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The Effects of Exercise Training on Insulin Supply and Demand in Breast Cancer Survivors

Richard Viskochil

THE EFFECTS OF EXERCISE TRAINING ON INSULIN SUPPLY AND DEMAND IN BREAST CANCER SURVIVORS

A Dissertation Presented

By

RICHARD VISKOCHIL

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ABSTRACT

THE EFFECTS OF EXERCISE TRAINING ON INSULIN SUPPLY AND DEMAND IN BREAST CANCER SURVIVORS

MAY 2017

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Elevated insulin concentrations may influence cancer and cardiometabolic disease onset and prognosis, and lower insulin levels after exercise may contribute to disease prevention and overall health. The effect of exercise training on systemic and tissue-specific insulin supply and demand in breast cancer survivors and adults at risk for cardiometabolic disease is unclear. The objective of this dissertation was to evaluate the effects of exercise training on postmeal insulin concentrations in breast cancer survivors, and identify mechanisms responsible for changes to insulin supply and demand following exercise training in breast cancer survivors and adults at risk for cardiometabolic disease.

Study 1 investigated differences between systemic and tissue-specific responses to exercise training and/or the anti-diabetes drug metformin in adults with prediabetes. Fasting proinsulin concentrations were lower following combined exercise and metformin (-24%), and insulin clearance was higher in the metformin and combined exercise and metformin groups (+19% and +17%). There were no differences in the exercise or placebo group, and taken together with previous work from our lab, suggests that exercise regulates insulin supply and demand systemically, while pharmacological adaptations may be tissue-specific.

Study 2 evaluated the effects of physical activity on postmeal insulin concentrations in breast cancer survivors. Fifteen women completed 12 weeks of exercise training with pre- and post-intervention oral glucose tolerance testing. Insulin concentrations 120 minutes following glucose ingestion decreased (68.8±34.5 vs. 56.2±31.9 uU/ml, $p<0.05$), along with leptin (-22.7%) and estrogen (-20.9%), biomarkers of cancer risk. This postmeal insulin response may have been blunted by the use of aromatase inhibitors.

Study 3 assessed the specific components of insulin supply and demand that may contribute to the blunted or absent postmeal insulin response observed in study 2. There was a significant increase in estimated skeletal muscle glucose uptake following exercise training (5.7±1.8 vs. 7.2 ±1.8, mmo*pmol*kg/m2 $p<0.05$), however there were no changes to systemic measures of insulin supply and demand. This, combined with reductions in leptin and estrogen (study 2), suggests that exercise training was sufficient to induce tissue-specific adaptations but was unable to alter systemic insulin supply and demand in breast cancer survivors.

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CHAPTER I

INTRODUCTION

Statement of the problem

The American Society of Clinical Oncology expects newly diagnosed cases of cancer to double by the year 2030 and, as a result, cancer will dethrone heart disease as the number one cause of death in the United States (ASCO, 2013). One in eight American women will be diagnosed with breast cancer in their lifetime, and the majority of these diagnoses will occur during or after menopause (Britt, 2012). The high rate of breast cancer incidence, improvements in screening/treatment, and an aging population have resulted in a large (and growing) number of postmenopausal breast cancer survivors living in the United States, with recent estimates approaching 3 million women (Toriola & Colditz 2013). While the majority of breast cancer survivors have been declared cancer free, breast cancer treatment often induces physiological and psychological changes that increase the risk of both cancer recurrence and cardiometabolic disease, such as type 2 diabetes.

Three specific aspects of breast cancer treatment may influence breast cancer recurrence and/or the risk of cardiometabolic disease. First, many chemotherapy drugs impair cardiac function (Kirkham et al. 2015), which reduces aerobic fitness and increases the risk of cardiovascular disease (Jones et al. 2016). These effects are not confined to the periods of chemotherapy delivery alone, since most breast cancer survivors immediately start a multi-year plan of secondary pharmacotherapy (e.g. aromatase inhibitors) that can have detrimental effects on cardiovascular and bone health

(Zagar et al. 2016, Hadji et al. 2011). Additionally, women undergoing chemotherapy and radiation are given glucocorticoids (e.g. Dexamethasone) as anti-emetics, which often raise glucose concentrations into the range of diabetes during treatment (Lu et al. 2014, JuanJuan et al. 2015). Finally, cancer treatment often induces physical and psychological fatigue (Berger et al. 2012, Stagl et al. 2014), which leads to lower levels of physical activity and increased sedentary behavior both during and after treatment. Given the large and growing number of breast cancer survivors in the United States, developing a greater understanding the relationship between modifiable cancer and cardiometabolic risk factors and using that knowledge to design precision interventions that improve prognosis has clear public health implications.

Being physically active reduces the risk of cancer recurrence and improves the prognosis of cancer survivors (Moore et al. 2016). Epidemiological evidence suggests that women who are meeting the US physical activity guidelines (150 min/wk of moderate to vigorous PA) are 20% less likely to develop breast cancer, and breast cancer survivors meeting the physical activity guidelines are 40% less likely to have a cancer recurrence or cancer related death (Ballard Barbash et al. 2012). The mechanisms behind this risk reduction are multi-factorial (e.g. weight control, reduced inflammation), however the results of several large cross-sectional studies and randomized controlled trials suggest that insulin may play a role (Ahern et al. 2013, Goodwin et al. 2012, Irwin et al. 2005). The amount of insulin released into the circulation (insulin supply) is based largely on the amount of insulin required by the body to maintain blood glucose concentrations within a relatively tight physiological range (insulin demand). Obesity and sedentary behavior reduce the effectiveness of insulin to regulate blood glucose, and this insulin resistance (i.e. increased insulin demand) causes a compensatory increase in insulin supply in order to match the higher demand and maintain glycemic control. Without this compensatory hyperinsulinemia most individuals with insulin resistance (approximately 30-40% of the population in the United States) would likely develop frank type 2 diabetes.

Although hyperinsulinemia helps restrain blood glucose and prevent T2D, it may be detrimental to cancer risk. In addition to regulating blood glucose levels, insulin stimulates cell growth and proliferation in both normal and cancerous cells (Gallagher et al. 2013). In a study by Irwin et al. women in the highest quartile of circulating insulin have a 3x greater risk of cancer mortality compared to women in the lowest quartile (Irwin et al. 2011). Several recent epidemiological studies have also suggested a role for insulin in cancer prevention. While the causal mechanisms are unclear, women who regularly engage in physical activity or those taking the anti-hyperglycemia drug metformin have a reduced risk of developing cancer, which is potentially mediated by reductions in circulating insulin concentrations following each treatment (Goodwin et al. 2015, Del Giudice et al. 1998). Despite epidemiological and cell/animal model evidence supporting a role of insulin in carcinogenesis, results from exercise training studies in breast cancer survivors have been largely equivocal (Fairey et al. 2003, Ligibel et al. 2008, Irwin et al. 2009, Campbell et al. 2012 and Guinan et al. 2013). This has lead some researchers to conclude that elevated insulin concentrations may contribute to carcinogenesis, however exercise-induced reductions in insulin are either absent or play a negligible role in cancer prognosis and/or the risk of recurrence in breast cancer survivors (Irwin et al. 2009).

One limitation of the prior interventions designed to evaluate the mediating/moderating effect of insulin on cancer risk and prognosis is the use of fasting insulin concentrations as the sole representation of glycemic control. Maintaining appropriate glucose concentrations involves complex regulation of insulin supply and demand on both a systemic and tissue-specific level across different metabolic states (e.g. fasting, postmeal), all of which have unique responses to physical activity. For example, fasting insulin concentrations primarily reflect the volume of insulin required for the liver to maintain appropriate glucose production and prevent low blood glucose. After a large meal however, insulin supply increases dramatically as insulin demand shifts from restraining liver glucose production to inducing skeletal muscle glucose uptake. While lean, physically active individuals tend to have lower fasting <u>and</u> postmeal insulin demand compared sedentary obese individuals, there can be a large degree of discordance <u>between</u> fasting and postmeal muscle insulin demand both within and across different populations (Faerch et al. 2008). It is therefore possible for two individuals with very similar fasting insulin concentrations to have significantly different postmeal insulin concentrations due to differences in postmeal insulin demand (i.e. skeletal muscle insulin sensitivity). Since insulin is a mitogenic, dose-dependent contributor to cancer risk, the relationship between insulin and cancer likely *<u>cannot</u>* be extrapolated from fasting insulin concentrations alone, as postprandial insulin represents an equal, and in some cases greater, contributor to daily insulin exposure.

Additionally, changes to insulin supply and demand following interventions are not similar in magnitude across all tissues and in all metabolic states. For example, exercise training appears to reduce skeletal muscle insulin demand (i.e. insulin resistance) to a much greater degree than liver insulin demand. Since skeletal muscle insulin sensitivity plays a significant role in postmeal, but not fasting, glycemic control, the magnitude of reductions in postmeal insulin are significantly greater than the reductions in fasting insulin following exercise training (Jenkins & Hagberg 2011). This response is in contrast to other interventions like metformin, which primarily reduces fasting insulin concentrations (Goodwin et al. 2008). Given the unique tissue-, and metabolic state-specific changes to insulin demand and supply, it is possible that studies evaluating the efficacy of physical activity interventions on cancer risk and prognosis may be undervaluing the impact of exercise training by failing to account for changes in postmeal insulin concentrations.

