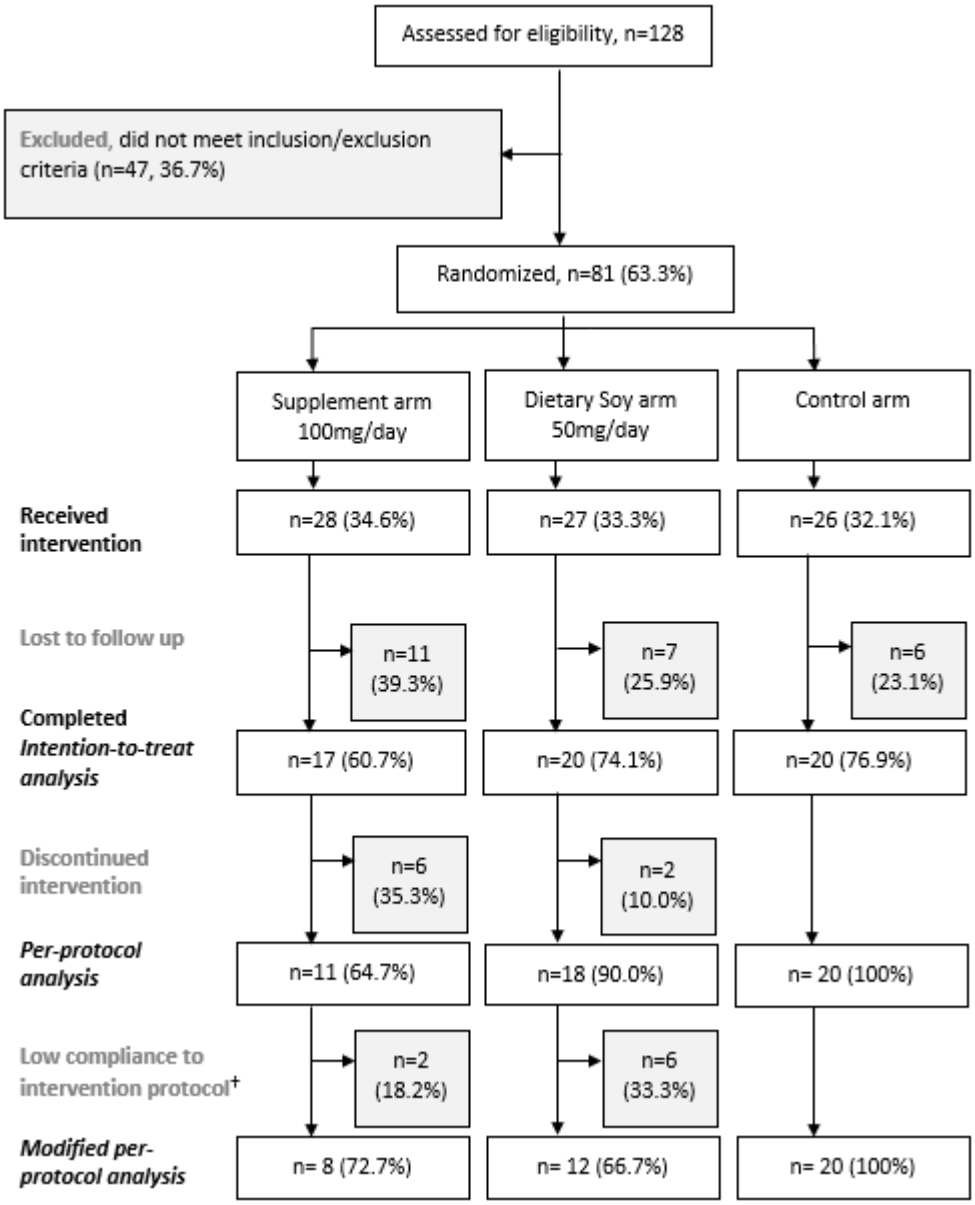


Figure 6-2 CONSORT flow diagram for participant enrolment and follow up.
†Low compliance is defined at study completion (Month 12) as <80mg/day of isoflavones in the Supplement arm, <40mg/day in the Dietary Soy arm, and ≥40mg/day in the Control arm.

6.3.2. Follow up and compliance to the intervention

The duration of intervention for participants who were lost to follow up and who discontinued the intervention is presented in Figure 6-3. Most women who were lost to follow up did so after 6 months in the study, while 4 women were lost after Month 3 follow up and 2 were lost right after enrolment. On the other hand, women who discontinued intervention (but completed final study assessments) did so within 6 months, with 2 women discontinuing intervention after enrolment.

Table 6-3 Participants who were lost to follow up (n=81)

Reasons	Overall (n=81)	Distribution by study arm			
		Supplements (n=28)	Dietary Soy (n=27)	Control (n=26)	
	n (%)	n (%)	n (%)	n (%)	*p* value
Total lost to follow up	24 (29.6)	11 (39.3)	7 (25.9)	6 (23.1)	0.402
Primary reasons:					
Adverse events	10 (41.7)	6 (54.5)	3 (42.9)	1 (16.7)	*0.084*
Covid-19 pandemic	10 (41.7)	5 (45.5)	1 (14.2)	4 (66.6)	
No longer interest	4 (16.6)	0 (0.0)	3 (42.9)	1 (16.7)	

Annotations: ***p* value < 0.001, **p* value < 0.05, n = number; % = column proportion.

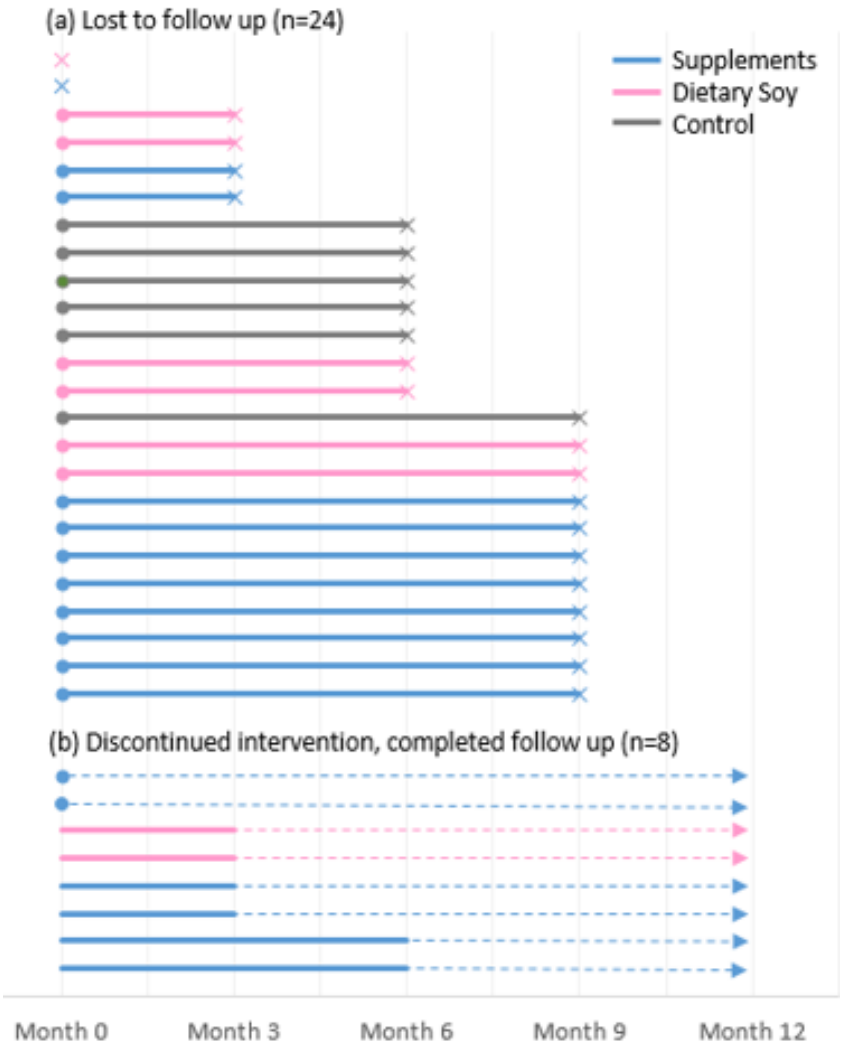


Figure 6-3 Duration of intervention for participants (a) who were lost to follow up and (b) who discontinued the intervention and completed follow up.

