

INSULIN RESISTANCE, METABOLIC SYNDROME AND TYPE 2 DIABETES IN
WOMEN AT HIGH RISK FOR BREAST CANCER

by

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List of abbreviations**T2D:** Type 2 diabetes**SD:** Standard deviation**IR:** Insulin resistance**N/A :** Not applicable**MetS:** Metabolic syndrome**TG :** Triglycerides**BMI:** Body mass index**WHO :** World health organisation**HDL:** High density lipoprotein**TNF :** Tumor necrosis factor**LDL:** Low density lipoprotein**IL :** Interleukin**Bp:** Blood pressure**BRCA1 or 2 :** Breast Cancer Gene 1 or 2**IGF-1:** Insulin like growth factor-1**MRI :** Magnetic resonance imaging**C-peptide:** Connecting peptide**FBG :** Fasting blood glucose**HTN:** Hypertension**CDA :** Canadian diabetes association**ADH:** Atypical ductal hyperplasia**IDF :** International diabetes federation**LCIS:** Lobular carcinoma in-situ**CVD :** Cardiovascular disease**DCIS:** Ductal carcinoma in-situ**CRP :** C reactive protein**WBHC:** Women's Breast Health Center**HOMA:** Homeostasis model assessment**BW:** Blood work**WC:** Waist circumference

Abstract

Purpose: The overall objective of this prospective study was to quantify the prevalence of insulin resistance (IR), metabolic syndrome (MetS) and type 2 diabetes (T2D) in women at high risk for breast cancer, stratified by menopausal status. We also aimed to calculate the sample size required for a future case-control study comparing these prevalences to those of the general population. **Methods:** Participants consisted of 100 Caucasian women above the age of 35 with an estimated 5yr risk of breast cancer $\geq 1.7\%$. A comprehensive metabolic profile was obtained for each participant based on a questionnaire, fasting blood sample and biophysical measurements. **Results:** In comparison to published prevalence's of IR, MetS and T2D in the general population, the prevalence of IR and MetS is higher in our study sample. High risk postmenopausal women have a higher prevalence of body mass index, waist circumference, MetS and hypertension than premenopausal women. We have shown a significant correlation between Gail score and high-density lipoprotein cholesterol. **Conclusions:** The sample size calculation, based on the prevalence's obtained in the present study, support the significance and feasibility of a future case control study comparing the prevalence of IR and MetS in women at high risk and average risk for breast cancer. Further studies are needed to clarify the underlying mechanisms for the association between IR, MetS, T2D and breast cancer risk.

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CHAPTER 1

1. Introduction

Although the Surveillance, Epidemiology and End Results (SEER) cancer statistics review shows a decreasing trend in both the incidence and mortality for women with breast cancer in the last decade, it remains the most common cancer among women with a lifetime risk of 12% (Altekruse et al., 2010). Type 2 diabetes (T2D), which is also a common disease in the Western population, often occurs in the same patients (Giovannucci et al., 2010). The association between T2D and breast cancer has been subject to extensive epidemiologic research in the last few decades. Recent meta-analyses report a positive association between T2D and breast cancer, with a stronger association in post-menopausal women (Xue & Michels, 2007; Vona-Davis, Howard-McNatt, & Rose, 2007). These authors also report an increase in breast cancer mortality associated with T2D. Even though T2D shares several risk factors with breast cancer such as hyperglycaemia and obesity, studies have shown that the association observed cannot be entirely attributed to the clustering of these 2 diseases with common risk factors (Xue & Michels, 2007).

The pathophysiology that explains the association between these 2 diseases, however, is not yet well established. It is the avid subject of ongoing research. Recent studies have investigated insulin and insulin-like growth factor-1 (IGF-1) in the context of breast cancer pathophysiology and several plausible pathways have been proposed (Xue & Michels, 2007; Key, Appleby, Reeves, & Roddam, 2010). Moreover, the association between T2D and breast cancer has set the stage to study the role of metformin in the prevention and/or management of breast cancer (Goodwin, Ligibel, & Stambolic, 2009).

Positive associations have also been observed between markers of insulin resistance (IR) such as hyperglycaemia (Rapp et al., 2006; Muti et al., 2002) and hyperinsulinemia (Muti et al.,

2002; Kabat et al., 2009; Gunter et al., 2009) and breast cancer. C-peptide, a marker of insulin secretion, has also been linked to breast cancer risk (Verheus et al., 2006; Yang et al., 2001). Considerable attention has been given, in the recent literature, to the potential role of IGF-1 and cancer development, namely because of the potential therapeutic implications.

Insulin-like growth factor-I (IGF-I) is a hormone, which mediates not only growth hormone, but also various anabolic responses in multiple human tissues. For instance, it will improve insulin sensitivity (IS) in patients with T2D (Clemmons, 2012). Recently, IGF-1 concentrations were shown to be positively associated with the risk of estrogen-receptor-positive breast cancer. (Key, Appleby, Reeves, & Roddam, 2010). This led to ongoing studies of inhibition of IGF-1 in women at high risk for breast cancer (Smith, Axelrod, Singh, & Kleinberg, 2011).

Metabolic syndrome (MetS) and its specific features, in association with breast cancer, is a relatively new area of research. MetS is a cluster of risk factors for T2D and cardiovascular diseases, including abdominal obesity, dyslipidemia, hypertension (HTN) and hyperglycaemia (Cardiometabolic Risk Working Group, 2011). It is associated with an increased risk of breast cancer (Agnoli et al., 2010) and with aggressive tumour biology (Healy et al., 2010). The specific components of MetS have also been independently associated with an increased risk of breast cancer (Huang et al., 2010; Connolly, Barnett, Vogt, Li, & Boyde, 2002; Han et al., 2005; Soler, Chatenoud, Negri, Parazzini, Franceschi & La Vecchia, 1999).

Many factors contribute to the risk of developing breast cancer, among which some are innate such as ethnicity and family history. Others, such as lifestyle, dietary and environmental factors are acquired and may be modified in efforts to reduce the associated increase risk (Costanza & Chen, 2010). A sub-group of women are identified as being at significantly higher risk of developing breast cancer and are followed in a high risk clinic for heightened screening

and risk management interventions (Gray, 2010). These women have various predisposing factors. Some will have known genetic mutations to explain their high risk status, while others will be considered high risk on the basis of family history, previous biopsy showing atypical ductal hyperplasia (ADH) or lobular cancer in situ (LCIS), history of mantle radiation or a high score on risk calculation models (Gray, 2010). This sub-group of women at high risk for breast cancer has been the subject of many studies in efforts to better understand the link between their specific risk factors and breast cancer (Costanza & Chen, 2010).

Because of the important metabolic changes that occur during menopausal transition (Duval et al., 2013; Abdalnour et al., 2012; Mauriege et al., 2000), it is important to take in consideration the menopausal status to investigate the relationship between IR, MetS and T2D and risk of breast cancer (Soules et al., 2001). Also, since estrogen obviously plays an intimate but complex role in breast cancer development, studies typically stratify the risk of breast cancer by menopausal status (Xue & Michels, 2007).

Despite the growing body of research on the association between IR, MetS, T2D and breast cancer, the prevalence of these metabolic conditions has yet to be explored in women at high risk for breast cancer. Delineating the numerous modifiable factors that contribute to these women's risk may play a role in risk management in this vulnerable population. This warrants exploring whether IR, MetS and T2D should be added to the list of factors that contribute to this population's high risk status, especially since these metabolic alterations and disease may be modifiable by lifestyle intervention and/or medication to a certain degree.

1.1 Purpose of the Study

The overall objective of the present study is to answer the question: What is the prevalence of IR, MetS and T2D in women at high risk for breast cancer? We aim to describe

the distribution of this prevalence by menopausal status. We will also define the prevalence of the individual components of MetS in women at high risk for breast cancer.

Because the prevalence of IR, MetS and T2D in women at high risk for breast cancer have, to our knowledge, never been described in the literature, it is difficult to estimate the power of an epidemiological study comparing this high risk sub-group to women at average risk for breast cancer. This study will generate the data necessary to determine the sample size required for a case-control study comparing the prevalence of IR, MetS and T2D in women at high risk and average risk for breast cancer.

1.2 Significance of the Study

Clinical decisions about risk reduction strategies are made by weighing risks versus benefits of interventions against likelihood of developing cancer. Accurate estimates of risk are therefore of great value. Given the association between IR, MetS and T2D with an increased risk of breast cancer in women, a description of the prevalence of these phenotypes in this population would help further characterise their risk and the most effective preventive and management interventions.

Furthermore, since many of the risk factors for IR, MetS and T2D are modifiable, if the high risk women are found to have a higher prevalence of these conditions, this will add weight to the dietary and physical activity recommendations already used, and set grounds for additional risk reduction strategies to be studied in randomized control trials.

Finally, this study will serve to fill the dearth in literature on the prevalence of IR, MetS and T2D in a high risk population for breast cancer.

CHAPTER 2

2. Literature Review

2.1 Breast Cancer

2.1.1 Risk factors for breast cancer

Many factors have been associated with an increased risk of breast cancer, including demographics, personal history of benign or malignant breast diseases, family history and genetic predisposition for breast cancer, estrogen exposure, lifestyle and dietary factors, and exposure to radiation (Costanza & Chen, 2010). Among these risk factors, some are innate and others are acquired through life exposures. Certain acquired risk factors are modifiable and are the target of recommendations and interventions in a high risk population for breast cancer.