Objectives and significance

Despite the disproportional relationship between fasting and postmeal insulin concentrations and the unique tissue- and metabolic state-specific effects of interventions on insulin supply and demand, many studies in breast cancer survivors only measure insulin in the fasted state, and thus may be underestimating the role of insulin in cancer risk and prognosis. Fasting insulin concentrations are only one component of insulin supply and demand, and one that is not particularly reflective of the overall impact of interventions on glycemic control. This limitation is especially relevant for exercise training, as reductions in skeletal muscle insulin resistance are primarily reflected through changes to postmeal insulin concentrations. While several clinical trials investigating the role of pharmacology- and/or exercise-induced changes in metabolism in cancer survivors are underway (Patterson et al. 2013), the health recommendations derived from these studies are predicated on the changes to fasting insulin concentrations observed following each treatment. Before physical activity, dietary and pharmacological interventions can be optimally combined into a personalized intervention to prevent or reverse chronic disease, a full understanding of independent and combined effects of these interventions must be elucidated in the populations that would most benefit from them. Without a greater understanding of the tissue- and metabolic state- specific effects of exercise training on insulin supply and demand in cancer survivors, the optimal utility of physical activity as means to improve cancer prognosis and reduce the risk of recurrence remains unknown.

The overall goal of this dissertation was therefore to address the current limitations in our understanding of the interactions between physical activity, insulin supply and demand and cancer risk. **Study one evaluated the effects of 12-weeks of exercise training and/or metformin on systemic and tissue-specific measures of fasting insulin supply and whole-body insulin demand.** This study used a fasting blood draw and the hyperinsulinemic-euglycemic clamp technique to better classify the responses to exercise training and/or metformin in men and women at risk for developing cardiometabolic disease. **Study two investigated the relationship between physical activity, insulin and cancer by measuring fasting and postmeal insulin concentrations and their relationship with cancer biomarkers prior to and following a personalized 12-week aerobic exercise training program in breast cancer survivors**. This involved the use of a five-sample oral glucose tolerance test prior to and following the completion of the exercise training protocol, with a primary focus on the aspects of physical activity and insulin supply and demand that modify cancer risk. Finally, **in study three we investigated the potential influence of exercise training on**

cardiometabolic health in breast cancer survivors in order to identify any potential changes to insulin supply and demand through which exercise training may reduce cancer-specific and all-cause mortality in breast cancer survivors. The information derived from this series of studies will shed light on the relationship between insulin supply and demand, exercise training and cardiometabolic disease risk, as well as their impact on cancer recurrence in breast cancer survivors, and guide future studies attempting to develop precise and personalized interventions to improve cancer prognosis.

CHAPTER II

REVIEW OF THE LITERATURE

Overview of cancer development and treatment

This project pertains to exercise training in women who have been previously diagnosed with breast cancer, received treatment, and have been deemed in remission or "cancer-free" by their oncologist. Cancer diagnosis and treatment can have profound effects on physical and psychological health and well being, and it is therefore necessary to provide background information regarding the development and treatment of cancer. This brief overview is by no means extensive, however it is important to review breast cancer development and treatment in order to understand why the effects of an exercise training program may differ between postmenopausal breast cancer survivors and non-cancer survivors of similar age, BMI and cardiometabolic health profile.

Cancer develops due to genetic or epigenetic mutations that confer a selective growth advantage to specific cells by 1) stimulating the cellular signaling pathways that lead to cell proliferation and/or 2) inhibiting the cellular signaling pathways that suppress tumor growth (Hanahan & Weinberg 2009). In most cases it takes many small genetic mutations (over the course of many years) to "add up" to a mutation large enough to overcome the inherent cellular defense mechanisms and manifest as a distinct physiological cancer phenotype (Vogelstein et al. 2013). Most solid tumor (e.g. breast) cancers can therefore be viewed as a *stepwise genetic disorder associated with aging*. This long process of genetic mutation can be exacerbated through many physiological mechanisms within individual control. For example, an individual may, after many years, have enough random mutations within his or her lung tissue to develop lung cancer. The length of time required to develop this "mutation load" may be extensive, and the likelihood of this mutation load reaching the point of metastasis, impairing breathing to a degree that would result in death, or outpacing other potentially fatal diseases (e.g. cardiovascular disease) is quite low. If however, he or she chooses to introduce a potent mix of carcinogens into his lungs via smoking, the likelihood of a mutation increases steeply and lung cancer is much more likely to develop over the course of his or her lifetime.

The modification of cancer risk through environmental factors within an individuals control represents an arm of cancer research that is equally important as the work being done on a genomic level, and equally as challenging (Vogelstein et al. 2013). The most appropriate primary outcome for studies investigating how environment and lifestyle (e.g. physical activity levels) may modify cancer risk is the development of cancer. Despite the increasing prevalence of cancer in America, the randomized controlled trials needed to establish causality or efficacy of prevention require very large sample sizes and many years of follow-up, which is quite burdensome on participants and researchers alike. Compounding the difficulty associated with evaluating cancer risk is the fact that cancer is not a singular disease originating from a singular location, but rather a collection of many different diseases with vastly discordant development trajectories (Baird & Caldas 2013). While the goal of cancer treatment is to halt the unregulated cell growth, prevent metastatic spread and eliminate (or vastly prolong) recurrence, the ways in which oncologists accomplish this task is highly dependent on the individual characteristics of the cancer. In order to understand the relationship between

PA and breast cancer recurrence it is necessary to delineate some of the differences and similarities in breast cancer development and treatment.

Breast cancer development

Breast cancer is a carcinoma arising from a mutation in the epithelial cells of the breast tissue, either in the lactiferous ducts or the lobular cells that supply milk to the ducts. The specific cause of the mutation can be multifactorial, however the primary manifestation of the mutation is disruption of one (or more) proliferative signaling pathways, such as PI3K/Akt (Dillon et al. 2007) or Ras-MAPK (Dunn et al. 2005), ***AND*** disruption of tumor suppressor pathways such as p53 or PTEN (Weng et al. 1999). The combination of enhanced cellular proliferation and loss of tumor suppression leads to unregulated cell growth, forming a solid and often palpable tumor within the breast tissue. Once detected, the size and growth rate of the solid tumor are then used to stratify the development into a specific stage (0-IV). Stage 0, or carcinoma in situ, is a non-invasive carcinoma that has not spread to surrounding tissue, stages I-III are breast cancers localized to the breast tissue, axillary lymph nodes and chest area, and stage IV is metastatic breast cancer that has spread to other organs of the body (Matsen & Neumayer 2013). These stages represent the progression of the disease, predict much of the physiological manifestations of treatment, and highly influence prognosis.

Since cancer is typically driven by a single mutated cancer stem cell (or several from within the same line of cells), receptors on the surface of the mutated cancer stem cell will be expressed ubiquitously on almost all of the cancerous cells throughout the tumor (Gupta et al. 2009). Tumors expressing the estrogen receptor (ER) and progesterone receptor (PR) fall under a similar category of hormone receptor positive cancers, which can be used for treatment as well as prevention by binding to that receptor and delivering drugs or preventing cell replication. The majority of breast cancers are hormone receptor positive cancers (>85%, Britt 2012), and it is common for women diagnosed with ER+ cancer to be given endocrine therapy, designed to inhibit estrogen production or binding, for five to ten years after successful primary treatment (Goss et al. 2016). An additional receptor known as the HER2/neu receptor is not responsive to estrogen-based therapy but does respond to other chemotherapy, notably herceptin. A breast tumor that is negative for ER, PR or HER2 expression is referred to as triple negative breast cancer, a much more worrisome diagnosis due to the lack of chemotherapeutic treatment options and in many cases a much more aggressive mutation.

The specific differences in staging and receptor status are the primary components of cancer diagnosis, and play the largest role in prognosis for successful treatment. More advanced stage (e.g. IIIA) is typically associated with reduced levels of PA following treatment (Mason et al. 2013), which may be due in part to greater fatigue induced by more aggressive chemotherapy (Blaney et al. 2013) or more intensive surgical procedures and lymphedema (Schmitz & Speck, 2010).

Breast cancer treatment

Treatment for breast cancer involves surgery and (when necessary) radiation therapy to remove the cancer localized within the breast and axillary lymph nodes, as well as the use of chemotherapy based on the specific expression of receptors on the surface of the cancerous cells.

Surgery represents the most common treatment for breast cancer, and can range from orthoscopic surgery with minimal invasiveness to a full mastectomy (including the removal of lymph notes and non-breast tissue) that could impact range of motion and lymphatic function (Boquiren et al. 2016). Extensive removal of axillary lymph nodes increases the risk for the development of lymphedema during physical activity, and may contribute to the lower physical activity levels observed in breast cancer survivors (Paskett et al. 2012). Radiation therapy involves targeted use of ionizing radiation to disrupt replication of cancer cells. Fatigue, nausea and localized discomfort or burning may accompany radiation therapy, and can contribute to the cancer-related fatigue and increase in sedentary behavior observed in individuals undergoing cancer treatment (Taunk et al. 2011), albeit to a lesser degree than surgery and chemotherapy.

Chemotherapy involves delivery of compounds and toxins designed to prevent cell replication and thus disrupt tumor growth and viability. Unlike surgery and radiation, chemotherapy is delivered systemically, and therefore has the largest impact on systemic physiology, cardiometabolic disease and physical activity. Chemotherapy may impact physical activity and exercise training both during and after treatment via cardiovascular impairment, nausea-induced anti-emetic use and fatigue. The impact of these three detrimental aspects of chemotherapy on physical activity, cardiometabolic health and cancer recurrence will be discussed in greater detail later in this review.

Women who have been treated for estrogen receptor positive breast cancer will often be prescribed drugs designed to disrupt estrogen binding for five to ten years after conclusion of primary treatment in order to reduce the risk of cancer recurrence. These drugs, often referred to as secondary treatment, fall into two broad classes that have wide-ranging effects of metabolism and health.