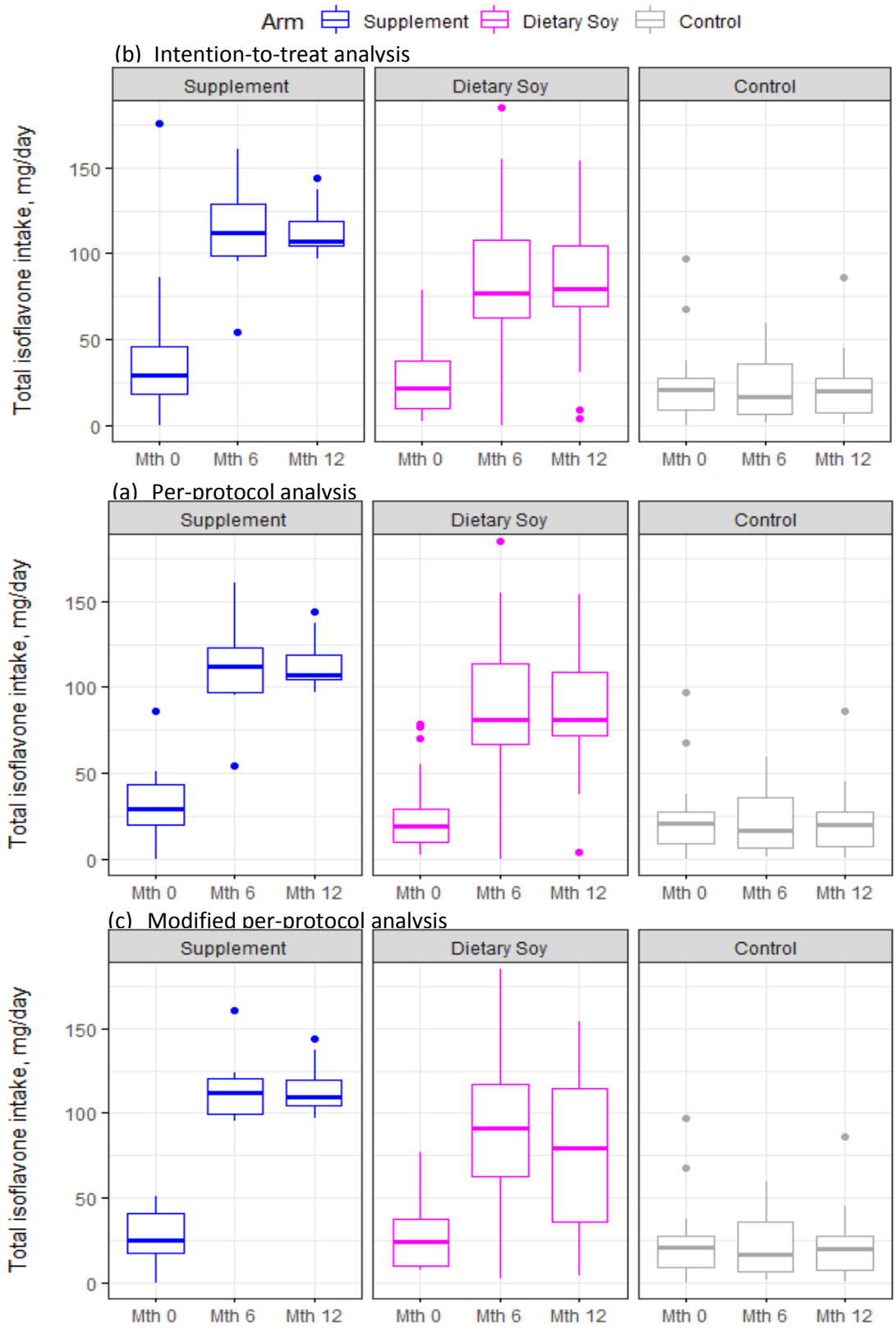


Figure 6-4 Boxplot representation of distribution of total isoflavones consumed through supplements and/or diet (mg/day)by study arm for (a) intention-to-treat analysis, (b) per-protocol analysis, and (c) modified per-protocol analysis.

Among the women who completed the study, there was good and consistent compliance over the study period in the study arms (**Figure 6-4**). There is a marked increase in total isoflavone intake in the Supplement arm (from supplements and diet) and the Dietary Soy arm (from diet alone), while isoflavone intake through diet remained low over time for the Control arm. Interestingly, there was much greater variation in isoflavone intake among women in the Dietary Soy arm, even in the modified per-protocol analysis which was restricted to compliant participants.

6.3.3. Participant characteristics

There were no significant differences in demographic, reproductive, or lifestyle risk factor distribution across the three arms for the intention-to-treat, per-protocol, and modified per-protocol analyses (**Table 6-4).** The average age of participants in the study were between 56-58 years old, and most women were post-menopausal at enrolment (88-95%). Between 65-83% of the women in the study were Chinese. It is important to note that there were more Indian women in the Supplement arm compared to the Diet arm and Control arm, but this was not statistically significant.

Table 6-4 Distribution of participant characteristics by study arm for the (a) intention-to-treat analysis (n=57), (b) per-protocol analysis (n=49), and (c) modified per-protocol analysis (n=40)

Characteristics	(a) Intention-to-treat				(b) Per-protocol			(c) Modified per-protocol		
	Supple-ments (n=17)	Dietary Soy (n=20)	Control (n=20)	p_{group}	Supple-ments (n=11)	Dietary Soy (n=18)	p_{group}	Supple-ments (n=8)	Dietary Soy (n=12)	p_{group}
Age in years, mean±SD	57.7±4.7	56.8±4.0	56.2±4.7	0.593	56.9±4.7	56.7±4.2	0.905	57.6±3.5	57.8±3.5	0.529
Ethnicity, n (%)										
Chinese	11 (64.7)	16 (80.0)	16 (80.0)	0.315	8 (72.7)	14 (77.8)	0.389	6 (75.0)	10 (83.3)	0.864
Indian	5 (29.4)	1 (5.0)	2 (10.0)		3 (27.3)	1 (5.6)		2 (25.0)	1 (8.3)	
Malay	1 (5.9)	3 (15.0)	2 (10.0)		0 (0)	3 (16.7)		0 (0)	1 (8.3)	
Post-menopause, n (%)	15 (88.2)	19 (95.0)	19 (95.0)	0.673	10 (90.9)	17 (94.4)	0.999	7 (87.5)	12 (100)	0.449
Menopause age[‡], mean±SD	50.4±4.1	51.1±3.2	49.8±2.8	0.504	48.9±4.3	51.0±3.2	0.277	49.5±3.7	50.9±3.7	0.607
Age at menarche, mean±SD	12.4±0.9	12.3±1.5	12.4±1.5	0.970	12.4±0.7	12.6±1.3	0.921	12.4±0.7	12.4±1.4	0.921
Live births[†], mean±SD	2.2±0.7	2.2±1.3	2.7±1.0	0.291	2.1±0.8	2.3±1.3	0.371	2.2±0.8	2.1±1.4	0.318
Nulliparous, n (%)	3 (17.6)	4 (20.0)	2 (10.0)	0.593	2 (18.2)	3 (16.7)	0.769	2 (25.0)	2 (16.7)	0.622
FH of breast cancer, n (%)	3 (17.6)	2 (10.0)	2 (10.0)	0.777	2 (18.2)	2 (11.1)	0.741	2 (25.0)	1 (8.3)	0.567
Education, n (%)										
Primary	1 (5.9)	1 (5.0)	0 (0)	0.843	1 (9.1)	1 (5.6)	0.533	1 (12.5)	1 (8.3)	0.283
Secondary	6 (35.3)	7 (35.0)	6 (30.0)		5 (45.5)	7 (38.9)		4 (50.0)	5 (41.7)	
Tertiary	9 (52.9)	12 (60.0)	14 (70.0)		5 (45.5)	10 (55.6)		3 (37.5)	6 (50.0)	
Monthly income, n (%)										
<RM5,000	9 (52.9)	9 (45.0)	7 (35.0)	0.351	8 (72.7)	8 (44.4)	0.448	7 (87.5)	5 (41.7)	0.207
RM5-10,000	5 (29.4)	3 (15.0)	5 (25.0)		1 (9.1)	3 (16.7)		0 (0)	2 (16.7)	
>RM10,000	2 (11.8)	7 (35.0)	8 (40.0)		2 (18.2)	6 (33.3)		1 (12.5)	4 (33.3)	
OC ever use, n (%)	5 (29.4)	9 (45.0)	8 (40.0)	0.655	3 (27.3)	8 (44.4)	0.698	2 (25.0)	4 (33.3)	0.907

Annotations: **p value < 0.001, *p value < 0.05, [†]among parous women, [‡]among post-menopausal women, x̄ = mean, SD = standard deviation, n = number; % = column proportion, FH = family history. For the distribution of the Control arm in analyses (b) and (c), please refer to the distribution in analysis (a).

Table 6-5 Distribution of time-dependent variables between the study arms and over time for the (a) intention-to-treat analysis (n=57), (b) per-protocol analysis (n=49), and (c) modified per-protocol analysis (n=40)

Characteristics	Distribution of time-dependent variables by study arm, median (IQR)									
	(a) Intention-to-treat				(b) Per-protocol			(c) Modified per-protocol		
	Supplements (n=17)	Dietary Soy (n=20)	Control (n=20)	p_{group}	Supplements (n=11)	Dietary Soy (n=18)	p_{group}	Supplements (n=8)	Dietary Soy (n=12)	p_{group}
Weight, kg										
Enrolment	61.2 (12.0)	59.0 (13.6)	57.1 (9.9)	0.725	59.9 (14.5)	58.0 (12.9)	0.845	60.5 (10.0)	57.8 (13.6)	0.502
12-months	61.5 (14.9)	58.1 (15.5)	58.2 (9.3)	0.902	61.5 (17.3)	57.0 (15.2)	0.897	61.8 (13.4)	56.6 (14.6)	0.531
p_{time}	0.979	0.313	0.165		0.894	0.500		0.944	0.470	
BMI, kg/m^2										
Enrolment	24.2 (5.1)	23.9 (3.5)	22.9 (5.2)	0.842	24.2 (5.7)	23.7 (3.5)	0.874	25.5 (4.9)	23.7 (2.8)	0.693
12-months	23.6 (5.6)	23.4 (5.8)	23.6 (5.1)	0.993	23.4 (6.4)	23.1 (5.4)	0.956	24.7 (5.5)	23.0 (4.3)	0.662
p_{time}	0.782	0.261	0.177		0.700	0.325		0.641	0.380	
Waist-to-hip ratio										
Enrolment	0.85 (0.06)	0.86 (0.06)	0.85 (0.05)	0.457	0.83 (0.06)	0.87 (0.06)	0.134	0.82 (0.07)	0.87 (0.07)	0.287
12-months	0.83 (0.10)	0.86 (0.09)	0.88 (0.08)	0.631	0.82 (0.06)	0.85 (0.09)	0.389	0.85 (0.07)	0.84 (0.10)	0.889
p_{time}	0.818	0.812	*0.090*		0.700	0.799		0.382	0.791	
Physical activity, MET-hours/week										
Enrolment	10.0 (11.0)	13.8 (22.2)	10.0 (9.6)	0.157	7.5 (12.0)	13.7 (20.0)	*0.092*	7.5 (13.0)	10.0 (20.0)	0.630
12-months	7.5 (7.0)	11.5 (10.0)	11.2 (10.0)	0.690	10.5 (6.5)	8.5 (10.0)	0.833	9.0 (6.0)	11.5 (10.0)	0.811
p_{time}	0.977	0.102	*0.066*		0.722	*0.070*		0.553	0.755	

Annotations: **p value < 0.001, *p value < 0.05, p_{group} = p value from Kruskal-Wallis test for between group differences, p_{time} = p value from paired Wilcoxon-rank-sum test for within-woman differences. For the distribution of the Control arm in analyses (b) and (c), please refer to the distribution for the Control arm in analysis (a).