2.1.1.1 Demographics

Gender is one of the strongest risk factor for breast cancer with women being affected 100 times more frequently than men (Jamal et al., 2009). Age is also a strong risk factor for the development of breast cancer. In the US population, the incidence of breast cancer rises with age with the steepest slope between ages 35-49 yrs. It then progressively decreases and reaches a near plateau in the 75-80 years age group (Surveillance, Epidemiology and End Results [SEER], 2007). This is thought to reflect hormonal changes, with the initial decrease in incidence seen in the peri-menopausal women (Costanza & Chen, 2010).

There are racial differences in the incidence of breast cancer related to a combination of lifestyle, genetics, socioeconomic status and access to medical care. The SEER data (2007) shows a higher incidence in white women and a progressively decreasing incidence rates for Blacks, Asians/Pacific Islanders, and Hispanics, with the lower incidence seen among American Indians/Alaskan Natives women.

2.1.1.2 Personal history of benign or malignant breast disease

Benign breast diseases that are nonproliferative, including fibrocystic changes, papillomas and fibroadenomas are generally not associated with an increased risk of breast cancer. Proliferative benign conditions without atypia such as radial sclerosing lesions and ductal hyperplasia are associated with a 1 to 2-fold increase in the risk of breast cancer. Proliferative lesions with atypia including atypical ductal hyperplasia and atypical lobular hyperplasia are associated with a higher risk of developing breast cancer (4-fold) (Brunicardi & Schwartz, 2005).

Malignant breast lesions, whether in situ or invasive, increase the risk of a second breast cancer. Invasive breast cancer survivors have a 2-6-fold increase of developing a second primary breast cancer (Chen, Thompson, Semenciw & Mao, 1999). In a study by Innos and Horn-Ross (2008), women with ductal carcinoma in situ (DCIS) had an increased risk of contralateral DCIS (standardized incidence ratio [SIR] 4.2 and 95% confidence interval [CI] 3.7-4.7), contralateral invasive cancer (SIR 1.4, 95% CI 1.2-1.5), ipsilateral DCIS (SIR 4.2, 95% CI 3.5-5.0), and ipsilateral invasive cancer (SIR 1.7, 95% CI 1.4-2.1) in comparison to the general population.

There is debate regarding lobular carcinoma in situ (LCIS) being a risk factor for breast cancer or a true precursor of breast cancer. A recent systematic review of the literature concluded that LCIS is both a risk factor for invasive breast cancer in both breasts (relative risk ranging between 2-12%) and a precursor for invasive breast cancer (Ansquer et al., 2010).

2.1.1.3 Family history and genetic predisposition for breast cancer

The presence of a first degree relative who developed breast cancer before the age of 50 is associated with a two or more times higher risk of developing breast cancer. Furthermore, the

risk is inversely proportional to the age of the relative at diagnosis (McPherson, Steel, & Dixon, 2000).

A genetic predisposition for breast cancer is inherited by autosomal dominant pattern with limited penetrance. Women who develop breast cancer at a very early age, those with bilateral breast cancers, and women with breast cancer as well as a personal or family history of ovarian, colon or prostate cancer are at higher risk of carrying a genetic mutation predisposing them to develop a breast cancer (McPherson et al., 2000).

BReast CAncer gene 1 (BRCA1) and *BReast CAncer gene 2* (BRCA2) are two of the most common susceptibility genes identified in families at high risk for cancer. They are associated with an increased risk for both breast and ovarian cancer. Although encountered more frequently than other known susceptibility genes, they remain infrequent at approximately 0.1 to 0.8 percent in the general population (Fletcher, 2010). The impact of being a mutation carrier for one of these genes, is however profound. As a comparison, the lifetime risk of developing breast cancer in the general population is 11-12%, compared to 20-25% in women with a positive family history, and to 65-85% in women with a positive BRCA1 or carrying 2 mutation (Fletcher, 2010).

2.1.1.4 Endogenous and exogenous estrogen

Breast cancer risk is proportional to the plasma concentration and length of exposure to estrogen. Factors of endogenous estrogen exposure that have been associated with an increased risk of breast cancer include early menarche, higher age at first live birth, late menopause, low parity and not breastfeeding (Costanza & Chen, 2010).

Menopausal transition leads to changes in endogenous estrogen exposure (Xue & Michels, 2007) and has been shown to have an effect on fat distribution (Mauriege et al., 2000),

body composition and cardiometabolic risk factors (Abdulnour et al., 2012). Furthermore, in women there is an association between visceral adiposity and plasma glucose/insulin homeostasis (Lemieux, et al. 1996). It is therefore important to stratify participants according to menopausal status before evaluating the risk of breast cancer.

The association between exogenous hormones and breast cancer risk was first demonstrated by the results of the Women's Health Initiative in the 1990s (Chlebowski et al., 2009). While post-menopausal hormone replacement therapy has been linked to an increased risk of developing breast cancer, the literature on oral contraceptives and infertility treatment remains controversial (Costanza & Chen, 2010).

2.1.1.5 Lifestyle and dietary factors

Obesity has been associated with an increased risk of breast cancer in post-menopausal women (Costanza & Chen, 2010). The postulated explanation for this relationship is based on the peripheral conversion of estrogen precursors to estrogen in adipose tissue. In pre-menopausal women however, obesity is associated with a reduced incidence of breast cancer (Costanza & Chen, 2010). Studies attempting to clarify this relationship are conflicting and the link between obesity and breast cancer in the pre-menopausal population remains a subject of ongoing research (Costanza & Chen, 2010).

Several meta-analyses have demonstrated a relatively consistent positive association between alcohol consumption and the risk of breast cancer (Smith-Warner et al., 1998; Longnecker, 1994). This association has been most consistently shown in hormone receptor positive breast cancer (Costanza & Chen, 2010). In a meta-analysis of 110 epidemiologic studies, a small but significant association was shown between breast cancer and even light alcohol intake (less than one drink per day) compared to abstainers (Bagnardi et al., 2013).

Physical activity has been shown in some studies to slightly decrease the risk of breast cancer but the relationship is complex, relating many overlapping factors such as reduction in estrogens levels and weight (Costanza & Chen, 2010). Many studies have investigated the association between dietary fats and risk of breast cancer. However, the results are inconsistent (Costanza & Chen, 2010). The results of the prospective cohort Nurses Health Study II showed a positive association between the consumption of more than 5 servings of red meat per week and estrogen/progesterone positive premenopausal breast cancer (Cho et al., 2006). The Nurses Health Study results also showed a negative association between the consumption of low fat dairy products and the risk of breast cancer in premenopausal women (Shin et al., 2002). The relationship between smoking and breast cancer is complex but a minimally increased risk has been suggested overall (Costanza & Chen, 2010).

2.1.1.6 Exposure to radiation

Henderson et al. (2010) reported that women treated with chest radiation for childhood cancers (most commonly for Hodgkin lymphoma), have a considerably elevated risk of breast cancer. Furthermore, these women tend to be younger at diagnosis and have a higher incidence of bilateral cancers. Because the previous radiation limits or negates additional radiation therapy, breast cancer survival is also lower in this population (Henderson et al., 2010).

2.1.2 Breast cancer risk prediction models

Various models of breast cancer risk assessment have been proposed, most of which take into account family history, personal history of breast disease and hormonal factors. A commonly used tool is the Gail Model (Gail et al., 1981). It is a simple and easily accessible tool that estimates 5 year and lifetime risk of breast cancer. It was developed by the National Cancer Institute (NCI) and the National Surgical Adjuvant Breast and Bowel Project (NSABP). It can be used in women older than 35yrs. It has been validated in white women (Costantino et al., 1999;

Rockhill, Spiegelman, Byrne, Hunter, & Colditz, 2001) and updated for African American women using data from the Contraceptive and Reproductive Experiences (CARE) study (Gail et al., 2007). It however does not take into consideration paternal family history, extended family history, and age at onset in affected relatives, and has not been validated in ethnicities other than Caucasian and African American.

2.1.3 High risk population for breast cancer

The purpose of breast cancer risk assessment is to identify patients who would benefit from heightened surveillance and risk reduction interventions. Women with an estimated lifetime risk of breast cancer $\geq 20\text{-}25\%$ or a 5-year risk of $\geq 1.7\%$, calculated with a risk assessment tool such as the Gail model (Gail et al., 1981), are generally considered to be at high risk (Newman & Vogel, 2007; Hooks, 2010; Tirona, Sehgal & Ballester, 2010). These thresholds were also used for the inclusion criteria in the National Surgical Adjuvant Breast and Bowel Project (NSABP) chemoprevention trials (Fisher et al., 1998; Fisher et al., 2005; Vogel et al., 2006). In addition, the high risk population at which risk reduction strategies are aimed, also includes women with previous breast biopsy showing ADH or LCIS, women who are carriers of gene mutations such as BRCA1/2, and women with history of chest radiation before the age of 30 (Tirona et al., 2010). Women meeting these criteria should be referred to a specialty clinic for counselling, genetic testing, heightened surveillance and risk management interventions.

2.1.3.1 Surveillance for high risk patients

The National Comprehensive Cancer Network (NCCN) Guidelines for Breast Cancer Screening and Diagnosis have detailed recommendations by high risk subgroup (NCCN 2011). In general, the recommendations for the high risk population are for yearly MRI as an adjunct to yearly mammography, physical examination and breast awareness (self-exam). The American

Cancer Society specifically recommends Screening MRI for women with 20-25% or greater lifetime risk of breast cancer (Saslow et al., 2007). This is supported by a recent study that has demonstrated that MRI can help detect mammographically and clinically occult breast cancer in high-risk populations (Kuhl et al., 2000). Among carriers of BRCA1 and BRCA2 mutations, Warner et al. (2011) showed that annual surveillance with MRI significantly reduces the incidence of advanced-stage breast cancer at detection.