For many years SERMs (e.g. Tamoxifen) were the primary means by which the potentially mitogenic effects of estrogen were controlled in breast cancer survivors (Chlebowski 2000). This class of drugs binds to the estrogen receptor and exhibits pro- or anti-estrogenic activity, depending on the tissue (Huang et al. 2015). In breast tissue, SERMs play and anti-estrogenic role, inhibiting the cellular activity of estrogen within breast cells. There are several known side effects of SERM use, including clotting disorders and elevated risk of other types of cancer, including endometrial cancer (Chen et al. 2014). Most studies support a beneficial role of SERM use for the prevention of cancer recurrence, and they are currently the most commonly prescribed secondary cancer prevention drugs to premenopausal women (Chojecki et al. 2014), due to their ability to block estrogen binding in certain tissues while allowing estrogen production to continue in the ovaries.

Aromatase inhibitors block the conversion of testosterone and anabolic precursors into estrogens, and, as a result, circulating concentrations of estrogen can be reduced to levels virtually undetectable in circulation. Longitudinal and case-control studies suggest that AI's are more effective in reducing cancer risk than SERMS, with fewer high-risk side effects (e.g. stroke), and they are currently used as the primary means of endocrine therapy for postmenopausal women (Amir et al. 2011). Recently, several studies have advocated for breast cancer survivors to remain on AIs for up to 10 years following remission, with some oncologists recommending lifelong adherence for those that can tolerate the drug and do not manifest many of the side effects (Goss et al. 2016). These side effects are primarily related to bone and joint health, including an increased risk of osteopenia/osteoporosis (Becker et al. 2012), as well as increased risk of arthralgia and joint pain (Niravath 2013). There is no evidence that AIs interfere with cardiovascular or metabolic health (Younus et al. 2011), however the evidence has primarily focused on their safety, and the long-term evaluation of their efficacy as well as their interaction with other pharmacological agents or lifestyle interventions is still being evaluated (Foglietta et al. 2016).

Summary of cancer development and treatment

Cancer is a multifaceted disease that has at its core genetic mutations that confer a selective growth advantage by increasing cell proliferation and inhibiting or abrogating tumor suppression. These mutations take many years to develop, and solid tumor cancers are often viewed as a disease of aging exacerbated by lifestyle choices (e.g. smoking, inactivity). Breast cancer is a specific type of carcinoma in which the mutation develops in the mammary epithelial tissue. Breast cancer treatment varies based on progression (stage 0-IV) and receptor status, (ER, PR, HER2 +/-; triple negative), and the techniques used to treat breast cancer (surgery and radiation with or without chemotherapy) are employed on a personalized basis with the overall goal of halting progression and preventing recurrence. The physiological manifestations of this personalized treatment include large inter-individual differences in the response to exercise interventions among breast cancer survivors.

<u>Mediators and moderators of cancer development and prognosis</u>

Overview of cardiometabolic health and cancer

While cancer is primarily a genetic disorder associated with aging, 30-50% of cancer cases are directly caused, or exacerbated by, environmental factors and lifestyle choices that may be within individual control (McKenzie et al. 2015). To evaluate the impact of a lifestyle intervention designed to increase physical activity (e.g. exercise training) on cancer recurrence, it is important to understand how physical activity levels and obesity moderate and/or mediate cancer risk. Additionally, the third aim of this project is to evaluate the impact of exercise training on cardiometabolic health in cancer survivors, which requires some background on the overlap between cancer and cardiometabolic disease.

Both incidence and prevalence of obesity and inactivity have risen exponentially over the last 50 years in the United States and abroad (Yang & Colditz, 2015). When combined with an aging population, these twin epidemics of obesity and sedentary behavior have lead to a significant increase in the prevalence and incidence of both cancer-specific and all-cause mortality, which is often the result of cardiometabolic disease (Chang et al. 2013). Investigators who focus on systemic physiology and the response to obesity, sedentary behavior, and lifestyle interventions have recently begun to envelop factors that impact both cardiovascular and metabolic disease under the blanket term “cardiometabolic health.” This phrase is beneficial due to the strong relationship between diabetes and cardiovascular disease, the similarity between the general pathology of each disease, and the shared beneficial response to lifestyle interventions targeting obesity and inactivity. The use of cardiometabolic health and disease within this section and throughout the rest of the document can therefore be interpreted as "cardiovascular and/or metabolic" health and disease, specifically as it pertains to obesity, inactivity, and lifestyle interventions such as exercise training.

Postmenopausal women have a significantly higher risk of developing cancer and/or cardiometabolic disease than premenopausal women (Britt 2012), and both obesity and inactivity contribute to this increased risk (Su et al. 2013). Additionally, postmenopausal women with cardiometabolic disease also have a higher risk of developing cancer or having a cancer recurrence (Eulenberg et al. 2016) compared to postmenopausal women without cardiometabolic disease. The primary aim of this section of the literature review is twofold; 1) to establish the impact of inactivity and obesity on cancer and cardiometaboic disease risk in postmenopausal women and 2) to identify potential areas where a previous cancer diagnosis and treatment places postmenopausal women at greater risk of cardiometabolic disease or cancer than age- and BMI- matched women without a cancer diagnosis. It is important to note that cancer survivors remain susceptible to cancer even if they have been aggressively treated for cancer in the past and been declared cancer free. While a double mastectomy may virtually eliminate the risk of a subsequent breast cancer diagnosis, the risk of developing other cancers remains at least the same, and in many cases may be greater due to side effects of the breast cancer treatment.

Inactivity

Many postmenopausal women do not meet the physical activity guidelines (McTiernan et al. 1998), and this number is even greater in postmenopausal breast cancer survivors (Bluthmann et al. 2015). Sabiston et al. found that breast cancer survivors spend an average of 78% of waking hours sedentary and just 2% of each day engaged in moderate-to-vigorous physical activity (MVPA). Not only is this volume of sedentary time consistent throughout the first year after treatment, the levels of MVPA decrease as the year progresses (Sabiston et al. 2015). The specific mechanism behind the increased sedentary time and reduced physical activity observed in breast cancer survivors is unclear, however it appears to be closely related to fatigue severity (Bower et al. 2000). Inactive breast cancer survivors are also more likely to report having poor quality of life compared to inactive age- and BMI-matched non-cancer controls (Meeske et al. 2007), which increases the likelihood of developing cardiometabolic disease (Rozenberg et al. 2007).

Paradoxically, women receiving chemotherapy in addition to surgery have significantly lower objectively measured sedentary time and higher MVPA than women who received surgery alone (Phillips et al. 2016), despite the greater fatigue induced by chemotherapy. While the specific nature of the association between objectively measured physical activity and sedentary time and cancer recurrence is still unclear, randomized controlled trials and case-control studies have clearly demonstrated both increased sedentary time and decreased physical activity in cancer survivors contributes to elevated risk of cardiometabolic disease compared to age- and BMI- matched postmenopausal women without a cancer diagnosis (Colditz et al. 2016, Jones et al. 2016, Foraker et al. 2016). Specific interventions designed to increase physical activity through exercise training, and their effects on cardiometabolic health, will be discussed later in this review.

Obesity

Obesity is a significant contributor to both cancer and cardiometabolic disease development (McTiernan 2005, Ndumele et al. 2016), and increases the risk of both all cause and cancer specific mortality after cancer diagnosis (Chang et al. 2013, McTiernan et al. 2010). Recently, the World Cancer Research Fund estimated that 17% of breast cancer diagnoses were a direct result of obesity (WCRF Food, nutrition, physical activity and the prevention of cancer global report, 2014), and Howell et al. estimated that weight loss, coupled with increased physical activity and a reduction in alcohol consumption, would reduce the risk of cancer development by 30% (Howell et al. 2014).

While the increased risk of cardiometabolic disease due to obesity is fairly consistent across the lifespan, this is not the case for the relationship between obesity and breast cancer risk. Overweight and obesity may have a protective effect on breast cancer risk in premenopausal women, but significantly increase the rate of breast cancer development in postmenopausal women (Hsieh et al. 1990, Chlebowski et al. 2015). The direct mechanisms for the discordant relationship between obesity and cancer risk based on menopause status are unclear, but it appears to impact and overlap with several systemic aspects of obesity. For example, one of the hallmarks of obesity-induced increases in cardiometabolic disease risk is the development of insulin resistance and elevated circulating insulin levels. The mechanisms behind insulin resistance and hyperinsulinemia will be discussed (in great length) later in this review, however it is important to note that while there is a significant association between insulin and both cancer development and recurrence in postmenopausal women (Goodwin et al. 2008), no such relationship exists between cancer development, insulin resistance and/or hyperinsulinemia in premenopausal women (Eliassen et al. 2007). The difficulty of studying the relationship between cancer, obesity, and the bioenergetics behind obesity has been well documented, and several large clinical trials are currently underway with the express purpose of clarifying the complex mechanisms behind the obesity-induced increase in cancer risk (Patterson et al. 2010).

Chemotherapy-induced cardiometabolic disease

The primary goal of chemotherapy is to deliver chemicals that will significantly impair or halt the ability of the cancerous cells to grow and/or replicate, however the systemic delivery of chemotherapy can induce profound changes to cardiometabolic health. These changes not only affect women during the months of chemotherapy, but also may impact health outcomes for years.