Table 6-6 Changes in energy, macronutrient and isoflavone intake over the study period for the (a) intention-to-treat analysis (n=51), (b) per-protocol analysis (n=45), and (c) modified per-protocol analysis (n=38)

Intake variable	Distribution of average daily intake over time, median (IQR)									
	(a) Intention-to-treat				(b) Per-protocol			(c) Modified per-protoco		
	Supple-ments (n=13)	Dietary Soy (n=19)	Control (n=19)	p_{group}	Supple-ments (n=9)	Dietary Soy (n=17)	p_{group}	Supple-ments (n=7)	Dietary Soy (n=12)	p_{group}
Calorie (kCal)										
Enrolment	1333.5 (502.1)	1598.9 (679.8)	1552.5 (615.7)	0.871	1278.0 (285.1)	1546.4 (798.2)	0.515	1278.0 (203.0)	1632.7 (1025.7)	0.447
6-months	1352.7 (1063.5)	1472.7 (829.1)	1303.7 (916.4)	0.703	1048.5 (1354.0)	1472.7 (1037.3)	0.182	1048.5 (718.9)	1337.5 (683.2)	0.366
12-months	1557.4 (572.3)	1738.1 (759.8)	1580.5 (947.3)	0.862	1535.3 (650.8)	1738.1 (838.3)	0.469	1535.3 (449.3)	1770.9 (775.7)	0.511
p_{time} ‡	0.636	0.418	0.768		0.359	0.120		0.297	0.569	
Carbohydrate (g)										
Enrolment	219.2 (91.2)	272.0 (115.2)	244.7 (137.0)	0.885	178.3 (77.9)	265.0 (110.1)	0.573	178.3 (62.5)	268.5 (153.6)	0.493
6-months	223.3 (229.8)	233.6 (116.5)	232.2 (134.6)	0.739	162.3 (235.3)	234.4 (131.9)	0.296	162.3 (154.5)	208.4 (90.8)	0.589
12-months	242.4 (132.6)	240.3 (88.3)	270.4 (183.9)	0.818	234.6 (147.6)	240.3 (87.6)	0.882	234.6 (103.9)	232.1 (114.6)	0.938
p_{time}	0.216	0.829	0.798		0.203	0.712		0.219	0.999	

Data missing for 6 participants. *Annotations:* **p value < 0.001, *p value < 0.05, p_{group} = p value from Kruskal-Wallis test for between group differences, p_{time} = p value from paired Wilcoxon-rank-sum test for within-woman differences. For the distribution of the Control arm in analyses (b) and (c), please refer to the distribution in analysis (a).

Table 6-6 (cont'd) Changes in energy, macronutrient and isoflavone intake over the study period for the (a) intention-to-treat analysis (n=51), (b) per-protocol analysis (n=45), and (c) modified per-protocol analysis (n=38)

Intake variable	Distribution of average daily intake over time, median (IQR)									
	(a) Intention-to-treat analysis (n=51)				(b) Per-protocol analysis (n=45)			(c) Modified per-protocol analysis (n=38)		
	Supple-ments (n=13)	Dietary Soy (n=19)	Control (n=19)	p_{group}	Supple-ments (n=9)	Dietary Soy (n=17)	p_{group}	Supple-ments (n=7)	Dietary Soy (n=12)	p_{group}
Fat (g)										
Enrolment	51.7 (21.2)	43.0 (25.9)	49.7 (14.0)	0.704	51.7 (19.7)	43.0 (26.6)	0.703	51.7 (21.1)	42.4 (30.4)	0.687
6-months	55.5 (26.0)	47.3 (28.6)	48.6 (34.9)	0.816	35.0 (28.1)	47.3 (28.7)	0.363	35.0 (26.5)	28.1 (44.7)	0.547
12-months	49.4 (45.3)	62.3 (20.2)	51.3 (31.1)	0.657	40.0 (20.8)	62.3 (20.0)	0.324	40.0 (21.4)	62.4 (24.3)	0.526
p_{time}	0.244	*0.066*	0.709		0.301	0.020*		0.688	0.470	
Protein (g)										
Enrolment	63.0 (26.4)	63.5 (19.9)	62.0 (18.9)	0.984	63.0 (13.2)	61.1 (22.7)	0.840	63.0 (16.0)	69.4 (53.8)	0.741
6-months	59.7 (45.5)	72.6 (26.9)	60.4 (41.2)	0.268	42.1 (35.7)	75.7 (32.3)	*0.071*	42.1 (31.2)	69.7 (26.3)	0.308
12-months	68.3 (50.7)	83.3 (25.3)	67.7 (35.7)	0.210	51.5 (22.9)	83.3 (26.9)	*0.057*	51.5 (22.8)	81.6 (32.8)	0.214
p_{time}	0.946	0.036*	0.768		0.99	0.005*		0.999	0.301	
Isoflavone (mg)										
Enrolment	29.6 (27.8)	21.6 (27.4)	20.6 (18.4)	0.330	29.6 (24.0)	19.5 (19.5)	0.413	25.4 (23.6)	23.9 (27.5)	0.592
6-months	14.6 (22.9)	77.1 (45.0)	16.8 (29.2)	<0.001**	19.0 (19.4)	81.1 (46.7)	<0.001**	17.1 (19.0)	90.9 (53.8)	<0.001**
12-months	18.9 (25.9)	79.7 (35.9)	20.2 (19.9)	<0.001**	18.9 (19.8)	80.8 (36.9)	<0.001**	13.9 (15.8)	79.5 (78.6)	0.004*
p_{time}	0.031*	<0.001**	0.956		*0.067*	<0.001**		*0.055*	0.009*	

6.3.4. Distribution of variables over time

Table 6-5 shows that there were no significant differences in the distribution of time-dependent variables across the study groups and over time for the three datasets for analysis. For intention-to-treat analysis, it appears that BMI at enrolment was lower in the Control arm (22.9kg/m^2) compared to the Dietary Soy arm (23.9kg/m^2) and Supplement arm (24.2kg/m^2), but this was not statistically significant (*p* value = 0.843). This trend was similarly observed in the per-protocol and modified per-protocol datasets.

WHR was similar at enrolment. Among women in the Control arm, however, there appears to be a marginal increase in WHR over 12 months, from 0.85 to 0.88 units (*p* value = 0.090, intention-to-treat analysis). Physical activity, as measured in MET hours/week, was non-significantly higher at enrolment for the Dietary Soy arm (13.8 units), compared to the Supplement arm or Control arm (10.0 units, *p* value = 0.157). Interestingly, women in the Control arm experienced a slight increase in physical activity level, to 11.2 units (*p* value = 0.066), while women in the Dietary soy arm experienced a decrease in physical activity levels over time, particularly in the per-protocol dataset (*p* value=0.070)

6.3.5. Dietary changes over time

Table 6-6 shows the changes in diet over the study period, by study arm for the three datasets for analysis. At enrolment, there were no significant differences in calorie and macronutrient intake across the study arms. Average calorie intake in this cohort was between 1333-1633 kCal/day at baseline, while carbohydrate intake was between 178-272 g/day. These variables did not change significantly over time, and were similar across datasets.

At enrolment, protein intake was between 62-69 g/day and fat intake was between 43-52 g/day. Over the study period, protein intake increased significantly for women in the Dietary Soy arm, from 61-69 g/day to 82-83 g/day, likely due to increase in dietary soy intake. This increase was statistically significant in the per-protocol dataset (*p* value = 0.005). Unexpectedly, there was a marginal increase in fat intake in the Dietary Soy arm, from 43 g/day to 62 g/day, which was more marked in the per-protocol analysis (*p* value = 0.020. Protein and fat intake did not change significantly in the other study arms.

At enrolment, the average intake of soy isoflavones from diet was between 20-30mg/day, approximately one cup of soymilk or less (**Table 6-6**). There were no significant

differences in daily soy isoflavone intake at enrolment across the three datasets. Women in the Dietary Soy arm reported a great increase in isoflavone intake through diet, up to 91mg/day soy isoflavones at 6 months and 81mg/day at 12 months. On the other hand, women in the Supplement arm experienced a significant decrease in daily soy isoflavone intake (by 10-12 mg/day), while there were no differences in isoflavone intake in the Control arm (*p* value = 0.956). This data shows that there was good compliance in the Dietary Soy arm, whilst women in the Supplement and Control arm did not increase dietary soy intake over the 12 months, as instructed.

6.3.6. Differences in mammogram acquisition parameters over time

Table 6-7 Distribution of mammogram acquisition parameters over time (n=55)

Mammogram acquisition parameters over time	**Distribution by study arm**			
	Supplements (n=16)	**Dietary Soy (n=19)**	**Control (n=20)**	
	Median (IQR)	Median (IQR)	Median (IQR)	p_{group}
Duration (months)	12.4 (2.0)	12.5 (1.2)	12.5 (1.4)	0.957
Compression force (N)				
Enrolment	95.6 (17.2)	100.1 (27.2)	105.7 (26.7)	0.251
12-months	84.6 (15.6)	89.0 (18.9)	93.4 (16.1)	0.627
			p_{time}	0.004*
Applied pressure (kPa)				
Enrolment	10.9 (5.8)	9.1 (4.3)	9.8 (5.4)	0.555
12-months	9.2 (2.8)	9.0 (2.7)	9.2 (3.5)	0.930
			p_{time}	0.001*
Compressed breast thickness (mm)				
Enrolment	59.0 (11.8)	60.0 (15.0)	53.0 (16.2)	0.808
12-months	59.0 (12.0)	57.0 (12.0)	60.5 (16.5))	0.558
			p_{time}	0.697
Paddle tilt (mm)				
Enrolment	14.2 (6.4)	12.9 (11.4)	13.7 (6.2)	0.876
12-months	11.4 (8.7)	11.9 (4.4)	12.1 (6.6)	0.964
			p_{time}	0.021*

Data missing for 2 participants. *Annotations:* ***p* value < 0.001, **p* value < 0.05, p_{group} = *p* value from Kruskal-Wallis test for between group differences, p_{time} = *p* value from paired Wilcoxon-rank-sum test for within-woman differences.

Table 6-7 shows that there was no significant difference in the duration of study across study arms, which was 12.5 months on average (*p* value = 0.957). While there were

no significant differences in mammogram acquisition parameters by study arm, there were significant within-women differences over time.

Specifically, compression force, applied pressure, and paddle tilt were significantly lower at the 12-month mammogram (*p* value =0.004, 0.001, and 0.021, respectively). Since compression force is a determinant of applied pressure and paddle tilt, it will be considered as a confounding variable in primary analysis. There were no differences in compressed breast thickness over time.

6.3.7. Primary analysis for volume-based mammographic density

At enrolment, women in the Supplement arm appear to have lower dense volume (49.4cm^3 vs 50.5cm^3 in the Dietary Soy arm and 53.0cm^3 in the Control arm, *p* value = 0.712), but higher percent density (8.8% vs 7.2% and 6.9%, respectively, *p* value =0.521, **Table 6-8**). Dense volume decreased by 1.3-1.9cm^3 over the study period, and there were no differences by study arm in unadjusted analysis (*p* value = 0.759).

