The Effect of Physical Activity and Body Size on BRCA1 mRNA Expression

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Abstract
Prevention of BRCA1-associated cancer is limited to prophylactic surgery and chemoprevention. Factors that increase BRCA1 expression and normalize protein levels may impact cancer risk. The objective of this study was to evaluate whether physical activity and/or body size correlate with BRCA1 mRNA expression. Women (n=50) were asked to wear the GT3X activity monitor for 7 days. Anthropometric measurements were obtained and blood samples were collected. BRCA1 mRNA was quantified using the NanoString nCounter Analysis System. Mean BRCA1 expression was significantly lower for mutation carriers vs. non-carriers (139 counts vs. 157 counts; \( P = 0.02 \)). No significant relationship was observed between physical activity, anthropometrics, and BRCA1 expression (\( P \geq 0.05 \)). A borderline significant inverse relationship was observed between mean sedentary time and BRCA1 expression (158.2 vs. 154.7 counts; \( P_{\text{trend}} = 0.05 \)), suggesting that prolonged sedentary behavior may be associated with lower BRCA1 expression. Whether this finding translates into an increased risk for BRCA1-associated cancer warrants further investigation.
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1.1 The Breast Cancer Susceptibility Gene-1 (BRCA1) and Cancer

Approximately 5-10% of breast cancers and 5-15% of ovarian cancers are hereditary in nature\(^1,2\). Since the discovery of the BRCA1 gene in 1994, and BRCA2 subsequently in 1995, it has been established that deleterious mutations in one of these two genes account for the large majority of hereditary cancers\(^3\). The following introduction provides an overview of the literature regarding BRCA1-associated cancer risk and prevention, the BRCA1 gene and protein, and factors known to affect BRCA1 expression.

### 1.1.1 Prevalence of BRCA1 Mutations

The frequency of BRCA1 mutations is approximately 1 in 250 women, or about 0.4%, however mutations occur more frequently in certain ethnic populations including the Ashkenazi Jewish (present in 2% of these individuals), French Canadians, Bahamians, Icelandic, Polish, and Dutch\(^4,5\). Specific mutations known as founder mutations, account for the large majority of BRCA mutations within these genetically isolated groups. For example, within the Ashkenazi Jewish population, two founder BRCA1 mutations, 184delAG and 5382insC, account for over 90% of mutations observed. This has somewhat simplified the process of genetic testing, as this eliminates the need for full gene sequencing\(^4\).

### 1.1.2 Risk of BRCA1-Associated Cancers

The inheritance of a BRCA1 mutation is associated with a high lifetime risk of developing breast cancer, estimated at 80% by the age of 70\(^6\text{-}^8\). BRCA1 mutations also increase the lifetime risks of ovarian cancer to approximately 36% to 66% compared to a risk of less than 2% for women without a mutation\(^9\text{-}^{11}\). Families with BRCA1 mutations are also at a slightly increased risk for male breast, pancreatic, uterine body, cervix, prostate, and colon cancers\(^7,^{10}\).

Women with BRCA-associated breast cancers are diagnosed at a young age (30-50 years of age)\(^4\), have an elevated risk of developing contralateral\(^12\) or ipsilateral breast cancer\(^13\) and exhibit pathological characteristics suggestive of an aggressive phenotype (e.g., triple negative)\(^14\).
1.1.3 Mutation Classification
Since the identification of BRCA1, over 3,000 sequence variations have been reported. These sequences fall within three categories 1) pathogenic mutation 2) polymorphism (neutral affect on risk), and 3) variant of unknown significance (VUS). VUS are named as such because the effect on protein function is not known and therefore breast and ovarian cancer risks cannot be accurately estimated\textsuperscript{15}. Pathogenic mutations include frameshift (which account for the majority of known mutations) and missense mutations (point mutations)\textsuperscript{16}. Large deletions and insertions/duplications comprise 15-27\% of known mutations. Variations in penetrance have been reported based on the position of the BRCA1 mutation, with mutations in the 5’ end of the gene being associated with a higher risk of ovarian cancer and those in the 3’ end of the gene being associated with lower risk\textsuperscript{17,18}. With respect to breast cancer risk, Thompson \textit{et al.} reported a lower risk for mutations located in the central region of the gene compared to those in the outer regions (relative risk [RR] = 0.71; 95\% CI 0.58-0.86; \(P = 0.0002\))\textsuperscript{18}. This increased penetrance for mutations found in the outer regions of the gene emphasizes the importance of the protein-interacting domains in the BRCA1 gene.

1.1.4 BRCA1 Location and Structure
BRCA1, breast cancer susceptibility gene 1, is a tumour suppressor gene whose protein assists in maintaining genomic stability\textsuperscript{19}. The gene is located on chromosome 17q21 and is 24 exons long, 22 of the 24 exons translating into a protein of 1863 amino acids\textsuperscript{16}. BRCA1 contains two highly conserved functional domains: a RING finger domain at the N-terminus and two tandem BRCT domains at the C-terminus (Figure 1). After the description of BRCT in BRCA1, the domain has been found in many proteins involved in DNA damage repair pathways, while the RING domain is found in many E3 ubiquitin ligases and mediates protein ubiquitylation\textsuperscript{19}.

Although there is no evidence that somatic BRCA1 mutations play a role in the development of sporadic breast cancer, decreased expression of BRCA1 has frequently been shown in sporadic tumours and the degree of reduction is associated with disease progression\textsuperscript{20,21}. This highlights the important roles that the BRCA1 protein plays within the cell.

1.1.5 Functions of the BRCA1 Protein
BRCA1, a tumour suppressor gene, encodes the BRCA1 protein which primarily prevents a carcinogenic event through its involvement in DNA damage repair and in cell cycle checkpoint
control\textsuperscript{19}. Following damage from either endogenous or exogenous sources, including free radicals and radiation, the genome becomes susceptible to DNA damage. In healthy, normally functioning cells, DNA damage triggers a cascade of events that cells have evolved to sense the damage, halt cell cycle progression, and initiate DNA repair during the S and G\textsubscript{2} phases of the cell cycle\textsuperscript{19}. When cells lack the required mechanisms to repair this damage, tumourigenesis may occur. Given the increased incidence of cancer associated with a \textit{BRCA1} mutation, it is not surprising that the protein plays a crucial role in the prevention of carcinogenesis. In fact, biallelic \textit{BRCA1} mutations result in murine embryonic lethality, given that one wild-type \textit{BRCA1} allele is required during embryogenesis. Only once has a biallelic \textit{BRCA1} mutation been reported in the literature, in a woman with one deleterious mutation in one allele and a variant of unknown significance (VUS) in the second allele\textsuperscript{22}.

Following a double stranded DNA break, BRCA1 associates with BARD1 (BRCA1-associated RING domain protein), its binding partner: both proteins are then phosphorylated by ATM (ataxia telangiectasia mutated) or ATR (ataxia telangiectasia and Rad3-related), prompting the initiation of the DNA damage response. BRCA1/BARD1 also binds to BRIP1 (BRCA1-interacting protein 1), to regulate histone deacetylases, thereby opening up chromatin for access of repair enzymes. BRCA1/BARD1 is recruited to the site of DNA damage where it then interacts with MRN (MRE11-RAD5—NBN) and CtIP (C-terminal binding protein interacting protein) to resect DNA ends. This complex binds to BRCA2, PALB2 (partner and localizer of BRCA2), and RAD51 which associates with the ssDNA for homologous repair\textsuperscript{23}. In vitro experiments confirm the importance of BRCA1 in homologous repair as cells that lack BRCA1 are very sensitive to DNA crosslinking agents that induce double-stranded breaks in DNA\textsuperscript{5}.

A second function of BRCA1 is within the cytoplasm as a cell cycle checkpoint control. During this period, BRCA1 associates with NBS1 (Nijmegen breakage syndrome 1), the RAD50-MRE11 complex, ATM, and several other proteins to form the BRCA1-associated genome-surveillance complex (BASC), associating with centrosomes to regulate the cell cycle after DNA damage. Outside of its role in double-stranded break repair and cell cycle checkpoint control, BRCA1 also plays a role in ubiquitylation (targeting proteins for degradation), apoptosis, and mitochondrial genome repair\textsuperscript{5,24}. 
Although BRCA1 is not a sequence-specific transcription factor, it has been shown to stimulate or inhibit the action of many transcription factors, and thus is involved in transcriptional regulation. BRCA1 participates in transcriptional complexes that activate genes involved in cell cycle arrest, DNA repair, and those that are pro-apoptotic. BRCA1 also participates in inhibitory transcriptional complexes that inhibit growth promotion genes\textsuperscript{25}.

Hereditary mutations in the \textit{BRCA1} gene predispose women to cancers in hormonally-sensitive tissues (see section 1.1.2). It is likely that estrogen plays an important role in the development of \textit{BRCA1}-associated breast cancer given the fact that oophorectomy significantly reduces the risk of breast cancer and pregnancy increases the risk of \textit{BRCA1}-associated breast cancer\textsuperscript{26,27}. Findings from Kienan \textit{et al.} suggest that estrogen and its metabolites are capable of causing S-phase specific DNA double stranded breaks (DSBs). Intact BRCA1 is required for the repair of estrogen/estrogen metabolite-mediated DSBs. Heterozygote mutations in \textit{BRCA1} and therefore lack of functional BRCA1 would lead to defective repair of these breaks and genomic instability in tissues with high levels of estrogen (i.e. breast tissue)\textsuperscript{25}.

\section*{1.2 Primary Prevention of and Modifiers of Risk of \textit{BRCA1}-Associated Cancers}

\subsection*{1.2.1 Prevention of \textit{BRCA1}-Associated Cancers}

Predictive genetic testing permits the identification of these high-risk women; however, prevention is limited to prophylactic surgery and chemoprevention with tamoxifen and uptake of these choices is low due to fear of disfigurement and side effects\textsuperscript{4}. Prophylactic surgery, including mastectomy and oophorectomy, has been shown to be the most effective prevention method for \textit{BRCA1} mutation carriers. Bilateral prophylactic mastectomy, or removal of the breasts, provides a 95\% reduction in breast cancer risk\textsuperscript{28}. Bilateral prophylactic oophorectomy, removal of the ovaries, has been shown to reduce breast cancer risk by 56\% for \textit{BRCA1} mutation carriers. The greatest risk reduction is seen when the oophorectomy is performed before the age of 40\textsuperscript{29}. Furthermore, Finch \textit{et al.} recently reported an 80\% reduction in risk of ovarian, fallopian tube, or peritoneal cancer in \textit{BRCA} mutation carriers (HR = 0.20 95\% CI, 0.13 to 0.30; \textit{P} < .001) and a 77\% reduction in all cause mortality to age 70 (HR = 0.23 95\% CI, 0.13 to 0.39; \textit{P} < .001) for mutation carriers with oophorectomies, compared to those without\textsuperscript{30}. 
Tamoxifen, an estrogen receptor (ER) antagonist, has been used as a treatment for breast cancer since the 1970s. Tamoxifen was also found to reduce the risk of contralateral breast cancer in BRCA1 mutation carriers and was therefore recommended for women at high risk for the disease. For women at high risk, a five year course of tamoxifen or raloxifene (a selective estrogen receptor modulator [SERM]) is recommended to reduce the risk of developing cancer. Nevertheless, tamoxifen use among BRCA mutation carriers has been very limited due to feared side effects including invasive endometrial cancer, deep vein thrombosis, pulmonary embolism, and cataracts. Finally, although tamoxifen has been shown to reduce breast cancer recurrence in BRCA carriers, no prospective studies have reported on tamoxifen as a chemopreventive for primary breast cancer.

1.2.2 Reproductive Modifiers of Risk

The incomplete penetrance associated with a mutation suggests that non-genetic modifiers may play an important role. Given the tissue-specificity of BRCA-associated cancers, it is logical that hormonal exposures influence risk. The hypothesized mechanism by which prophylactic oophorectomy reduces breast cancer risk is through the reduction of hormonal exposure. This reduction in risk is seen even though the majority of BRCA1-associated cancers are estrogen-receptor negative. Exogenous hormone exposure, including use of oral contraceptives (OCs) and hormone replacement therapy (HRTs), has also been shown to increase breast cancer risk. In a recent case-control study of 2,492 matched pairs of BRCA1 mutation carrier women, Kotsopoulos et al. reported a significant increase in early onset (before age 40) breast cancer risk in women who started using the birth control pill prior to age 20 (OR 1.45; 95% CI 1.20-1.75; \( P = 0.0001 \)) compared to never users. A meta-analysis by Iodice et al., revealed that OC use was associated with a significant reduction in ovarian cancer risk for BRCA1 mutation carriers (SRR = 0.50; 95% CI 0.33-0.74) and the magnitude of decrease was proportional to the duration of use (\( P_{\text{trend}} < 0.01 \)). Therefore, mutation carriers must be counseled on how to effectively balance the various risks associated with oral contraceptive use with pregnancy prevention.

A positive association between HRT use and sporadic breast cancer risk was first reported in the Women’s Health Initiative over ten years ago and has been confirmed in many other studies. However, the effect on cancer risk specifically within BRCA1 mutation carriers is not
well understood. Understanding the risks associated with HRT use within high-risk women is of extreme importance, as HRTs are often used following prophylactic oophorectomy to prevent decreases in bone density and cardiovascular health that are associated with premature estrogen deprivation.\(^{41}\)

As previously discussed, lifetime exposure to ovarian hormones, particularly estrogen, is hypothesized to influence cancer risk in \textit{BRCA1} mutation carriers. Thus, factors that alter the number of menstrual cycles, including early age at menarche, nulliparity, and breastfeeding, may modify risk. Early menarche is associated with an increased risk of hereditary breast cancer.\(^{42}\) A matched case-control study of 945 \textit{BRCA1} mutation carriers reported a 54% reduction in risk (OR = 0.46; 95% CI 0.30-0.69) in women who experienced a late age at menarche (14-15 years of age) compared to women whose age at menarche was \(\leq 11\)\(^{42}\).

The relationship between parity and \textit{BRCA1}-associated cancer risk has not been fully elucidated and the association has been shown to be weak at best. A recent meta-analysis of ten studies found no association between parity and breast cancer risk.\(^{43}\) Furthermore, Cullinane \textit{et al}. reported a decrease in risk with pregnancy in \textit{BRCA1} carriers only after four births compared to nulliparous women, and the protective effect was only modest (OR = 0.62; 95% CI = 0.41-0.94).\(^{44}\) In the general population, both parity and an early first birth (before the age of 30) are associated with a decrease in breast cancer risk. The lack of effect of parity on breast cancer risk in \textit{BRCA1} mutation carriers may be explained by the fact that \textit{BRCA1} plays an important role in the branching pattern of the breast during lobular development. Within the breast tissue of carriers, the lack of functional \textit{BRCA1} may prevent lobular differentiation that is normally induced during pregnancy.\(^{45,46}\)

Finally, breastfeeding is inversely related to both sporadic and hereditary breast cancer risk.\(^{47-49}\) Specifically, in a case-control study of 1,243 pairs of women who carried a deleterious \textit{BRCA1} mutation, increasing duration of breastfeeding conferred a reduction in risk. Breastfeeding for one year offered a 32% reduction in risk (OR = 0.68; 95% CI 0.52 to 0.91; \(P = 0.008\)), while breastfeeding for two years or more offered an even greater reduction in risk (OR = 0.51; 95% CI 0.35 to 0.74; \(P = 0.0003\)).
The relationship between breastfeeding and age at menarche with BRCA1-associated cancer risk confirms the impact of prolonged or increased estrogen exposure for developing cancer; early age at menarche increases the number of menstrual cycles while longer periods of breastfeeding decreases the number of cycles\(^{16}\).

### 1.2.3 Dietary and Lifestyle Modifiers of Risk

Various reproductive factors have been shown to influence BRCA-associated cancer risk\(^{42,50}\); however, a role of dietary and lifestyle factors is much less clear\(^ {51}\). There is some evidence that coffee\(^ {52}\), selenium\(^ {53,54}\), iron and antimony\(^ {55}\) as well as weight loss in early adult life\(^ {56}\) protect against BRCA1-related breast cancer. Coffee consumption has been shown to stimulate cell differentiation and inhibit mitosis. Nkondjock et al. investigated the association between lifetime coffee consumption and breast cancer risk among 652 BRCA1 mutation carriers. For women who consumed 6 or more cups of coffee per day, the odds ratio of developing breast cancer was 0.31 (95% CI 0.13-0.72; \(P_{\text{trend}} = 0.02\))\(^ {52}\). This study suggests that coffee consumption is not harmful for mutation carriers and may actually confer a reduction in risk.

Selenium is a cofactor of various antioxidant enzymes and aids with DNA repair. Kowalska et al. examined the impact of selenium supplementation on chromosome breakage within BRCA1 mutation carriers. An increased mean frequency of bleomycin-induced chromosome breaks in cultured blood lymphocytes was seen in carriers compared to non-carrier relatives (0.58 versus 0.39; \(P < 10^{-4}\)). Oral selenium supplementation for three months also reduced the number of chromosome breaks in carriers to a normal level\(^ {53}\).