Several recent studies have identified an unusually high percentage of women who have blood glucose concentrations that move from the normal range into the range of T2D during chemotherapy (JuanJuan et al. 2015). Interestingly, the majority of these cases of chemotherapy-induced transient diabetes are only identified through postprandial glucose and/or hemoglobin A1c values (Lu et al. 2014), suggesting that the common measure of glycemic control during cancer treatment (fasting blood glucose) may be vastly underestimating the impact of chemotherapy on glucose homeostasis. The most likely culprit for this transient diabetes is a combination of reduced physical activity and glucocorticoids regularly given during treatment as anti-emetics. Glucocorticoids increase glucose concentrations through a combination of increased glucose production and decreased insulin sensitivity, and the elevated glucose concentrations mostly return to normal following cessation of treatment (Wu et al. 2015). While there have been no longitudinal studies investigating the role of glucocorticoid use or elevated blood glucose during cancer treatment as risk factors for the subsequent development of frank T2D, evidence from other situations that induce transient diabetes and/or insulin resistance (e.g. gestational diabetes, polycystic ovary syndrome) suggests this would be highly likely (Appelman et al. 2015).

Cardiovascular fitness and maximal aerobic capacity (VO_2max) represent key predictors of cardiometabolic disease risk, and emerging evidence suggests that many chemotherapeutic agents have a detrimental effect on aerobic fitness. A recent meta-analysis by Peel et al. that evaluated 27 different studies in which VO2max was determined prior to and after chemotherapy found a significant reduction in aerobic fitness due to the chemotherapy, and this could only be partially explained by reduced levels of physical activity (Peel et al. 2014). Jones et al. recently quantified the decline in VO2max following cancer treatment in terms of the age-related decline in cardiorespiratory fitness, and found that a group of women who enter chemotherapy with the average aerobic capacity within the normal range for 60 year old women have aerobic capacity more reflective of 80 year old women following an average of 6 months of treatment (Jones et al. 2016). As discussed in the previous paragraph, this precipitous decline in aerobic fitness following cancer treatment is not met with a vigorous exercise-induced rebound following completion of treatment, but rather with a decline in MVPA in the year following treatment completion.

Summary of moderators of cancer progression

While the root of cancer is a genetic mutation that confers a selective growth advantage in certain cells, inactivity and obesity both can significantly contribute to cancer development, recurrence and cancer-specific mortality. Additionally, there appears to be a relationship between cardiometabolic disease and cancer recurrence, both as a direct relationship (e.g. women with diabetes are more likely to develop cancer) as well as the shared common root of obesity and inactivity. The systemic impact of inactivity and obesity, as well as the difficulty of measuring the "crosstalk" between many of the tissues and organs that link obesity and inactivity with cardiometabolic health (e.g. adipose tissue, skeletal muscle), have made it difficult to determine the precise mechanistic relationship between cancer and cardiometabolic health. This difficulty is compounded by the large sample size necessary for cancer research; while cancer rates are rising, evaluating cancer risk with the primary outcome of cancer development (or recurrence) still requires large sample sizes and many years of follow-up. For smaller studies designed to test the efficacy of interventions designed to target inactivity and/or obesity in order to determine the impact on cancer and cardiometabolic disease risk, surrogate measures, such as biomarkers, are required

<u>Biomarkers of cancer development and prognosis</u>

Over the last 20 years several cross-sectional and longitudinal epidemiological studies that are large enough to use cancer diagnosis as a primary outcome, such as the Women's Health Initiative (Thomson et al. 2014), the Nurses Health Study (Eliassen et al. 2010) and NHANES (Lynch et al. 2011), have contributed much to the understanding of cancer risk and prognosis, especially as they pertain to lifestyle factors (e.g. obesity, inactivity). These epidemiological studies are vital links in the research chain, and have served as the primary "jumping off point" for many subsequent randomized controlled trials, case-control studies and interventions. A major contribution of these longitudinal epidemiological studies was the identification of biomarkers associated with cancer. The strength of these biomarkers with respect to cancer-specific or all-cause mortality is in fairly constant flux, and the associations between these biomarkers and cancer risk range from moderate to weak. Despite this limitation, biomarkers of breast cancer risk and recurrence represent the most viable metric by which intensive short-term (e.g. 3-12 month) lifestyle interventions can study cancer risk in human beings. Many of the biomarkers are not localized to the cancerous tissue, and have wide-ranging effects that are not directly related to tumorigenesis or cellular growth. It is therefore not enough to simply identify the circulating quantities of the biomarker and block (or reduce) it's activity, as that may have wide-ranging detrimental (and potentially life-endangering) effects.

Given the difficulty of using systemic hormones and metabolites as cancer biomarkers, studies making use of them must also include 1) the mechanisms of action of a hormone/metabolite within the specific cancerous tissue 2) the effects of the biomarker on non-cancerous tissue and 3) the potential interaction of two or more biomarkers on cancerous and non-cancerous tissue. This three-pronged approach may appear arduous, especially when one considers that in many cases these biomarkers do not CAUSE cancer (i.e. are carcinogens), but instead increase the RISK of cancer development and/or ENHANCE tumor growth (i.e. are mitogens). However, until biomarkers directly associated with tumorigenesis and/or genetic profiling can be identified, understanding the nature and interaction of biomarkers associated with risk of recurrence is critical for interpreting projects in which a relationship between metabolism and cancer must be established.

Insulin

Insulin is a peptide hormone secreted from the pancreas that has wide-ranging metabolic effects on many different tissues throughout the body. Insulin is a primary anabolic hormone, and the majority of the effects of insulin on cellular tissue are to induce nutrient uptake, storage and cell growth. Several other studies have identified a relationship between hyperinsulinemia, poor breast cancer treatment prognosis (Ahern et al. 2013), and increased risk of breast cancer recurrence (Irwin et al. 2011), However, these findings are by no means uniform, and other researchers have either observed very weak or absent relationships (Minatoya et al. 2013, Sieri et al. 2012), or have been unable to disentangle hyperinsulinemia from the myriad inter-related concurrent symptoms of obesity and/or metabolic syndrome that have *all* been identified as potential mediators or moderators of breast cancer risk (Capasso et al. 2013). In order to clarify the potential role of hyperinsulinemia as a causal mechanism of increased risk of breast cancer recurrence, an investigation into the cellular relationship between insulin and cancer development is warranted.

The intricate relationship between insulin and insulin-like growth factors (IGFs) is often cited as the primary means by which circulating insulin can induce cellular proliferation, but insulin alone can provide enough impetus to cause aberrant cellular growth (Rostoker et al. 2013). The signaling pathways induced by insulin are far-reaching and varied, however the ones most associated with cellular proliferation are the

PI3K/Akt and MAPK pathways (Weinstein et al. 2009). Briefly, insulin binds to a tyrosine kinase receptor on the surface of the cell, which activates insulin receptor substrate 1 (IRS-1) and induces phosphorylation cascades through the activation of PI3K and MAPK. The phosphorylation of PI3K induces the phosphorylation of Akt, mTOR and p70S6k, while phosphorylation of MAPK induces the phosphorylation of the Ras/ERK/MEK pathway, both of which induce cell proliferation (Weinberg 2008). Mutations in these pathways are often present in all types of cancer, and can represent the acceleration of proliferation that is akin to "stepping on the gas pedal" which, when combined with loss of tumor suppressor genes, leads to unchecked cell growth and tumorigenesis.

Both insulin and the genetic mutations that induce cancer cell proliferation activate similar phosphorylation cascades, and indeed several studies have demonstrated a strong relationship between exposure to insulin and cell proliferation in cancer cell models (Milazzo et al. 1997, Rostoker et al. 2013). Additionally, blocking the PI3K signal cascade appears to reduce the size of mammary tumors in a hyperinsulinemic mouse breast cancer model (Gallagher et al. 2012, Novosyadlyy et al. 2010), albeit with the unfortunate side effect of severe hyperglycemia. The specific mechanism behind any potential mitogenic action of insulin is poorly understood, likely due to the intricacy of the signaling cascade. In addition to the role insulin may play in tumorigenesis through overactivation of these critical phosphorylase cascades, hyperinsulinemia may also increase the activity of insulin receptors themselves, another potential mechanism of cancer cell proliferation (Gallagher et al. 2013). Finally, it has recently been demonstrated that insulin suppresses the metabolic intermediate PTEN, a potent tumor suppressor that inhibits PI3K/Akt pathway expression (Liu et al. 2013). This suggests that hyperinsulinemia may be helping cells "step on the gas" through enhanced proliferation and "take the foot off of the brake" through inhibition of tumor suppression *at the same time*.

There are several limitations in generalizing the results of these cell culture and mouse model studies to systemic human cancer development. First, these studies often are demonstrating proof of concept through activation of a specific signal cascade intermediate, and therefore insulin exposure is often higher and of greater duration than what would normally be observed in even the most hyperinsulinemic individuals. Along similar lines, even those studies that reduce the insulin exposure to physiological levels use static exposure, which does not represent the cyclical insulin release in humans.

While the precise mechanisms are unclear there is consensus that insulin represents a mitogenic, rather than carcinogenic, compound on a cellular level (Call et al. 2010). If we equate the spread of cancer to an uncontrolled wildfire, insulin likely does not provide the spark that triggers the mutation leading to cancer. But hyperinsulinemia may add fuel to that spark, allowing a fire to grow where it would otherwise not via overexpression of PI3K/MAPK pathways, enhanced insulin receptor activity, and inhibition of tumor suppressor activity. Additionally, it appears that the mitogenic response to hyperinsulinemia is highly variable (Beelen et al. 2014, Baxi et al. 2012). To extend the wildfire analogy to its terminus, in some cases the hyperinsulinemia plays a minimal role, such as a few leaves on a blazing bonfire, however in others hyperinsulinemia behaves more like dumping lighter fluid on a candle. It is unclear what specifically causes insulin to show such a high degree of variability, and further research in this area is required before any definitive conclusions may be made regarding the direct cellular influence of insulin on cancer development. In addition to this direct role of insulin as a biomarker of cancer development, the myriad roles of insulin throughout the body create several other mechanisms by which hyperinsulinemia can mediate or modify cancer risk. The two areas that appear to contribute the most in breast cancer are through the homology and cross-reactivity between insulin and insulin-like growth factors (IGFs) and through the effects of insulin on the activity of the sex hormones, specifically estrogen.