Table 6-8 Unadjusted distribution of volume-based mammographic density over the study period (n=54)

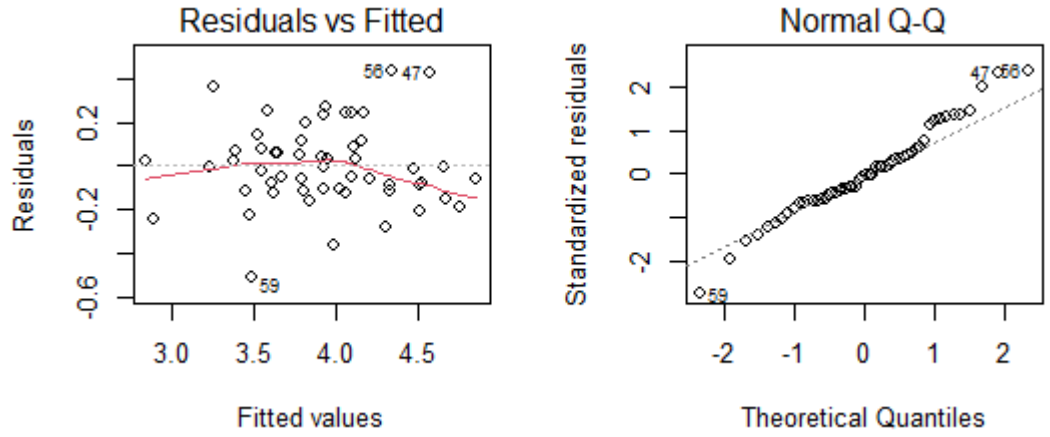
Study time point	Distribution of mammographic density by study arm			
	Supplements (n=16)	**Dietary Soy (n=19)**	**Control (n=19)**	
	Median (IQR)	Median (IQR)	Median (IQR)	p_{group}
Dense volume (cm^3)				
Month 0	49.4 (19.3)	50.5 (38.2)	53.0 (29.2)	0.712
Month 12	50.2 (30.8)	53.3 (27.2)	52.2 (38.1)	0.794
Absolute change (cm^3)	-1.9 (16.7)	-1.3 (7.4)	-1.8 (8.8)	0.759
Percent density (%)				
Month 0	8.8 (2.8)	7.2 (5.4)	6.9 (6.2)	0.521
Month 12	7.7 (3.0)	7.5 (4.6)	6.3 (6.9)	0.681
Absolute change (%)	0.1 (2.4)	-0.4 (1.0)	-0.4 (1.5)	0.867

Data missing for 3 participants: missing raw mammogram image (n=2), errors in volume-based mammographic density measurement (n=1). *Annotations:* ***p*** value < 0.001, ***p*** value < 0.05, p_{group} = *p* value from Kruskal-Wallis test for between group differences

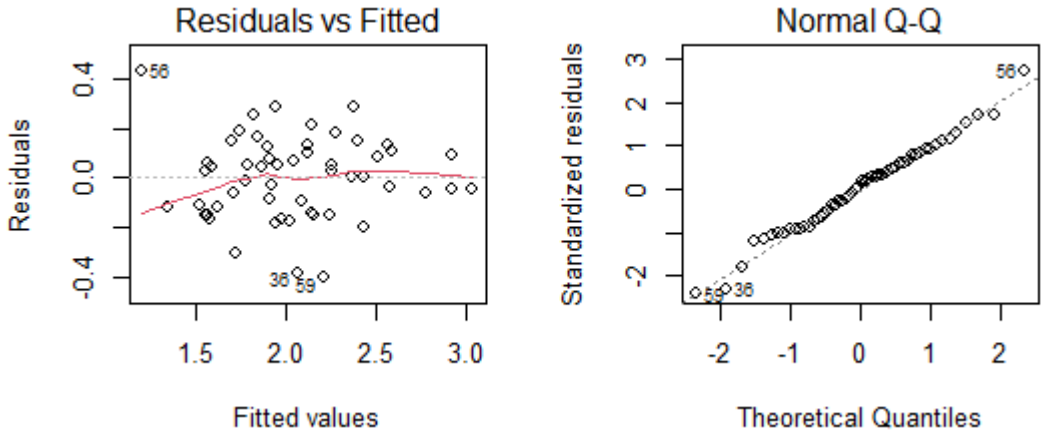
Table 6-9 Intention-to-treat analysis for volume-based mammographic density (n=54)

Type of measure	Difference in mammographic density at Month 12, compared to the Control arm			
	Supplement (n=16)		Dietary Soy (n=19)	
	Δ [95% CI]	p_{group}	Δ [95% CI]	p_{group}
Dense volume (cm^3)				
Null model	-2.7 [-17.0, 17.2]	0.751	-1.8 [-15.8, 17.5]	0.826
+ Baseline dense volume	2.7 [-4.2, 10.5]	0.457	-0.1 [-6.3, 7.0]	0.978
+ Compression force difference	3.7 [-3.3, 11.8]	0.311	1.1 [-5.4, 8.5]	0.752
Percent density (%)				
Null model	0.6 [-1.2, 3.0]	0.574	0.0 [-1.6, 2.2]	0.960
+ Baseline dense volume	0.1 [-0.6, 0.8]	0.891	0.0 [-0.7, 0.7]	0.968
+ Compression force difference	0.0 [-0.7, 0.8]	0.910	0.0 [-0.7, 0.7]	0.946

Annotations: **p value < 0.001, *p value < 0.05, p_{group} = p value from linear regression models, Δ = Mean-centered difference in mammographic density compared to the Control arm.



(a) Dense volume



(b) Volume-based percent density

Figure 6-5 Assessment of linearity and normality of residuals in primary intention-to-treat analysis for (a) dense volume or (b) volume-based percent density. The assumption for linearity is met if the red line of the "Residuals vs Fitted" plot is approximately horizontal. The assumption for normality of residuals is met if there are no large deviations from the diagonal line in the "Normal Q-Q" plot.

Linear regression models were used to test for differences in mammographic density measures after 12 months across the three intervention groups. As shown in **Figure 6-5**, the assumptions for linearity and normality of residuals for the analysis of $\log_{10}$ volume-based mammographic density measures were met.

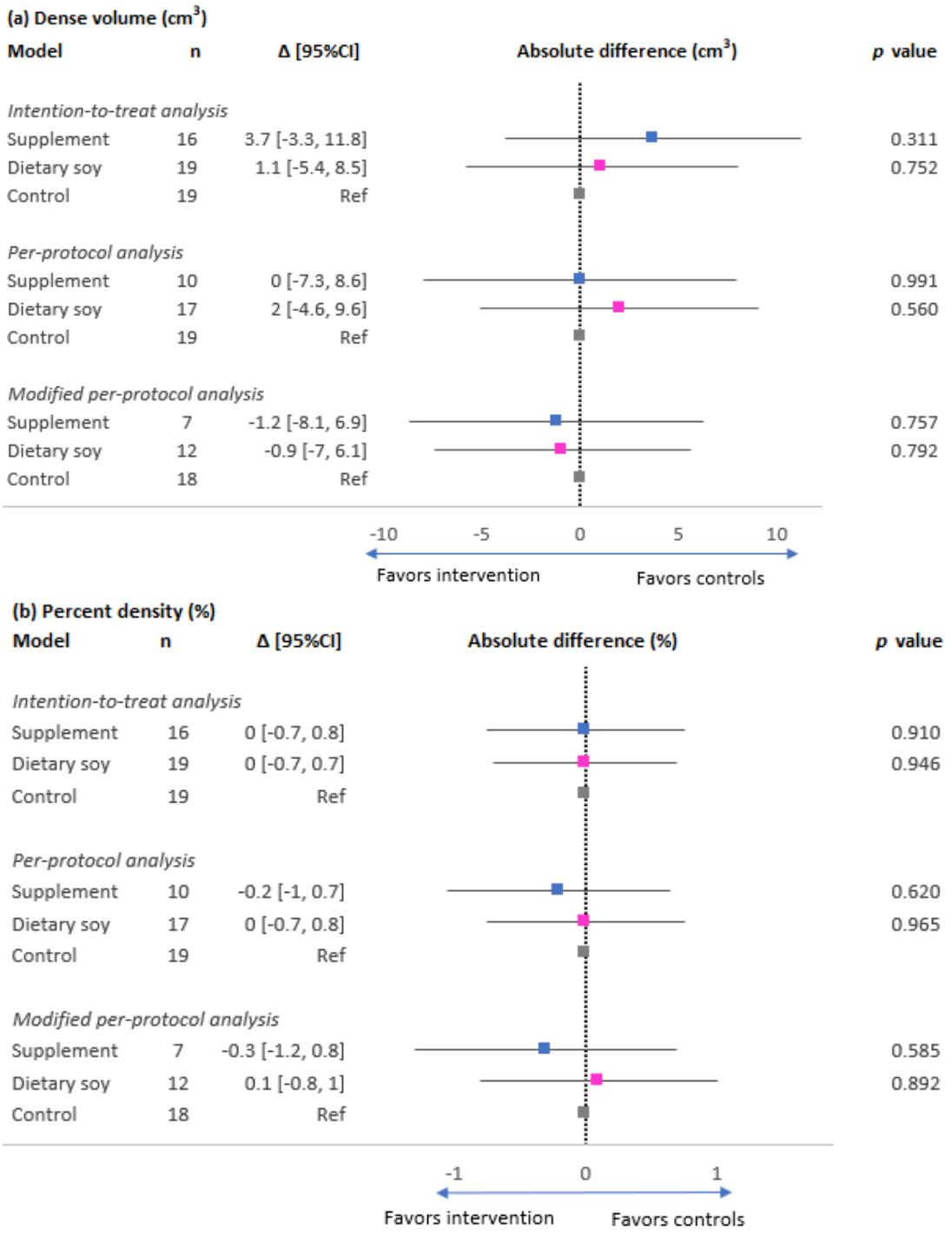


Figure 6-6 Intention-to-treat and per protocol analyses for (a) dense volume and (b) volume-based percent mammographic density. All models were adjusted for baseline mammographic density and difference in compression force.

In adjusted intention-to-treat analysis, there were no significant difference in mammographic density after 12 months of intervention across the study arms (**Table 6-9**). Dense volume at 12 months was higher in the Supplement arm (by 3.7, 95% CI = [-3.3, 11.8]) and Dietary soy arm (by 1.1, 95% CI = [-5.4, 8.5]), compared to the Control arm, but the results were not statistically significant.

The per-protocol analysis did not lead to large changes in the magnitude or direction of effect size. In modified per protocol analysis, on the other hand, women in the Supplement arm observed 1.2cm^3 lower dense volume (95% CI = [-8.1, 6.9]) compared to the Control arm, while women in the Dietary arm observed 0.9cm^3 lower dense volume (95% CI = [-7.0, 6.1], **Figure 6-6**). Interestingly, for percent density measures, inverse associations were only observed for women in the Supplement arm (by -0.3, 95% CI = [-1.2, 0.8]), and not for women in the Dietary Soy arm (by 0.1, 95% CI = [-0.8, 1.0]). All these results were however not statistically significant

6.3.8. Exploratory analyses for volume-based mammographic density

Figure 6-7 shows the subgroup analysis for BMI, WHR, and dietary fat intake at 12 months. Overall, there were no significant associations between intervention arm and mammographic density at 12 months in subgroup analyses, and no significant interactions were observed for BMI, WHR, and dietary fat intake. However, as a hypothesis generating exercise, some interesting findings were noted.