Iron and antimony have also been shown to affect BRCA-associated cancer risk. Kotsopoulos et al. investigated the role of micronutrients and trace elements in breast development among BRCA1 mutation carriers. A matched case-control design was utilized (48 cases, 96 controls) and plasma levels of various micronutrients were measured. Women with the highest levels of plasma iron had a 57% lower risk of breast cancer compared to those in the lowest quartile (OR = 0.43; 95% CI 0.18-1.04; \(P_{\text{trend}} = 0.06\)) whereas a positive association was seen between plasma antimony levels and breast cancer risk (\(P_{\text{trend}} = 0.05\))\(^ {55}\).
1.3 Physical Activity, Body Size, and Breast Cancer Risk

1.3.1 Physical Activity and Premenopausal Breast Cancer Risk in the General Population
It has been well established by evidence from a multitude of studies that a relationship exists between physical activity and sporadic breast cancer risk. Women from the Nurses’ Health Study II reporting the highest levels of leisure-time physical activity between the ages of 12-22, experienced a 25% reduced risk of premenopausal breast cancer (RR=0.75, 95% CI= 0.57 to 0.99; $P_{trend} =0.05$).57

Physical activity during adolescent years may influence risk as breast tissue is developing; lobule formation begins at puberty and ducts grow and divide into terminal end buds.46 Therefore, breast tissue may be more susceptible to risk factors, including physical activity, during this developmental period.46 Accordingly, physical activity during pubertal and adolescent years has been shown to affect premenopausal breast cancer risk.57

1.3.2 Physical Activity and Postmenopausal Breast Cancer Risk in the General Population
A recent review by Lynch et al. found an average decrease in postmenopausal breast cancer risk of 25% amongst physically active women, compared to the least active women. This review included 73 studies: 33 cohort studies and 44 case-control studies involving women around the world. The greatest reduction in risk was seen for recreational activity versus household or occupational, activity performed regularly and maintained over the lifetime, and moderate-to-vigorous activity versus light activity.58

However, even light activity has been shown to modify risk; the American Cancer Society recently published their findings including over 73,000 postmenopausal women followed over 17 years. Women who reported $\geq 7$ hours/week of walking compared to $\leq 3$ hours/week experience a 14% lower risk of breast cancer occurrence (95% CI, 0.75-0.98).59

Results have been conflicting regarding adolescent physical activity and risk of postmenopausal breast cancer. A case-control study involving 1527 non-Hispanic white cases and 1601 controls, reported a reduction in postmenopausal breast cancer risk for high total MET (metabolic equivalent of task) hours of activity at age 15 (OR: 0.57; 95% CI 0.38, 0.88;
However, a recent study by Boeke et al. evaluated the association between adolescent physical activity and postmenopausal breast cancer risk among over 75,000 women in the Nurses’ Health Study II and no decrease in risk was found even for the most active of adolescents (72 MET-hours per week) compared to the least active (<21 MET-hours per week) (HR = 0.95, 95% CI: 0.78-1.16; \( P_{\text{trend}} = 0.84 \)).

1.3.3 Hypothesized Mechanisms
Although the exact mechanism by which physical activity modulates cancer risk is not fully understood, several hypothesized mechanisms have been proposed. Increased activity may lower breast cancer risk by increasing insulin sensitivity, reducing adiposity, affecting menstruation patterns and therefore decreasing levels of circulating sex hormones, or by reducing inflammation and the production of cytokines \(^{58,59}\). In reality, it is likely that physical activity modulates risk through a combination of these mechanisms.

1.3.4 Physical Activity and Breast Cancer Risk in \textit{BRCA1} Mutation Carriers
In total, three epidemiological studies have evaluated whether physical activity influences risk in this high-risk population \(^{36,62-64}\). Table 1 summarizes the key characteristics of the published studies to date investigating the association between physical activity and breast cancer risk in \textit{BRCA} mutation carriers. In the first study, King et al. reported a significant delay in breast cancer onset among 104 \textit{BRCA} mutation carriers who were physically active as teenagers compared to those who were not \((P = 0.03)\). A second study of 89 \textit{BRCA} mutation carriers found no association between levels of physical activity (calculated as metabolic equivalents of task [MET] and breast cancer risk \(^62\). The odds ratios (ORs) and 95\% CIs comparing the highest versus lowest tertile of MET for moderate, vigorous or total physical activity were 1.40 (95\% CI 0.58-3.40), 0.73 (95\% CI 0.27-1.94) and 1.05 (0.42-2.60), respectively. In a recent retrospective cohort study of 725 \textit{BRCA} mutation carriers, Pijpe et al. reported a significant reduction in risk with increasing levels of sports prior to, but not after, age 30 \(^63\). The hazard ratios (HRs) comparing the highest versus lowest tertile of MET hours/week were 0.58 (95\% CI 0.35-0.94) and 1.24 (95\% CI 0.70-2.19), for sports activity before and after age 30, respectively. Collectively, results from these three studies provide important preliminary evidence for a protective role of physical activity among \textit{BRCA} mutation carriers, particularly if occurring during adolescence or early adulthood.
To date, one study has investigated a potential mechanism by which physical activity might affect breast cancer risk in BRCA1 mutation carriers. High estrogen levels are implicated in the development and growth of sporadic breast cancer and oophorectomy (which results in a sharp decline in estrogen) decreases the risk for BRCA-associated breast cancer. Therefore, factors that decrease estrogen levels in the healthy BRCA1 mutation carrier might also decrease BRCA-associated cancer risk. Similarly, breast mitotic activity is highest during the luteal phase when progesterone is also at its peak, indicating a positive association between breast cell activity and progesterone. Kossman et al. recruited 7 healthy eumenorrheic women at high risk for breast cancer (i.e. BRCA1/2 mutation carriers or women who have a lifetime risk of ≥16.5%) for a seven menstrual cycle study. Daily baseline measurements of urinary estrogen and progesterone were collected for two menstrual cycles, followed by an exercise intervention (300 min/week at 80-85% of maximum aerobic capacity) throughout the next five menstrual cycles. Urinary estrogen and progesterone levels were again measured daily throughout the last two cycles. The authors reported an 18.9% decline in estrogen exposure ($P = 0.03$) and a 23.7% decline in progesterone exposure ($P = 0.05$) following exercise intervention. This study provides preliminary evidence that physical activity is able to modulate hormone levels in high-risk women.

Given the importance of determining the relationship between physical activity and BRCA-associated cancer risk, it is not surprising that two research groups have recently published proposals to investigate this relationship. Pasanisi et al. have proposed a randomized controlled trial (RCT) to investigate whether calorie and protein restriction with physical activity decrease insulin-like growth factor I (IGF-I) in BRCA mutation carriers. Evidence has recently been published that serum IGF-I is associated with a significantly increased penetrance in 308 high risk women; the OR for breast cancer risk for the upper tertile of serum IGF-I compared to the lowest tertile was 3.5 (95% CI: 1.4-8.8). Thus, the authors of this RCT will recruit a cohort of 300 non-affected BRCA carriers to investigate whether a comprehensive metabolic intervention may reduce IGF-I and thus BRCA-associated cancer risk.

Guinan et al. have proposed a prospective cohort study to examine the relationship between metabolic syndrome, body composition, physical activity, diet, and hormone measurements from 352 unaffected BRCA1/2 mutation carriers. Participants will complete baseline, two-year,
and five-year follow up assessments and cancer incidence will be recorded. This study aims to identify environmental factors that modify \textit{BRCA}-associated cancer penetrance\textsuperscript{68}.

### 1.3.5 Body Size and Premenopausal Breast Cancer Risk in the General Population

Considerable experimental and epidemiological evidence supports an association between energy balance, obesity, and risk of breast cancer\textsuperscript{71}. Obesity is an independent positive predictor of risk among postmenopausal women, but inversely related to risk among premenopausal women\textsuperscript{72}.

Before the onset of menopause, estrogen production occurs in the ovaries. Circulating estrogen levels are under tight regulation and thus are not affected by estrogen production in adipose tissue. The mechanism by which obesity leads to a reduction in premenopausal breast cancer is not fully understood. However, it is hypothesized that lower levels of circulating estrogens and therefore a reduction in breast tissue exposure to estrogen might mediate the decrease in risk. Obese women frequently experience irregular and anovulatory menstrual cycles due to the loss of normal ovary function. This loss of function leads to a decrease in estrogen and progesterone production, which may prevent breast carcinogenesis in premenopausal women\textsuperscript{73}.

### 1.3.6 Body Size and Postmenopausal Breast Cancer Risk in the General Population

In obese postmenopausal women, higher circulating levels of estrogen are seen as a result of hormone production in the adipocytes of the body. Within adipose tissue, estrogen is produced through the aromatization of androgens. Postmenopausal women of a healthy weight have been shown to have lower levels of circulating estrogen than overweight or obese women\textsuperscript{74}. It has been hypothesized that these increased levels of proliferative estrogen may overcome the protective effects of \textit{BRCA1} and lead to an increased risk for sporadic breast cancer\textsuperscript{75}. Furthermore, obesity is associated with poor prognosis (more aggressive breast tumours, an increased risk of recurrence, and higher mortality) following breast cancer diagnosis, regardless of menopausal status\textsuperscript{76}.  

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1.3.7 Mechanisms Mediating the Relationship between Body Size, Physical Activity and Breast Cancer

Mechanisms by which obesity influence cancer development include metabolic consequences (i.e. hyperinsulinemia, insulin resistance) and alterations in the IGF-I axis, altered reproductive hormone profiles (i.e. estrogen, testosterone) and chronic inflammation.\(^7^7\)

1.3.8 Body Size and Breast Cancer Risk in BRCA1 Mutation Carriers

Relatively few studies have evaluated the effect of body size on breast cancer risk specifically among women with a BRCA mutation. Table 2 summarizes the key characteristics of the studies published to date investigating the association between body size and breast cancer risk in BRCA mutation carriers. In the largest study published to date (n = 797 matched pairs), it was reported that weight loss of \(\geq 10\) pounds between the ages 18-30 was associated with a decreased risk of breast cancer between ages 30-49 (odds ratio [OR]=0.47; 95% confidence intervals [CI] 0.28-0.79); while weight gain did not influence risk.\(^5^6\) This reduction in risk with weight loss was limited to women with a BRCA1 mutation (OR=0.35; 95%CI 0.18–0.67; \(P = 0.002\)).

A second large study (n = 719 BRCA1/2 mutation carriers) investigated the association between self-reported anthropometric measures and both pre- and postmenopausal breast cancer.\(^7^8\) For premenopausal breast cancer, no statistically significant associations were found for any of the anthropometric measures (i.e., weight at age 18 and adult changes in weight). However, a trend of increasing body mass index (BMI) at age 18 and decreasing risk was observed (HR\(_{\geq 25.00\text{ vs.} 18.5-22.49} = 0.41; 95\% \text{ CI} 0.13-1.27\)). For postmenopausal breast cancer, adult height of greater than 1.67 meters was associated with a 1.7-fold increase in risk (HR = 1.67, 95\% CI = 1.01-2.74), adult weight of greater than 72 kg was also associated a 2.1-fold increase in risk (HR = 2.10, 95\% CI = 1.23-3.59). No associations were observed between body measurements at age 18 and postmenopausal breast cancer risk.\(^7^8\) This retrospective cohort study provides evidence that teenage weight may affect premenopausal BRCA-associated breast cancer risk, while adult weight may affect postmenopausal BRCA-associated breast cancer risk.

Results from earlier studies with smaller sample sizes have been inconsistent. King et al. reported that a healthy weight at menarche and age 21 were associated with a significant delay
in breast cancer onset\textsuperscript{36}, while Chang-Claude \textit{et al.} found no association between BMI and age at onset among 419 \textit{BRCA1} mutation carriers\textsuperscript{79}. A small case-control study of 137 French-Canadian mutation carriers reported that a later age at which the subjects reached their maximum BMI, as well as, maximum weight gain since both ages 18 and 30, were all associated with an increased risk of breast cancer\textsuperscript{62}. These limited findings suggest that maintenance of a healthy weight, particularly early in adult life, may protect against \textit{BRCA}-associated breast cancer.

1.4 Haploinsufficiency and Factors Known to Influence \textit{BRCA1} mRNA Expression

1.4.1 Knudson’s Two Hit Hypothesis and Haploinsufficiency

When identifying possible lifestyle interventions that may help in the prevention of \textit{BRCA1}-associated breast cancer, it is important to consider that the mechanism underlying the predisposition is likely different from that in the general population\textsuperscript{80}. In all analyzed tumours of \textit{BRCA1} mutation carriers, a homozygous \textit{BRCA1} genotype is detected in which the normal allele has been deleted or inactivated via methylation. This substantiates Knudson’s two-hit hypothesis, the classical theory that explains the increased incidence of cancer seen in dominantly inherited cancer susceptibility syndromes\textsuperscript{81}. Women who carry germline \textit{BRCA1} mutations remain cancer free until they experience a “second hit” or loss of the single normal copy of the \textit{BRCA1} gene. However, evidence also exists to suggest that presymptomatic \textit{BRCA1} mutation carriers have altered mRNA profiles compared to non-carriers. This altered mRNA profile likely also confers an increased risk for tumourigenesis, either by promoting the loss of the second normal \textit{BRCA1} allele or by altering the DNA repair mechanisms of the cell\textsuperscript{82}. This phenomenon, known as haploinsufficiency, refers to a state in which a heterozygous individual has only one functional copy of a gene and thus only produces half the amount of protein needed to prevent disease development\textsuperscript{82,83}.

It is believed that a haploinsufficient state contributes to the predisposition for breast cancer among \textit{BRCA1} mutation carriers. \textit{BRCA1} helps maintain genomic integrity through participating in the cellular response to DNA damage, specifically in the repair of double-stranded DNA breaks\textsuperscript{84}. Thus, it is believed that DNA repair is an intrinsic problem among \textit{BRCA1} mutation carriers. Although limited, there is evidence to support the idea that the
predisposition to breast and ovarian cancer among \textit{BRCA1} mutation carriers is the haploinsufficiency associated with heterozygosity, increasing genomic instability and accelerating the mutation rate of other critical genes, including the second copy of \textit{BRCA1} \textsuperscript{80}. It is likely that the increased risk for breast and ovarian cancer seen in \textit{BRCA1} mutation carriers operates on a continuum where tumour formation occurs as a result of haploinsufficiency that led to loss of the normal \textit{BRCA1} allele\textsuperscript{82}. Thus, factors which might increase the physiologic expression of the normal copy of the gene and normalize protein levels may mitigate the effect of the mutation. Of particular relevance for \textit{BRCA1} mutation carriers is that physical activity during puberty in rodents resulted in a significant increase in \textit{BRCA1} mRNA levels in the mammary glands \textsuperscript{64}. If haploinsufficiency predisposes mutation carriers to cancer development, ultimately, the ability to up-regulate \textit{BRCA1} expression in female \textit{BRCA1} heterozygotes should translate into a reduced cancer risk.

\textbf{1.4.2 Lifetime \textit{BRCA1} Expression}

\textit{BRCA1} is broadly expressed during development, becomes increasingly tissue-specific with age, and is cell cycle regulated \textsuperscript{85,86}. The highest expression of \textit{BRCA1} is during the late G1 phase and early S phase, while the lowest expression is during the G0 and early G1 phases \textsuperscript{86}. During embryogenesis, \textit{BRCA1} is highly expressed in rapidly dividing cells that are undergoing proliferation and differentiation. Postnatally, increases in \textit{BRCA1} expression are seen in the breast, gastrointestinal tract, and thymus during puberty and pregnancy \textsuperscript{85}. With the onset of pregnancy, the terminal end buds in the adult mammary gland proliferate extensively to form the alveolar sacs where \textit{BRCA1} expression remains high until birth of the child. Throughout breastfeeding, \textit{BRCA1} expression levels fall as the alveolar cells become terminally differentiated. Upon completion of breastfeeding, \textit{BRCA1} levels first rise to trigger apoptosis of the alveolar cells, and then remain low until a second pregnancy \textsuperscript{87}. In the murine model, \textit{BRCA1} mRNA levels also decrease with age \textsuperscript{85}. Interestingly, \textit{BRCA1} expression patterns mirror those of another tumour suppressor gene, \textit{p53} \textsuperscript{85}.

\textbf{1.4.3 Dietary Factors}

Several dietary factors have been shown to affect \textit{BRCA1} mRNA expression in both animal and \textit{in vitro} experiments. An \textit{in vivo} study by Vissac-Sabatier \textit{et al.} demonstrated that flax seed consumption over 90 days significantly increased \textit{BRCA1} mRNA expression in the mammary glands of ovariectomized adult Wistar rats \textsuperscript{88}. 
Childhood dietary factors may also affect BRCA1 expression and consequently cancer risk for mutation carriers. An in vivo study by Olivo-Marston et al. found a 30% reduction of BRCA1 expression in rats who were fed a high-fat n-3 polyunsaturated fatty acid (PUFA) diet during postnatal day 5-25, compared to those fed a low-fat n-3 PUFA diet or a low-fat n-6 PUFA diet ($P < 0.001$). The rats on the high-fat n-3 PUFA diet also displayed increased tumourigenesis compared to the other two diets ($P = 0.0148$), perhaps caused by a decrease in functional BRCA1 protein. In a study investigating phytoestrogens, prepubertal genistein supplementation in rodents increased mammary gland BRCA1 mRNA expression in wild type mice compared to mice fed the control diet ($P = 0.04$); however, no increase was seen in BRCA1 expression with the genistein diet for the BRCA$^{+/-}$ mice. Furthermore, wild-type mice supplemented with genistein also experienced a significant decrease in tumourigenesis ($P = 0.029$) while BRCA$^{+/-}$ mice did not. This study further supports the haploinsufficiency hypothesis that increased BRCA1 expression decreases tumour incidence.