2.1.3.2 Prevention measures for high risk patients

The modifiable risk factors for breast cancer previously mentioned are all potential risk reduction measures that should be included in the counselling of high risk women. In addition, there are specific risk reduction interventions that are aimed at this high risk population, which include chemoprevention (Tamoxifen and Raloxifen) and surgical prophylaxis (bilateral mastectomy and bilateral oophorectomy). These procedures all have risks and complications associated with their risk reduction benefit. These are important to consider and to discuss with patients since physiological as well as psychological issues may influence the appropriateness of one treatment over another in a given woman. The treatment plan should be individualised based on the woman estimate risk, age, menopausal status, concurrent and past medical conditions, as well as personal preference.

Although chemoprophylaxis and surgical prophylaxis are an important part of the management of women at high risk for breast cancer, they are generally reserved for those women with an identified cause (BRCA mutation, LCIS, etc). For the remainder of the women whose risks are determined by a risk calculation model, the prevention measures are limited and further studies attempting to identify specific modifiable risk factors, such as this one, are much needed.

2.2. Insulin Resistance

IR refers to an abnormal glucose response to a given concentration of insulin. It can result from defects intrinsic to target cells such as mutations in the insulin-receptor gene or other defects in genes important for insulin physiology (Moller & Flier, 1991). IR can also result from secondary factors such as abnormal physiologic states (sepsis, starvation, obesity), normal physiologic states (puberty, pregnancy), and specific hormonal and metabolic factors (Cushing's syndrome, pheochromocytoma, diabetes) (Moller & Flier, 1991).

2.2.1 Pathophysiology of insulin resistance

In obese and diabetic patients, the number of insulin receptors is decreased, possibly secondary to down-regulation of the receptors secondary to the hyperinsulinemic state. The insulin receptor function is also affected in obese and diabetic patients with a decreased ability of insulin to stimulate autophosphorylation and tyrosine kinase activity (Moller & Flier, 1991). It was suggested that the activation of IGF-1 receptors may also explain the association between IR and various physiologic states such as MetS, T2D, polycystic ovarian syndrome and infection (Mantzoros, Holman, & Mulder, 2010).

2.2.2 Measuring insulin sensitivity

The value of quantifying insulin sensitivity in the clinical setting is not well established, but may be useful to identify those at increased risk of developing T2D and cardiovascular disease and possibly cancer. There is a growing need for effective measurements of insulin sensitivity in the research setting as the importance of insulin resistance in various pathologic states is investigated.

The gold standard technique for the measurement of insulin sensitivity is the euglycaemic hyperinsulinaemic clamp. The high cost and complexity of this test has led to the development of alternate techniques, such as the intravenous glucose tolerance test and the

insulin suppression test. The indices of insulin sensitivity derived from these tests correlate well with those derived from the euglycaemic hyperinsulinaemic clamp studies. However, these tests are also impractical in research studies with a large number of participants (Borai, Livingstone, & Ferns, 2007).

Various biochemical markers have been evaluated and validated as more efficient and practical measures of insulin resistance. These include the oral glucose tolerance test, the homeostasis model assessment and the quantitative insulin sensitivity check index (Malita et al., 2006; Borai, Livingstone, & Ferns, 2007). C-peptide is also often used as a marker of insulin secretion since they are both secreted by the pancreatic β -cells in equimolar amounts, and C-peptide has a longer half-time in plasma than does insulin. Another advantage of using C-peptide as a marker of insulin secretion is that it is not as affected by diet as insulin and therefore is less variable from one person to another (Xue et Michels, 2007).

2.2.3 Insulin resistance and breast cancer

Most epidemiologic studies investigating the relationship between hyperglycaemia and breast cancer have shown a positive association between the two (Xue & Michels, 2007). The stratification by menopausal status has not been consistent in these studies, with some observing an association in the premenopausal group only, others in the postmenopausal group only and some studies showing no difference among menopausal status (Xue & Michels, 2007).

There is epidemiologic evidence of an association between elevated plasma insulin concentrations and the development of breast cancer with a stronger association in premenopausal women (Xue & Michels, 2007). In one study, insulin concentrations were found to correlate with cancer recurrence and mortality in breast cancer patients (Goodwin et al., 2002).

Because cancer cells use glucose for proliferation, it is thought that hyperglycaemia resulting from insulin resistance may create a favourable environment for the growth of

malignant cells (Simon & Balkau, 2010). By activating the insulin receptor, insulin exerts its effects of glucose uptake, glycogen, lipid and protein synthesis, activation of transcription of specific genes and modulation of cell growth and differentiation (Xue et Michels, 2007). Insulin receptors are found in higher concentrations in breast cancer tissue in comparison to normal breast tissue (Papa et al., 1990), as well as other normal tissues such as liver, muscle and fat (Pezzino et al., 1989). Insulin receptor content in breast cancer tissue correlates positively with tumour size and histologic grade (Papa et al., 1990).

C-peptide, when used as a marker of insulin resistance, has also been associated with an increased risk of breast cancer in multiple studies, with no distinction between premenopausal and post-menopausal women (Xue & Michels, 2007).

Insulin-like growth factor-1 (IGF-1) is a polypeptide with structural similarity to insulin. IGF-1 and insulin also have analogous receptors to which their ligands can cross-react at high concentrations. By binding to its receptor, IGF-1 modifies cell growth, differentiation and transformation. If at high concentration insulin can cross-react with the IGF-receptor, then these cellular effects may promote cancer development (Xue & Michels, 2007). A recent review by the Endogenous Hormones and Breast Cancer Collaborative Group has concluded that there is a positive association between IGF-1 and breast cancer, in both pre- and post-menopausal groups, although limited to estrogen receptor positive tumours (Key, Appleby, Reeves, & Roddam, 2010). There are now ongoing studies of the therapeutic effect of an inhibitor of IGF-1 in women at high risk for breast cancer (Smith, Axelrod, Singh, & Kleinberg, 2011).

2.3 Metabolic Syndrome

MetS is defined as a cluster of risk factors for T2D and cardiovascular diseases, including abdominal obesity, dyslipidemia, hypertension (HTN) and hyperglycaemia (Brien & Katzmarzyk, 2006). Approximately 20-25% of the world's adult population (IDF, 2006), 25% of

Canadians (Ohinmaa, Jacobs, Simpson, & Johnson, 2004) and 34% of Americans (Ervin, 2009) meet the criteria for metabolic syndrome. This cluster of metabolic abnormalities confers a substantial additional CVD risk over and above the sum of the risk associated with each abnormality. In fact, patients with MetS are three times as likely to have, and twice as likely to die from a myocardial infarction or stroke compared with people without the metabolic syndrome. It has been said that “*the clustering of CVD risk factors that typifies the metabolic syndrome is now considered to be the driving force for...the twin global epidemics of type 2 diabetes and CVD*” (IDF, 2006).

Other than T2D and CVD, MetS is associated with a higher risk of fatty liver and cirrhosis (Marceau, et al., 1999), chronic kidney disease (Chen et al., 2004), polycystic ovarian syndrome (Pasquali et al., 1999), gout (Choi, Ford, Li, & Curhan, 2007), colorectal adenomas and sporadic colorectal cancers (Lohsiriwat, Pongsanguansuk, Lertakyamane, & Lohsiriwat, 2010) as well as breast cancer (Agnoli et al., 2010; Connolly, et al., 2002; Han et al., 2005; Healy et al., 2010; Huang et al., 2010; Soler, et al., 1999).

2.3.1 Pathophysiology of metabolic syndrome

The pathogenesis of the MetS and each of its components is complex and not well understood. We know however, that it is predisposed by genetics, physical inactivity, ageing, as well as pro-inflammatory and pro-thrombotic states. Central obesity and IR are acknowledged as key causative factors (IDF, 2006).

The role of pro-inflammatory states is based on the association between MetS and various markers of inflammation such as increased high sensitivity-C-reactive protein (hs-CRP), pro-inflammatory cytokines (interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF- α), serum amyloid A and leptin as well as decreased levels of interleukin-10 (IL-10) and adiponectin. This

is thought to lead to endothelial cell dysfunction, which is the first step in formation of a thrombus, hence the risk of cardiovascular diseases (Devaraj, Rosenson, & Jialal, 2004).

The role of pro-thrombotic states is based on the association between MetS and various hematologic abnormalities such as impaired fibrinolysis (increased plasminogen activator inhibitor-1 (PAI-1)), and increases in fibrinogen, von Willebrand factor (vWF), factor VIII, vitamin K-dependent factors, and factor VII (Devaraj et al., 2004).

2.3.2 Limitations of the metabolic syndrome

The MetS model has been criticised for its limited practical utility. Conclusions of a World Health Organization (WHO) Expert Consultation that evaluated the utility of the MetS concept were that while it is not practical for its use as a diagnostic or management tool, it may be of clinical utility as an educational concept (Simmons et al., 2010).

Furthermore, the definition of MetS has been fairly inconsistent. There has been debate between various organizations, mainly regarding the central obesity criteria. Debates have focused on the actual diagnostic measurement for central obesity and whether it should be an obligatory component of the diagnostic criteria. Also some clinical researchers believe that there should be ethnic specificity within the central obesity (waist circumference) diagnostic measurements.