An inverse association was observed in the Dietary Soy arm for women with BMI greater than 25kg/m^2 (-2.4cm^3, 95% CI = [-12.6, 10.6]). Among women with lower BMI, however, positive associations were noted ($p_{interaction}$ = 0.363). Similar observations are observed when the analysis was stratified by WHR ($p_{interaction}$ = 0.265).

Interestingly, women in the Supplement arm observed 4.8cm^3 lower dense volume compared to women in the Control arm (95%CI = [-13.6, 6.0]) when the analysis was restricted to women with low dietary fat intake. This association was not observed for women with high fat intake ($p_{interaction}$ = 0.121) nor for women in the Dietary Soy arm ($p_{interaction}$ = 0.767).

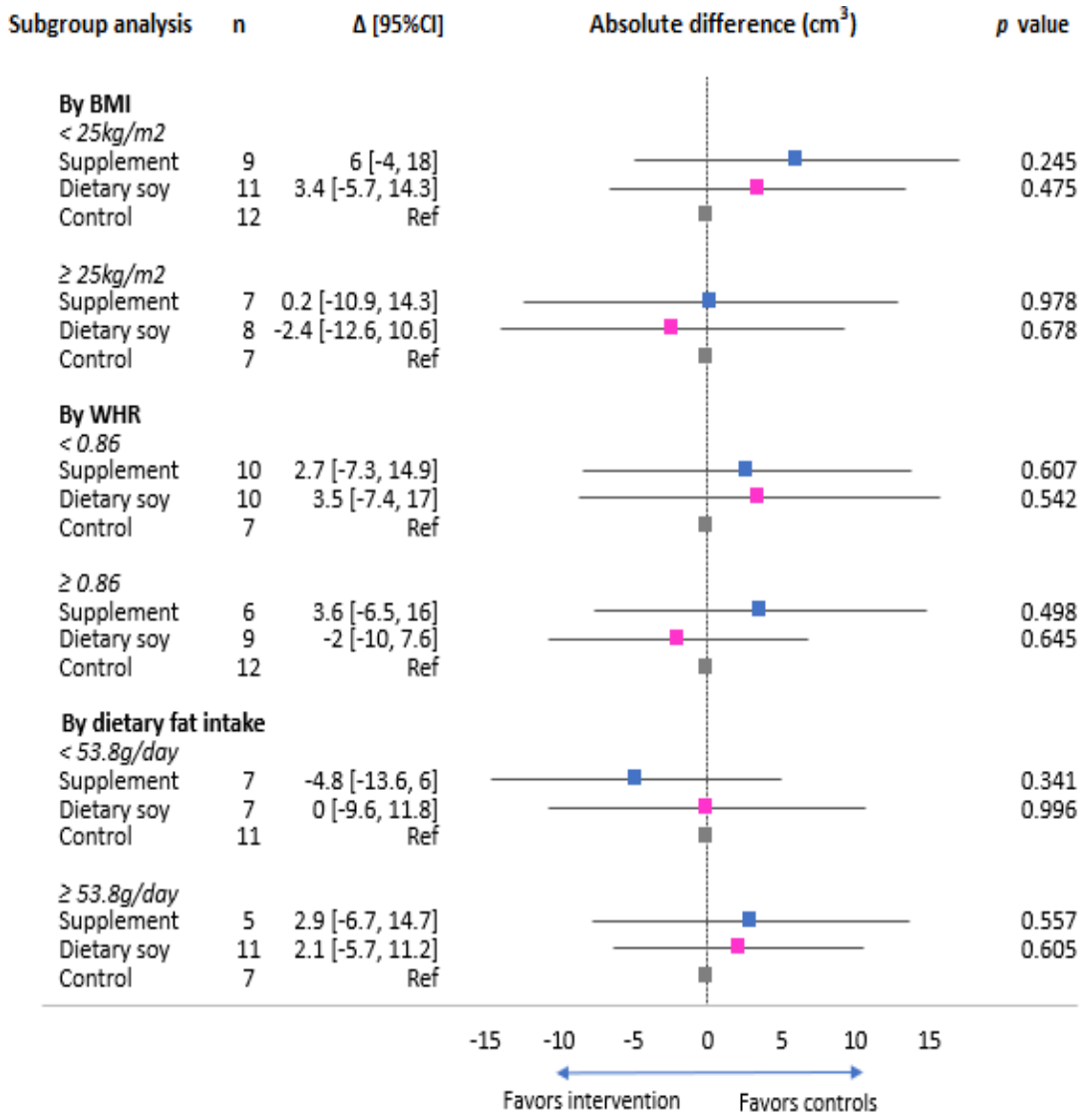


Figure 6-7 Linear regression analysis for volume-based measures, stratified by BMI, WHR, and dietary fat intake at 12 months. All models were adjusted for baseline mammographic density and difference in compression force.

6.3.9. Primary analysis for area-based mammographic density

Table 6-10 shows that there were no statistically significant differences in distribution of area-based mammographic density at enrolment. Notably, women in the Dietary Soy arm appear to have lower dense area (10.3cm^2 vs 16.1cm^2 in the Supplement arm and 17.5cm^2 in the Control arm, *p* value = 0.401) and percent density (7.6% vs 12.7% and 14.7%, respectively, *p* value = 0.367). The absolute change in dense area and percent density was small and there were no differences across the study arms in unadjusted analysis (*p* value = 0.741 and 0.826, respectively).

Table 6-10 Unadjusted distribution of area-based mammographic density over the study period (n=45)

Study time point	Distribution of mammographic density by study arm			
	Supplements (n=13)	**Dietary Soy (n=16)**	**Control (n=16)**	
	Median (IQR)	Median (IQR)	Median (IQR)	p_{group}
Dense area (cm^2)				
Month 0	16.1 (14.4)	10.3 (13.1)	17.5 (21.0)	0.401
Month 12	15.9 (7.3)	12.0 (13.9)	19.5 (20.7)	0.419
Absolute change (cm^2)	1.1 (7.3)	1.0 (10.5)	0.1 (3.9)	0.741
Percent density (%)				
Month 0	12.7 (14.7)	7.6 (13.6)	14.7 (19.7)	0.367
Month 12	15.5 (8.3)	10.2 (15.8)	13.3 (18.0)	0.547
Absolute change (%)	0.5 (7.2)	1.0 (7.2)	-0.9 (3.1)	0.826

Data missing for 12 participants: missing raw mammogram image (n=2), errors in volume-based mammographic density measurement (n=4), not measured on Stratus (n=6). *Annotations:* ***p*** value < 0.001, ****p*** value < 0.05, p_{group} = *p* value from Kruskal-Wallis test for between group differences.

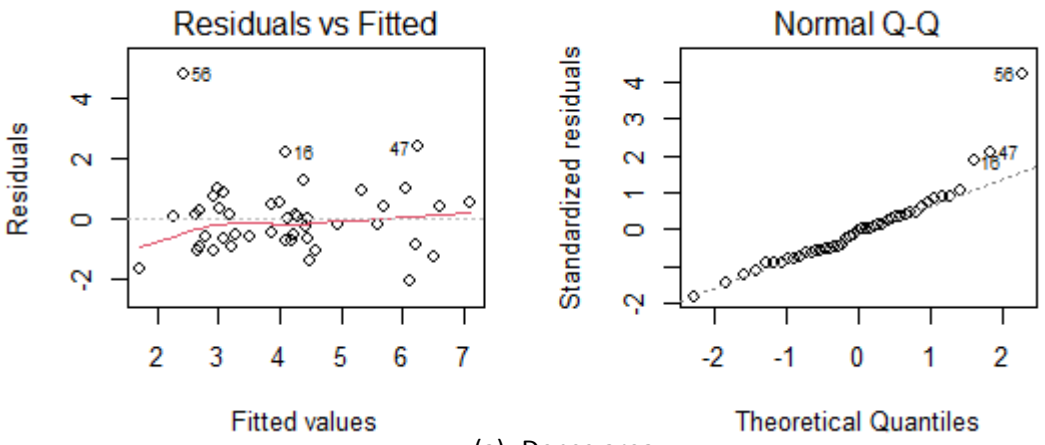
Linear regression models were used to test for differences in mammographic density measures after 12 months across the three intervention groups. As shown in **Figure 6-8**, the assumptions for linearity and normality of residuals for the analysis of square-rooted-based mammographic density measures were met. There appears to be one possible outlier in the dense area analysis.

Overall, there were no statistically significant differences in area-based mammographic density at 12 months across study arms in adjusted analysis (**Table 6-11**). However, women in the Supplement arm 4.1 lower dense area (95% CI = [-10.5, 4.1]) compared to the Control arm. Similar to the associations observed for dense area, women in the Supplement arm observed the lower percent density (by -2.4, 95% CI = [-6.4, 2.6]) in fully adjusted analysis. For women in the Dietary soy arm, strong inverse associations observed in the null model attenuated after accounting for the distribution of dense area at enrolment. This was observed for both area-based mammographic density measures.

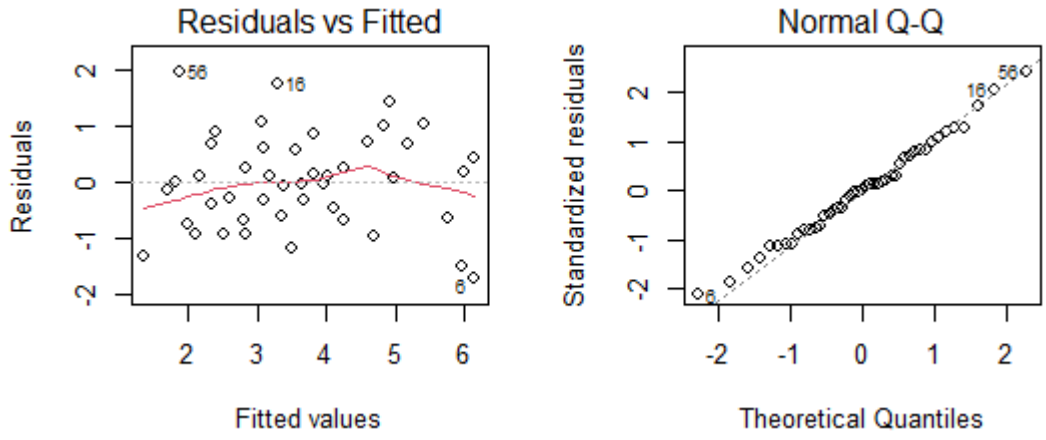
Table 6-11 Intention-to-treat analysis for area-based mammographic density (n=45)

Type of measure	Difference in mammographic density at Month 12, compared to the Control arm			
	Supplement (n=13)		Dietary Soy (n=16)	
	Δ [95% CI]	p_{group}	Δ [95% CI]	p_{group}
Dense area (cm^2)				
Null model	-3.7 [-12.4, 8.6]	0.507	-6.6 [-14.0, 3.9]	0.187
+ Baseline dense volume	-4.8 [-11.0, 3.2]	0.211	-2.7 [-9.2, 5.4]	0.476
+ Compression force difference	-4.1 [-10.5, 4.1]	0.290	-1.4 [-8.4, 7.2]	0.719
Percent density (%)				
Null model	-1.4 [-8.1, 8.1]	0.743	-4.4 [-9.8, 3.5]	0.237
+ Baseline dense volume	-3.0 [-7.0, 1.9]	0.211	-1.5 [-5.6, 3.6]	0.535
+ Compression force difference	-2.4 [-6.4, 2.6]	0.320	-0.3 [-4.6, 4.9]	0.893

Annotations: **p value < 0.001, *p value < 0.05, p_{group} = p value from linear regression models, Δ = Mean-centered difference in mammographic density compared to the Control arm.