Maternal dietary factors have also been shown to influence BRCA1 expression in rat offspring. In utero exposure to a whole-wheat diet, compared to a control diet, upregulated BRCA1 expression in the mammary glands of both pubertal and adult rats ($P < 0.05$). These offspring also had a lower incidence of mammary tumourigenesis compared to pups from mothers fed a control diet ($P < 0.07$) indicating that maternal diet can influence offspring cancer risk.

Dietary factors have also been reported to affect BRCA1 mRNA expression in in vitro designs. Kachhap et al. reported greater expression of BRCA1 mRNA in MCF-7 breast cancer cells exposed to linoleic acid (an n-6 PUFA) or estradiol compared with controls. However, cells exposed to both linoleic acid and estradiol had decreased BRCA1 expression when compared with controls indicating that a diet rich in both of these factors may promote breast cancer. Furthermore, MCF-7 cells treated with pomegranate extract (PE) had decreased BRCA1 expression compared to MCF-7 cells grown without PE ($P < 0.001$). This study suggests that PE may assist in the prevention of the survival of cancer cells.
1.4.4 Hormones
Reproductive hormones, including progesterone and estrogen, may also affect \textit{BRCA1} mRNA expression. Prepubertal hormone levels have been shown to affect \textit{BRCA1} expression later in life; Cabanes \textit{et al.} reported higher \textit{BRCA1} mRNA levels in the mammary glands of adult rats supplemented prepubertally with estrogen (17β-Estradiol), compared to control rats ($P = 0.006$)\textsuperscript{94}. Estrogen and progesterone also regulate \textit{BRCA1} expression in breast cancer cell lines; increased \textit{BRCA1} mRNA expression was seen in MCF-7 and C7-MCF-7 cell lines in response to estrogen treatment by Romagnolo \textit{et al.}\textsuperscript{95}.

After findings were published reporting that incidence of breast cancer is higher in Israeli Jewish women exposed to the Holocaust than among those who were not (RR = 2.44, 95% CI = 1.46 to 4.06), Ritter \textit{et al.} investigated the relationship between the primary steroid hormone, cortisol, and \textit{BRCA1} expression\textsuperscript{96,97}. The authors of this study illustrated that loss of the unliganded glucocorticoid receptor from the \textit{BRCA1} promoter, due to the presence of cortisol, results in decreased \textit{BRCA1} expression. In simpler terms, the presence of stress hormones leads to a decrease in \textit{BRCA1} expression\textsuperscript{97}.

1.4.5 Physical Activity
To date, two studies have investigated the relationship between physical activity and \textit{BRCA1} expression. An \textit{in vivo} study by Wang \textit{et al.} investigated whether prepubertal physical activity (postnatal day 14 to day 32) was associated with an alteration of \textit{BRCA1}, \textit{p53}, estrogen receptor (ER)-\textalpha{} and ER-\textbeta{} mRNA expression in the mammary glands of adult rats (100 days old)\textsuperscript{64}. Wang \textit{et al.} found that levels of all four genes, including \textit{BRCA1}, were significantly higher in the mammary glands of exercised versus the sham control rats ($P < 0.03$). Further, the mammary glands of the exercised rats had fewer terminal end buds and a higher number of differentiated alveolar buds and lobules compared with the controls, suggestive of reduced targets for neoplastic transformation\textsuperscript{64}. A second study by Magbanua \textit{et al.}, found increased prostate tissue \textit{BRCA1} mRNA expression ($P = 0.005$), as part of an upregulated gene set, in men (n=71) who reported engaging in 3 hours of vigorous physical activity per week compared to those who did not\textsuperscript{98}.

The studies of Wang and Magbanua provide important insight into possible mechanisms that might mediate the protective effects of physical activity on breast cancer risk in \textit{BRCA1}
mutation carriers. To our knowledge, whether physical activity and/or body size affects BRCA1 mRNA expression in women has never been evaluated.

1.5 Methods of Measuring Physical Activity
The accurate assessment of physical activity is important for evaluating and understanding the relationship between health and physical activity. A number of methods are used to measure physical activity, including both subjective methods (i.e. recall questionnaires and physical activity logs) and objective methods (i.e. indirect calorimetry, measurement with doubly labeled water (DLW), and the use of a heart rate monitor, accelerometer or pedometer). The DLW technique is considered the gold standard for measuring energy expenditure under free-living conditions. However, this method is very expensive and provides no information on the timing, type, and intensity of physical activity. Physical activity is defined as any body movement that increases energy expenditure. Motion sensors are tools used to measure body movement and include pedometers and accelerometers. Triaxial accelerometers, validated with the DLW method, are widely used tools used to measure physical activity and sedentary patterns over longer periods of time.

1.6 Rationale
Women who carry pathogenic BRCA1 mutations are at an increased risk for both breast (up to 80% by age 70) and ovarian cancer (approximately 36 to 66% increased risk). Although prevention methods are available for mutation carriers, many women choose to forego these procedures until childbearing is complete. Therefore, it is important to be able to provide mutation carriers with lifestyle prevention options that complement current prevention options (oophorectomy/mastectomy). Certain reproductive factors have been shown to influence the BRCA-associated cancer risk; however, a role of dietary and lifestyle factors is much less clear. Although evidence exists that physical activity and body size, particularly weight loss early in adult life, may influence BRCA-associated cancer risk, the mechanism by which these factors mediate a decrease in risk is not known. Therefore the aim of this study is to evaluate whether physical activity and body size may decrease BRCA-associated cancer risk through an increase in BRCA1 mRNA expression. Increased BRCA1 mRNA expression would result in increased translation and production of the BRCA1 protein, a tumour suppressor. Finally, increased BRCA1 protein may mitigate the effects of haploinsufficiency and prevent a “second hit,” thereby reducing a woman’s risk for BRCA-associated cancer.
The findings from this pilot study will contribute to a program of research aimed at providing novel lifestyle prevention strategies that complement current prevention methods with the overall goal of decreasing the incidence of breast cancer cases and deaths attributed to a \textit{BRCA1} mutation.
Figure 1. BRCA1 domains and protein interactions Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Molecular Cell Biology] \(^9\), copyright (2009).

Figure 2. BRCA1 tumour suppressor function and associated proteins Reproduced with permission from Journal of Pathology \(^23\) copyright (2013) John Wiley and Sons.
Table 1. Summary of retrospective studies investigating the relationship between physical activity and *BRCA*-associated breast cancer risk

<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Type of Study</th>
<th>Number of Cases/controls</th>
<th>Type and age period of physical activity</th>
<th>Measurement of Physical Activity</th>
<th>OR, HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>King <em>et al.</em> 36</td>
<td>Retrospective</td>
<td>N = 104 <em>BRCA</em> mutation carriers</td>
<td>Sports, dance, or casual exercise during teenage years</td>
<td>Participation in sports, dance, or casual exercise</td>
<td>Delay in cancer onset</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>Nkondjock <em>et al.</em> 62</td>
<td>Retrospective; case-control</td>
<td>N = 89 affected <em>BRCA</em> mutation carriers N = 48 non-affected carriers</td>
<td>Seasonality, frequency, and duration engaged in 12 most common types of leisure-time physical activity in Canada Participation in sports or exercise 2 years before diagnosis (cases) or interview (controls)</td>
<td>Metabolic equivalent of task (METs)</td>
<td>Highest vs. lowest MET for moderate activity: 1.40 (0.58-3.40) Highest vs. lowest MET for vigorous activity: 0.73 (0.27-1.94) Highest vs. lowest MET for total activity: 1.05 (0.42-2.60)</td>
<td>P&lt;sub&gt;trend&lt;/sub&gt; = 0.4 P&lt;sub&gt;trend&lt;/sub&gt; = 0.56 P&lt;sub&gt;trend&lt;/sub&gt; = 0.91</td>
</tr>
<tr>
<td>Pijpe <em>et al.</em> 63</td>
<td>Retrospective cohort</td>
<td>N = 725 <em>BRCA</em> mutation carriers</td>
<td>Activity (type of sport, frequency, and duration) stratified by age (before and after 30 years)</td>
<td>METs</td>
<td>Activity before age 30: highest versus lowest tertile of MET hours/week were 0.58 (0.35-0.94) Any activity after age 30: 0.63 (0.44-0.91)</td>
<td>P&lt;sub&gt;trend&lt;/sub&gt; = 0.053</td>
</tr>
</tbody>
</table>
Table 2. Summary of studies investigating the relationship between body size and BRCA-associated breast cancer risk

<table>
<thead>
<tr>
<th>Study Reference</th>
<th>Type of Study</th>
<th>Number of Cases/controls</th>
<th>Timing and type of anthropometric measurement</th>
<th>OR, HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kotsopoulos et al. 56</td>
<td>Case-control</td>
<td>N = 797 matched pairs</td>
<td>Questionnaire, lifetime weight</td>
<td>0.35 (0.18–0.67)</td>
<td>P = 0.002</td>
</tr>
</tbody>
</table>
| King et al. 36   | Retrospective; case-control | N = 104 BRCA mutation carriers | Questionnaire, lifetime weight                | Delay in cancer onset for women who were a healthy weight at menarche  
Delay in cancer onset for healthy weight at age 21 | P = 0.017   |
| Chang-Claude et al. 79 | Cross-sectional       | N = 419 BRCA1 mutation carriers | Current BMI (stratified by < 25 or >25)       | No effect on age of disease onset | P = 0.54       |
| Nkondjock et al. 62  | Case-control           | N = 89 affected BRCA mutation carriers 
N = 48 non-affected carriers | Lifestyle core questionnaire               | Age at maximum BMI 2.90 (1.01-8.36) 
(compared to lowest tertile)  
Max. weight gain since age 18 4.64 (1.52-14.12) 
(compared to lowest tertile)  
Max. weight gain since age 30 4.11 (1.46-11.56) 
(compared to lowest tertile) | P_trend = 0.043   |
| Manders et al. 78 | Retrospective cohort   | N = 719 BRCA1/2 mutation carriers | Self-reported anthropometric measures (at age 18 and current) | Premenopausal risk: 
BMI at age 18 HR_{\text{BMI at age 18}} vs. 18.5-22.49 = 0.41 (0.13-1.27)  
Postmenopausal risk:  
Adult height (>1.67 m)  
HR = 1.67, 95% CI = 1.01-2.74  
Adult weight (>72 kg)  
HR = 2.10 (1.23-3.59) | P_trend = 0.011   |


Chapter 2
Objectives and Hypotheses

The overall goal of this study was to evaluate the relationship between physical activity, anthropometric measures (i.e., BMI, Index of central obesity, etc.) and \textit{BRCA1} gene expression in women with and without a \textit{BRCA1} mutation. Findings from this pilot study will help support further investigation into the possible role of physical activity and/or body size in the etiology of \textit{BRCA1}-associated breast cancer and may provide the impetus to perform a prospective examination in women with a \textit{BRCA1} mutation.

\textbf{2.1 Objectives:}

1) To evaluate the relationship between physical activity and \textit{BRCA1} mRNA expression.
2) To evaluate the relationship between body size and \textit{BRCA1} mRNA expression.
3) To evaluate \textit{BRCA1} mRNA expression differences between \textit{BRCA1} mutation carriers and non-carriers.

\textbf{2.2 Hypotheses:}

1) Physical activity will be positively associated with \textit{BRCA1} mRNA expression.
2) Body size will be inversely associated with \textit{BRCA1} mRNA expression.
3) \textit{BRCA1} mRNA expression will be significantly lower in \textit{BRCA1} mutation carriers versus non-carriers.
Chapter 3
Materials and Methods

3.1 Study Design and Population
The current study is a cross-sectional study (Figure 3). Eligible subjects were women who were at least 18 years of age, had no personal history of cancer, and who were not pregnant or breastfeeding. There were two distinct study populations included in this thesis study. The first group (n=36) included women from the general population (\( BRCA1^{WT} \)). These women were recruited using study posters advertised throughout Women’s College Hospital (WCH), on the WCH website, through the hospital social media networks, including Facebook and Twitter, and in Connect and Women’s Health Matters, the internal newsletter and the WCH health information website respectively. Given the low carrier frequency of \( BRCA1 \) mutations in the general population (0.32%), these women were assumed to have two normal copies of the \( BRCA1 \) allele\(^{102} \). The second group (n=14) included women who have a confirmed \( BRCA1 \) mutation (\( BRCA1^{+/}\)). These women were identified from an existing database at the Familial Breast Cancer Research Unit, Women’s College Research Institute (WCRI) and included women who were already enrolled in previous and ongoing clinical research protocols. Eligible women recruited from the Familial Breast Cancer Research Unit database were invited to participate in the study by letter. Interested individuals then contacted the study coordinator over the phone or through email to confirm their participation in the study and to schedule two 30-minute study appointments at the WCRI, 790 Bay Street, Toronto, ON. This research received approval from the Research Ethics Board at Women’s College Hospital (# 2012-0055-B). All patients gave written informed consent to participate in the study.

3.2 Data Collection
During the first study visit, participants completed the informed consent form (Appendix I) and a personal and physical activity questionnaire (Appendix II), which is detailed below.

3.2.1 Questionnaire
This questionnaire collected information on reproductive and medical history, lifestyle factors including lifetime physical activity, and family history of cancer. This questionnaire has been used over the last ten years by the ‘Risk factor analysis for Hereditary Breast Cancer’ study, which currently has over 15,000 women enrolled. The questionnaire included an additional
section on current and past physical activity. This excerpt was taken from the Nurses’ Health Study Questionnaire and includes activity questions regarding number of hours per week spent walking, TV watching, and performing strenuous and moderate recreational activity from grade seven until age 34. This section also collects information on physical activity throughout the past year including participation in moderate and vigorous activities, usual walking pace, and number of stairs climbed per day. The reproducibility and validity of this section of the questionnaire has been described in detail previously103.

3.3 Biological Sample Collection and Anthropometric Measurements
At the first visit, standardized procedures were used to collect anthropometric measurements including weight, height, waist and hip circumference. Measurements were taken in duplicate and the average of two measures was calculated. The four measurements were used to calculate body mass index (BMI), waist-to-hip-ratio (WHR), and the index of central obesity (ICO). ICO, a ratio of waist circumference to height, was proposed by Parikh et al. as a better measure of central adiposity as compared to waist circumference; ICO eliminates the need for gender and race specific WC cutoffs104. At the second study visit, a phlebotomist drew blood into one labeled EDTA containing tube (~8mL) by venipuncture. The sample was placed on ice and delivered immediately to the Narod laboratory (WCRI) for RNA extraction.

3.4 Physical Activity Assessment
During the first study visit, participants were provided with a detailed explanation on how to use the GT3X accelerometer (ActiGraph, USA). Assessment of physical activity and sedentary behaviour using devices such as accelerometers provides an objective measure of activity in comparison to self-report. Self-reporting of physical activity are subject to recall and response bias from memory difficulties and social desirability105. Participants were asked to wear the accelerometer on an elasticized belt on the right hip for seven consecutive days during waking hours except during bathing and water activities. The GT3X is a triaxial monitor that detects movement in three planes (i.e. vertical, horizontal and anterior-posterior), and converts these data into counts that are incorporated into proprietary algorithms to calculate energy expenditure106. Each monitor was fully charged and programmed to record activity information for one second epochs. The GT3X monitor has been validated for use in an adult population107.
In order to evaluate the validity of the GT3X accelerometer, participants were also asked to keep a log of their daily exercise and wear of the accelerometer (Appendix III). Each individual’s physical activity routine, recorded utilizing the log, was compared to results obtained from the GT3X accelerometer. At the second study visit, participants returned the accelerometer to the WCRI and data from the GT3X was downloaded onto a secure server.

3.5 RNA Quantification

3.5.1 RNA Isolation
RNA was isolated from one of the two EDTA tubes using the LeukoLOCK Total RNA Isolation System (Ambion, USA). This system is optimized for use with human blood and offers the isolation of total RNA from the leukocyte population. Comparative studies have revealed similar results in terms of quality and yield, between the LeukoLOCK Total RNA Isolation System and other RNA extraction methods. White blood cell BRCA1 mRNA, which has been shown to be expressed in peripheral blood cells, was used as a surrogate marker of BRCA1 expression in the breast and ovaries. In order to maximize RNA isolation yield, all samples were stabilized with RNA later within 35 minutes of blood draw. Standard protocol was followed excluding the optional TURBO DNase as primers that cover exon-exon junctions were used in downstream analysis. Resulting extracted RNA was stored in several aliquots at -80°C until required for downstream analysis.