2.3.3 Diagnosing metabolic syndrome

In 2009 a meeting was held with various major organizations including the International Diabetes Federation (IDF), the American Heart Associations, and other international organizations, which led to the following consensus regarding the definition of MetS.

Table 1. Criteria for diagnosis of MetS with diagnosis requiring any 3 of 5 criteria (Alberti et al., 2009).

Measures	Categorical cut points
↑ Waist circumference*	Population specific recommendations.
↑ Triglycerides or treatment for ↑ triglycerides	≥150 mg/dL (1.7 mmol/L)
↓ HDL-C or treatment for ↓ HDL-C	Men <40 mg/dL (1.0 mmol/L); Women <50 mg/dL (1.3 mmol/L)
↑ Bp or antihypertensive drug therapy in a patient with history of HTN	Systolic ≥130 and/or diastolic ≥85mm Hg
↑ Blood glucose or drug Tx for ↑ blood glucose	≥100 mg/dL (5.6 mmol/L)

HDL-C=High Density Lipoprotein cholesterol, Bp=Blood Pressure, HTN=Hypertension

*Recommended use of IDF cut points until further data available.

While it was decided that an increased waist circumference would not be an obligatory criteria, the argument that the diagnostic measurement should be specific to ethnicity was sustained. The IDF cut points of ethnic specific values for waist circumference were therefore set at 94cm and 80cm respectively for men and women of European, Middle Eastern and Sub-Saharan African descent, and at 90cm and 80cm respectively for men and women of Asian and Central/South American descent (Alberti et al., 2009).

2.3.4 Metabolic syndrome and breast cancer

Very few studies have investigated the relationship between MetS and breast cancer. However, in a recent prospective study by Agnoli et al (2010), MetS was significantly associated with the risk of breast cancer in a group of postmenopausal Italian women. When analysing the components of MetS independently, only serum HDL-cholesterol and triglycerides were significantly associated with breast cancer. However, a significant increased risk was shown with a cumulative number of MetS components. In another recent study, MetS was associated with more aggressive breast tumour biology in postmenopausal women with newly diagnosed breast cancer (Healy et al., 2010). Other studies have also shown an association between specific components of MetS and breast cancer, such as abdominal obesity (Huang et al., 2010; Connolly, et al., 2002) elevated triglycerides and low HDL-cholesterol (Han et al., 2005), as well as high blood pressure (Soler, et al., 1999).

2.4 Type 2 diabetes

The incidence of T2D in industrialised countries has been increasing exponentially in the last few decades. Based on the Canadian Chronic Disease Surveillance System, 5.1% of Canadians aged 20 yrs and older in 2006/7 had a diagnosis of diabetes (Dai et al., 2010). Furthermore, it is believed that up to 30% of diabetes is undiagnosed (Kelly & Booth, 2004). Projection estimates for the Canadian population state that the number of Canadians with T2D in the general population will increase from approximately 1.4 million patients in 2000 to 2.4 million patients in 2016 (Ohinmaa, Jacobs, Simpson, & Johnson, 2004).

Various hormonal processes such as insulin, insulin-like growth factors, estrogen and various cytokines and growth factors play a role in T2D development (Xue & Michels, 2007). Based on these, many possible pathways have been proposed to explain the epidemiologic association between T2D and breast cancer (Gonzalez-Angulo & Meric-Bernstam, 2010; Vona-Davis, Howard-McNatt & Rose, 2007; Xue & Michels, 2007). Contributing to the complexity of this relationship is the fact that T2D and breast cancer have many risk factors in common, confounding the epidemiologic analysis of the association between the two.

2.4.1 Pathophysiology of type 2 diabetes

As cells in the liver, skeletal muscle and adipose tissue become less sensitive and eventually resistant to insulin, glucose uptake is reduced in the cells (transported by glucose GLUT4 transporters in response to insulin PIP3 – PKB/Akt signaling) and increases plasma glucose levels (IDF, 2006; The Metabolic Syndrome Institute, 2011). This triggers the need for increased insulin production by the pancreatic beta cells, which eventually weaken and wear out. When the pancreas can no longer produce sufficient insulin, hyperglycemia results. When this hyperglycemia reaches a certain level, patients are diagnosed with T2D (IDF, 2006).

2.4.2 Diagnosing type 2 diabetes

Fifty percent of North-Americans with T2D are undiagnosed (Harris et al., 1998). In 1998, the clinical practice guidelines for the diagnosis of T2D in Canada were reduced from 7.8 to 7.0 for fasting blood glucose (FBG) in order to improve the sensitivity of the main diagnostic criterion and reduce the number of missed diagnoses (Meltzer et al., 1998). These guidelines were reviewed in 2008 by the Canadian Diabetes Association (CDA, 2008). They were last updated in 2013, after the data collection for the present study, to include $A1C \geq 6.5\%$ as an additional diagnostic criterion (CDA, 2013).

Table 2. Diagnostic criteria for diabetes (T2D) (CDA, 2008).

A confirmatory test must be done on another day in all cases.
Random plasma glucose value ≥ 11.1 mmol/L + Symptoms of T2D*
OR
Fasting plasma glucose ≥ 7.0 mmol/L [†]
OR
Plasma glucose in the 2h sample of the oral glucose tolerance test ≥ 11.1 mmol/L

*Fatigue, polyuria, polydipsia and unexplained weight loss.
[†]No caloric intake ≥ 8 h.

2.4.3 Type 2 diabetes and breast cancer

A number of epidemiologic studies have shown a positive association between T2D and breast cancer. These have been highlighted in many reviews studies (Vona-Davis, Howard-McNatt & Rose, 2007; Wolf, Sadezi, Catane, Karasik & Kaufman, 2005; Xue & Michels, 2007). A meta-analysis revealed a risk ratio and 95% CI of 1.15 (1.12, 1.19) for the development of breast cancer in patients with a history of T2D (Xue & Michels, 2007). This meta-analysis also observed a higher overall summary risk ratio for post-menopausal women (RR: 1.19; 95%CI: 1.15-1.23) in comparison to pre-menopausal women (RR:0.94; 95%CI: 0.80-1.10). In addition, these epidemiologic studies observed increases mortality in breast cancer patients with T2D compared to non-diabetic controls.

2.4.3.1 Common risk factors

Obesity, sedentary lifestyle and a high fat diet are risk factors for both T2D and breast cancer (Xue & Michels, 2007). This confounds the association between these two diseases observed in epidemiologic studies. However, this problem has been addressed by accounting for the time lag between the diagnosis of T2D and the subsequent diagnosis of breast cancer (Wideroff et al., 1997; Weiderpass et al., 1997), by matching groups for weight and height (Muck, Trotnow & Hommel, 1975), by adjusting for body mass index (BMI) (Michels et al., 2003; Talamini et al., 1997), or by controlling for physical activity (Michels et al., 2003). While part of the association between T2D and breast cancer is due to the clustering of the diseases by their common risk factors, it was shown that T2D independently affects the risk of developing breast cancer in women (Xue & Michels, 2007).

CHAPTER 3

3. Method

This study has consisted of a descriptive design with the goal of quantifying the prevalence of IR, MetS and T2D in women at high risk of breast cancer. This has allowed to generate data required for sample size calculation for a subsequent case-control study comparing the prevalence of these metabolic states in women at high risk vs. average risk for breast cancer.

3.1 Participants

Participants were Caucasian women above 35 years of age who are at high risk of developing breast cancer. The exclusion of non-Caucasian women below the age of 35 reflects limitations from our risk calculation model (Gail model), which has not been validated in these subgroups of the population. Also, the threshold for IR based on the homeostasis assessment model varies between ethnicities and cut-offs have not been established for many non-Caucasian

populations. Ethnicity was defined based on self-reported data from the High Risk Breast Assessment Clinic Questionnaire, which was completed by all women prior to their initial consultation to the High Risk Clinic of the Women's Breast Health Center. In this questionnaire, women were asked to check their race/ethnic background from 5 choices: (a) White, (b) Black, (c) Oriental/Asian, (d) Jewish, and (e) Other-specify. Recognising the limitations of self-reported data and the complexity of defining ethnicity, the decision to use this self-reported answer to a questionnaire in order to satisfy our inclusion criteria was based on feasibility and on respect for participants since non-Caucasian participants would not be recruited instead of excluded after recruitment.

We have included women with an estimated 5-year risk of breast cancer $\geq 1.7\%$. This criterion was met by obtaining this score on a risk calculation model (the Gail model), or by having specific risk factors that are known to be associated with a 5-year risk of breast cancer $\geq 1.7\%$. The participants included in the study therefore consisted of women (a) who are BRCA mutation carriers, (b) with LCIS on a previous breast biopsy, (c) with ADH on previous breast biopsy, (d) with a history of mantle radiation, or (e) with a calculated 5-year risk of breast cancer $\geq 1.7\%$ using the Gail model (Gail et al., 1981).

The recruitment was done by sending letters to eligible patients who were or had been followed in the High Risk Clinic at the Women's Breast Health Center in Ottawa, Ontario. Letters were sent to those who satisfy the risk of breast cancer (5-year $\geq 1.7\%$), age (>35) and ethnicity (Caucasian) inclusion criteria based on data from The High Risk Breast Assessment Clinic Questionnaire.

Because the prevalence of IR, MetS and in patients at high risk for breast cancer have, to our knowledge, never been described in the literature, it was difficult to calculate the required sample size for this pilot study. Base on the prevalence of IR (11%), MetS (19%) and T2D (5%)

in the general population (IDF, 2011; Riediger & Clara, 2011; Statistics Canada, 2012) we have therefore aimed to recruit approximately 100 women. The enrolment rate of previous studies among this group of women was approximately 45%. We therefore randomly select 250 patients meeting the selection criteria from the High Risk Clinic, by randomly sampling the High Risk database.