(a) Dense area



(b) Area-based percent density

Figure 6-8 Assessment of linearity and normality of residuals in primary intention-to-treat analysis for (a) dense volume or (b) volume-based percent density. The assumption for linearity is met if the red line of the "Residuals vs Fitted" plot is approximately horizontal. The assumption for normality of residuals is met if there are no large deviations from the diagonal line in the "Normal Q-Q" plot.

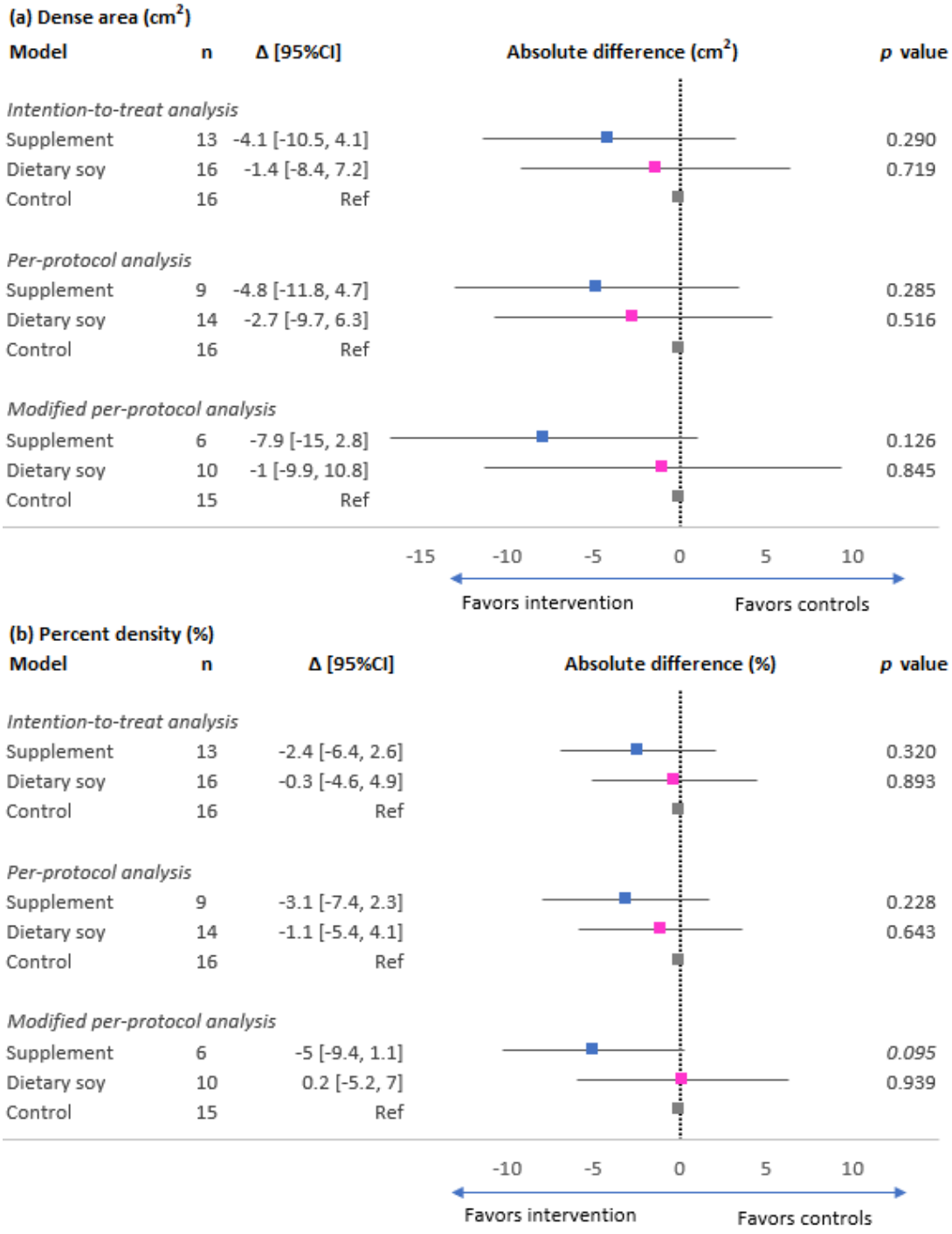


Figure 6-9 Intention-to-treat and per protocol analyses for (a) dense area and (b) area-based percent mammographic density. All models were adjusted for baseline mammographic density and difference in compression force.

Restricting the analysis to women who were compliant to the study intervention led to greater, albeit non-significant, effect sizes for the Supplement arm (**Figure 6-9**). In the modified per protocol analysis, women in the Supplement arm observed 7.9cm^2 lower dense area (95% CI = [-15.0, 2.8]) and 5.0% lower percent density (95% CI = [-9.4, 1.1]) compared

to women in the Control arm. It is important to note that the sample size available for this analysis was small (n = 6 in the Supplement arm).

6.3.10. Exploratory analyses for area-based mammographic density

Consistently, stronger inverse associations were observed for women in the Supplement arm or Dietary Soy arm when the analysis was conducted among women with higher BMI or higher WHR (**Figure 6-10**). However, the associations were not statistically significant in this small sample, and there were no significant interactions.

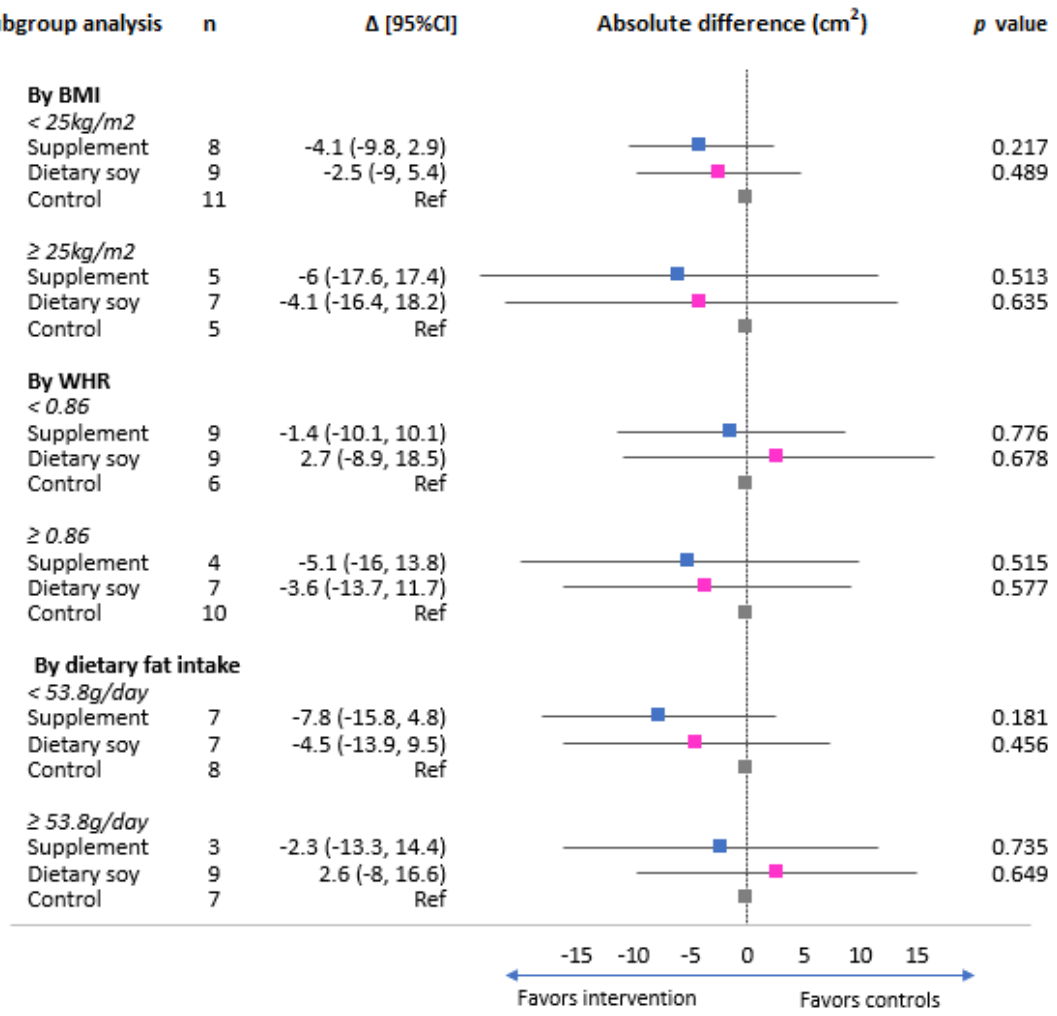


Figure 6-10 Linear regression analysis for area-based measures, stratified by BMI, waist-to-hip ratio, and dietary fat intake at 12 months. All models were adjusted for baseline mammographic density and difference in compression force.

Furthermore, inverse associations were observed when the analysis was restricted to women with low dietary fat intake, where dense area was lower in the Supplement arm by 7.8cm^2 (95% CI = [-15.8, 4.8]) and in the Dietary Soy arm by 4.5cm^2 (95% CI = [-13.9, 9.5]) Among women with high fat intake, on the other hand, weaker inverse associations were

noted in the Supplement arm, while weak positive associations were observed for women in the Dietary Soy arm intake ($p_{interaction}$ = 0.607).

6.3.11. Power calculation

A total of 81 women were enrolled between November 2018 and August 2019, excluding women who were not eligible or did not complete screening. After 30% of women were lost to follow up, 57 women were included in the primary analysis. In volume-based analysis, where only small differences were observed in intention-to-treat analysis (1.8-2.7cm^3), an analysis of 54 women only had 8% power to detect a true difference (**Table 6-12**). Statistical power was higher for area-based measures of 45 women, approximately 16%, to detect a difference of 3.7-6.6cm^2 between the intervention arms and Control arm.