3.5.2 RNA Yield and Quality
Two aliquots per participant were used to assess the quality and quantity of RNA. The nucleic acid content was quantified in one aliquot per sample using the Nanodrop spectrophotometer (ThermoScientific). Total RNA quality and quantity was then determined using the Agilent 2100 Bioanalyzer (The Centre for Applied Genomics, 686 Bay Street, Toronto). The mean sample RNA concentration was 173.7ng/µL (range: 85-368 ng/µL) which is more than sufficient required for downstream analyses. The RNA Integrity Number (RIN) indicates the extent of degradation and absence of contaminants. RIN values range from 1 for poor quality RNA or 10 for fully intact RNA. The mean RIN for the samples of interest was 8.7 (range: 7.9-9.3), indicating good quality RNA for downstream application.
3.5.3 Quantification of BRCA1 mRNA
BRCA1 mRNA levels were quantified using the NanoString nCounter Analysis System and the Human Cancer Reference Kit, a panel of 230 cancer-related genes, including the BRCA1 gene. This study only investigated BRCA1 expression and thus the remaining information obtained from this panel will be used in future studies. The nCounter Analysis System, by NanoString Technologies, was used to measure BRCA1 mRNA expression (expressed as counts). A detailed explanation of the nCounter Analysis System can be found in the 2006 publication by Geiss et. al. Figure 4 illustrates the concept of NanoString’s two probe-transcript design. Briefly, the nCounter Analysis System probe library contains two sequence-specific probes, the capture probe and the reporter probe, for each gene of interest. The digital multiplexed NanoString nCounter Human Cancer Reference panel was performed with 200ng of total RNA as input material for each sample. The capture probe is complementary to the target RNA and to an affinity tag (biotin). The reporter probe, also complementary to one gene of interest, is coupled to a unique colour-coded tag (detection signal). Probe pairs are mixed with total RNA in one hybridization reaction and then the structures are imaged with the use of fluorescent microscopy. Expression is measured by counting the number of unique colour tags within the gene-probe tripartite structures. This technology provides advantages over existing methods of quantifying RNA expression, including a digital readout and a lack of enzymatic reactions or bias. The nCounter Analysis System is a counting technology that provides an output of counts (a direct measure of the number of RNA transcripts of each gene of interest).

3.6 Sample Size Considerations
The overall goal of this thesis was to evaluate whether two lifestyle factors, physical activity and body size, affected BRCA1 mRNA expression. BRCA1 mRNA has never before been measured in lymphocytes using NanoString, and as such this study was a pilot, as the expected effect size was unknown.

3.7 Statistical Analyses

3.7.1 BRCA1 Expression
All data analysis was performed using the nSolver software (NanoString Technologies). Positive control normalization was used to normalize platform-associated sources of variation. The geometric mean of the six positive controls for each sample was calculated to estimate the
efficiency of hybridization and recovery for each lane (sample). The average of the geometric means across all samples was calculated and then used as a reference against which each lane was normalized. A lane-specific scaling factor was then calculated for each lane based on the geometric mean of the lane and overall average. The scaling factor was within the guideline and expected range of 0.3-3, allowing for adjustment of each target gene and negative control in each lane. Reference housekeeping gene normalization was then performed to adjust counts relative to probes that are not expected to vary between samples or replicates, allowing meaningful comparisons between samples. Reference housekeeping genes included CLTC (clathrin, heavy chain (Hc)), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), GUSB (glucuronidase, beta), HPRT1 (hypoxanthine phosphoribosyltransferase 1), PGK1 (phosphoglycerate kinase 1), and TUBB (tubulin, beta). These six housekeeping genes were chosen based on NanoString’s analysis guidelines; compared to fewer housekeeping genes, more accurate normalization is expected when six are used in the calculation of the geometric mean. The geometric mean of the six housekeeping genes for each lane was calculated and utilized as the scaling factor. Eight negative controls (probes for which there was no transcript) were also included in the gene panel and the average of all the negative controls in each lane was calculated as an indicator of background (NanoString Technologies, nCounter Expression Data Analysis Guide)

3.7.2 Accelerometer-Derived Variables
For the current analysis, a valid day was defined as at least 600 minutes (ten hours) or more of accelerometer wear. ‘Non-wear’ time was defined as an interval of at least ten consecutive minutes of zero activity counts. This resulted in all 50 participants having six or more valid days of monitor wear. ‘Wear-time’ was validated using the Troiano 2007 cut-points. Five physical activity variables (Table 3) were selected for analysis based on their prevalence in the literature and use in previous research where ActiGraph’s GT3X accelerometer was utilized. Raw accelerometer counts were used to determine the percent of time each participant spent in sedentary, light, moderate, and vigorous intensity activity using the manufacturer’s software in conjunction with the Troiano 2008 cut points. The percentage time was calculated by adding the minutes within each activity level (i.e. sedentary, light, and moderate to vigorous physical activity [MVPA]) and then dividing by the total minutes of wear per day. These five variables included the percent of time spent in MVPA (%) and the mean MVPA per day (min/day; defined as the average amount of time spent per day in MVPA), and the Metabolic Equivalents
of Task (MET) rate (calculated using the Swartz Adult Overground and Lifestyle cut points \(^{115}\)). METs are an estimation of the energy cost of physical activity defined as the ratio of work metabolic rate to a standard resting metabolic rate of 1.0 (4.184 kJ·kg\(^{-1}\)·h\(^{-1}\)). MET values range from 0-23, while a value of 1 MET represents resting metabolic rate during quiet sitting while 18 represents running at 16.1 km·h\(^{-1}\). Two sedentary variables were also included for analysis: the percent of time spent sedentary (%) and the mean sedentary (hours/day; defined as the average amount of time per day spent sedentary).

### 3.7.3 Descriptive Characteristics and Identifying Confounders

The Anderson-Darling test was used to verify the normality of \(BRCA1\) mRNA expression; \(BRCA1\) mRNA expression was normally distributed \((P > 0.25)\). The Student’s t-test was used to compare continuous variables between mutation carriers and non-carriers, while the chi-square test was used to test for differences in categorical variables (Table 4).

Linear regression was used to generate univariate betas and \(P\) values to test for the association of potential confounders including age (continuous), parity (parous vs. non-parous), and menopausal status (premenopausal vs. postmenopausal), with \(BRCA1\) expression (counts) (Table 8). A significance level of <0.2 and/or biological relevance based on the existing literature (see Chapter 1 pages 12-16) was used as criteria for including variables in the multivariate models. Given the small sample size of this study, only three variables were included in the multivariate model: mutation status and age, as well as WHR for the physical activity analysis and percent MVPA for the body size analysis. \(BRCA1\) mutation status was included in the multivariate model given its correlation with \(BRCA1\) expression \((P = 0.02, \text{Table 8})\). Age was also included in the multivariate model given its correlation with \(BRCA1\) expression \((P = 0.14)\) and since there is evidence that in a murine model, \(BRCA1\) expression decreases with age\(^{85}\). Lastly, WHR (a measure of central adiposity) was adjusted for in the physical activity analysis given its potential to act as a confounder. Similarly, percent MVPA (as a measure of physical activity level) was adjusted for in the body size analysis. Although other variables were correlated with \(BRCA1\) expression (Table 8), such as menopausal status, HRT use, and oophorectomy, these variables were also associated with \(BRCA1\) mutation status (see Discussion) and so were not included in the multivariate model.
Batch effect for each cartridge used with the nCounter Analysis System was also assessed to ensure that technical sources of variation did not affect BRCA1 expression. Each cartridge contained 12 wells (one well for each RNA sample) and the five cartridges were run at the same time. Linear regression was used to generate univariate betas and P values to test for the association between each cartridge and BRCA1 expression (Table 7).

3.7.4 Physical Activity, Body Size, and BRCA1 Expression

Univariate and multivariate linear regression were used to examine the association between measures of physical activity and body size with BRCA1 mRNA expression levels. The multivariate model used to examine the relationship between physical activity and BRCA1 expression adjusted for BRCA1 mutation status (carrier/non-carrier), age (years), and waist-to-hip ratio. The multivariate model used to examine the relationship between body size and BRCA1 expression adjusted for BRCA1 mutation status (carrier/non-carrier), age (years), and percent MVPA.

Dichotomous variables of the five physical activity variables were then created, using the 75th percentiles, based on the distribution in the entire study population. Table 3 is a summary of the physical activity and anthropometric variables used in the analyses as well as the mean, standard deviation, and 75th percentile for each variable. The adjusted geometric mean levels for BRCA1 mRNA expression by exposure category (0-75th percentile, >75th percentile) was determined using generalized linear models. The student’s t-test was used to compare mean BRCA1 mRNA expression levels between the high (>75th percentile) and low (0-75th percentile) exposure groups. P-trend for each relationship was calculated using linear regression.

Statistical significance was defined at the level of $P \leq 0.05$ and all analyses were carried out using SAS Version 9.4 (SAS Institute, Cary, NC, USA).
Table 3. Physical activity and anthropometric measurement variables used in final analyses

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Variable (units)</th>
<th>Mean (SD)</th>
<th>75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Activity</td>
<td>Percent of time spent in moderate-to-vigorous physical activity (MVPA) (%)</td>
<td>5.65 (0.03)</td>
<td>7.20</td>
</tr>
<tr>
<td></td>
<td>Mean MVPA/day (min)</td>
<td>44.9 (21.7)</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>Percent of time spent sedentary (%)</td>
<td>64.6 (0.08)</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>Mean sedentary/day (hours)</td>
<td>8.60 (1.5)</td>
<td>9.55</td>
</tr>
<tr>
<td></td>
<td>MET (Metabolic equivalent of task) rate</td>
<td>1.83 (0.2)</td>
<td>1.96</td>
</tr>
<tr>
<td>Body Size</td>
<td>Body Mass Index (BMI) (kg/m²)</td>
<td>24.6 (5.0)</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>Waist Circumference (inches)</td>
<td>33.2 (4.8)</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Hip Circumference (inches)</td>
<td>39.1 (4.1)</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>Index of Central Obesity</td>
<td>20.5 (3.0)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Waist-to-Hip Ratio</td>
<td>0.85 (0.06)</td>
<td>0.88</td>
</tr>
</tbody>
</table>
Figure 3. Study design overview
Figure 4. NanoString’s nCounter Analysis System; reporter probe and capture probe bind the target molecule; complex is immobilized for data collection; quantity of genes of interest are reported as expression counts. Reproduced with permission from NanoString nCounter Workflow © 2014 NanoString Technologies, Inc. All rights reserved.
Chapter 4

Results

Table 4 outlines selected characteristics stratified by BRCA1 mutation status (n = 14 BRCA1 mutation carriers versus n = 36 non-carriers). Women with a BRCA1 mutation were significantly older (44.4 years vs. 34.4 years; *P* = 0.02), were more likely to be parous (64% vs. 31% *P* = 0.05), postmenopausal (64% vs. 8%; *P* < 0.001), of Ashkenazi Jewish descent (64% vs. 8%; *P* = 0.001), and were more likely to have had a prophylactic mastectomy (21% vs. 0%; *P* = 0.02) or oophorectomy (57% vs. 2%; *P* < 0.0001) than women without a BRCA1 mutation.

Table 5 outlines the mean, median, range, and standard deviation (SD) of normalized BRCA1 mRNA expression in the total study population and stratified by BRCA1 mutation status. BRCA1 mRNA expression was significantly lower in mutation carriers compared to non-carriers (139.9 vs. 164.1 counts; *P* = 0.02) (Figure 5). BRCA1 mRNA expression was normally distributed as assessed by the Anderson-Darling test (*P* > 0.25).

The distribution of the physical activity and anthropometric variables are summarized in Tables 6 and 7. BRCA1 mutation carriers were slightly less sedentary (7.90 hours/week vs. 8.87 hours/week; *P* = 0.03) and had a higher MET rate (1.94 vs. 1.79; *P* = 0.01) compared to non-carriers (Table 6). There were no significant differences between BRCA1 mutation carriers and non-carriers with respect to any of the other physical activity variables (% MVPA, mean MVPA/day, and % sedentary; *P* ≥ 0.07) or anthropometric variables (i.e., BMI, waist and hip circumference, WHR, and ICO; *P* ≥ 0.77) (Table 7).

Table 8 outlines the results from the univariate linear regression analysis between BRCA1 expression and various exposures to evaluate what other factors are associated with BRCA1 expression. *P* values of variables that were ≤ 0.20 were considered for inclusion in the multivariate models. These variables included age, menopausal status, parity, lifetime use of HRT, oophorectomy status, BRCA1 mutation status, and history of breastfeeding. Three of these variables were included in the multivariate model: mutation status and age, as well as WHR for the physical activity analysis and percent MVPA for the body size analysis. These three variables were carefully selected given their strength of association with BRCA1 expression (*P* ≤ 0.20) and/or due to their biological relevance (see Chapter 3).
Table 9 summarizes the univariate and multivariate relationship between high ($\geq 75^{th}$ percentile) vs. low levels ($< 75^{th}$ percentile) of physical activity variables (% MVPA, mean MVPA/day, % sedentary, mean sedentary per day, and MET rate) and $BRCA1$ mRNA expression in all the women. There was no significant difference in mean $BRCA1$ mRNA expression among women in the low vs. highest level of any of the five physical activity variables ($P \geq 0.05$). After adjustment for relevant covariates, there was a borderline significant inverse relationship between the percent of time spent sedentary and $BRCA1$ expression ($P_{\text{trend}} = 0.07$) as well as the mean number of hours spent per day spent sedentary and $BRCA1$ expression ($P_{\text{trend}} = 0.05$). Following adjustment, there was a non-significant positive relationship between MET rate and $BRCA1$ mRNA expression levels ($P_{\text{trend}} = 0.08$). An increased percent of time spent in MVPA was associated with lower $BRCA1$ expression ($P_{\text{trend}} = 0.07$). Similarly, increases in mean MVPA led to a decrease in $BRCA1$ expression ($P_{\text{trend}} = 0.07$).

Table 10 depicts the relationship between measures of anthropometric variables (BMI, waist circumference, hip circumference, WHR, and Index of Central Obesity) and $BRCA1$ mRNA expression. Mean $BRCA1$ mRNA expression did not differ significantly between the low and high categories for any of the five anthropometric variables ($P \geq 0.05$). Although not statistically significant, there was an inverse relationship between waist circumference and $BRCA1$ expression, both in the univariate ($P_{\text{trend}} = 0.35$) and multivariate analyses ($P_{\text{trend}} = 0.15$). Similarly, as ICO increased, $BRCA1$ expression decreased, as seen in the univariate model ($P_{\text{trend}} = 0.28$) and in the multivariate analyses ($P_{\text{trend}} = 0.13$). However, there was no evidence of trends between the other body size variables (BMI, hip circumference, and WHR) and $BRCA1$ expression.
### Table 4. Characteristics by *BRCA1* mutation status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BRCA1&lt;sup&gt;+&lt;/sup&gt; (n=14)</th>
<th>BRCA1&lt;sup&gt;WT&lt;/sup&gt; (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean, range</td>
<td>44.4 (27-62)</td>
<td>34.4 (18-62)</td>
<td>0.02</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other white</td>
<td>5 (36)</td>
<td>20 (56)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>9 (64)</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>East Asian</td>
<td>0</td>
<td>6 (17)</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>0</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>Parous, n (%)</td>
<td>9 (64)</td>
<td>11 (31)</td>
<td>0.05</td>
</tr>
<tr>
<td>Age at menarche (years), mean (SD)</td>
<td>12.8 (1.4)</td>
<td>12.2 (1.7)</td>
<td>0.16</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>9 (64)</td>
<td>4 (8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current oral contraceptive use, yes, n (%)</td>
<td>2 (4)</td>
<td>4 (8)</td>
<td>1.0</td>
</tr>
<tr>
<td>Current smoking status, yes, n (%)</td>
<td>1 (7)</td>
<td>0 (0)</td>
<td>0.28</td>
</tr>
<tr>
<td>Current alcohol consumption, yes, n (%)</td>
<td>12 (86)</td>
<td>31 (78)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mastectomy, yes, n (%)</td>
<td>3 (21)</td>
<td>0 (0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Oophorectomy, yes, n (%)</td>
<td>8 (57)</td>
<td>1 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current Hormone Replacement Therapy (HRT) use, n (%)</td>
<td>5 (36)</td>
<td>3 (8)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Table 5. Normalized *BRCA1* mRNA counts<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Carriers</td>
<td>36</td>
<td>164.0</td>
<td>163.1</td>
<td>109.8 - 265.6</td>
<td>34.4</td>
</tr>
<tr>
<td>Carriers</td>
<td>14</td>
<td>139.9</td>
<td>147.1</td>
<td>98.7-172.0</td>
<td>20.6</td>
</tr>
<tr>
<td>All</td>
<td>50</td>
<td>157.3</td>
<td>154.8</td>
<td>98.7 - 265.6</td>
<td>32.8</td>
</tr>
</tbody>
</table>

<sup>1</sup> Normalized with housekeeping genes: *CLTC, GAPDH, GUSB, HPRT1, PGK1*, and *TUBB*. 
Figure 5. Normalized BRCA1 Expression (counts) stratified by mutation status; box-and-whisker plot depicting median, lower and upper quartiles, and range.