The recruitment letter asked participants who were interested to contact us by telephone or e-mail. We then responded with further information about the study and offered to book the meeting if they chose to participate. We also let them know that we encouraged and supported the accompaniment of family members for translation in order to help overcome language barriers for participants whose first language was not French or English.

The 250 recruitment letters were sent in batches of 50 over a 2-month period in order to be able to keep up with scheduling the meetings and to accommodate participants in a timely fashion. Several responding participants declined participation on the basis of having to attend a specific lab for the blood work. However, because allowing participants to get their blood work done at random labs would have introduced significant variability in our data, we opted to keep this requirement and instead to persist with our recruitment efforts. One month after sending the recruitment letters, 115 reminder letters were sent to those who had not responded. Three letters were returned to us by return mail because of wrong/changed recipient address. Addresses were obtained from The Ottawa Hospital electronic charts. In order to compensate for these, three additional letters were sent to women meeting the selection criteria by following the same random sampling as for the original 250 women. An additional 30 letters were also sent after a 2 month period to reach our goal of 100 participants. The overall response rate of 36% (100/280 women) was slightly lower than the response rate of previous studies.

In order to maximise the accuracy of measurement of breast cancer risk using the Gail model (Gail et al., 1981), our questionnaire included questions allowing to recalculate the Gail score on all of the participants. This was compared to the previously calculated score in the database and any discrepancies were verified from the patient hospital charts. The High Risk clinic will occasionally broaden its inclusion criteria in order to accommodate for risk factors that are not well measured by calculation models and for physician's subjective opinion of a patient's risk despite not meeting the risk model cut-offs. In order to prevent this from introducing sampling error in our study, we had planned to exclude from the data analyses all participants who's 5-year risk of developing breast cancer on final score was less than 1.7. Fortunately none of the calculated 5-year risk scores were less than 1.7 and therefore we did not have to exclude any participants on this basis.

Once the initial lab results were received, we noticed that there were missing details from the lipid profile. After discussion with the laboratory director, this was due to an out-dated blood work requisition being provided by the Women's Breast Health Center. This was addressed in 4 ways: a) Calling all participants who had already received their requisitions but had not gone to the lab yet and faxing them an updated form, b) Asking the lab to do the additional analyses on those women who would present with an old version of the requisition in the meantime, c) Assuring only updated requisitions were used for distribution from that point on at the Women's Brest Health Center, and d) Calling all participants who had already done their blood work to explain the situation and ask them to get a second blood test done. Most participants were agreeable but they did not all accept to repeat the blood work and therefore those with missing data were excluded from the corresponding analyses. There were also a few missing blood work results for certain participants from laboratory errors such as failure to report certain results. This resulted in 96, 88 and 97 women being included in the analysis to determine the prevalence of IR, MetS and T2D respectively.

3.2 Measurements

3.2.1 Gail model

The Gail model was used for calculating estimates risk of breast cancer (Gail et al., 1981). This risk assessment tool is the most commonly used model. It is accessible through the National Cancer Institute website (<http://www.cancer.gov/bcrisktool/Default.aspx>). It is simple to use, requiring electronic entries for seven questions, which once submitted, yields an estimated 5-year and lifetime risk of breast cancer. The seven questions ask about (a) medical history of breast cancer, DCIS or LCIS, (b) age, (c) age at menarche, (d) age at time of first live birth of a child, (e) number of first degree relatives with breast cancer, (f) number of breast biopsies and presence of ADH, and (g) ethnicity.

The model can be used in women older than 35 years and has been validated in Caucasian women (Costantino et al., 1999; Rockhill, Spiegelman, Byrne, Hunter, & Colditz, 2001) and updated for African American women (Gail et al., 2007). Limitations of the Gail model include that it does not account for paternal family history, extended family history, or age at onset in affected relatives. Also, it is not validated in non-Caucasian, non-African American women.

3.2.2 Anthropometric assessment

Weight and height were taken using a mechanical scale and height rod. Waist circumference was taken using a flexible measuring tape placed horizontally just superior to the uppermost border of the iliac crest, at end of normal expiration.

3.2.3 Resting Blood Pressure

Blood pressure measurements were taken using a mercury sphygmomanometer and auscultation of the brachial artery at the antecubital fossa. Two measurements were taken at rest

after the participant had completed their questionnaire. The average of these two measurements was used for analysis.

3.2.4 Blood sampling

Fasting blood samples were taken at a Dynacare laboratory after a 7-10 hour overnight fast. Blood samples were taken for glucose, insulin, total cholesterol, HDL, LDL and triglycerides.

3.2.5 Homeostasis model assessment (HOMA)

The diagnosis of IR was based on the Homeostasis model assessment (HOMA) (Wallace, Levy, & Matthews, 2004). This model provides the following formula for calculation of IR derived from fasting blood glucose and insulin measurements.

$$\text{HOMA-IR} = \text{Insulin (mU/L)} \times \text{Glucose (mmol/L)} / 22.5$$

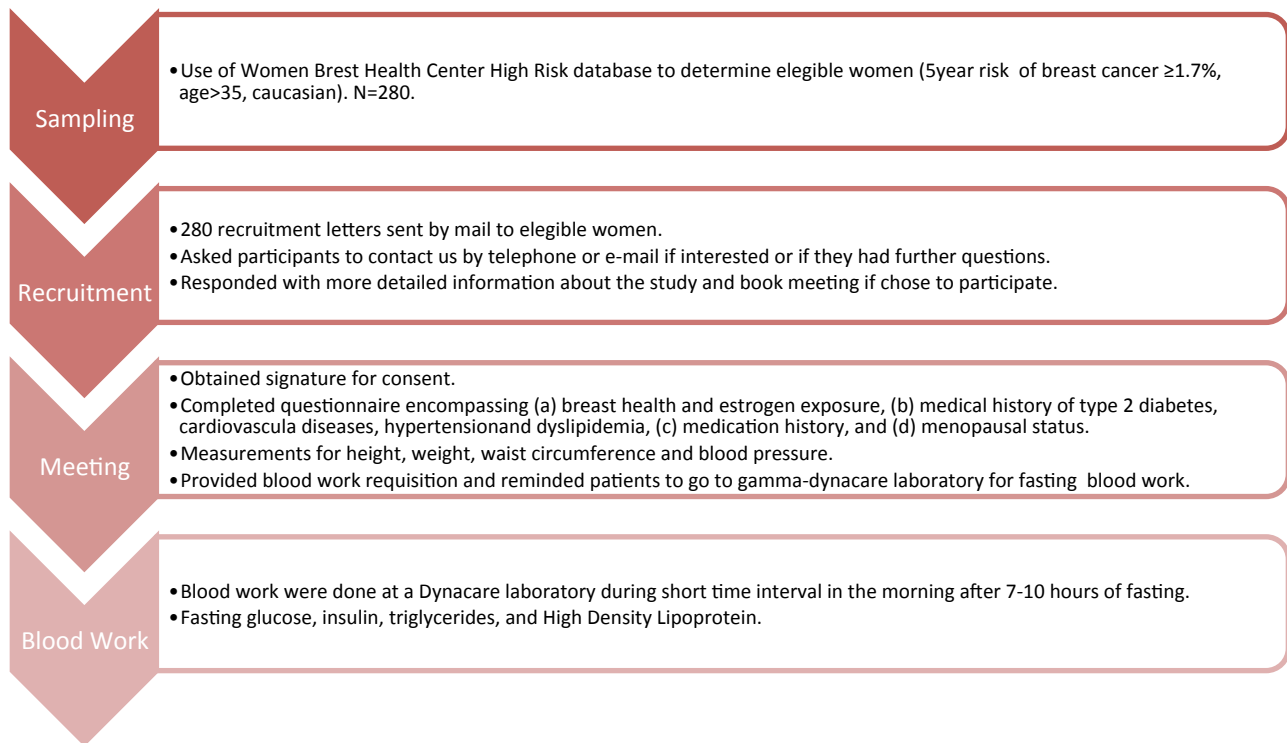
We chose to use this index to measure IR because of its reported practicality and good correlation with the hyperglycaemic clamp test (gold standard) (Malita et al., 2006; Borai, Livingstone, Gordon, & Ferns, 2007). It is less precise than the euglycaemic-hyperinsulinemic clamp technique but less invasive and more applicable to a large number of participants (Bonora et al., 2002). The optimal threshold of homeostasis model assessment for insulin resistance has been shown to vary between ethnicities (Esteghamati et al., 2009). The cut-off point to define IR in a Caucasian population was set at a HOMA-IR value of 2.29 by Radikova et al. (2006) based on a sample of Caucasian rural population with no previous evidence of diabetes or other dysglycemias.

3.3 Protocol

Data collection consisted of a questionnaire, physical measurements and a blood sample (fig 1). Meetings were held at the Women's Breast Health Center. They were booked at the

participants' convenience over a 3-month period. After the initial booking, participants had access to a research voice box in order to request changes to their scheduled meeting and were re-contacted accordingly.

Figure1. Flow chart of study protocol.