Insulin-like growth factors and binding proteins

IGFs represent a distinct class of molecules with high sequence homology to insulin (hence the name), but with markedly different physiological roles and regulation. Structurally these molecules exist in a complex composed of one of the two specific factors (IGF-1 and IGF-2) and are typically associated with one of six binding proteins (IGFBP1-6) (Rosen et al. 1991). Unbound IGFs bind to two different types of receptors (IGFR1 and IGFR2) that induce cellular proliferation and growth through interaction with growth hormone (Vottero et al. 2013). IGF-1 is the primary circulating IGF in adults, and is found in almost every tissue in the body. The ubiquitous nature of IGF-1 as one of the, if not the, primary regulators of cell growth (including exercise-induced skeletal muscle hypertrophy) results in widely discordant and opaque views on the role of IGF-1 in health, aging and disease (Berryman et al. 2008). Liver production and secretion is primarily responsible for circulating (as opposed to tissue specific) IGF-1 and the proportion of IGF-1 bound to associated IGFBPs dictates its physiological activity.

IGFBP1 and IGFBP3 appear to bind to IGF-1, hindering its ability to bind to the IGF1R and induce cell proliferation and growth (Yeap et al. 2011).

Results have been mixed (Shernhammer et al. 2006), but current epidemiological evidence suggests that elevated IGF-1 levels positively correlate with ER+ breast cancer development in women over 50 (Key et al. 2010, Kaaks et al. 2013). Additionally, the ratio of IGF-1/IGFBP3 has been widely used as a metric to assess the bioactivity of the IGF system in the development of all forms of breast cancer, with positive associations between IGF-1/IGFBP3 ratio and mortality in breast cancer survivors (Duggan et al. 2013). The reasons behind a specific type (ER+) and age range being more susceptible to breast cancer are unknown, however it is possible that there is some interaction between IGF-1 and estrogen, which will be discussed later in this section. Elevated IGF-1 and Reduced IGFBP also appear to be related to hyperinsulinemia and may combine synergistically to increase the risk of cancer development (Malin et al. 2004). The precise nature of the relationship between insulin binding to the insulin receptors and IGF-1 secretion from the liver is unclear, however recent studies suggest that it may be a result of *hyperinsulinemia interfering with the actions of IGFBP3 and its binding affinity with IGF-1*, thus increasing the bioavailable supply of circulating IGF-1 (Yamada et al. 2010, Kaaks et al. 2001).

The close homology between insulin and IGF-1, as well as their cellular receptors, may serve as an additional means by which hyperinsulinemia increases the risk for breast cancer development and/or recurrence. For many years it was a mystery how vast differences between insulin signaling (nutrient uptake, metabolism) and IGF-1 signaling (cell proliferation and growth) could exist despite the close homology between insulin receptors (IRs) and IGF receptors (IGFRs) and the virtually identical signal transduction cascades. Work in the late 1990s identified a specific set of pre-receptor ligand binding interactions between IGF and IGFBPs that effectively “set up” the system for the appropriate phosphorylation and cellular result (Mynarcik et al. 1997). However, just as this mystery was being solved IR/IGFR hybrids were identified (Federici et al. 1998), and these receptors were responsive to both insulin and IGF-1 (Belfiore et al. 2009). These receptors typically exist in extremely small quantities, however they become hyperactive and overexpressed in many cancer cells, including breast cancer cells (Pandini et al. 1999). Results from several studies using a breast cancer mouse model generated by LeRoith and colleagues suggests that the overexpression of IR/IGFR hybrids in the presence of systemic hyperinsulinemia results in enhanced risk of breast cancer development and greater tumor load (Novosyadlyy et al. 2010).

The specific role that these IR/IGFR hybrids may play after diagnosis and treatment of breast cancer in humans is unclear, however it provides a secondary mechanism by which high insulin and/or high IGF-1 may activate the PI3K/Akt and MAPK pathways and increase the risk for recurrence of breast cancer after treatment.

Sex hormones

There has been extensive research into the role of sex hormones, specifically estrogens (e.g. 17-b estradiol) and their receptors, on the development of breast cancer. Thoroughly delineating the relationship between estrogen and breast cancer would encompass an entire literature review, however there are several key concepts that have bearing on this project, and therefore warrant discussion. For simplicity, all subsequent information presented in this section will be with respect to postmenopausal women.

There are several key manifestations of this distinction. First, while premenopausal women produce estrogen in the ovary (which may enhance cardiometabolic protection), postmenopausal women produce estrogen primarily in adipose tissue. This estrogen production is under the primary regulation of a complex of enzymes known as aromatase, which converts androgens into estrogen within the adipocytes (Bulun et al. 2012). In addition to the activity in the adipose tissue, aromatase can become highly active in the adipocytes of the breast, greatly increasing the local concentrations of estrogen (Sebastian et al. 2002). Since this aromatase activity is localized in adipose tissue and adipocytes, there is a high degree of correlation between fat tissue and circulating estrogen concentrations in postmenopausal women (Perry et al. 1998). Additionally, a strong correlation exists between circulating estrogens and breast cancer risk in postmenopausal women (Zhang et al. 2013, Licznerska et al. 2008), suggesting that adipocyte-derived estrogen plays a significant role in breast cancer development and recurrence.

This correlation also appears to hold for androgen levels (Folkerd, 2013), which represent the precursors for estrogen. Approximately 75-85% of breast cancers are ER+ or PR+ (Glass et al. 2007), and often exhibit overexpression of estrogen receptors on the surface of cancer cells. The elevated circulating estrogen concentrations derived from adipose tissue may initiate a signaling cascade by activating these receptors, leading to cell proliferation and growth which is enhanced through the localized estrogen production in the adipocytes of breast tissue. In support of this correlation between obesity, estrogen and cancer risk, weight loss appears to significantly reduce circulating estrogen concentrations in postmenopausal women as well as significantly reduce the risk of cancer development. This risk reduction occurs regardless of whether that weight loss is achieved via diet (Wasserman et al. 2004), physical activity (Friedenreich et al. 2011) or their combination (Carpenter et al. 2012), provided the primary means of this weight loss is through reductions in adiposity.

Insulin appears to play an active role in estrogen production within the adipocytes by increasing a specific type of aromatase activity that converts androstenedione to estrone (McTernan et al. 2000), which can then either be released into the general circulation as estrone or undergo an additional conversion to estradiol prior to subsequent release. The magnitude of this increased production of estrone and estradiol due to hyperinsulinemia is not clear, however it appears to occur in all adipose tissue and breast cancer cell models (Lisztwan et al. 2008), indicating that the specific magnitude of effect would be increased with higher degrees of insulin and/or greater volume of adipose tissue. In addition to regulating production of estrogen, hyperinsulinemia can also modify bioactive adipose-derived estrogen concentrations by reducing the activity of sex hormone binding globulin (SHBG), which attaches to estrogens and androgens and deactivates them (Tymchuk et al. 2000). SHBG is produced by the liver in response to circulating estrogen and androgen concentrations, however it has a high degree of negative regulation through the activity of several primary hormones and growth factors, most significantly insulin and IGF-1 (Kaaks et al. 2005). This downregulation of SHBG occurs in the liver at the site of production, and can partially explain results from studies in which a change in circulating insulin concentrations can drive a change in circulating estrogen concentration with no apparent change in body fat (Zeleniuch-Jacquette et al. 2004).

The significant variability of cancer development, essentially a progressive genetic disease, and the interrelatedness of symptoms associated with obesity and metabolic disease causes much debate over the impact of biomarkers in the progression of cancer. There is a positive association between circulating estrogen concentrations on the risk for developing postmenopausal (ER+) breast cancer, despite the low levels of estrogens within this population. Aromatase inhibition via pharmacotherapy has become quite common, and one of the outcomes of aromatase inhibition is to drive concentrations of circulating estradiol well below detectable assay limits, a significant challenge to studies attempting to address the relationships between postmenopausal estradiol concentrations and health outcomes after primary breast cancer treatment (Rosner et al. 2013). Additionally, aromatase inhibition may significantly alter SHBG concentrations. Several studies have identified both significant increases and significant decreases in SHBG following aromatase inhibition (Bajetta et al. 1999, Boeddinghaus et al. 2001) and the appropriateness of using SHBG as a surrogate measure for estradiol in postmenopausal women is unclear. Thus, while estradiol represents a prime candidate for the assessment of intervention efficacy (based on the high correlation with cancer risk and recurrence), that rapidly increasing number of women taking aromatase inhibitors and the difficulty of assessing postmenopausal estradiol concentrations may necessitate using alternative measures, such as SHBG and estrone. This limitation will be discussed further in chapter III.