\[ P = 0.02 \]
<table>
<thead>
<tr>
<th>Accelerometer-Derived Variable</th>
<th>BRCA1&lt;sup&gt;wt&lt;/sup&gt; (n=14)</th>
<th>BRCA1&lt;sup&gt;wt&lt;/sup&gt; (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of time in sedentary mode, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60%</td>
<td>6 (43)</td>
<td>7 (19.5)</td>
<td></td>
</tr>
<tr>
<td>60%-70%</td>
<td>7 (50)</td>
<td>16 (44.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;70%</td>
<td>1 (7)</td>
<td>13 (36)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sedentary hours/day, mean (SD)</td>
<td>7.90 (1.2)</td>
<td>8.87 (1.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>MVPA (min), mean (SD)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>44.7 (29.0)</td>
<td>45.0 (18.7)</td>
<td>0.96</td>
</tr>
<tr>
<td>Percent of time in MVPA, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5%</td>
<td>7 (50)</td>
<td>15 (42)</td>
<td></td>
</tr>
<tr>
<td>5%-8%</td>
<td>3 (21)</td>
<td>16 (44)</td>
<td></td>
</tr>
<tr>
<td>&gt;8%</td>
<td>4 (29)</td>
<td>5 (14)</td>
<td>0.26</td>
</tr>
<tr>
<td>MET Rate, mean (SD)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.94 (0.2)</td>
<td>1.79 (0.2)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>1</sup> MVPA; moderate-to-vigorous physical activity

<sup>2</sup> MET Rate; metabolic equivalent of task rate
<table>
<thead>
<tr>
<th>Anthropometric Measurements</th>
<th>BRCA1^[+/-] (n=14)</th>
<th>BRCA1^[WT] (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m^2 (SD)</td>
<td>24.4 (5)</td>
<td>24.7 (5)</td>
<td>0.90</td>
</tr>
<tr>
<td>Normal (19.0-24.99) (%)</td>
<td>8 (57)</td>
<td>21 (58)</td>
<td>1.0</td>
</tr>
<tr>
<td>Overweight (25.0-29.99) (%)</td>
<td>4 (29)</td>
<td>10 (27)</td>
<td></td>
</tr>
<tr>
<td>Obese (&gt;30) (%)</td>
<td>2 (14)</td>
<td>5 (14)</td>
<td></td>
</tr>
<tr>
<td>Waist Circumference, inches (SD)</td>
<td>33.0 (4.9)</td>
<td>33.2 (4.8)</td>
<td>0.85</td>
</tr>
<tr>
<td>Hip Circumference, inches (SD)</td>
<td>38.8 (3.6)</td>
<td>39.2 (4.3)</td>
<td>0.77</td>
</tr>
<tr>
<td>Waist to Hip Ratio, (SD)</td>
<td>0.85 (0.07)</td>
<td>0.85 (0.05)</td>
<td>0.96</td>
</tr>
<tr>
<td>Index of Central Obesity, (SD)</td>
<td>0.52 (0.08)</td>
<td>0.52 (0.07)</td>
<td>0.77</td>
</tr>
<tr>
<td>Variable</td>
<td>Univariate β (95% CI)</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.52 (-1.2, 0.2)</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Menopausal status (postmenopausal vs. premenopausal)</td>
<td>-25.45 (-45.6, -5.3)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Age at menopause (years)</td>
<td>1.45 (0.5, 3.4)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Current coffee user (yes vs. no)</td>
<td>9.58 (-11.2, 30.4)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Current tea user (yes vs. no)</td>
<td>-5.56 (-15.9, 4.8)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Current alcohol user (yes vs. no)</td>
<td>0.80 (-26.4, 28.0)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Parity (parous vs. nulliparous)</td>
<td>-16.80 (-35.4, 1.8)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>-1.97 (-9.5, 5.6)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Current OC use (yes vs. no)</td>
<td>-2.33 (-31.3, 26.7)</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>OC use (ever vs. never)</td>
<td>-0.46 (-22.5, 21.6)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Current HRT use (yes vs. no)</td>
<td>-7.42 (-33.1, 18.2)</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>HRT Use (ever vs. never)</td>
<td>-24.04 (-45.0, -3.1)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Oophorectomy (yes vs. no)</td>
<td>-24.64 (-48.1, -1.2)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Mastectomy (yes vs. no)</td>
<td>-15.76 (-55.2, 23.7)</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.44 (-2.4, 1.47)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>-140.71 (-307.4, 25.9)</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Percent of time in MVPA (%)</td>
<td>-0.11 (-3.6, 3.4)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Percent of time sedentary (%)</td>
<td>0.43 (-0.7, 1.6)</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Time elapsed between blood draw and NanoString (days)</td>
<td>0.11 (-0.08, 0.3)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>BRCA1 mutation status (carrier vs. non-carrier)</td>
<td>-24.13 (-43.9, -4.3)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding (ever vs. never)</td>
<td>-13.37 (-32.6, 5.9)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Increasing duration of breastfeeding (months)</td>
<td>0.68 (-0.2, 1.6)</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>% MVPA (%)</td>
<td>0.47 (-28.5, 29.0)</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Cartridge Number</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cartridge 1</td>
<td>3.01 (-47.9, 54.0)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Cartridge 2</td>
<td>-1.52 (54.0, 49.4)</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Cartridge 3</td>
<td>-1.07 (-52.0, 49.9)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Cartridge 4</td>
<td>-0.44 (-51.4, 50.5)</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Cartridge 5</td>
<td>-4.23 (-55.2, 46.7)</td>
<td>0.87</td>
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</table>
Table 9. Adjusted geometric mean levels and 95% CI of BRCA1 mRNA for high (≥ 75th percentile) versus low levels (<75th percentile) of physical activity for entire study population (n=50)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Univariate¹</th>
<th>Model 1²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low % MVPA</td>
<td>36</td>
<td>158.2 (147, 169)</td>
<td>158.9 (148, 169)</td>
</tr>
<tr>
<td>High % MVPA</td>
<td>14</td>
<td>155.0 (137, 173)</td>
<td>153.2 (136, 170)</td>
</tr>
<tr>
<td>(P)</td>
<td>0.77</td>
<td>0.58</td>
<td>0.95</td>
</tr>
<tr>
<td>(P_{\text{trend}})</td>
<td>0.95</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Low Mean MVPA/day(min)</td>
<td>37</td>
<td>158.6 (148, 170)</td>
<td>159.4 (149, 170)</td>
</tr>
<tr>
<td>High Mean MVPA/day(min)</td>
<td>13</td>
<td>153.6 (135, 172)</td>
<td>151.4 (133, 170)</td>
</tr>
<tr>
<td>(P)</td>
<td>0.64</td>
<td>0.47</td>
<td>0.07</td>
</tr>
<tr>
<td>(P_{\text{trend}})</td>
<td>0.84</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Low % Sedentary</td>
<td>41</td>
<td>156.6 (146, 167)</td>
<td>158.0 (148, 168)</td>
</tr>
<tr>
<td>High % Sedentary</td>
<td>9</td>
<td>160.4 (138, 183)</td>
<td>153.9 (132, 175)</td>
</tr>
<tr>
<td>(P)</td>
<td>0.76</td>
<td>0.73</td>
<td>0.07</td>
</tr>
<tr>
<td>(P_{\text{trend}})</td>
<td>0.46</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Low Mean Sedentary/day</td>
<td>37</td>
<td>155.8 (145, 167)</td>
<td>158.2 (148, 169)</td>
</tr>
<tr>
<td>High Mean Sedentary/day</td>
<td>13</td>
<td>161.5 (143, 180)</td>
<td>154.7 (137, 173)</td>
</tr>
<tr>
<td>(P)</td>
<td>0.60</td>
<td>0.74</td>
<td>0.05</td>
</tr>
<tr>
<td>(P_{\text{trend}})</td>
<td>0.95</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Low MET Rate</td>
<td>36</td>
<td>156.4 (145, 167)</td>
<td>156.2 (146, 167)</td>
</tr>
<tr>
<td>High MET Rate</td>
<td>14</td>
<td>159.7 (142, 178)</td>
<td>160.2 (143, 177)</td>
</tr>
<tr>
<td>(P)</td>
<td>0.75</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>(P_{\text{trend}})</td>
<td>0.50</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

¹ Univariate geometric means and 95% Confidence Intervals
² Multivariate geometric means and 95% CI adjusted for BRCA1 mutation status (carrier/non-carrier), age (years), and WHR (waist-to-hip ratio)
Table 10. Adjusted geometric mean levels and 95% CI of *BRCA1* mRNA for high (≥ 75th percentile) versus low levels (<75th percentile) of anthropometric variables for entire study population (n=50)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Univariate¹</th>
<th>Model 1²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low % BMI</td>
<td>37</td>
<td>157.9 (147, 169)</td>
<td>157.6 (147, 168)</td>
</tr>
<tr>
<td>High % BMI</td>
<td>13</td>
<td>155.6 (137, 174)</td>
<td>156.4 (137, 175)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.83</td>
<td>P = 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend = 0.65</td>
<td>P_trend = 0.19</td>
</tr>
<tr>
<td>Low Waist Circumference</td>
<td>37</td>
<td>160.0 (149, 171)</td>
<td>160.1 (149, 171)</td>
</tr>
<tr>
<td>High Waist Circumference</td>
<td>13</td>
<td>149.7 (131, 168)</td>
<td>149.3 (130, 168)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.33</td>
<td>P = 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend = 0.35</td>
<td>P_trend = 0.15</td>
</tr>
<tr>
<td>Low Hip Circumference</td>
<td>37</td>
<td>157.1 (146, 168)</td>
<td>156.4 (146, 167)</td>
</tr>
<tr>
<td>High Hip Circumference</td>
<td>13</td>
<td>157.8 (139, 176)</td>
<td>160.0 (142, 178)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.95</td>
<td>P = 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend = 0.99</td>
<td>P_trend = 0.19</td>
</tr>
<tr>
<td>Low Waist/Hip</td>
<td>37</td>
<td>157.6 (147, 169)</td>
<td>157.3 (146, 168)</td>
</tr>
<tr>
<td>High Waist/Hip</td>
<td>13</td>
<td>156.4 (138, 175)</td>
<td>157.4 (138, 176)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.91</td>
<td>P = 0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend = 0.10</td>
<td>P_trend = 0.07</td>
</tr>
<tr>
<td>Low Index of Central obesity</td>
<td>38</td>
<td>159.3 (149, 170)</td>
<td>159.8 (149, 170)</td>
</tr>
<tr>
<td>High Index of Central obesity</td>
<td>12</td>
<td>151.0 (132, 170)</td>
<td>149.2 (129, 169)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.45</td>
<td>P = 0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_trend = 0.28</td>
<td>P_trend = 0.13</td>
</tr>
</tbody>
</table>

¹ Univariate geometric means and 95% CI
² Multivariate geometric means and 95% CI adjusted for *BRCA1* mutation status (carrier/non-carrier), age (years), and percent MVPA
Chapter 5
Discussion

This study is the first to show lower \textit{BRCA1} expression in the white blood cells of healthy, unaffected \textit{BRCA1} mutation carriers. Also of particular interest was the borderline significant decrease in \textit{BRCA1} mRNA expression observed in women who were the most sedentary or who had the highest levels of central adiposity.

\textbf{5.1 \textit{BRCA1} mRNA Expression by \textit{BRCA1} mutation status}

\textit{BRCA1} mRNA expression was significantly lower in mutation carriers versus non-carriers (139.9 counts vs. 164.1 counts; $P = 0.02$). The observed difference in \textit{BRCA1} expression between the two groups is an important one for our hypothesis that increasing \textit{BRCA1} expression may play a role in \textit{BRCA}-associated cancer prevention. The concept of haploinsufficiency (i.e. \textit{BRCA1} mutation carriers produce insufficient levels of \textit{BRCA1} protein to prevent a carcinogenic event and cancerous cell development) is currently a hypothesis that rationalizes the increased incidence of cancer experienced by mutation carriers. The phenotype of decreased mRNA expression due to a mutated and non-functioning allele has never before been observed in healthy mutation carriers and no evidence exists to prove that decreased expression leads to a carcinogenic event.

Lower \textit{BRCA1} mRNA expression has previously been reported in the tumour cells of \textit{BRCA1} mutation carriers. Kainu \textit{et al.} investigated \textit{BRCA1} mRNA expression in the breast cancer tumours in women who were \textit{BRCA1} mutation carriers ($n = 25$) and non-carriers ($n = 29$). The expression levels of \textit{BRCA1} mRNA were six times higher in the sporadic tumours versus the \textit{BRCA1} tumours (480 counts/mm$^2$ versus 4530 counts/mm$^2$; $P < 0.0001$). \textit{BRCA1} expression in the normal breast epithelium was similar throughout all of the patients regardless of \textit{BRCA1} mutation status, signifying an inactivation of the wild type \textit{BRCA1} allele in mutation carriers within the tumours. The authors of this study conclude that mutation carriers may be identified through RNA expression profiling of the tumour with 95% accuracy$^{118}$. Nevertheless, this would not allow the phenotypic identification of healthy pre-cancerous \textit{BRCA1} mutation carriers.

To our knowledge, decreased expression of \textit{BRCA1} mRNA has never been previously shown in healthy (pre-cancerous) women who carry \textit{BRCA1} mutations. Two previous studies have
provided preliminary evidence substantiating the theory of haploinsufficiency. The first was an in vivo study which reported significantly lower mammary gland \textit{BRCA1} mRNA expression in \textit{BRCA1}+/\sim mice (n =30) compared with wild type mice ($P < 0.001$)\textsuperscript{90}. The second study investigated \textit{BRCA1} protein levels within healthy \textit{BRCA1} mutation carriers. Baldeyron \textit{et al.} reported a three to five fold reduction in \textit{BRCA1} protein levels in lymphoblastoid cell lines derived from three \textit{BRCA1} mutation carriers, compared to the \textit{BRCA1} protein levels in cell lines from non-carriers\textsuperscript{119}.

Although \textit{BRCA1} mRNA expression was significantly lower in \textit{BRCA1} mutations carriers, there was overlap by mutation status. Table 11 outlines the mutation types and location within the gene and protein of 13 of the 14 \textit{BRCA1} mutation carriers in this study. One of the 14 carriers was referred to the FBCRU from another centre; thus, her specific mutation type is unknown to our clinic. The NanoString probe for \textit{BRCA1} mRNA quantification targeted a 100 base pair sequence spanning exon 6 and exon 7 of the \textit{BRCA1} transcript\textsuperscript{120}. None of the five mutation types were located in either exon 6 or exon 7; therefore NanoString quantified both normal and mutant \textit{BRCA1} mRNA.

Germline mutations in the \textit{BRCA1} gene may be located anywhere within the 22 exons, with the majority of known pathogenic mutations generating premature termination codons (PTCs). mRNA transcripts with PTCs are typically degraded in a mechanism called nonsense-mediated mRNA decay (NMD) in order to prevent the synthesis of potentially harmful truncated protein products. The decay of mutant \textit{BRCA1} mRNA has been shown to result in a 1.5 to 5-fold decrease in mRNA abundance. However, PTCs located in the last exon (i.e. 5382insC) or very close to the translation initiation codon in exon 2 (185delAG) may escape NMD\textsuperscript{121}. Table 11 also outlines whether the mutation type results in NMD. The destruction of mutant \textit{BRCA1} mRNA as a result of NMD may explain the difference in \textit{BRCA1} expression observed between the two groups (mutation carriers and non-carriers) in this study. Future studies should utilize RNA sequencing, in order to differentiate wild type versus mutant transcripts. This method of quantification might explain the overlap in expression between mutation carriers and non-carriers observed in this study.

Although \textit{BRCA1} expression was significantly lower in \textit{BRCA1} mutation carriers, it is important to note and explain the differences observed between the two groups. Women who
BRCA1 mutations are part of a unique population and the small number of BRCA1 mutation carrier participants in our study is a reflection of the recruitment criteria and difficulty in enrolling unaffected mutation carriers. Dr. Narod and his study team at the Familial Breast Cancer Research Unit (FBCRU) have compiled a database of over 15,000 BRCA1 and BRCA2 mutation carriers from multiple centres and countries around the world. However, many of these women visited the FBCRU for genetic counseling after receiving a cancer diagnosis. The eligibility criteria for the current study included participants that were free from a personal history of cancer. Furthermore, women were required to visit the study clinic on two separate occasions. These two criteria limited the number of women who were eligible for the study and who were available and willing to travel to the FBCRU in Toronto.

Several other differences were observed between BRCA1 mutation carriers versus non-carriers: BRCA1 mutation carriers were significantly older than non-carriers in this study (44.4 years vs. 34.4 years; \(P = 0.02\)), likely contributing to the differences in parity and menopausal status. BRCA1 mutations are more prevalent in women of Ashkenazi Jewish descent compared to other ethnicities, and thus, a greater number of the mutation carriers in this study were of Jewish background\(^4,5\). It was expected that BRCA1 mutation carriers would have higher rates of prophylactic mastectomies and oophorectomies compared to non-carriers as these surgeries present primary prevention options for this high-risk population\(^5\).