During the meeting, we initially explained the study, went over the consent form and obtained a signature for consent. We then asked participants to complete the questionnaire. Following this, measurements of height, weight, waist circumference and resting blood pressure were obtained. Finally, we offered to answer any additional questions on the study and provided the blood work requisition and directions to the Gamma-Dynacare laboratories. Meetings were led by a medical professional or research staff in a private and comfortable environment, at the Women's Breast Health Center. Meetings were held in either official languages, and were facilitated by family member translation for participants who didn't speak French or English.

The questionnaire consisted of questions regarding (a) breast health and estrogen exposure in order to collect the information required for the Gail model, (b) medical history of T2D, CVD, HTN and dyslipidemia, (c) medication history with specific questions on cholesterol lowering and anti-hypertensive drugs, (d) menopausal status, and (e) lifestyle including exercise, smoking and alcohol consumption. For comparison purposes, questions regarding lifestyle were worded exactly the same way in our questionnaire as they were in the original WBHC database questionnaire (with no threshold for exercise regularity).

Blood samples were drawn for fasting blood glucose, insulin, triglycerides, and HDL-CHOL. All participants' blood samples were measured by Gamma-Dynacare laboratories, in a short time interval difference in the morning (between 7:30 and 9:30 AM) after a 7-10 hour overnight fasting in order to minimise the effect of laboratory variability and length of fasting on the blood work values.

The extracted data served to calculate the estimated risk of breast cancer with the Gail model (Gail et al., 1981), and as diagnostic criteria for IR, MetS and T2D. The diagnostic criteria used for T2D were those of the Canadian Diabetes Association previously described (CDA 2008). Because the data collection was done prior to the 2013 revision of these criteria, the A1C was not collected. We therefore could not use the updated criteria in the present study. The threshold for IR reflected those suggested for Caucasians at a HOMA-IR value of 2.29 (Radikova et al., 2006). The MetS diagnostic criteria were those set by the IDF where 3 of 5 criteria from elevated waist circumference, elevated triglycerides, decreased HDL-C, HTN and hyperglycemia must be met (Alberti et al., 2009). The criteria for post-menopausal were based on the Stages of the Reproductive Aging Workshop (STRAW) (Soules et al., 2001). Women were considered post-menopausal based on self-reported amenorrhea for 12 consecutive months. Women who were undergoing menopausal transition were therefore included in the

premenopausal group. Women with surgically or medically induced menopause were not included in the study by verifying these factors from the Women Breast Health Center database.

3.4 Statistical Analysis

This cross-sectional study used mainly descriptive statistics. In order to first characterise our recruited population, we calculated the frequency and percentages of participants for each of the following categorical variables: age, BMI, menopausal status, Gail 5-year risk, reason for high risk, smoking, alcohol consumption and exercise. The cut-points for the different variables were chosen based on the same cut-points used in the original Women's Breast Health Center database from which our population was recruited. These were based on self-reported data from the questionnaire. The questions were worded exactly the same way in our questionnaire as it was in the original database questionnaire (with no threshold for exercise regularity). The BMI and exercise variables were further analysed by comparing the pre and post-menopausal groups using the Fisher's exact test for the categorical data and the unpaired T-test when comparing the means.

As a measure of sampling accuracy we also compared our recruited sample to the overall population of women followed at the Women's Breast Health Center (from the Database from which our recruitment was done), for the following characteristics: age, smoking, exercise and BMI. We used the unpaired t-test to compare the means for age and BMI, the Fisher exact test for the smoking and exercise comparisons. Results are presented as mean \pm standard deviation.

For our overall objective, which was to determine the prevalence of IR, MetS and T2D, in women at high risk for breast cancer, we calculated the frequency and percentage of women meeting the diagnostic criteria for each of these phenotypes. This was described for the overall group of high risk women and stratified by menopausal status using the Fisher exact test.

For our secondary objective of defining the prevalence of the individual components of MetS, we calculated the frequency and percentage of MetS and its components stratified by menopausal status using the Fisher exact test.

To further analyse the relationship between the risk of breast cancer and metabolic factors, we did correlational analyses using the Spearman test between the following variables: fasting glucose, insulin, triglycerides, HDL-C, LDL-C, waist circumference, systolic Bp, diastolic Bp and BMI and the Gail score

Finally, to meet our objective of sample size calculation for a future case-control study comparing the prevalence of IR, MetS and T2D in women at high risk and average risk for breast cancer, we used G-Power program to calculate the number of participants required in each group to achieve power of 80% and 90%.

CHAPTER 4

4. Results

Participants' characteristics are presented in Table 3. Women's mean age was 58.2 ± 8.3 years, and the median age 58 (range: 42 to 82). They presented a BMI of 25.7 ± 4.8 kg/m². Based on WHO (2012) BMI categories, 49% of the participants were of normal weight, 48% were either overweight or obese and 3% were underweight. The majority of women (83%) were post-menopausal. The mean Gail score was $4.5 \pm 2.2\%$ and the median was 3.8%. Most women were non-smoking (98%), consumed between 1-5 alcoholic drinks per week (46%) and exercised between 1-5 hours per week (60%).

Table 3. Participant characteristics (n=100).

Characteristics	Number (Percentage)
Age (year)	
≤ 49	17 (17)

≥ 50	83 (83)
<hr/>	
BMI* (Kg/m ²)	
≤ 18.4	3 (3)
18.5-24.9	49 (49)
25.0-29.9	32 (32)
≥ 30.0	16 (16)
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Menopausal status	
Pre-menopausal	17 (17)
Post-menopausal	83 (83)
<hr/>	
Gail score 5yr risk (%)	
Mean \pm SD	4.5 \pm 2.2
<hr/>	
Reason for high risk	
BRCA	7 (7)
LCIS	10 (10)
ADH	8 (8)
LCIS & ADH	2 (2)
Thoracic radiation	2 (2)
Gail score >1.7	71 (71)
<hr/>	
Smoking	
No	98 (98)
Yes	2 (2)
<hr/>	
Alcohol (drinks/wk)	
<1	32 (32)
1-5	46 (46)
>5	22 (22)
<hr/>	
Exercise (h/wk)	
<1	20 (20)
1-5	60 (60)
>5	20 (20)
<hr/>	

*The International Classification of adult underweight, overweight and obesity according to body mass index (BMI) (WHO, 2012).

†N/A refers to participants who are at high risk due to LCIS on previous biopsy or BRCA mutations. The Gail model does not provide risk estimates for women with history of LCIS. The Gail model is not optimal for risk calculation in BRCA mutation carriers.

ADH=Atypical Ductal Hyperplasia.

LCIS=Lobular Carcinoma In-situ.

In table 4 we have further classified the participants based on menopausal status. There was no significant difference in the frequency of BMI categories between the pre and post-menopausal women. The mean BMI was significantly higher in the post-menopausal women as compared to the premenopausal group. The exercise frequency intervals were not significantly

different between the pre and postmenopausal groups. The mean exercise frequency also did not show a significant difference between the pre and postmenopausal groups.

Table 4. Body mass index and exercise frequency by menopausal status.

	Premenopausal* (n=17)	Postmenopausal* (n=83)	P value
BMI (Kg/m ²)			
≤18.4	1 (0.06)	2 (0.02)	0.28
18.5-24.9	11 (64.7)	38 (45.8)	
25.0-29.9	4 (23.5)	28 (33.7)	
≥30.0	1 (0.06)	15 (18.1)	
Mean BMI (Kg/m ²)	23.6±3.5	26.1±4.9	0.05
Exercise (h/wk)			
<1	4 (23.5)	16 (19.3)	0.76
1-5	11 (64.7)	49 (59)	
>5	2 (11.8)	18 (21.7)	
Mean exercise (h/wk)	2.8±2.5	3.4±2.6	0.34

*Body mass index (BMI) categories and exercise categories are expressed as number (%) and their comparison between menopausal groups were calculated using the Fisher exact test. Mean BMI and mean exercise are expressed as mean ± standard deviation and their comparison between menopausal groups calculated using the T-test.

Compared to the Ottawa Hospital Women's Breast Center, our study sample mean age and exercise frequency are higher but there are less smokers (table 5).

Table 5. Comparison between the study population and the Women's Breast Health Center (WBHC) Database population.

Characteristics*	Study sample (n=100)	WBHC database (n=698)	P value
Age(years)	58.2±8.3	49.1±11.1	<0.0001
BMI (Kg/m ²)	25.7±4.8	25.0±4.9	0.20
Smoking n (%)	2 (2.0)	72 (11.3)	0.001
Exercise n (%)	86 (86.0)	383 (65.3)	<0.0001

*Age and body mass index (BMI) data are expressed as means ± standard deviation and their comparison between populations were calculated using the unpaired t-test. The comparisons between populations for smoking and exercise were calculated using the Fisher exact test.

Table 6 describes the prevalence of IR, MetS and T2D by menopausal status. Overall, the MetS was the most prevalent phenotype followed by IR and T2D. Very few premenopausal women meet the diagnostic criteria for IR, MetS and T2D. A statistically different prevalence of MetS was observed between the pre and postmenopausal groups but not for IR and T2D.

Table 6. Prevalence of insulin resistance, metabolic syndrome and type 2 diabetes by menopausal status.

	Overall (n=88)	Premenopausal (n=16)	Postmenopausal (n=72)	P value*
Insulin resistance	17 (18.0)	1 (5.9)	16 (20.2)	0.29
Metabolic syndrome	29 (33.0)	1 (6.3)	28 (38.8)	0.01
Type 2Diabetes	5 (5.0)	0 (0)	5 (6.3)	0.58

Data are present as number (%),

*Calculated using the Fisher exact test with the number of participants and not the percentage.