Adipokines

The role of leptin and adiponectin in breast cancer has recently generated large amounts of research. One contributing factor to this is the identification of leptin expression in mammary epithelial cells, a primary location for mutations leading to breast cancer development. Leptin has wide-ranging systemic effects on a variety of tissues, but is currently viewed as both a regulator of energy balance and an inflammatory adipokine. Estrogen production in adipose tissue is highly promoted by leptin (Binai et al. 2013), and circulating leptin levels are correlated with aromatase activity (Maccio et al. 2010). Leptin could therefore serve as a surrogate marker for the effects of exercise on aromatase activity in postmenopausal women taking aromatase inhibitors. These women would not register significant levels of circulating estrogen, but may have locally active estrogen acting in a paracrine fashion to enhance breast cancer risk. Another interesting and potentially relevant aspect of leptin is the signaling pathway activated by the binding of leptin to its receptor. While insulin and IGF-1 enhance cellular proliferation through PI3K/Akt and MAPK pathways, leptin appears to enhance cell proliferation through the JAK/STAT pathway (Sharma et al. 2006). This would therefore suggest that a mutation residing on this pathway may not be enhanced by hyperinsulinemia but may be enhanced by hyperleptinemia. As researchers begin to view leptin as hormone that shares some similarity with insulin with respect to disease development and impact (however with daily peaks and nadirs much more narrow than those of insulin), investigations into the role of leptin as a contributor to metabolism in both health and disease are critical.

Adiponectin is also a hormone secreted from adipocytes that may play a role as a biomarker for breast cancer development, however it appears to have an inverse relationship with cancer risk (Liu et al. 2013). The mechanisms by which adiponectin may influence breast cancer development are unclear, however it has been proposed to be related to insulin and IGF-1 activity (Duggan et al. 2011). Adiponectin also displays an inverse relationship with circulating estrogen levels (Cleary et al. 2009), but any potential causal interactions have not been identified. Similar to leptin, it appears that adiponectin may work via a PI3K/Akt independent pathway, in this case through direct modification of AMPK (Kim et al. 2009). The adipokines therefore represent a highly intriguing secondary area of interest in studies in which the primary focus centers on insulin/IGF-1 because these hormones often alter cellular metabolism independently of the PI3K/Akt phosphorylation cascade.

Summary of biomarkers

The current literature on biomarkers associated with physical activity and breast cancer recurrence centers around three overlapping and interrelated pathways with two potential alternatives. First, insulin itself may act as a potent mitogenic compound, directly activating the PI3K/Akt pathway and potentially blocking the tumor suppressors that regulate this pathway. Secondly, high levels of IGF-1 may stimulate cell proliferation, and this may be in part regulated via insulin's' effects on IGFBP3 activity and shared sequence and receptor homology. Finally, aromatization of androgens to estradiol in adipose tissue represents a significant risk factor for breast cancer development, and this process may also be modulated in part by the degree of insulin and IGF-1 exposure. In addition to these primary mediators and moderators of breast cancer risk, systemic low-grade inflammation and adipokines may provide alternative means by which the environment may modify breast cancer development. *In all of these cases it is not necessarily the exposure to a specific hormone/factor that induces breast cancer, but rather the* ***magnitude and/or combinations of exposure***. Unlike specific (and avoidable) carcinogens, it is virtually impossible to eliminate these hormones and factors from the body, and in many cases it would be unwise to try and do so.

Assessing the degree of systemic exposure to biomarkers traditionally involves measuring them at a singular, well-controlled timepoint. This is not unreasonable, and while there may be slight variations in the majority of these hormones over the course of the day it is typically on a relatively small order of magnitude. This is not the case however when it comes to insulin. The role of insulin to induce glucose uptake and initiate many of the cellular processes involved in nutrient storage require it to be highly variable over the course of the day. Indeed, the pancreas is capable of increasing insulin production up to 25x that of basal levels (Ferrannini, 2010), and upwards of 70% of the insulin to which an individual may be exposed is primarily in response to a meal, as opposed to the fasted state when insulin is generally assessed. In order to thoroughly understand the relationship between insulin and breast cancer risk, as well as the modification of that relationship through exercise training, it is necessary to understand the behavior of insulin as a hormone, especially in its primary role as the regulator of glycemic control.

Postprandial insulin and insulin supply and demand

Several epidemiological studies have identified a potential relationship between insulin and breast cancer risk and/or risk of recurrence (Irwin et al. 2011, Goodwin et al. 2012). Most notably, Goodwin and colleagues have consistently shown over the last decade that not only is there a relationship between circulating insulin concentrations and breast cancer (Goodwin et al. 2009, Goodwin et al. 2002), but that immediate treatment with the anti-diabetes drug metformin after breast cancer diagnosis leads to a significantly better prognosis in hyperinsulinemic women (Niraula et al. 2012, Goodwin & Stambolic 2011). This data is consistent with an unexpected finding that individuals taking metformin for its anti-hyperglycemic properties were somewhat less likely to develop breast cancer (Bodmer et al. 2010). Insulin may play a role in the regulation of cancer risk, but studies investigating this role have traditionally fit into either cellular mechanistic studies or population-based epidemiological studies, which have potential limitations in their applicability to case-control or randomized controlled trials.

Concentrations of insulin, as mentioned previously, do not exist as a fixed and stable value throughout the day. Instead, insulin rises and falls due to meals given its role as a secretory hormone responsible for nutrient uptake and metabolism. While certainly not exclusive, the primary role of insulin in systemic physiology is glycemic control, or the maintenance of glucose homeostasis. In skeletal muscle, this involves initiating the translocation of GLUT-4 glucose transporters to the surface of the cell, inducing glucose uptake (Goodyear & Kahn 1998). In the liver, insulin causes cessation of hepatic glucose production by inhibition of gluconeogenenesis and hepatic glycogenolysis (Wahren & Ekberg 2008). *The degree of insulin required by these tissues to accomplish these tasks represents <u>the insulin demand</u>, or insulin sensitivity*. Given the negative health outcomes associated with hyperglycemia, the beta cells of the pancreas are quick to modify insulin supply to match any changes in muscle and/or liver demand.

Should insulin supply exceed insulin demand (as is often the case with pancreatic tumors), glucose levels will decline and there is severe risk of hypoglycemia. Likewise, should insulin supply fail to match insulin demand, hyperglycemia and Type II Diabetes (T2D) is likely. Diagnoses of T2D have reached epidemic proportions through a combination of obesity- and age-associated increases in incidence and prevalence. Estimates now suggest that by the year 2050 one in three people in America over the age of 50 will be present some form of dysglycemia (either prediabetes or frank T2D) (Boyle et al. 2010), making it one of the nations' most pressing health concerns. Given the increased prevalence of both T2D and cancer, by 2050 breast cancer survivors presenting with prediabetes or T2D *may become the norm, rather than a minority subset of the breast cancer survivor population.* The development of T2D centers on the dynamic closed-loop relationship between insulin secretion and both hepatic and peripheral (e.g. skeletal muscle) insulin action, and this relationship may play a role in the risk of breast cancer recurrence.

The role of insulin in the progression of T2D

The closed-loop relationship between insulin supply and insulin demand has made isolating the physiological mechanisms of worsening glucose tolerance difficult, however the development of the hyperinsulinemic-euglycemic clamp "opened the loop" and provided a direct assessment of whole body insulin sensitivity (DeFronzo et al. 1979). This newfound ability to isolate whole body insulin demand contributed to the idea of a singular "pathophysiology" of T2D, in which skeletal muscle insulin resistance induced by obesity, inactivity, and/or genetic predisposition was the initial insult to glycemic control (Bonadonna et al. 1990). To compensate for this reduced insulin action, adaptations occur within the beta cells of the pancreas that increase circulating insulin concentrations and "overcome" both the ineffective insulin-mediated glucose uptake and reduced suppression of hepatic glucose production in order to maintain circulating glucose concentrations within a tight range (Festa et al. 2006).

Insulin-resistant individuals can thus maintain normal glucose tolerance, provided that they maintain enough compensatory hyperinsulinemia to match the higher insulin demand. Eventually however, some combination of genetic factors, aging and consistent hypersecretion of insulin causes the pancreatic beta cells to fail to produce enough insulin to match the prevailing insulin demand (Kanat et al. 2012). In this period, commonly referred to as 'beta cell dysfunction,' blood glucose begins to rise into the range of prediabetes, and the elevated blood glucose exacerbates the beta cell dysfunction. This has led to a view of a homeostatic negative feedback loop between insulin sensitivity and insulin secretion that is maintained until the beta cells, weary after (in some cases) years and decades contending with elevated insulin demand, cannot maintain glycemic control, and glucose homeostasis enters into a positive feedback loop that invariably results in the development of T2D (DeFronzo & Abdul-Ghani 2009). While this linear disease trajectory may be appropriate with respect to the development of T2D, several interactions between exercise training and insulin supply/demand make T2D prevention a more complicated situation.

Insulin demand (sensitivity)

The unique ability of insulin to both induce glucose uptake (primarily) in the skeletal muscle and suppress glucose production in the liver manifest as two distinct metrics of insulin demand. Given the critical nature of insulin resistance as a primary link between obesity/inactivity and T2D, there has been much research into how insulin resistance develops in both skeletal muscle and the liver. The specific mechanism is still unclear, but insulin resistance in skeletal muscle appears to manifest downstream from both the surface receptor and the upper aspects of the phosphorylase cascade (PI3K/Akt)

(Johnson & Olefsky 2013), such that insulin binds normally to its receptor, PI3K/Akt gets turned on, but the signal gets lost along the way to the GLUT-4 receptors and they never translocate to the surface. Glucose uptake can also be hindered by a loss of total GLUT-4 receptors in the case of obesity and inactivity (Bienso et al. 2012). It is unclear what molecular signal induces the compensatory increases insulin supply to overcome this insulin resistance, however the additional insulin binding to receptors on the surface of skeletal muscle provides enough impetus to drive enough GLUT-4 translocation to maintain adequate glucose uptake. Since these GLUT-4 receptors are only active in the insulin stimulated (i.e. postmeal) state, changes to skeletal muscle insulin demand have a much larger effect on postprandial insulin secretion than on fasting insulin secretion(Daily 2003). It is common for highly inactive people to have ***relatively normal*** fasting insulin levels but require large amounts of insulin during and after a meal to induce glucose uptake lower blood glucose and.