BRCA1 mutation carriers were significantly less sedentary (7.90 hours/week vs. 8.87 hours/week; \(P = 0.03\)) and more active (higher MET rate: 1.94 vs. 1.79; \(P = 0.01\)) than non-carriers. Although the differences were statistically significant, they were small. This may be due to the small sample size of mutation carriers (\(n = 14\)). A few relatively active BRCA1 mutation carriers may have increased the mean MET rate and decreased the percent of time spent sedentary of the mutation carrier group. Another possibility is that mutation carriers, due to their status, might be aware of the association between physical activity and sporadic breast cancer risk and thus might engage in these health-promoting behaviours. Conflicting findings exist in the literature regarding whether or not BRCA1 mutation carriers engage in more health-promoting behaviours than non-carriers\(^122-124\). Spector et al. interviewed ten BRCA1/2 mutation carriers regarding their current lifestyle behaviours. Most of the women reported making lifestyle changes after learning about their elevated risk for breast cancer. Four of the ten women reported engaging in 30 minutes or more of moderate activity five times per week.
However two of the ten women were smokers and one of ten women ate the recommended five or more servings of fruits and vegetables per day\textsuperscript{122}.

Emmons \textit{et al}. investigated behavioural risk factors including smoking status, physical activity level, sun protection, and fruit and vegetable consumption for women presenting for genetic testing. The authors reported that women presenting for genetic testing have substantially better health behaviours than women in the general population. Specifically, less than 10\% of the women were smokers (vs. 23\% in the general population), 67\% engaged in regular physical activity (vs. 16\% in the general population), and 67\% reported consuming sufficient amounts of fruits and vegetables per day (vs. 30\% in the general population)\textsuperscript{123}. In contrast, findings published by O’Neill \textit{et al}. reported no changes in diet or physical activity level following genetic testing in 115 women. Participants completed diet and physical activity questionnaires prior to testing, one month following results, and six months following results. No differences were observed across the three time points for diet or physical activity, with most of the participants failing to meet recommended guidelines for fruit and vegetable intake\textsuperscript{124}. Thus given the conflicting findings present in the literature, it is not possible to conclude that \textit{BRCA1} mutation carriers engage in more health promoting behaviours than non-carriers.

The current study and its finding of lower mRNA \textit{BRCA1} expression in mutation carriers provides preliminary evidence that mutation carriers are in fact haploinsufficient for \textit{BRCA1} mRNA. This finding has important implications for women and families who carry \textit{BRCA1} mutations; it provides mechanistic evidence for the increased cancer risk observed in these individuals. Future studies can focus on investigating factors that affect \textit{BRCA1} expression, with the aim of increasing \textit{BRCA1} mRNA expression to prevent cancer.

\textbf{5.2 Physical Activity and \textit{BRCA1} mRNA Expression}

We observed a borderline significant inverse relationship between sedentary time and \textit{BRCA1} expression. This finding is in line with current literature outlining the detrimental and possibly additive effects of sedentary time, physical inactivity and television watching on chronic disease\textsuperscript{125}. Prolonged sedentary behavior (including sitting, watching television and using a computer) has been associated with increased cancer incidence and mortality, independent of physical activity levels, making this an important public health concern\textsuperscript{125,126}. Whether sedentary behaviour affects \textit{BRCA}-associated cancer has yet to be determined. Although the
deleterious effects of increased sedentary time on cancer risk may be due to reduced physical activity along with the resultant metabolic and hormonal consequences including hyperinsulinemia and insulin resistance, elevated levels of circulating growth factors and sex hormones, this study’s findings suggest that alterations in gene expression may also be involved.

No significant relationship ($P \geq 0.05$) was observed between any of the other measures of physical activity and $BRCA1$ expression. However, conflicting results were observed between increasing physical activity and $BRCA1$ expression; although not statistically significant, MET rate was positively associated with $BRCA1$ expression while MVPA was negatively associated with expression. The lack of concordance and significant association between physical activity and $BRCA1$ expression points towards perhaps an even more important role of sedentary behavior per se rather than physical activity with respect to influencing gene expression. However, the lack of significant association observed in this study may be explained by several factors including a small sample size. Perhaps with a greater number of participants, a larger effect would have been observed. Additionally, the study population may have been too homogeneous in terms of physical activity level (i.e., the range of physical activity level was not great enough to observe a difference in $BRCA1$ expression). Although there were some individuals (both mutation carriers and non-carriers) who were more active, even the most active woman was relatively sedentary. Perhaps a more dichotomous group, in terms of physical activity level, may have yielded a larger difference in $BRCA1$ expression.

Given the lack of association observed in this study between current physical activity level and $BRCA1$ mRNA expression, it may be plausible that adult $BRCA1$ expression is influenced by childhood or adolescent physical activity. However, the current study was not able to assess this relationship. Evidence in the literature suggests that adolescent physical activity may affect both $BRCA1$ expression and $BRCA$-associated cancer risk. Wang et al. investigated the relationship between prepubertal physical activity and adult $BRCA1$ expression in rodents. Higher $BRCA1$ expression was observed in the rats who engaged in prepubertal activity compared to those who were sedentary. King et al. reported a delay in the onset of $BRCA$-associated breast cancer with increasing levels of physical activity during teenage years. $BRCA1$ expression therefore may be affected by physical activity level at earlier stages in life (i.e., during childhood, puberty, or adolescence) rather than by current activity.
Collectively these findings point towards the need for two large-scale studies specifically in mutation carriers. The first study should aim to evaluate the relationship between childhood/adolescent physical activity (and inactivity) and adult $BRCA1$ expression, while the second study should aim to further investigate how current physical activity and inactivity affect $BRCA1$ expression. This second aim might be accomplished by assessing a greater number of $BRCA1$ mutation carriers with a wider level of physical activity. To our knowledge, this represents the first evaluation of physical activity/sedentary time and gene expression in women with a hereditary predisposition. Nonetheless, given the multiple comparisons and relatively small sample sizes, these findings should be interpreted with caution and require replication in a larger study population.

5.3 Anthropometrics and $BRCA1$ mRNA Expression
No significant relationship ($P \geq 0.05$) was observed between $BRCA1$ mRNA expression and the five anthropometric variables (BMI, waist circumference, hip circumference, WHR, ICO). However, there was evidence of a trend between increasing central adiposity (waist circumference and ICO) and $BRCA1$ expression, both in the univariate and multivariate analyses. These findings provide preliminary evidence that central adiposity (abdominal fat) may influence $BRCA1$ expression.

Of the women in the highest quartile for ICO ($\geq 0.55$) nine of the total thirteen (69%) were premenopausal and four (31%) were postmenopausal. Of the women in the highest quartile for waist circumference ($\geq 35$ inches) eight of the total thirteen (62%) were premenopausal and five (38%) were postmenopausal. As previously discussed, in the premenopausal obese woman, circulating estrogens may be lower due to irregular and anovulatory menstrual cycles. This decrease in circulating estrogen may mediate the decrease in $BRCA1$ expression observed in this study. However, within the postmenopausal obese woman, estrogen levels may be higher than in the normal weight postmenopausal woman. Given that 31% of the women in the highest quartile for ICO and 38% of the women in the highest quartile for waist circumference were postmenopausal, lower levels of estrogen may not be the mechanism by which high abdominal obesity mediates a decrease in $BRCA1$ expression.
As previously mentioned, adiposity increases the risk for postmenopausal breast cancer in the general population\textsuperscript{72}. Increased risk is thought to be conferred by the increased exposure of breast tissue to endogenous estrogen released from adipose tissue\textsuperscript{128}. Although general adiposity (as measured by BMI) is associated with an increased risk, central adiposity (as measured by waist and hip circumference) has been shown to have the strongest associations with PM breast cancer risk\textsuperscript{129}. Central adiposity may confer an increased risk to postmenopausal breast cancer via the presence of insulin resistance\textsuperscript{128}. This hypothesis is supported by findings that aberrant insulin signaling and higher concentrations of insulin-like growth factor 1 (IGF-1) are associated with increased risk of estrogen-receptor positive breast cancer\textsuperscript{130}. IGF-1 and aberrant insulin signaling mediate events that encourage neoplastic transformation. Interestingly, the BRCA1 protein has been found to negatively regulate IGF-1 expression and \textit{BRCA1} knockdown induces the expression of IGF-1\textsuperscript{131}. IGF-1 has also been shown to modulate BRCA1 abundance via AKT, a downstream mediator of IGF-1, suggesting a complex feedback loop between IGF-1 and BRCA1. These results indicate that increased central adiposity may lower the expression of the tumour suppressor gene \textit{BRCA1} due to increases in insulin and changes in the IGF-1-BRCA1 feedback loop, thereby increasing risk of \textit{BRCA}-associated cancers.

This finding does have important clinical implications (following confirmation in a larger sample size); \textit{BRCA1} mutation carriers may be counseled on the importance of maintaining a healthy body weight in order to maintain increased levels of \textit{BRCA1} and decrease \textit{BRCA}-associated cancer risk. Furthermore, the increase in endogenous estrogen production due to central adiposity may lead to an increase in estrogen mediated DSBs and therefore increased genomic instability\textsuperscript{25}. As BRCA1 is required to repair these DSBs, mutation carriers with high central adiposity may be at increased risk for \textit{BRCA}-associated cancer compared to mutation carriers with a healthy level of central adiposity, perhaps due to higher levels of endogenous estrogen and therefore increased levels of estrogen-mediated DSBs. Given the trend of decreased \textit{BRCA1} expression in women with higher levels of central adiposity observed in this study, the relationship between obesity, or more specifically abdominal obesity, and risk of \textit{BRCA}-associated cancers warrants further investigation.
5.4 Other factors associated with BRCA1 mRNA expression

Within this study, several variables were found to be associated with BRCA1 mRNA expression (Table 8, \( P \leq 0.20 \)) including age, menopausal status, parity, HRT use, oophorectomy, and breastfeeding. An inverse relationship was observed for all six of these variables and BRCA1 expression. Both parity and breastfeeding were found to be associated with lower BRCA1 expression. As previously discussed, BRCA1 expression in the breast tissue increases during pregnancy\(^85\) and levels then fall during breastfeeding\(^87\). Estrogen levels, which increase steadily during pregnancy\(^132\), have also been shown to increase BRCA1 expression\(^94,95\). Therefore, it is somewhat counterintuitive that parity was associated with a decrease in BRCA1 expression rather than an increase. Oophorectomy and menopausal status were also both associated with a decrease in BRCA1 expression in this study. It is well established that following oophorectomy or menopause, circulating estrogen levels decrease\(^133\). This decrease in circulating estrogen is a plausible explanation for the decrease in BRCA1 expression observed in this study.

BRCA1 mutation carriers differed significantly from non-carriers with respect to the six variables listed above. Given the decreased BRCA1 expression also seen in carriers (versus non-carriers), it is reasonable to hypothesize that the decreased expression observed with increased parity and breastfeeding, menopausal status, oophorectomy, HRT use, and age is closely linked to mutation status. The relationship between BRCA1 expression and the six variables above may actually be a reflection of the effect of BRCA1 mutation status on expression. Futures studies need to validate these findings by investigating factors that affect peripheral blood cell BRCA1 expression within a larger group of mutation carriers.

Within the literature, several factors have been reported to influence BRCA1 expression. Puberty and pregnancy are associated with increased BRCA1 expression while breastfeeding is associated with a reduction\(^85,87\). BRCA1 expression decreases with age, oophorectomy, and menopause\(^85\). Several dietary factors consumed at various points throughout the lifespan in both in vivo and in vitro experiments have been shown to affect BRCA1 expression. Flax seed, genistein, whole wheat, and either linoleic acid or estradiol (separately), have been shown to have a positive association with BRCA1 expression\(^88,90-92\). Dietary factors shown to have an inverse association with BRCA1 expression are a high-fat n-3 PUFA diet, linoleic acid and...
estradiol (in conjunction), and pomegranate. Reproductive hormones, including estrogen and progesterone, have also been shown to up-regulate BRCA1 expression and cortisol was shown to down-regulate BRCA1 expression. Lastly, Wang et al. and Magbanua et al. reported a positive associated between physical activity and BRCA1 expression.

5.5 Strengths, Limitations, and Future Directions
There are many strengths associated with the current study including the evaluation of the effect of multiple exposures on BRCA1 mRNA expression, the use of validated and accurate measurement tools to assess physical activity and RNA expression, and the consistency of the methodology of RNA isolation (RNA was stabilized within 30 minutes of blood draw) and the high quality RNA used for quantification. The current study also possesses certain weaknesses including small sample size and homogeneity of the study population, unmeasured confounding, use of peripheral blood lymphocyte BRCA1 expression as a surrogate for breast or ovarian BRCA1 expression, as well as limitations associated with accelerometers.

It is probable that BRCA1 expression may be affected by variables not measured within the context of this study. One variable that may hypothetically affect BRCA1 expression is the stage of menstrual cycle. Estrogen levels vary significantly throughout the menstrual cycle: during the early follicular phase, estrogen levels are at their lowest. Estrogen levels gradually increase and peak just before ovulation (day 14). Following ovulation, estrogen levels are at a moderate level throughout the luteal phase and decline to the lowest level just before menstruation. As previously mentioned, estrogen may affect the expression level of BRCA1 and therefore the stage of menstrual cycle may also affect BRCA1 expression. This information was not collected and thus the relationship between these two variables cannot be assessed within this study.

Another variable that was not measured in this study, which may affect BRCA1 expression, is stress. Ritter et al. reported an inverse association between the stress hormone cortisol and BRCA1 expression in mice. Neither current nor lifetime levels of chronic or acute stress were measured in this study; however they may have affected BRCA1 expression. Considering the reduced level of BRCA1 expression observed in BRCA1 mutation carriers in this study, it is plausible that this decrease may have been mediated by stress. Conflicting findings exist in the
literature regarding stress and anxiety levels as a result of genetic testing. Several studies in the United States and Norway showed no changes in anxiety in $BRCA1$ mutation carriers 6 months post testing\textsuperscript{136,137}. However, studies from the United Kingdom and Australia have reported an increase in anxiety in women who tested positive for $BRCA1$ mutations compared to those who tested negative\textsuperscript{138,139}. Therefore, whether the women in the current study had higher levels of stress due to their mutation status and whether this stress may have mediated the decrease in $BRCA1$ expression is unknown.

$BRCA1$ expression was measured in the peripheral blood lymphocytes (white blood cells) as a surrogate measure of $BRCA1$ expression in the breast or ovarian tissue. Blood samples rather than breast biopsies were used as blood collection is minimally invasive and is associated with little risk. As this is a pilot study, it would have been unethical to take breast biopsy samples from healthy participants. Measuring $BRCA1$ expression outside of the tissues where decreased expression may lead to cancer (breast and ovaries) represents a limitation of the current study. Additionally, although no relationship was observed between physical activity and $BRCA1$ expression in white blood cells, expression may be changing within the breast, ovaries, or other tissue.

Assessing physical activity level in participants is challenging in terms of accurately and reliably measuring true activity. Activity monitors, such as accelerometers, provide an objective method of measuring activity that escapes the subjectivity of questionnaires. In fact the reliability and validity of the measurement of physical activity by questionnaires is low\textsuperscript{140}. Accelerometers are useful tools in that they are able to objectively measure long-term physical activity without over-burdening the participant. Inter-instrument reliability of ActiGraph’s GT3X accelerometer has consistently been found to be high\textsuperscript{141}. Santos-Lozano \textit{et al.} investigated the GT3X inter-instrument reliability in a participant that wore 8 GT3X devices on the right or left hip; coefficients were high for vector magnitude outputs of all three axes ($r \geq 0.925$)\textsuperscript{141}. Furthermore, within this study, physical activity results obtained from the GT3X accelerometer aligned with each participant’s physical activity log, indicating that the accelerometer was a valid tool.
However, accelerometers generate less accurate measurements for light activity or activity that does not involve the whole body (i.e., cycling). Accelerometer data is also not able to portray the energy cost associated with standing, upper body movements, static work, or vertical lift. It was assumed in this study that the one week of body movement monitoring with the GT3X accelerometer represented a typical week of habitual exercise for each participant. However, participants may have altered their physical activity level throughout this particular week, as they were aware they were being monitored for level of activity.

Isolating high quality RNA and accurate measurement of RNA from whole blood can be challenging. RNA is not as stable as DNA, and thus degrades at a fast rate after blood collection. RNA quality, as measured by the Agilent 2100 Bioanalyzer, was high for all of the samples in this study (mean sample RNA concentration was 173.7ng/µL, range: 85-368 ng/µL, mean RIN=8.7, range; 7.9-9.3). Samples were stabilized within 30 minutes of isolation, however, RNA yield and therefore BRCA1 mRNA expression may have been affected in the time between isolation and stabilization with RNA later.