In the overall study sample, more than two-third of the women met the MetS criteria for WC, and approximately one-third met the criteria for elevated TG, low HDL-C, HTN and hyperglycemia (Table 7). The post-menopausal women had a significantly higher prevalence of increased WC, HTN and a trend of higher TG in comparison to the pre-menopausal participants.

Table 7. Prevalence of metabolic syndrome components by menopausal status.

Characteristic	Overall (n=88)	Pre- menopausal (n=16)	Post- menopausal (n=72)	P value*
↑ WC	60 (68.2)	7 (43.8)	53 (73.6)	0.02
↑ TG	23 (26.1)	1 (6.3)	22 (30.4)	0.06
↓ HDL-C [‡]	22 (29.7)	2 (16.7)	20 (32.3)	0.49
HTN	33 (37.5)	1 (6.3)	32 (44.4)	0.004
↑ BG	27 (30.7)	2 (12.5)	25 (34.7)	0.13

[‡]N=74 for HDL analyses.

*Differences in characteristics between pre- and post-menopausal women were calculated using the Fisher exact test.

WC= waist circumference, TG= triglycerides, HDL-C= high-density lipoprotein - cholesterol, HTN= hypertension, BG= blood glucose.

Values presented are number and percentages (%).

Table 8 presents the Spearman correlations between the Mets components and Gail score. A modest but significant correlation was observed only between HDL-C and Gail score.

Table 8. Correlation between body mass index, waist circumference, cardiometabolic profile (lipids, Bp, BG, insulin) and Gail score (n=100).

Characteristics	r	p value
BMI	0.03	0.82
WC	0.01	0.93
TG	0.06	0.62

HDL-C	0.35	0.01
LDL	0.04	0.77
Systolic Bp	0.20	0.07
Diastolic Bp	0.07	0.52
BG	0.11	0.33
Insulin	0.08	0.48

r= correlation coefficient calculated with the Spearman correlation test.

WC= waist circumference, TG= triglycerides, HDL-C= high-density lipoprotein-cholesterol, Bp= blood pressure, BG= blood glucose.

The sample size calculation shown in table 9 was performed with the assumption that the prevalence of IR in women at high risk is 18% (based on the results of the present study) and the prevalence of 11% in the general population is (IDF, 2011). A study comparing the prevalence of IR between women at high risk for breast cancer and women at average risk would therefore require a sample size of 312 or 864 per group to achieve power of 80% and 90% respectively.

For MetS, the assumption is that the prevalence in high risk women is 33% (based on the observed prevalence in the present pilot study) and the prevalence of 19% reported for Canadian women (Riediger & Clara, 2011). Therefore, a study comparing the prevalence of MetS between women at high risk for breast cancer and women at average risk would require a sample size of 121 or 167 per group to achieve power of 80% and 90% respectively.

The prevalence of T2D in Canadian women between the ages of 35 and 64 year old is 5% (Statistics Canada, 2012). The prevalence of T2D observed in our sample of women above the age of 35 who are at high risk for breast cancer is also 5%. A study comparing these groups would therefore not be significant.

Table 9. Sample size calculations for a comparative study of the prevalence of insulin resistance, metabolic syndrome and type 2 diabetes between women at high risk for breast cancer and women at average risk.

	No. of participant required in each group to achieve power of:	
	80%	90%
Insulin resistance	312	864
Metabolic syndrome	121	167
Type 2 Diabetes	N/A	N/A

CHAPTER 5

5. Discussion

The purpose of this study was to determine the prevalence of IR, MetS and T2D in women at high risk for breast cancer, to stratify this prevalence by menopausal status, and to determine the prevalence of the individual components of metabolic syndrome. By establishing these prevalences in our sample, we ultimately aimed to generate the data to determine the sample size required for a case-control study comparing the prevalence of IR, MetS and T2D in women at high risk and average risk for breast cancer.

5.1 General conclusions

5.1.1 Overall prevalence of IR, MetS and T2D

In our sample of women at high risk for breast cancer, we have obtained a prevalence of 18%, 33% and 5% for IR, MetS and T2D respectively. The magnitude of these prevalences is reflective of the reported prevalences of IR, MetS and T2D in the general population with MetS being the most prevalent condition, followed by IR and T2D (Riediger & Clara, 2011; IDF, 2011; Statistics Canada 2012). However, the proposed case-control study would allow to further validate the prevalence for these conditions in high risk women for breast cancer and to determine if they are higher than the general population.

5.1.2 Stratification by menopausal status

The proportion of pre vs post-menopausal women observed in our sample is partially reflective of the overall Women's Breast Health Center high risk population due to the increasing risk of breast cancer with age and menopause (Suveillance, Epidemiology and End Results [SEER], 2007; Costanza & Chen, 2010). Pre-menopausal women had a statistically

significant lower prevalence of MetS in comparison to the post-menopausal women. This could be explained by the body composition and metabolic changes that are known to occur around menopausal transition. Studies have shown that menopausal transition has an effect on BMI (Pasquali et al. 1994), body composition and fat distribution (Mauriege et al., 2000, Abdulnour et al. 2012). Our postmenopausal participants did have a significantly higher BMI and WC in comparison to the premenopausal women. This could be a consequence of the decreased energy expenditure that occurs around menopausal transition, which has been attributed to decreased physical activity measured by accelerometry and a shift to a more sedentary lifestyle (Duval et al., 2013). However, based on our self-reported exercise frequency data, there is no significant difference between the pre and postmenopausal women. This could be explained by the limitations of self-reported exercise frequency (Prince, Adamo, Hamel, Hardt, Connor-Gorber, & Tremblay, 2008).

5.1.3 Prevalence of the components of MetS

In our study population of women at high risk for breast cancer, the most prevalent MetS criterion was waist circumference. Because the majority of our participants were post-menopausal, this could again be explained by the changes in body composition and body fat distribution, especially an increase in abdominal fat that occur during the menopausal transition (Mauriege et al. 2000; Sowers et al, 2007; Abdulnour et al., 2012). Supporting this explanation is the fact that we also observed a significantly higher mean WC in our post-menopausal women. In the present study waist circumference and hypertension were the only two components of MetS for which the prevalence was significantly higher in postmenopausal women. Various studies have shown a higher prevalence of MetS components in post-menopausal women compare to pre-menopausal women (Abdulnour et al., 2012; Sowers et al., 2007; Agrinier, Cournot & Ferrieres, 2009). One study followed women over menopausal transition for 6 years and observed an increased waist circumference (Sowers et al., 2007).

Another longitudinal study showed an increase in truncal and visceral obesity after menopause transition (Abdulnour et al., 2012). A review of the literature revealed an effect of menopause on total cholesterol, low-density lipoprotein-C and TG which persisted after age adjustments (Agrinier, Cournot & Ferrieres, 2009). However, the independent effect of menopause on HTN is inconsistent and controversial (Lee et al., 2013; Pimenta, 2012).

5.1.4 Correlations between cardiometabolic factors and risk for breast cancer

Our results show a modest significant correlation between HDL-C and Gail score. In the current study, the positive correlation between HDL-C and Gail score is surprising given that other sources have shown that high HDL-C concentrations decrease the risk of breast cancer, more so in post-menopausal women (Han et al., 2005, Xue & Michels, 2007). The proposed mechanism for this association is that a low HDL-C is a marker of androgen excess, and aromatization of androgens into estrogens in adipose tissues is a stimulus for breast carcinogenesis (Xue & Michels, 2007). We cannot exclude a type I error (false positive) to explain this contradiction with the results reported in literature. However, given that HDL is increased by physical activity (Kokkinos & Fernhall, 1999), and that our study population had a significantly increased level of physical activity in comparison to the WBHC database population, this could have influenced the higher HDL in our population. Also, since the median age of our population is 58, it is likely that many of our participants were in menopausal transition. It has been shown that during menopausal transition, there is a peak in HDL (Abdulnour et al., 2012).

We have not observed any statistically significant correlations between BMI, WC, TG, LDL-C, systolic Bp, diastolic Bp, glucose, insulin and breast cancer risk. However, previous studies have shown associations between elevated TG (Agnoli et al., 2010; Kabat et al., 2009), abdominal obesity (Huang et al., 2010; Connolly, et al., 2002), elevated LDL-C (Kumar,

Sachdanandam & Arivazhagan, 1991), hypertension (Largent et al., 2006; Soler et al., 1999) and hyperglycemia (Kabat et al., 2009) and risk of breast cancer. This discrepancy could be related to the small sample size of the study. A future well-powered study could serve to rule out a type II error (false negative).

Most studies that have shown a link between metabolic factors such as IR, MetS, T2D and breast cancer were epidemiologic in nature (Xue & Michels, 2007; Vona-Davis, Howard-McNatt, & Rose, 2007). Possible pathways have been proposed, mostly relating various hormonal factors including insulin, insulin-like growth factors, estrogen and their respective effect on cell growth, proliferation and differentiation (Xue & Michels, 2007). Further studies are needed to confirm these correlations and to clarify the underlying physiologic mechanisms.