Liver insulin resistance is different from skeletal muscle in that abnormalities to insulin demand are reflected in both fasting and postmeal glycemic regulation. In the fasted state, the liver is responsible for producing sufficient amounts of glucose in order to maintain adequate circulating concentrations. The importance of this task cannot be overstated; if you do not produce glucose from the liver by creating it (gluconeogenesis) and/or breaking down stored liver glycogen, hypoglycemia can be common and debilitating. Hyperinsulinemia in the fasted state is therefore a reflection of the amount of insulin required to maintain this normal production of glucose from the liver (Basu et al. 2013). Because glucose concentrations following a meal are high enough to keep the brain operating, the role of liver insulin demand in the postmeal state shifts towards suppressing the (now superfluous) production of glucose. This leads to a fairly clear distinction between the role of liver and skeletal muscle insulin sensitivity, based on the presence or absence of elevated circulating glucose concentrations. The insulin levels observed in the fasted state represent the interaction between liver insulin demand and pancreatic beta cell insulin supply, whereas the insulin levels observed in the fed state are a reflection of the interaction between; 1) liver insulin demand (to suppress endogenous glucose production), 2) skeletal muscle insulin demand (to initiate glucose uptake) and 3) beta cell insulin supply (Matveyenko et al. 2008). We, and others, have observed discordance between skeletal muscle insulin sensitivity and hepatic insulin sensitivity (Braun et al. in preparation, Faerch et al. 2010). The correlation between liver and skeletal muscle insulin sensitivity is not strong enough that one may serve as a surrogate for the other, and this may have wide-ranging implications on the conclusions drawn in many studies investigating the influence of insulin and diabetes on breast cancer risk or recurrence.

Insulin supply and beta cell function

Insulin demand is the primary driver of insulin supply, but there are several distinct ways that the pancreas and liver can accomplish the task of meeting the demand. Insulin, as mentioned previously, is formed in the beta cells of the pancreas through the process of cleaving proinsulin into insulin and C-peptide. This cleaving takes place inside the vesicles, and therefore C-peptide and insulin leave the beta cells of the pancreas in an equimolar ratio (Polonsky & Rubenstein 1984). A certain percentage of these vesicles lie attached to the cell membrane of the beta cells and are released extremely quickly in response to a rise in circulating glucose, but other vesicles are located deeper within the beta cells and take longer to release their stored insulin in response to both glucose and other signals, such as free fatty acids and incretins (Hatakeyama et al. 2006). This results in a distinct secretory pattern in which insulin is released very quickly with the first exposure of glucose (known as the acute insulin response, or AIR), followed by a second slower phase of insulin secretion. The relationship between these phases of insulin secretion and diabetes risk are fairly well established (Cobelli et al. 2007), however their role in breast cancer risk or risk of recurrence is unknown.

Prior to reaching the general circulation, a portion of the insulin produced by the pancreas is extracted and degraded by the liver in a process referred to as first-pass hepatic insulin extraction (HE) (Campioni et al. 2009). The percentage of insulin extracted through HE is highly variable, such that severely insulin resistant individuals (i.e. those requiring a high degree of hyperinsulinemia to maintain glycemic control) may extract minimal amounts of insulin (Andreev et al. 2009, Tura et al. 2001). In order to get an accurate assessment of insulin secretion from the beta cells (beta-cell function), interventions often use C-peptide instead of insulin to quantify changes to insulin secretion and the effects of interventions on the pancreas. The precise metabolites and signaling pathways that influence hepatic extraction are poorly understood, and several researchers have noted that HE and insulin degradation in general represents a remarkably understudied area of research (Bonnet et al. 2011). Given the importance of the liver in relation to many of the risk factors associated with cancer development (e.g. SHBG, IGF-1), the assessment of HE along with insulin may represent a fruitful area of research with respect to interventions.

To return to the study in which the relationship between insulin resistance and biomarkers of breast cancer risk was determined based on the change in fasting C-peptide (Fairey et al. 2003), it should now be evident that these conclusions may be an oversimplification of the actual effect of the exercise intervention. The C-peptide concentration reflects the function and adaptation of the beta cell and not necessarily the action of the affector hormone (insulin). Additionally, the assessment of insulin resistance in the fasted state may not be capturing the change to insulin demand in the postprandial state, which may be important considering the role of exercise to modify postmeal hyperinsulinemia and the uncertainty about total or fasting insulin exposure in the risk for breast cancer recurrence.

Clinical role of the disruption of insulin supply and demand

In order to assess the impact of insulin supply and demand on the risk for breast cancer, it would make the most sense to include individuals with prediabetes who display marked hyperinsulinemia. Prediabetes exists as an intermediate metabolic state between normal glucose homeostasis and T2D, and is primarily characterized by mild hyperglycemia and exaggerated hyperinsulinemia. Compared to T2D, insulin levels are higher in this prediabetic stage, and it has been recently reported that *the risk for breast cancer development is higher in individuals with prediabetes than in those with normal glycemic control* ***or frank T2D*** (Onitilo et al. 2014). Additionally, the largest magnitude of change in insulin concentrations following exercise training is seen in individuals with prediabetes, which is likely due to a confluence of factors associated with the pathophysiology of T2D. Individuals with prediabetes are close to maximally insulin resistant, however they retain the ability to secrete considerable amounts of insulin

(Abdul-Ghani et al. 2006). Reducing insulin supply by reducing insulin demand (in the form of exercise induced increase in insulin sensitivity) will preserve beta cell function and significantly prolong the time in which normal circulating glucose concentrations can be maintained (Utzschneider et al. 2004). Additionally, the reduction in insulin concentrations in individuals with prediabetes following exercise training often approaches 10-20% in the fasted state and 30-50% in the postprandial state (Jenkins & Hagberg 2011, Malin et al. 2013), which may represent a significant reduction in risk of breast cancer recurrence and warrants a more complete understanding of the precise mechanisms involved.

Interventions to better match insulin supply and demand

Given the role of mismatched insulin supply and demand in diabetes pathophysiology, it is not surprising that researchers have focused a large volume of research on interventions that may better match insulin supply and demand. While exercise training significantly increases whole body insulin sensitivity (Malin et al. 2013) and likely leads to concomitant reductions in insulin supply, pharmacological agents such as metformin likely exert a tissue-specific effect (Viollet & Foretz, 2013). While it is unlikely that metformin and exercise training work together to improve specific components of systemic insulin supply and demand (Malin et al. 2013, 2014, Jenkins et al. 2014), it is possible that they have unique tissue specific effects. **Therefore, the purpose of Study 1 of this dissertation was to investigate the role of exercise training and/or metformin on tissue-specific components of insulin supply and demand. This may provide insight into the specific means by which exercise training can modify breast cancer risk and/or improve prognosis in breast cancer survivors.**

Summary of insulin supply and demand

Insulin is the primary hormone responsible for maintaining glycemic control, and circulating insulin concentrations have wide-ranging applicability in health and disease. While the assessment of insulin concentrations in the fasted state may present a snapshot of metabolic health, it is by no means exclusive and all encompassing. There is evidence that insulin levels may play a role in cancer recurrence, however an individuals insulin exposure may be widely discordant based on that individuals particular supply and demand. Insulin demand can be identified through skeletal muscle, liver, fasting and postmeal effects. Insulin supply includes not only the actions of the beta cell increasing or decreasing secretion, but also that of the liver breaking down a certain percentage before it reaches the general circulation. Conclusions and inferences based on relationships between insulin supply and demand and markers for breast cancer risk may be failing to capture areas in which hyperinsulinemia, glycemic control and breast cancer risk overlap. Both hyperinsulinemia and insulin resistance have been suggested to be moderators of breast cancer recurrence, however not all insulin resistance and hyperinsulinemia are created equal. Many aspects of the interactions between hyperinsulinemia and insulin resistance and breast cancer risk have not been thoroughly investigated. This lack of information can have direct effects on several aspects of glycemic control not traditionally assessed in breast cancer survivorship studies, including those using exercise training.

Exercise, hyperinsulinemia and breast cancer

The relatively recent obesity epidemic has triggered interest in the relationship between physical activity, metabolic outcomes, and diseases such as cancer. As mentioned previously, the link between physical activity and cancer development appears to be fairly consistent across all types of solid tumor cancers (Courneya et al. 2013, McTiernan 2003), and the consensus based on these large cross-sectional epidemiological studies is that individuals who engage in physical activity have approximately 10-25% lower risk of developing breast cancer than highly sedentary individuals (Ballard-Barbash et al. 2012, Irwin et al. 2003). Additionally, several observational studies suggest that breast cancer survivors who habitually engage in walking or other aerobic activities reduce their risk of all cause and cancer specific death by up to 70% (Holmes et al. 2005, Holick et al. 2008). This relationship between PA and cancer recurrence has generated research into prospective mechanisms behind this risk reduction.