If sample size were increased to 220 for area-based analysis, statistical power would be approximately 90%, assuming that the true effects are similar to the associations observed in modified per protocol analyses. For volume-based analysis, however, a larger sample size will be required, up to 330 women.

Table 6-12 Post-hoc power calculations

Sample size	**Power to detect a significant difference in mammographic density at Month 12 among the study arms (%)**			
	Dense volume analysis		**Dense area analysis**	
	Model 1	Model 2	Model 1	Model 2
45 (current, area-based)			15.7	26.9
54 (current, volume-based)	8.2	17.3	17.4	32.0
110	11.8	31.2	35.1	60.3
220	20.2	57.4	61.4	90.1
330	27.5	80.7	78.7	98.1
440	36.0	90.4	89.6	99.7

Annotations: Model 1 = statistical power is estimated using the effect size from the unadjusted analysis (null models), Model 2 = statistical power is estimated using the effect size from modified per protocol analysis, the standard deviation from unadjusted analyses are used in Model 1 and Model 2.

6.3.12. Adverse events

Almost half of the participants enrolled in the study reported at least one adverse event, and they were predominantly from the Supplement (75.0%) and Dietary Soy (70.4%) arms (**Table 6-13**). Specifically, women in the Supplement arm were more likely to report joint pains and numbness in the extremities (21.4%) compared to women in the Dietary Soy

arm and Control arm (*p* value = 0.036). Women in the Dietary Soy arm, on the other hand, were more likely to report gastrointestinal issues (22.2%), such as bloating, diarrhoea or constipation, compared to the other arms (*p* value = 0.028). There were three serious events in this study, including two cases of brain aneurysm rupture/stroke and one incidence of breast cancer.

Table 6-13 Adverse events reported during the study (n=81)

Adverse events	Overall (n=81)	Supplements (n=28)	Soy diet (n=27)	Control (n=26)	
	n (%)	n (%)	n (%)	n (%)	p_{group}
All events	44 (54.3)	21 (75.0)	19 (70.4)	4 (15.4)	<0.001**
Common cold/flu	11 (13.6)	6 (21.4)	3 (11.1)	2 (7.7)	0.362
Gastrointestinal complaints	9 (11.1)	3 (10.7)	6 (22.2)	0 (0)	0.028*
Joint pain/numbness	9 (11.1)	6 (21.4)	3 (11.1)	0 (0)	0.036*
Weight changes	6 (7.4)	2 (7.1)	4 (14.8)	0 (0)	0115
Increased blood indices†	6 (7.4)	2 (7.1)	2 (7.4)	2 (7.7)	0.999
Breast changes	2 (2.5)	0 (0)	1 (3.7)	1 (3.8)	0.542
Others	11 (13.6)	6 (21.4)	5 (18.5)	0 (0)	0.036*
Serious events					
Aneurysm rupture/stroke	2 (2.5)	2 (7.1)	0 (0)	0 (0)	
Breast cancer	1 (1.2)	1 (3.6)	0 (0)	0 (0)	

Annotations: †Measurement of glucose and uric acid in blood, n = number; % = column proportion, p_{group} = *p* value from Kruskal-Wallis test for between group differences.

6.4. Discussion

6.4.1. Summary of main findings

In this RCT of soy isoflavone intake on mammographic density, as a biomarker of breast cancer risk, women in the Supplement arm (100mg/day isoflavones) observed 4.1cm^2 lower dense area and 2.4% lower area-based percent density compared to women in the Control arm at 12 months. The associations were weaker for women in the Dietary Soy arm (50mg/day isoflavones). In contrary, volume-based analysis yielded positive associations for dense volume and no differences for volume-based percent density. Modified per protocol analysis suggests that strong inverse associations may be observed for both mammographic density measures if all women consistently complied to the study intervention. However, these associations were not statistically significant in this small sample of peri- and post-menopausal Malaysian women.

Body fatness, measured by BMI and WHR, appears to modify the association between soy intake and mammographic density measures. Interestingly, this study also suggests that women with low dietary fat intake may benefit more from a soy intervention, compared to women with a high fat diet.

6.4.2. Effect of the soy intervention on mammographic density as a biomarker of breast cancer risk

Whilst not statistically significant, consistent inverse associations were noted for area-based mammographic density measures after 12 months of intervention. Six previous clinical trials in Caucasian populations have demonstrated an intervention of 50-120mg/day of isoflavones did not lead to significant changes in mammographic density, compared to the natural decline of mammographic density over time (87–92). It is difficult to compare the effect observed in this study to previous studies due to differences in the analyzed outcome. For example, some studies have used mixed effect models to test for differences in mammographic density measures between study arms and over time (88,89), while others examine differences in baseline-to-final mammographic density change (87) or ratio of mean mammographic density at 12 months to baseline (91). Contrary to the RCTs reported thus far, this study suggests that with sufficient statistical power and high compliance to the intervention, a soy isoflavone intervention could potentially lead to significant reductions in mammographic density among Asian women.

One theory for the lack of association in previous clinical trials of soy is that the isoflavone supplements used in these RCTs are less potent compared to foods made from whole soybeans, such as those traditionally consumed in Asia (76,98). To date, no other RCT has concurrently tested the effect of isoflavones obtained through supplement and through diet on biomarkers of breast cancer risk. Here, stronger inverse associations were observed for women in the Supplement arm compared to the Dietary Soy arm, which suggests that the above theory is false. This study also demonstrates that a dosage of up to 80mg/day of soy isoflavones, as observed in the Dietary Soy arm, may be insufficient to reduce mammographic density measures in 1 year.

It is possible that the composition of the isoflavone supplement used in this study led to a favorable effect on biomarkers of breast cancer risk. In many of the previous RCTs, the isoflavone supplement used have near equal amounts of daidzein and genistein (88,89,91). In others, the proportion of genistein was higher compared to daidzein (87,92).

Here, daidzein was the predominant compound of the isoflavone supplement (46mg in aglycone weight per 50mg isoflavone tablet). A meta-analysis of observational studies has shown that high daidzein concentrations were associated with 34% decrease in relative risk to breast cancer, compared to a 28% decrease in relative risk observed for high genistein concentrations (207). Similarly, data mining approaches show strongest inverse associations for daidzein compared to genistein (208). Interestingly, the concentration of daidzein is higher than genistein in commonly consumed soy foods in Asia, such as tofu and soymilk (209). Taken together, this suggests that a supplement high in daidzein may more closely mimic dietary soy consumption in Asia. Given the higher rates of adverse events in the Supplement arm, however, further studies are required to robustly test for the efficacy and safety of long-term high-dose daidzein intake.

It is important to note that there is currently no consensus on what constitutes a clinically significant reduction in mammographic density for breast cancer prevention. Therefore, it is not known if the effects observed in this study can translate to clinically meaningful reductions in breast cancer risk. In tamoxifen studies, a 10% decrease in percent density over 1-1.5 years was associated with 63% lower relative risk of breast cancer (210). More recently, a study of East Asian women showed that reduction in visually assessed mammographic density consistently led to a decrease in 5-year breast cancer risk (211). For example, 5-year risk decreased from 1.24% (95% CI = [1.19, 1.28]) to 0.92 (95% CI = [0.76, 1.08]) when women experienced a change from BI-RADS 4 to BI-RADS 1 (211). The impact of more subtle mammographic density change on absolute breast cancer risk, however, is not known and remains a critical gap in breast cancer prevention research.

6.4.3. The role of BMI in the association between soy intake and mammographic density

Non-significant inverse associations were observed for the soy intervention arms when the analysis was restricted to women who were overweight or obese, for both volume-based and area-based analysis. Previous RCTs have typically incorporated BMI into the analysis as a confounder to the association between soy intake and mammographic density, but have not shown stratified analysis by BMI (87,89–92). Only one RCT showed no significant differences when the analysis was stratified by normal vs high BMI (88). It that study, however, women were excluded if their BMI was greater or equal to 30kg/m^2 (88). Here, at least 12% of participants had a BMI that was greater than 30kg/m^2. Therefore, it is possible that the protective effect of soy may be most apparent for women who are the

heaviest, but this theory warrants further investigation in a study sample that includes more overweight and obese women.

6.4.4. The role of dietary fat intake in the association between soy intake and mammographic density

Reductions in dietary fat intake have been shown to modestly reduce relative risk of breast cancer, particularly among post-menopausal women (212–214). There is some evidence that dietary fat intake, in early life or in adulthood, is positively correlated to mammographic density (122,215), although some reports suggest no significant association (216). To date, there have been few intervention studies that assess both dietary fat and soy intake on biomarkers of breast cancer risk (79,83,217), but none have explored the interaction between the two dietary factors. The findings in this study suggests that women on a soy isoflavone intervention may observe greater reductions in breast cancer risk if they were also on a low-fat diet. Despite the small sample size for analysis, the replication of this finding across two independent methods of mammographic density measures gives some confidence that there may be a real effect of dietary fat intake on the soy-breast cancer risk relationship, at least among post-menopausal Asian women. This finding will require confirmation in a larger trial that is powered to test for the joint effects of soy isoflavone and low dietary fat intake on breast cancer risk.

6.4.5. Adverse events

In this study, more than 70% of the women in the Supplement arm and Dietary Soy arm reported an adverse event, compared to only 15% in the Control arm. It is important to bear in mind that this was an unblinded, non-placebo-controlled study, and therefore, participants who were in the intervention arms may be more likely to report any changes to their body.

The main adverse events reported were joint pains and numbness in the Supplement arm, as well as gastrointestinal issues in the Dietary Soy arm. Gastrointestinal issues, such as diarrhea, bloating and nausea, have been reported in previous clinical trials (78,91) and in the feasibility study described in **Chapter 5**. The incidence of joint pains in the Supplement arm, however, were not commonly reported in the literature. It is possible that a 100mg/day dose of isoflavones, while able to produce stronger inverse associations with mammographic density, may have led to more adverse events in this population. Furthermore, women in the Supplement arm also consumed some isoflavones through diet, up to 20mg/day, thereby

increasing their total soy isoflavone intake to 120mg/day. There are no reports on safety for high isoflavone intake, but intake of up to 60mg/day was ascertained to be safe on breast tissues and endometrial tissues (200).

6.4.6. Strengths

This is the first RCT to investigate the causal association between soy isoflavone intake on the biomarkers of breast cancer risk among Asian women, for whom there is strong and consistent protective associations in observational study. This study is also the first to concurrently study the effect of soy isoflavones consumed through supplements on breast cancer risk.

Mammographic density was used as a biomarker of breast cancer risk in this study, and was estimated by two independent fully-automated, high-throughput software. All women were assessed using the same mammography system at the same hospital, thereby reducing potential confounding factors in the primary analysis.