5.1.5 Sample size calculation for a future case-control study

The prevalence of T2D in our sample of high risk women was exactly the same as the reported prevalence in average risk women between the ages of 35 and 64 (Statistics Canada 2012) and therefore a comparative study would not be feasible. On the other hand, the reported prevalences of IR and MetS in the general population (IDF, 2011; Riediger & Clara, 2011), appear to be lower than those observed in our high risk population. Based on our sample size calculations, a comparative study of the prevalence of IR and MetS between women at high risk and women at average risk would be feasible. The ideal design for such a study would be a case-control design. The total number of women in the original Women's Breast Health Center database, which reflects the approximate number of patients followed in the center at any given time, was 698. Using the enrolment rate for this study as a guide, which was 36% within a 2-month period, 250 participants could easily be recruited within 2 months. Previous similar studies on this same population have reported an enrolment rate of 45% possibly by persisting with enrolment over a longer period or with additional recruitment tactics. It would therefore

likely be reasonable to aim to recruit a minimum of 312 high risk participants from the center to achieve a power of 80% to determine the prevalence of IR and a power of 90% for MetS.

Furthermore, a multicenter study would provide a greater sample of high risk patients to recruit from for a minimum of 864 participants in each group for a power of 90% to determine the prevalence of both IR and Met. Such a multicenter study would also allow recruiting a more representative sample of the general high risk population.

Ultimately, any association that may result from such a case-control study would be very valuable information for this high risk population because of the potential for modification of these cardiometabolic risk factors.

5.2 Study limitations

5.2.1 Sampling

Our study population was recruited from the Ottawa Hospital Women's Breast Health Center High Risk Clinic by using their database. This database is created with self-reported data from the High Risk Breast Assessment Clinic Questionnaire, which was completed by all women prior to their initial consultation at the center. The study population was therefore recruited at a point in time where all of the women had benefitted from at least one visit with counselling regarding risk reduction strategies. This evidence-based counselling is given by specialised nurses, oncologists and breast surgeons. In ideal sampling we would therefore expect our study sample to have a similar mean age to the original population, but not necessarily a similar BMI or percentage of smoking and exercise frequency, given the benefit of counselling for these modifiable factors. Our results showed that there is no statistical difference between the BMI of the sample and original population. Our results also showed that our study sample has a statistically significant lower percentage of smokers and higher percentage of women who stated

they exercised regularly, in comparison to the original population. The specific frequency of exercise was not measured in the original WBHC database and therefore a more detailed comparison to our study population was not possible in regards to the frequency of exercise. Nevertheless, this is encouraging data underlining the benefits of the counselling received through the High Risk Clinic. This could also be explained by behavioural changes simply related to being labeled as high risk. However, the mean age of our study population is 9.1 years higher than the original population. This is recognised as a limitation of our study results since the prevalence of IR, MetS and T2D all increase with age (Utzschneider et al., 2004; Hildrum, Mykletun, Hole, Midthjell & Dahl, 2007; Centers for disease control and prevention, 2013). The more readily available post-menopausal, retired women to participate in a study can also explain this sampling error. We tried to limit this by facilitating after hours meetings for the working participants and by allowing blood work to be done at the Gamma Dynacare laboratory, which was the most convenient to the participant. However, we did not allow women to have their blood work drawn at laboratories other than Gamma Dynacare in order to limit the variability in sample processing. The higher mean age in our study population can also be explained by the fact that we had a lower age cut-off of 35 but no higher age cut-off. The reason for this is that the Gail model (Gail et al., 1981) is validated in women above the age of 35 without an upper age limit. This nevertheless is a limitation of our study because High Risk Clinics don't have a lower age cut-off and therefore this limits external validity.

The objective of this study was to determine the prevalence of IR, MetS and T2D in this subgroup of patients who are identified and followed for their high risk of developing breast cancer. Given that our sampling was done randomly, but among an enriched population, which is followed clinically in a specialised clinic, the results are limited to this specific population. Major cities across the country have similar high risk clinics with similar, although not standardised, inclusion criteria. In addition, the genetic component of the risk for breast cancer

leads to groupings of high risk families within various communities. The results of the current study can therefore serve as a basis for similar studies, in similar populations but the specific prevalence of the different conditions cannot be extrapolated to other high risk groups across the country or to those who are not followed by health care professionals.

5.2.2 Defining ethnicity

Defining ethnicity is challenging in any study, and even more so in a study where the independent variable (risk of breast cancer) can vary with ethnicity. Because of this, the measurement tools in this type of study are ethnicity specific (Gail model and HOMA-IR) which led to the limitation of defining ethnicity as an inclusion criterion. Defining ethnicity is difficult because of the sensitive social aspect of its classification. Furthermore, in individuals with mixed ethnic backgrounds, measuring contributions of various ethnicities is impossible and defining ethnicity comes down to the individual's personal identity. We have therefore opted to define ethnicity in this study based on self-reported data. We then included only those who self-reported being Caucasian. Only participants who met this inclusion criterion a priori were approached to participate in the study. In order to minimise this limitation, we asked our participants the exact same question in our questionnaire as they were asked in the initial Women's Breast Health Center database questionnaire and confirmed the same reported ethnicity in both. Further studies are warranted to document the prevalence of IR, MetS and T2D in women at high risk for breast cancer of other ethnic groups.

5.2.3 Risk calculation models

Estimating risk of developing breast cancer is complex due to the various contributing factors and to the varying definition of high risk. There are numerous risk calculation models, which have been developed and validated to estimate the risk of developing breast cancer. Each

of these models has advantages and limitations and no model can be applied to every women. In this study, we chose to use the Gail model (Gail et al., 1981). This tool is well known and validated and is simple to access (from developer's website) and to apply (includes 7 variables). High Risk Clinics and studies of High Risk populations very commonly use it as an inclusion criterion. It was also the main instrument used to calculate risk in the Women's Breast Health Center database from which our population was selected. It can be used in women older than 35yrs and has been validated in Caucasian women (Costantino et al., 1999; Rockhill, Spiegelman, Byrne, Hunter, & Colditz, 2001) and updated for African American women (Gail et al., 2007). Limitations of the Gail model include that it does not account for paternal family history, extended family history, or age at onset in affected relatives. Also, it is not validated in non-White and non-African American women. As discussed above, these limitations, in addition to the ethnic specific HOMA-IR tool, have shaped our inclusion criteria.

5.2.4 Recollection reliability

Certain items in the Gail model (Gail et al., 1981) rely on patient's individual memory of events such as menarche and family history. Although studies show that the sensitivity of self-reported family history of breast cancer is high (83-97%) (Kerber & Slattery, 1997), the recollection of menarche is reported to vary by as much as 4.5 years even in adolescents who are closer to menarche than many of our postmenopausal participants (Dorn, Sontag-Padilla, Pabst, Tissot & Susman, 2013). Nevertheless, the inaccuracy in the Gail score that this may have led to is no different in our sample than it was in the population that the Gail score was validated in.

5.2.5 Metabolic syndrome and breast cancer

Obesity can lead to IR, which can also lead to T2D. The hyperglycaemia which is associated with IR also contributes to atherosclerotic and CVD through various factors including abnormal lipid profiles and hypertension. This constellation of risk factors for T2D and CVD has

been referred collectively as metabolic syndrome. The use of this term as a diagnosis has been criticised by those who believe it does not add anything more than the addition of each risk factor for T2D and CVD (Golden, 2002). However, others argue that its associated risk is above the cumulative risk of each of its components (Golden, 2002). Regardless of the value of the term as a diagnosis, the importance of its components as risk factors for T2D and CVD is undebatable. Furthermore, any association that could be made with the risk of breast cancer is valuable since many of its components are modifiable risk factors.

The relationship between MetS and breast cancer is further complicated by the fact that certain breast cancer risk reducing interventions, which are recommended for those women who are at significant risk of developing breast cancer, include bilateral oophorectomy and hormonal treatments, which can lead to premature ovarian deficiency, which may increase the risk of developing MetS. It is difficult to control for this confounding factor because excluding participants who have had an oophorectomy or who have received chemoprophylaxis would lead to exclusion of a significant proportion of women who are followed in High Risk Clinics and would therefore make the study sample not representative of the target population of women at high risk for breast cancer.

5.2.6 Sample size calculations

One of the objectives of this study was to serve as a pilot study to generate data necessary to calculate the required sample size for a future case-control study. In order to make this calculation, an estimate of the prevalence of IR, MetS and T2D in the general average risk population was necessary. However, to make an accurate comparison to our high risk study population, we would ideally require the prevalence of these metabolic diseases specifically in Canadian White women above the age of 35 who are at average risk for breast cancer. This specific data is obviously not available in such detail, which is a limitation of our power calculation analyses. For the prevalence of T2D in the general population, we used data from

Statistics Canada (2012), which reports the prevalence of T2D in Canadian women between the ages of 35 and 64 at 5%. For the prevalence of IR in the general population we used a reported 11% in the Canadian population (IDF, 2011). For the prevalence of metabolic syndrome in the general population we used 19% reported for Canadian Women (Riediger & Clara, 2011).

Recruiting case-controls should therefore be part of a subsequent study to confirm the prevalence of those conditions.

CHAPTER 6

6. Conclusion

In conclusion, our results suggest that in women at high risk for breast cancer, the prevalence of T2D is similar to that of the general population but the prevalence of IR and MetS appears to be higher which supports a follow-up case-control study. High risk post-menopausal women have a higher mean BMI and prevalence of MetS, WC and HTN than pre-menopausal women. We have shown a modest significant correlation between HDL and Gail score. Further studies are needed to clarify the physiologic mechanisms explaining the association between breast cancer risk and metabolic factors such as IR, MetS and T2D. Although the basis for the high risk status is obvious for some women (ex BRCA mutation), it is less evident for others (elevated Gail score). If it was determined that these women have a higher prevalence of IR or MetS in comparison to the general population, this would support the contribution of metabolic factors to the multifactorial risk calculation, which would in turn help understand and modify this risk.

CHAPTER 7

7. References

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