RNA was quantified using NanoString’s nCounter Analysis System, which provides advantages over existing methods of quantifying RNA expression (including a lack of enzymatic reactions or bias). The quantity of the gene of interest is also reported as counts, which is a direct measure of the number of RNA transcripts and requires no amplification of the target (unlike other methods of RNA quantification such as RT-PCR). Therefore, NanoString allows for the quantification of a target present in small quantity, and avoids the error and/or bias associated with amplification. However, measurement error within the system may have affected BRCA1 expression. Northcott et al. reported on the reproducibility of results by the nCounter Analysis System in the measurement of the expression level of 22 medulloblastoma subgroup-specific signature genes. NanoString assay was compared with Affymetrix expression data on n = 101 medulloblastomas and a high concordance rate was demonstrated (Pearson correlation r = 0.86). The validity of the nCounter Analysis System was then assessed on a second set of 130 medulloblastomas. The system was able to assign 98% of the medulloblastoma tumours to the correct subgroup. Finally, reproducibility was assessed by repeating the assay across three geographical sites (Toronto, Canada; Miami, the United States; Geneva, Switzerland). Scatterplot analysis was used to confirm the results produced in Toronto and a high degree of correlation was observed (Toronto/Miami r = 0.97; Toronto/Geneva r =
Furthermore, Geiss et al. investigated the sensitivity of the nCounter Analysis System by comparing signal levels of 14 genes as assessed on three platform 1) the nCounter Analysis System 2) GeneChip microarray and 3) TaqMan. The nCounter Analysis System was found to be more sensitive than the other two assays \(^{109}\).

### 5.6 Conclusion

In summary, results from this small exploratory study suggest \(BRCA1\) mutation carriers may have significantly lower \(BRCA1\) expression, which may predispose somatic cells to a “second-hit” or loss of the remaining functional \(BRCA1\) copy. It was also observed that prolonged periods of sedentary behavior, waist circumference, and ICO are all inversely associated with \(BRCA1\) mRNA expression. Whether these associations also exists at the protein level, or importantly, translates to enhanced cancer risk is unknown, but it is suggestive of a possible detrimental role of sedentary time and central adiposity on \(BRCA1\) gene expression.

Given the preliminary evidence that exists supporting a relationship between adolescent physical activity and \(BRCA\)-associated cancer risk \(^{127}\) as well as the results demonstrated by Wang et al. \(^{64}\), the next step at the FBCRU will be to collect information on lifetime physical activity (prepubertal, pubertal, adolescent, and adult physical activity). Lifetime and current physical activity questionnaires will be given to all \(BRCA1\) mutation carriers at baseline and every two years with follow-up. This information will be used to investigate the relationship between physical activity performed throughout development years on \(BRCA\)-associated cancer.

As this study was a pilot study, results should be confirmed in a larger study. Specifically, the decreased expression observed in \(BRCA1\) mutation carriers should be confirmed by measuring \(BRCA1\) expression in a greater number of mutation carriers. \(BRCA1\) mRNA expression should also be quantified utilizing RNA sequencing, in order to determine the quantity of both the mutant and wild type transcripts. The inverse relationship observed between \(BRCA1\) expression and ICO/waist circumference should also be confirmed in a larger study in \(BRCA1\) mutation carriers specifically. Findings from this study should be confirmed by measuring \(BRCA1\) protein expression. Protein will be isolated from white blood cells and \(BRCA1\) protein will be measured used Western Blots and the rabbit polyclonal IgG C-20 \(BRCA1\) Antibody from Santa Cruz Biotechnology.
Taken together, findings from this study provide important preliminary insight into how sedentary time and central adiposity may mediate cancer risk in this high-risk population. This study offers the potential for a clinically important paradigm shift in the prevention strategies available for *BRCA1* mutation carriers. The prospect of changing lifestyle for the purpose of preventing breast cancer in high-risk women, complemented by mechanistic evidence, warrants evaluation in large-scale prospective studies.
Table 11. *BRCA1* Mutation types and location within the transcript; mutation effect on mRNA and protein expression

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Participant Count</th>
<th>Exon Involved</th>
<th>Nonsense-mediated Decay</th>
</tr>
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<tbody>
<tr>
<td>185delAG</td>
<td>7</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>5382insC</td>
<td>2</td>
<td>20</td>
<td>No</td>
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<tr>
<td>1293del40</td>
<td>2</td>
<td>11</td>
<td>Yes</td>
</tr>
<tr>
<td>3867 G&gt;T</td>
<td>1</td>
<td>11</td>
<td>Yes</td>
</tr>
<tr>
<td>MLPA-detected deletion spanning exon 1-2</td>
<td>1</td>
<td>1-2</td>
<td>Yes</td>
</tr>
</tbody>
</table>
References


127. King M, Marks J, Mandell J. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2.


Appendices
Appendix I- Informed Consent Form
Appendix II- Questionnaire
Appendix III- Accelerometer Time Log
Appendix I
Informed Consent Form

The Effect of Physical Activity on BRCA1 Gene and Protein Expression

PRINCIPAL INVESTIGATOR
Dr. Joanne Kotsopoulos

SITE
Women’s College Research Institute

BACKGROUND
Women with a BRCA1 mutation have a high lifetime risk of developing breast cancer (80% vs. 12% for women without the mutation). Prevention in these women is an important part of their care. Women with a mutation have one normal copy of the BRCA1 gene and so only produce half the amount of the BRCA1 protein. Dr. Kotsopoulos and her team will try to determine whether physical activity may decrease may increase BRCA1 protein levels. To do so, they will measure daily physical activity levels and BRCA1 protein levels in 100 women. Since low BRCA1 protein levels are believed to contribute to cancer development in women with a mutation, physical activity might reverse the effect of the mutation by increasing the amount of BRCA1 protein.

PURPOSE OF RESEARCH
The purpose of this study is to determine if physical activity affects BRCA1 protein levels in women. The results from this research could have a significant impact if it can show that BRCA1 levels are affected by physical activity. Future studies could evaluate if this increase in BRCA1 levels is linked with a decreased risk of breast cancer. More importantly, the outcomes of this study and future studies will ensure that women with a BRCA1 mutation are provided with safe and effective lifestyle prevention methods.

RELEVANCE
This pilot study will enable us to perform a larger study to confirm that there is a potential for physical activity to decrease the risk of hereditary breast and ovarian cancer, specifically in individuals with a BRCA1 mutation.

WHO CAN PARTICIPATE IN THE STUDY?
- Individuals who fully understand the study and give informed consent to participate as demonstrated by signing the provided consent form.
- Women who are at least 18.
- Women with no personal history of cancer.
- Women who are not currently pregnant or breast-feeding.

WHO CANNOT PARTICIPATE IN THE STUDY?
- Women who do not meet the criteria above.
- Individuals who are unable to give voluntary, informed consent or who will not comply with study procedures.

STUDY DESIGN
The study will include 100 women that will be divided into two groups:
- **Women with a BRCA1 mutation:** this group will include 50 women who have a BRCA1 mutation.
- **General Study Control Group:** this group will include 50 women who do not have a BRCA1 mutation, or women who do not know whether or not they have a BRCA1 mutation.

WHAT WILL HAPPEN DURING THE STUDY?
All women will be asked to meet with the study coordinator for two short 15 minute visits at the Familial Breast Cancer Research Unit at Women’s College Research Institute, located at 790 Bay Street on the 7th Floor in Toronto, Ontario. Please bring your completed consent form (this form) and the research questionnaire that asks about fertility history, breast cancer screening, medical and reproductive history, vitamin use, sun exposure, physical activity and lifestyle factors as well as family history of cancer to your first appointment. We estimate that the questionnaire should take about 30 minutes to
complete. If you do not feel comfortable answering any of the questions in the questionnaire, please know you are not obliged to respond and may leave answers blank. If you prefer, the questionnaire may be completed over the telephone with our research coordinator. The study coordinator will also instruct you on how to use the accelerometer. You will be asked to wear an accelerometer on an elasticized belt on your right hip for 7 days during waking hours except during bathing and water activities. **You will also be asked to keep a log of the hours of the day that you wear the accelerometer.** The accelerometer measures movement and calculates amount of energy burned. Body measurements (weight, height, hip and waist circumference) will also be taken on your first visit.

After 7 days, participants will return to the Familial Breast Cancer Research Unit to return the accelerometer. During this visit, a phlebotomist (someone who is trained in taking blood) will draw 20 cubic centimetres of blood (about four tablespoons). Blood samples will be used to obtain DNA so we can determine whether physical activity affects BRCA1 gene and protein levels. To respect your privacy, no identifying data will accompany these samples.

You may stop participation at any time.

Please note: This study will NOT be testing participant **BRCA1** mutation status.

**WHAT SHOULD I DO TO PARTICIPATE IN THE STUDY?**

If you are willing to participate, please contact our study coordinator, Rachael Pettapiece-Phillips by email rachael.pettapiece-phillips@wchospital.ca or by phone 416-351-3800 x.2869 to arrange your visit to our clinic.

**CONFIDENTIALITY**

Your privacy will be respected at all times. Any of your personal information (information about you and your health that identifies you as an individual) collected or obtained, whether you choose to participate or not, will be kept confidential and protected to the fullest extent of the law. All personal information collected will be kept in a secure location. The study staff, the Women’s College Hospital Research Ethics Board or employees of Health Canada may look at your personal information for purposes associated with the study, but will only be allowed to see your records under the supervision of the Principal Investigator and will be obligated to protect your privacy and not disclose your personal information. None of your personal information will be given to anyone without your permission unless required by law.

**BENEFITS**

Participation in this research does not provide any direct benefit to you. There is no compensation for your participation in this study. However, the results from this study may confirm that there is a potential for physical activity to cause increased production of the **BRCA1** gene and/or protein in **BRCA1** mutation carriers. Future studies can determine if this means reduced breast cancer risk for women with a **BRCA1** mutation.

**RISKS, DISCOMFORTS, AND SAFETY**

I understand that there are possible discomforts, risks, and side effects associated with the study. I am aware that having a blood sample taken may cause some discomfort and/or bruising. I will be given all necessary medical care for injury or illness that results from giving a blood sample. Some participants may also experience anxiety about their risk of developing breast or ovarian cancer. I understand that if at any time throughout or following the study I feel discomfort or anxiety about any aspect of the study, I may contact the study coordinator. The study coordinator will then provide a referral for counselling at Women’s College Hospital.

There are no risks associated with wearing the accelerometer. I must not participate in the research if I am pregnant or breast-feeding.

**CONSENT TO STUDY**

I understand that I have been asked to participate in a research project of familial breast and ovarian cancer. I understand that if I agree to participate I will be asked questions about my medical and dietary history. I will be asked to allow body measurements to be taken at the first visit to the Familial Breast Cancer Research Unit at Women’s College Research Institute. I will then be asked to wear the accelerometer for a period of 7 days. I will also be asked to return to the research institute a second
time, one week after the first visit, to return the accelerometer and provide a blood sample of 20 cubic centimetres to the principal investigator.

I understand that testing will be done on this blood with the purpose of measuring BRCA1 gene and protein levels. The RNA and protein extracted from the blood sample will be stored in the laboratory of the principal investigator and will become the property of the principal investigator.

Blood samples may be sent to other academic institutions for additional studies of the hereditary basis of cancer, in which case no identifying information will be provided. I understand that the blood samples will not be used for purposes other than the study of familial breast and ovarian cancer.

WITHDRAWAL FROM STUDY PARTICIPATION
I understand that my participation is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation at any time without prejudice to or loss of my medical care or the benefits to which I, or my family, are otherwise entitled. I understand that my participation will not affect my choice of, or access to, treatment or screening. I understand that my participation may be terminated with or without my consent.

REQUEST FOR MORE INFORMATION
I understand that I may ask more questions about the study. Dr. Kotsopoulos is available to answer my questions and concerns (Tel. 416-351-3732 Ext 2126). If I have any questions about my rights as a research participant, I can contact Diana Raymond-Watts, Manager of the Women’s College Hospital Research Ethics Board at (416) 351-2535. This person is not involved with the research project in any way and calling her will not affect my participation in the study.

CONFIRMATION OF PARTICIPATION
I confirm that the purpose of the research, the study procedures that I will undergo and the possible discomforts that I may experience have been explained to me in sufficient detail.

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care.

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

YES _______
NO _______

Please check the appropriate box to indicate that you have read and understood this consent form and if you agree or not to participate in this study:

☐ I do not consent to participate in this study.
☐ I consent to participate in this study.

Participant’s Signature ______________________ Date ______________________

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

Investigator’s Signature ______________________ Date ______________________
Appendix II
Questionnaire
The effect of physical activity and body size on \textit{BRCA1} mRNA expression

This questionnaire is part of a research study to improve our understanding of the factors that influence risk of hereditary breast cancer.

Name: _________________________

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

Date Completed: ___/___/______

___/___/______

___/___/______

___/___/______

___/___/______
SECTION I – Fertility History

1. Have you ever been pregnant?
   - No → Go to question 6.
   - Yes

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

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<tr>
<th>#</th>
<th>Year of Pregnancy</th>
<th>Miscarriage</th>
<th>Therapeutic Abortion</th>
<th>Still Born</th>
<th>Live Born (Month/Year of Birth)</th>
<th>Length of Pregnancy (In Weeks)</th>
<th>Birth Weight (If known)</th>
<th>Months of Breast Feeding</th>
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*(for twins, enter ‘T’ at the corresponding cell)*

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

   Circle which pregnancies (1-9 in chart above):

   1 2 3 4 5 6 7 8 9

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

   Circle which child (1-9 in chart below) you experienced this with:

   1 2 3 4 5 6 7 8 9

5. Have you ever taken medication to stop milk production?
   - No
   - Yes → Name of medication (if known): __________________
           Method: ___ injections or ___ pills

   Circle with which child (1-9 in chart below) you took this medication.
6. How old were you when you had your first menstrual period? _____ YEARS OLD

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

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

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

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

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

12. What was the reason your periods stopped? (Select one only)
    - Natural Menopause (change of life)
    - Hysterectomy (uterus removed/ovaries not removed)
    - Uterus and ovaries removed
    - Oophorectomy (ovaries removed/uterus not removed)
    - Medication / Chemotherapy
    - Other (please specify): ____________________________________________

13. Have you ever seen a doctor for a problem of difficulty in getting pregnant or in carrying a pregnancy, such as several miscarriages?
    - No → Go to question 16.
    - Yes → What reason did the doctor give to explain why you had trouble getting or staying pregnant? (Please check all that apply.)
      - __ no problem was found
      - __ problem with cervix
      - __ problem with ovaries
      - __ partner has fertility problem
      - __ problem with fallopian tubes
      - __ endometriosis
      - __ other (please specify): ________________________________
14. Have you ever taken medication to increase your chances of becoming pregnant?
   □ No
   □ Yes → Name of medication(s): ____________________________
   For how many months did you take this medication? _____ months
   What years did you take this medication? _____, _____

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

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

<table>
<thead>
<tr>
<th></th>
<th>Name of Medication</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used</th>
<th>Method</th>
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16b. Are you currently using birth control pills, Depo-Provera or Norplant?
   □ No
   □ Yes

17a. Have you ever taken hormone replacement therapy for menopause (i.e. estrogen, progesterone)?
   □ No → Go to question 18.
   □ Yes → Complete table below:

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<th></th>
<th>Name of Hormone</th>
<th>Starting Year</th>
<th>Ending Year</th>
<th>Length of time used (Years)</th>
<th>Method</th>
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</table>
17b. Are you currently taking hormone replacement therapy?
☐ No
☐ Yes → Name of hormone: ___________________ dose? __________

SECTION II – Breast Cancer Screening/Prevention

18. Have you ever taken any drugs for the prevention of breast cancer (i.e. drugs taken before any diagnosis of breast cancer)?
☐ No
☐ I’m not sure
☐ Yes → Name of Drug: ___ Tamoxifen (Nolvadex)
      ___ Raloxifene (Evista)
      ___ Aromasin (Exemestane)
      ___ Femara (Letrozole)
      ___ Arimidex (Anastrozole)
      ___ Other (please name) ______________________
Number of pills per day: __________
Date started: ___ / ___ / ___ (mm / yyyy) to ___ / ___ / ___ (mm / yyyy)

19. Have you ever participated in a clinical trial for the prevention of breast cancer?
☐ No
☐ Yes → Which one? ___ Tamoxifen Trial (Tamoxifen vs. placebo)
      ___ STAR Trial (Tamoxifen vs. Raloxifene)
      ___ Other: __________________________
      ___ Unknown

Do you know which drug you took? ☐ No ☐ Yes
If yes, which one? ______________________

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

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

23. Have you ever had a breast biopsy?
   - [ ] No
   - [ ] Yes → Number of biopsies: _______
     Month/Year of last biopsy: _____/_______
     What was the result of the biopsy? (Check all that apply)
     [ ] normal [ ] DCIS [ ] atypical hyperplasia
     [ ] cancer [ ] LCIS [ ] fibroadenoma
     [ ] other (please specify): ____________________________

24. Approximately how many mammograms have you had in your lifetime? I have had _____ mammograms. If you do not recall the exact number, please provide a range.
   Age at first mammogram? ________ years old.
   Age at last mammogram? ________ years old.