Over the 1-year period, there was good compliance to the study intervention across study arms. Dietary soy isoflavone intake was robustly measured through comprehensive food frequency questionnaires, and served as an additional indicator of compliance to the study intervention. Additionally, extensive data collection throughout the study period has enabled the generation of new hypotheses that can be tested in future analyses. This includes the assessment of BMI and WHR as two measures of body fatness. Also, the assessment of calorie and macronutrient intake over time led to the investigation of dietary fat intake as a possible effect modifier of the relationship between soy intake and breast cancer risk.

6.4.7. Limitations

The main limitation in this analysis was statistical power. Power was estimated to be 8% for the analysis of 54 women in volume-based analysis and 17% for the analysis of 45 women in area-based analysis. The low statistical power is likely due to the small effect sizes observed in this study, particularly for the volume-based analysis. To be sufficiently powered to detect true difference between the soy intervention arms and Control arm, up to 330 women will have to enroll, comply, and complete the study. It is also important to note that this power calculation does not yet consider the complexity of analyses nor does it account for loss to follow up. Therefore, a larger number of women will be required.

Randomization is a key process which ensures that participants are evenly distributed across the study arms. Loss to follow up over the study period could lead to disruption of randomization and increases the risk of selection bias (218). Among 94 women enrolled in the study, up to 40% of women were considered lost to follow up. There was a higher percentage of loss to follow up in the Supplement arm due to onset of adverse events, which might have resulted in attrition bias. Despite this, participants appear to be evenly distributed across study arms based on socio-demographic variables, breast cancer risk factors, anthropometric measurements, and baseline mammographic density.

An unanticipated source of loss to follow up was the Covid-19 pandemic and national movement control orders in Malaysia, beginning in March 2020. In this analysis, 5 women chose to discontinue participation in the study due to reluctance to attend hospital follow up visits during the pandemic.

It is also important to consider the modified per-protocol with caution as compliance was measured based on self-reported information, which may not accurately represent womens' intake. The use of a second measure of compliance, such as the concentration of isoflavones in serum or urine (87,90–92), could overcome this barrier in future studies.

Another important limitation in this study is the possibility for non-differential misclassification. For example, cooking methods were not considered in the food frequency questionnaire administered in this study, and could have led to an underestimation of calorie and macronutrient intake. Also, the total calorie and macronutrient intake reported here is marginally higher compared to that reported by women in the MANS survey (203). However, authors of the report note that these parameters are likely underestimated in their report (203). Apart from dietary variables, misclassification could have also occurred when data was collected by different researchers (such as for body measurements) or when various radiographers conducted mammogram acquisition (90). A total of 14 radiographers collected mammogram data for this study. The radiographers were unaware of participant's study arm, and therefore, any misclassification is likely non-differential.

Differences in breast positioning may also be an important source of inaccuracy when studying mammographic density change over time. A recent study demonstrated great differences in the position of the breast, even when the mammograms were taken 2 minutes apart (56). To overcome this, Eriksson et al. have proposed alignment of mammogram images for each women prior to measuring mammographic density (56). This ensures that

the differences in mammographic density observed over time are not merely artefacts of differences in breast position.

6.5. Conclusion

High soy isoflavone intake, up to 100mg/day, may reduce biomarkers of breast cancer risk among post-menopausal Asian women, but this requires confirmation in a larger sample. This study refutes the theory that isoflavones obtained through supplements are less potent than traditional soy foods found in Asia. It also suggests that the isoflavone daidzein may exert a greater effect on breast cancer risk, compared to genistein. Furthermore, it proposes that women with high BMI and/or low dietary fat intake may benefit more from a soy isoflavone intervention, but this warrants thorough investigation in a well-powered RCT.

Chapter 7 : Overall Conclusions

Over the next 30 years, Asian countries are expected to experience a substantial increase in post-menopausal breast cancer incidence (2). While the use of SERMs and repurposed drugs are investigated for use among high-risk women in countries with high breast cancer incidence (8), there remains a critical need to effective primary prevention strategies for women at low-to-moderate risk in countries with growing breast cancer incidence. In **Chapter 1**, I hypothesize that there is an inverse causal association between soy isoflavone intake and breast cancer risk among post-menopausal Asian women. In **Chapters 2-6** of this thesis, I summarize the breadth of research on soy intake and breast cancer risk, and described four studies that were carried out to test my hypothesis. The overarching conclusions are presented here.

In **Chapter 2**, the current body of research suggests that there is sound biological plausibility for the association between soy and breast cancer risk (17). Furthermore, strong, consistent evidence from observational research show that a diet high in soy isoflavones is associated to lower breast cancer risk in post-menopausal Asian women (66–69). The evidence from RCTs of soy isoflavone supplements among Caucasian women, however, do not support this hypothesis (87–92). Some theories have been suggested to account for this inconsistency between observational and clinical studies, including differences in how soy isoflavones are consumed (traditional soy food versus isoflavone supplements), possible population differences in lifelong soy intake or ability to metabolize soy isoflavones, as well as the use of imprecise biomarkers of breast cancer risk. An RCT of soy isoflavones among post-menopausal Asian women may address some of these questions about the soy-breast cancer risk relationship, but has not been reported in the literature.

The data presented in **Chapter 3** suggests that some mammographic density measures are suitable biomarkers of breast cancer risk among Asian women. Volume-based percent density appears to be particularly futile for use among the Asian women. Instead, population difference in absolute dense volume more closely followed population trends in breast cancer risk and was attributable to differences in prevalence of traditional risk factors between the cohorts, such as height, weight, and parity.

Consistent with the limited evidence among Asian women (108,119–121), the data in **Chapter 4** suggests that mammographic density is a suitable biomarker of breast cancer risk for studies of soy intake. However, the inverse associations between mammographic

density and soy intake were small and not-statistically significant. Most notably, this study suggests that the benefit of a soy intervention may be greatest among the heaviest women, while caution should be employed in prescribing a soy isoflavone intervention to younger, leaner women. This finding warrants further investigation.

The feasibility of implementing a long-term dietary soy intervention was assessed in **Chapter 5**, in a small group of post-menopausal Asian women with low soy intake at baseline (approximately 12mg/day). The results demonstrate that Asian women were motivated to adhere to a dietary change for breast cancer prevention research. Importantly, this study showed that isoflavone intake of 100mg/day through diet was not feasible in a long-term trial. It also demonstrated that the key components of a successful dietary intervention trial include building good rapport with participants and developing tailored communication tools.

Finally, in **Chapter 6**, a randomized, three-armed, non-placebo-controlled trial was conducted among post-menopausal Asian women to test the effect of a year-long intervention of soy isoflavone supplements (100mg/day) or dietary soy isoflavones (50mg/day) on mammographic density measures. After 12 months of intervention, women in the Supplement arm experienced 4.1cm^2 lower dense area and 2.4% lower area-based percent density compared to women in the Control arm. Even though the association was not statistically significant, the data suggests that strong significant inverse associations may be observed in a larger trial. Also, stronger inverse associations were observed for women in the Supplement arm, rather than the Dietary Soy arm. In exploratory analysis, the effect of soy isoflavone intake on mammographic density appeared more prominent among women with high BMI or low dietary fat intake.

In conclusion, the data presented in this thesis demonstrate that soy isoflavone intake may be causally associated with lower breast cancer risk, and that this effect is likely mediated through mammographic density. However, the findings will require confirmation in a well-powered RCT. It refutes the theory that soy isoflavone supplements are less potent than isoflavones consumed through dietary soy, and suggests that the isoflavone daidzein may be more important for breast health. Also, it proposes that body fatness and dietary fat intake may be important factors to consider in assessing the association between soy isoflavone intake and post-menopausal breast cancer risk.

Chapter 8 : Future work

In view of the findings in this thesis and the existing body of literature, I propose the following four priority areas in studying soy isoflavones for primary prevention of breast cancer among Asian women.

Firstly, the findings presented in **Chapter 6** of this thesis will require confirmation in a larger RCT. This study of 57 women had less than 20% power to detect the small effects observed, particularly for volume-based measures. Furthermore, improving compliance to the study intervention could greatly strengthen the effect size and reduce variance in the study, thereby increasing statistical power. Also, the RCT should be powered to test primary and secondary outcomes, including possible effect modification by BMI or dietary fat intake. If the findings from **Chapter 6** are replicated in a larger trial, this will be the first clinical trial to show strong inverse associations between soy isoflavone intake and mammographic density as a breast cancer risk.

Secondly, the effect of soy isoflavone intake on breast cancer risk among pre-menopausal women warrants further investigation. A recent RCT of women at high risk of breast cancer reported that the effect of tamoxifen on mammographic density was strongest among premenopausal women, before the stabilization of natural mammographic density decline (219). There are few clinical trials of pre-menopausal women, which have shown null or positive associations (78,99). However, in light of the findings in **Chapter 4**, it is possible that a soy isoflavone intervention may benefit overweight or obese pre-menopausal women.

Thirdly, the implications of daidzein metabolism on breast cancer risk across populations requires further research. There has been interest in studying the interaction between soy intake and the metabolism of daidzein to (S)-equol on breast cancer risk. Several observational studies have shown a significant inverse association between soy intake and mammographic density among women who are able to produce (S)-equol efficiently (107,108,115,118), however this was not consistent across all studies (110). As part of the study described in **Chapter 6**, urine samples have been collected at baseline, 6-months and 12-months post intervention, and is available for future analyses of isoflavone intake, (S)-equol producing status, and breast cancer risk. Furthermore, there is an opportunity to test if the form of soy isoflavones consumed (either by supplement or in whole soy food) affects the ability to metabolise daidzein.

Lastly, there remains a need to find more opportunities for primary prevention of breast cancer among Asian women. For some cancers, such as lung cancer or cervical cancer, removing just one major risk factor (smoking or chronic human papilloma virus infection, respectively) could prevent a majority of those cancers (220,221). The prevention of breast cancer, however, will likely require population- and tumour-specific solutions. What is promising, however, is the research that suggests that 26-50% of breast cancers may be prevented if mammographic density was reduced to the minimum (46,47). Hence, interventions that target mammographic density could be key strategies for primary prevention of breast cancer. More recently, Eriksson et al. reported that a 2.5mg dose of tamoxifen had a similar effect on mammographic density as a 20mg dose, but with fewer side effects. Such an intervention could be considered for Asian women who are at high risk for breast cancer.

In summary, while there is convincing evidence that soy isoflavone intake could reduce breast cancer risk, more research is required to understand how these isoflavones affect breast cancer risk and who could benefit most from an isoflavone-rich diet. Furthermore, there is a need to find other strategies for primary prevention of breast cancer in Asia, particularly among women at highest risk.

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