25. Have you ever had any abnormalities detected by mammogram?
   - [ ] No
   - [ ] Yes → Please describe the abnormality:
     __________________________________________
     Month/Year of abnormality: _____/_______

26. Have you ever had MRI screening of your breasts?
   - [ ] No
   - [ ] Yes → Age at first MRI: ________
     How many MRIs have you had? ________________
     Where were they done: ___________________________
     Were there any abnormalities detected by MRI?
     - [ ] No
     - [ ] Yes → If yes, please describe:
       __________________________________________
       Year of abnormality: ________
       What year was your last MRI done? ________
SECTION III – Medical History

27. Are you taking or have you taken any medications (prescriptions or over-the-counter) on a regular basis?
   □ No
   □ Yes → Complete:

   1. Drug: ____________________________________________
      From: _______ (year) to _______ (year)
      Reason: _________________________________________

   2. Drug: ____________________________________________
      From: _______ (year) to _______ (year)
      Reason: _________________________________________

   3. Drug: ____________________________________________
      From: _______ (year) to _______ (year)
      Reason: _________________________________________

28. Have you ever been diagnosed with any cancer other than breast or ovarian?
   □ No
   □ Yes → What type? ___________________ Year of Diagnosis?

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

__________________________________________________
__________________________________________________

30. Have you received your genetic test results?
   □ No
   □ Yes → Date you received your results: __ __ / __ __ / __ __ __ __ (mm / dd / yyyy)

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

   __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ __ _______
SECTION IV – Reproductive/Abdominal Surgeries

33. Have you had one or both of your ovaries removed (oophorectomy) for reasons other than ovarian cancer? (e.g. preventative measures, fibroids, cyst, scar tissue, or pain)
   □ No
   □ Yes → Year of surgery: _________
   Reason for the surgery: _______________________
   Number of ovaries removed: ___ One ___ Two

34. Have you ever had a tubal ligation (fallopian tubes tied)?
   □ No
   □ Yes → Year: _________

35. Have you had surgery performed on your reproductive organs: including ovaries, fallopian tubes, or uterus? (e.g. hysterectomy, cervix removed, myomectomy, D & C)
   □ No
   □ Yes → Year: _________
   What operation was performed? _______________________
   Reason for the surgery: _______________________

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

SECTION V – Personal Information

37. Place of Birth: ____________________________
   City Province/State

38. Current Residence: ____________________________
   City Province/State

39. Ethnic Background: ____________________________

40a. What is the major ancestry of your father (paternal)? (Please circle one option.)
   African/Caribbean/African American Irish
   (country of origin: _________) Italian
   Ashkenazi Jewish Native American (Amer. Indian)
<table>
<thead>
<tr>
<th>Ancestry</th>
<th>Country of Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian/Pacific Islander</td>
<td>Polish/Slavic/Eastern</td>
</tr>
<tr>
<td>Dutch</td>
<td>Russian</td>
</tr>
<tr>
<td>(country of origin: ________)</td>
<td></td>
</tr>
<tr>
<td>English</td>
<td>Scandinavian (Swedish/Finnish/ Norwegian/Dane)</td>
</tr>
<tr>
<td>European Bloc countries</td>
<td>Scot-Irish or Scottish</td>
</tr>
<tr>
<td>French Canadian</td>
<td>Sephardic Jewish</td>
</tr>
<tr>
<td>German</td>
<td>Other (specify: __________)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>Unknown</td>
</tr>
<tr>
<td>(country of origin: ____________ )</td>
<td></td>
</tr>
</tbody>
</table>

40b. What is the major ancestry of your **mother** (maternal)? *(Please circle one option.)*

- African/Caribbean/African American
- Irish
  (country of origin: ____________ )
- Ashkenazi Jewish
- Native American (Amer. Indian)
- Asian/Pacific Islander
- Polish/Slavic/Eastern
  (country of origin: ____________ )
- Russian
- Dutch
- Scandinavian (Swedish/Finnish/ Norwegian/Dane)
- French Canadian
- Sephardic Jewish
- German
- Other (specify: ____________ )
- Unknown
  (country of origin: ____________ )

40c. Please indicate if your grandparents were:

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

41. What is your current work status? *(Mark all that apply)*

- ☐ Retired  ☐ Homemaker  ☐ Office job  ☐ Other (please specify) ____________________

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

- ☐ No  ☐ Yes

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

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

45. Think back to when you were **18 years old**, about the time you graduated from high school.
   - How much did you weigh then? _____ pounds
   - **at age 30? _____ pounds**
   - **at age 40? _____ pounds**
   - What is the **most** you have ever weighed (exclude pregnancy)? _____ pounds
   - How old were you when you weighed the **most? _____ years old**

46. Do you know how much you weighed when **you** were born?
   - [ ] No
   - [ ] Yes → _____ pounds _____ ounces **OR** _____ grams

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

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

49. Were you part of a multiple birth (i.e. twin, triplet)?
   - [ ] No
   - [ ] Yes → *(Please check one)*
     - ____ twin → Are you an identical twin? [ ] No [ ] Yes
     - ____ triplet
     - ____ other (please specify): __________

**SECTION VI – Lifestyle**
We would like you to describe your lifestyle since its modifications may result in a reduced risk of breast and ovarian cancer.

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

51. Do you or did you ever drink coffee regularly?
   - [ ] No
   - [ ] Yes → From: __________ (age first started), Until: __________ (age last used)
     - Caffeinated [ ] Yes [ ] No
     - Decaffeinated [ ] Yes [ ] No
If you answered yes, please check the best answer:

<table>
<thead>
<tr>
<th></th>
<th>1 – 2 cups/day</th>
<th>3 – 4 cups/day</th>
<th>5 or more cups/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 2 cups/day</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
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<tr>
<td>3 – 4 cups/day</td>
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</tr>
<tr>
<td>5 or more cups/day</td>
<td>[ ]</td>
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</tr>
</tbody>
</table>

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

- [ ] Yesterday
- [ ] Daily during the last week
- [ ] Occasionally during the last week
- [ ] Other. Please specify_____________________________ ____________

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

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

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

- [ ] Yesterday
- [ ] Daily during the last week
- [ ] Occasionally during the last week
- [ ] Other. Please specify_____________________________ ____________

53. Do you or have you ever had alcoholic beverages?
- [ ] No
- [ ] Yes → From: __________ (age first started), Until: __________ (age last used)

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

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<thead>
<tr>
<th></th>
<th>0-3</th>
<th>4-9</th>
<th>10-20</th>
<th>20 or more</th>
</tr>
</thead>
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<td>[ ]</td>
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</tbody>
</table>

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

- □ Beer
- □ Wine
- □ Hard liquor
Please indicate when was the last time you drank alcoholic beverages?

☐ Yesterday
☐ Daily during the last week
☐ Occasionally during the last week
☐ Other. Please specify__________________________________________

SECTION VII – Physical Activity

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

a) Walking to and from school or work

<table>
<thead>
<tr>
<th>Age Range</th>
<th>None</th>
<th>0.5</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-10</th>
<th>11+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grades 7-8</td>
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<tr>
<td>Grades 9-12</td>
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<td>Ages 18-22</td>
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<tr>
<td>Ages 30-34</td>
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b) TV watching

<table>
<thead>
<tr>
<th>Age Range</th>
<th>None</th>
<th>1</th>
<th>2-5</th>
<th>6-10</th>
<th>11-20</th>
<th>21-40</th>
<th>41-60</th>
<th>61-90</th>
<th>91+</th>
</tr>
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<tbody>
<tr>
<td>Grades 7-8</td>
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</table>

c) Strenuous Recreational Activity causing increased breathing, heart-rate or sweating (e.g., running, aerobics, lap swimming)

<table>
<thead>
<tr>
<th>Age Range</th>
<th>None</th>
<th>0.5</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-10</th>
<th>11+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grades 7-8</td>
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</tr>
</tbody>
</table>
Grades 9-12

Ages 18-22

Ages 23-29

Ages 30-34

d) Moderate Recreational Activity e.g., hiking, walking for exercise, casual cycling, yard work (do not count activities already reported)

<table>
<thead>
<tr>
<th></th>
<th>None</th>
<th>0.5</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-10</th>
<th>11+</th>
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<tbody>
<tr>
<td>Grades 7-8</td>
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</tbody>
</table>

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

| Activity                                      | Time per WEEK
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z er</td>
</tr>
<tr>
<td>Walking for exercise or walking to work</td>
<td></td>
</tr>
<tr>
<td>Jogging (slower than 6 minutes/Km)</td>
<td></td>
</tr>
<tr>
<td>Running (6 minutes/Km or faster)</td>
<td></td>
</tr>
<tr>
<td>Bicycling (include stationary machine)</td>
<td></td>
</tr>
<tr>
<td>Tennis, squash, racquetball</td>
<td></td>
</tr>
<tr>
<td>Lap swimming</td>
<td></td>
</tr>
<tr>
<td>Other aerobic exercise (aerobic, dance, ski or stair machine, etc.)</td>
<td></td>
</tr>
<tr>
<td>Lower intensity exercise (yoga, stretching, toning)</td>
<td></td>
</tr>
<tr>
<td>Other vigorous activities (e.g., Lawn mowing)</td>
<td></td>
</tr>
<tr>
<td>Weight training or resistance Arm weights</td>
<td></td>
</tr>
<tr>
<td>Other vigorous activities (e.g., Lawn mowing)</td>
<td></td>
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<tr>
<td>Weight training or resistance Arm weights</td>
<td></td>
</tr>
<tr>
<td>Lower intensity exercise (yoga, stretching, toning)</td>
<td></td>
</tr>
</tbody>
</table>
56. What is your usual walking pace outdoors?
- Easy, casual (less than 3 km/h)
- Normal, average (3-4.9 km/h)
- Brisk pace (5-6.9 km/h)
- Unable to walk

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

<table>
<thead>
<tr>
<th>Time per WEEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Hrs</td>
</tr>
<tr>
<td>Standing or walking around work or away from home? (hrs./week)</td>
</tr>
<tr>
<td>Standing or walking around at home? (hrs./week)</td>
</tr>
<tr>
<td>Sitting at work or away from home or while driving? (hrs./week)</td>
</tr>
<tr>
<td>Sitting at home while watching TV/VCR (hrs./week)</td>
</tr>
<tr>
<td>Other sitting at home (e.g., reading, meal times, at desk)? (hrs./week)</td>
</tr>
</tbody>
</table>

58. How many flights of stairs (not individual steps) do you climb daily?
- 2 flight or less
- 3-4
- 5-9
- 10-14
- 15 or more flights

59. The following items are about activities you might currently do during a typical day. Does your current health limit you in these activities? If so, how much? (Mark one response on each line)

<table>
<thead>
<tr>
<th>Yes, limited a lot</th>
<th>Yes, limited a little</th>
<th>No, not limited at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Lifting or carrying groceries</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Climbing several flights of stairs</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Climbing one flight of stairs</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Bending, kneeling, or stooping</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Walking more than 1.5 Km</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
SECTION VIII – Current Vitamin and Supplement Use

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

☐ No
☐ Yes → If yes, please specify in table below

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

61. Think back to the time you were pregnant. In the 12 months before pregnancy, did you take any dietary supplements (i.e., folic acid, vitamin B6, vitamin B12, multivitamin, prenatal vitamin)? Please indicate the type, brand name, frequency of intake and the month when intake was started/stopped for prior to each pregnancy. (If you have never had children, please proceed to question 62 in Section VIII).

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

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

63. Please complete the following chart on your outdoor activities. Check all that apply.

<table>
<thead>
<tr>
<th>Outdoor Activity</th>
<th>Time of Year</th>
<th>Time of Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity 1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td></td>
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<tr>
<td></td>
<td>Fall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td></td>
</tr>
<tr>
<td>How many hours/week?</td>
<td></td>
<td>9am – 11am</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 am – 3 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 pm – 6 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Past 6 pm</td>
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<td></td>
<td></td>
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<tr>
<td>Activity 2:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td></td>
</tr>
<tr>
<td>How many hours/week?</td>
<td></td>
<td>9am – 11am</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 am – 3 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 pm – 6 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Past 6 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity 3:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td></td>
</tr>
<tr>
<td>How many hours/week?</td>
<td></td>
<td>9am – 11am</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 am – 3 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 pm – 6 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Past 6 pm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
64. How many hours per week, on average, do you spend outdoors during the day (9am – 6 pm)? ______ hours

65. Please check the responses that apply to you during your outdoor activities:
   a) How often do you use sunscreen?
      □ All the time
      □ Most of the time
      □ Never

   If you use sunscreen, what strength do you typically use?
   SPF <15 □ SPF 15-30 □ SPF >30 □

   b) During outdoor activities, do you wear clothing that covers your arms, legs and torso?
      □ All the time
      □ Most of the time
      □ Never

   c) Do you use tanning beds? No □ Yes □ If yes, how often?
      □ Less than 3 times per year □ 2-3 times per month
      □ 1-3 times per week □ Once per month or less
      □ 3-6 times per week

If yes, what type(s) of tanning bed(s) do you use? (Please check all that apply.)

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Regular</td>
<td></td>
</tr>
<tr>
<td>□ High</td>
<td></td>
</tr>
<tr>
<td>□ Other: _____________</td>
<td></td>
</tr>
</tbody>
</table>

SECTION VII – FAMILY HISTORY OF CANCER

66. Have there been any cancers diagnosed on your mother’s side of the family:
   □ No
   □ Yes → If yes, complete the table below

<table>
<thead>
<tr>
<th>Name</th>
<th>Relationship</th>
<th>Type of Cancer</th>
<th>Year of Diagnosis</th>
<th>Age at Diagnosis</th>
</tr>
</thead>
</table>

67. Have there been any cancers diagnosed on your father’s side of the family:
   □ No
Yes  → If yes, complete the table below

<table>
<thead>
<tr>
<th>Name</th>
<th>Relationship</th>
<th>Type of Cancer</th>
<th>Year of Diagnosis</th>
<th>Age at Diagnosis</th>
</tr>
</thead>
</table>

Information for Follow-Up:

Availability for Telephone Interviews
Please select the day and the time of day when it would be best to reach you.

Weekday: Morning ______ Afternoon ______ Evening ______
Weekend: Morning ______ Afternoon ______ Evening ______

Contact Number:__________________________
Email: __________________________________
Address:   ______________________________________

In the case we cannot reach you, please provide an alternate contact:
Name: ________________________________ Relationship:_____________________________
Contact Number: ________________________
Email: _________________________________
Address:
_________________________________________________________________
_________________________________________________________________
_________________________________________________________________

Please call us if you are changing contact information – 416-351-3732 ext. 2920

FOR OFFICE USE
Date questionnaire received: ____________
Month – Day – Year
Appendix III
Accelerometer Time Log

*****This device is meant to measure your physical activity- so please try to wear it throughout the WHOLE day including when you exercise.******

During each of the 7 days that you wear the accelerometer, please record the start time (time accelerometer was affixed to your hip when you wake up) and the stop time (time accelerometer was removed at night). If you forget to wear the accelerometer on one of the following days (if for example you were sick), please write “N/A” under start time.

***Please do not wear the accelerometer while in the shower, while bathing, or while swimming as it is not waterproof.*****

<table>
<thead>
<tr>
<th>Accelerometer Number</th>
<th>Participant ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Date (dd/mm/yy)</th>
<th>Start Time (hh:mm)</th>
<th>Stop Time (hh:mm)</th>
<th>Did you do any of the following activities…?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>AM</td>
<td>AM</td>
<td></td>
<td>Swimming [ ] Bicycling [ ] Weights [ ]</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>PM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AM</td>
<td>AM</td>
<td></td>
<td>Time of day (e.g. 2-3pm):</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>PM</td>
<td></td>
<td>Length of time (minutes):</td>
</tr>
<tr>
<td>Day 2</td>
<td>AM</td>
<td>AM</td>
<td></td>
<td>Swimming [ ] Bicycling [ ] Weights [ ]</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>PM</td>
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<td>AM</td>
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<td></td>
<td>Time of day (e.g. 2-3pm):</td>
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<tr>
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<td>PM</td>
<td>PM</td>
<td></td>
<td>Length of time (minutes):</td>
</tr>
<tr>
<td>Day 3</td>
<td>AM</td>
<td>AM</td>
<td></td>
<td>Swimming [ ] Bicycling [ ] Weights [ ]</td>
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<td>PM</td>
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<td>AM</td>
<td>AM</td>
<td></td>
<td>Time of day (e.g. 2-3pm):</td>
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<tr>
<td></td>
<td>PM</td>
<td>PM</td>
<td></td>
<td>Length of time (minutes):</td>
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<tr>
<td>Day 4</td>
<td>AM</td>
<td>AM</td>
<td></td>
<td>Swimming [ ] Bicycling [ ] Weights [ ]</td>
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<tr>
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<td>PM</td>
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<td>AM</td>
<td>AM</td>
<td></td>
<td>Time of day (e.g. 2-3pm):</td>
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<tr>
<td></td>
<td>PM</td>
<td>PM</td>
<td></td>
<td>Length of time (minutes):</td>
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<tr>
<td>Day</td>
<td>AM</td>
<td>AM</td>
<td>PM</td>
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<tr>
<td>Day 5</td>
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<td>Day 6</td>
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<tr>
<td>Day 7</td>
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<tr>
<td>Day 8</td>
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<tr>
<td>Day 9</td>
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<tr>
<td>Day 10</td>
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