Randomized controlled trials of exercise training in breast cancer

In 2010 the American College of Sports Medicine released an evidence-based position stand on the relationship and efficacy of exercise training to improve outcomes related to cancer (Schmitz et al. 2010). In it, they identified 32 prospective randomized controlled trials (RCTs) that investigated the relationship between an exercise training program and health outcomes in breast cancer survivors. The results of these studies all seem to suggest that exercise training in breast cancer survivors is safe and leads to improvements in fitness and quality of life similar to those benefits seen in age and sex-matched controls. One of the limitations noted by the authors of this position stand was the relative paucity of information with respect to metabolic outcomes. Of the 32 RCTs included, less than 10 collected any type of blood metabolites, and very few of those did so with specific transdisciplinary goals in mind, such as the role of exercise training to modify the relationship between breast cancer survivorship and CVD. Indeed, it was this paucity of data that likely spurred on several aspects of the current Transdisiplinary Research on Energetics and Cancer (TREC) initiative (Patterson et al. 2013(a)), composed of several large RCTs designed to investigate the relationship between cancer and a host of physiological variables, such as insulin resistance. Several of these TREC projects include exercise training and/or increased PA as the means by which they modify energetics and potentially manipulate health outcomes, including a major wing of the project investigating the role of insulin in breast cancer (Patterson et al. 2013 (a)). These projects were all initiated in 2011 (with study designs published) and given a five-year window to complete before the information will be reduced and released in a singular document, and until 2016 we are left to speculate with respect to what they will find. Given the relatively few published studies investigating metabolic outcomes in breast cancer survivors it is beneficial to identify their specific methods and outcomes, specifically highlighting any relationship between exercise, insulin/glycemic control and markers of breast cancer risk.

One of the first studies to investigate the role of insulin to moderate the effects of exercise in breast cancer survivors was published in 2003 by Fairey and colleagues at the University of Alberta (Fairey et al. 2003). This study included 52 postmenopausal stage I-III breast cancer survivors randomized into 3x/week of cycle ergometry for 15 weeks (n=24) or control (n=28). The primary outcomes of this study were fasting insulin, IGF-1, IGFBP and insulin resistance (assessed by homeostasis model assessment, or HOMA, the product of fasting insulin and glucose and an algorithmic constant). They observed *no differences in insulin* or insulin resistance following the 15 weeks of exercise training, however they did observe statistically significant decreases in IGF-1 and increases in IGFBP3, leading to a significant change in the IGF-1/IGFBP3 ratio.

The results from this study would therefore suggest that exercise training may have a significant impact on risk of breast cancer recurrence, however this risk reduction does not occur as a result of a change in insulin concentrations or insulin sensitivity. This conclusion is limited in scope however, as the effects of insulin and insulin resistance were evaluated *only through **fasting** insulin concentrations*, which also serves as the basis for their modeling of insulin resistance. Since exercise training manifests primarily in reductions in postprandial insulin concentrations (as a reflection of increased skeletal muscle insulin sensitivity), it is likely that assessing fasting insulin concentrations alone was not enough to capture the full modifications to glycemic control brought on by the exercise training intervention. In fact, one of the interesting aspects of this study was that fasting insulin concentrations in the exercise group slightly increased following the exercise training, rather than decreasing (as was expected) or staying the same (as was the case with the control group). This was not commented upon by the researchers, however it is not a unique finding. Other researchers have observed slight increases in fasting insulin and/or glucose levels following the initiation of an exercise program as the mechanisms controlling fasting insulin supply and demand attempt to adjust to potentially large changes in postmeal insulin supply and demand (Winnick et al. 2008, Yates et al. 2010). This behavior of fasting insulin may therefore represent a manifestation of changes in postmeal insulin levels that were not addressed in the study.

Ligibel and colleagues (Ligibel et al. 2008, Ligibel et al. 2009) attempted to build on the conclusions of the previously described study by incorporating resistance training and including a slightly larger number of participants (n=40 for the exercise group). This study achieved remarkable control for an exercise training study in which 40 women did supervised resistance training, and the results from this intervention suggest that this program was enough to induce moderate changes in insulin concentrations. These changes in insulin and insulin resistance were significant over time, and were trending towards significance when compared to the control group. In addition to the standard limitation of *using fasting insulin concentrations* as a marker for daily glycemic control, the women in this study were remarkably metabolically healthy. While the criteria for enrollment in this study almost exactly matched the study by Fairey et al. (mostly postmenopausal breast cancer survivors with BMI>28 and exercising <15 min/wk), their fasting glucose (85 mg/dL) and insulin (8 uU/mL) are in the optimal range for this population. It is therefore possible that the results from this study may be difficult to interpret based on the health of the participants and the relative lack of improvement to insulin and glucose concentrations that may be gained through moderate aerobic and resistance training. The results of this intervention may have been different had the participants been hyperinsulinemic or prediabetic, which is a significant proportion of overweight, inactive postmenopausal women.

At roughly the same time, Irwin and colleagues published results from the Yale Exercise and Survivorship (YES) study, a large RCT also aimed at further establishing the metabolic pathways by which exercise may modify cancer recurrence. Metabolic results from this study were published in three papers (Irwin et al 2009 (a), Irwin et al.

2009(b), Jones et al. 2013), and served as much of the basis for this proposed project. The primary metabolic outcome investigated was the relationship between insulin, IGF-1 and IGFBP3 following a 6-month combined supervised and home-based aerobic exercise intervention in breast cancer survivors (Irwin et al. 2009(a)). This study is notable for its inclusion of several key measures that were not included in the previous studies, including an objective (albeit pedometer based) measure of PA and DEXA scanning to assess changes in BMD, body fat and central adiposity. The women enrolled in this study were remarkably similar to those from Fairey et al. and Ligibel et al with respect to BMI (30), age (57 years) and activity status prior to intervention (12 min/week). Participants *fasting insulin concentrations decreased* in the exercise training group (24.57 uU/mL to 22.9 uU/mL) and increased in the control group (25.69 uU/mL to 31.98 uU/mL), however this result was not significant (p=0.08). When compared to control, the exercise training group did have significantly lower IGF-1 concentrations following the exercise, in support of the results found by Fairey and colleagues. In addition to the changes observed in IGF-1 and trends towards change in insulin, this cohort also lost a significant amount of weight and body fat compared to control, leading the researchers to conclude that the primary impact of the exercise training on metabolic health and risk of recurrence is through weight loss in this population. Without a thorough investigation into the mechanisms of postmeal insulin supply and demand, that conclusion may not be representative of the exact role an exercise induced increase in insulin sensitivity plays in the reduction of breast cancer risk.

In addition to the direct measurement of insulin and IGF-1 the YES cohort was evaluated for changes in inflammatory markers, including TNF-a and IL-6 (Jones et al.

2013). While there was no role of inflammation in the overall group responses, there was a significant change in IL-6 in those who performed >80% of the training sessions. Based on this result and the results of several other exercise studies (Rogers et al. 2013), a potential role of inflammation with respect to exercise induced modulation of breast cancer recurrence risk cannot be ruled out and warrants further investigation. Absent from these previous studies is an investigation into the role of estrogen, SHBG and the adipokines (leptin and adiponectin). Roughly 30% of the participants of the YES study were on aromatase inhibitors, and that may have severely complicated any potential conclusions made with respect to the interaction between estrogen/SHBG levels and exercise.

Other exercise training in breast cancer survivor studies

Several studies investigating the role of exercise training in breast cancer survivors published after the 2010 ACSM position stand warrant further discussion. Campbell and colleagues have recently completed an exercise trial in which an exercise program similar to the one introduced by the United States Diabetes Prevention Program (USDPP) was used in breast cancer survivors (Campbell et al. 2012), while Goodwin and colleagues have published preliminary work on the role of metformin in breast cancer survivors (Niralua et al. 2012), which also draws partly from the USDPP. The USDPP was a large multi-site RTC investigating the role of a lifestyle intervention or metformin in the prevention of the transition from prediabetes to frank T2D. This trial was a resounding success, as the lifestyle intervention reduced the transition from prediabetes to T2D by well over 50% and metformin was also effective at a rate of approx. 30% (Knowler et al. 2002). The success of this program has lead to widespread follow-up studies, including by our lab (Malin et al. 2012). Of particular note, these follow-up studies have shown that while both exercise (the primary driver of the changes seen in the lifestyle group) and metformin improve metabolic health and reduce insulin concentrations, they do so through different mechanisms. Exercise training, as mentioned previously, induces metabolic adaptations to insulin supply and demand through skeletal muscle, and therefore manifests most prominently in postmeal hyperinsulinemia (Kirwan & Jing 2002), while metformin interacts primarily with the liver (McCormack et al. 2001), inducing changes to fasting insulin and glucose concentrations.

Results from Goodwin and colleagues work with metformin and breast cancer recurrence/prognosis have been mixed. They generally support a significant reduction in fasting insulin concentrations and insulin resistance (Niralua et al. 2012), but with minimal effect on prognosis in those with T2D (Lega et al. 2013). Several large clinical trials, including several arms of the TREC initiative, are underway to determine the exact role metformin may play in reducing the risk of cancer recurrence, with the results from these trials forthcoming. The study by Campbell et al. did not observe any relationship between a USDPP-based lifestyle intervention composed of exercise and dietary modification and biomarkers of breast cancer recurrence, however several limitations may have confounded their results. First, their fasting glucose concentrations were not in the prediabetic range (95 mg/dL) and their fasting insulin concentrations were normal (8.9 uU/mL), potentially running into a “basement” effect where the low levels of glucose and insulin prevent exercise training from having a measurable effect on glycemic control, similar to Ligibel et al. Secondly, while there was considerable reduction in insulin concentrations following the exercise training, the high degree of variability (+/- 9.1 uU/mL SD) in those insulin concentrations may have contributed to the lack of significant differences in the lifestyle intervention group. Finally, as with other studies investigating the role of exercise in breast cancer survivors, the metrics of hyperinsulinemia and insulin resistance were derived from a fasting measure of insulin (and glucose for the HOMA score), which may not be an appropriate reflection of the role of exercise in the modulation of hyperinsulinemia. Several of the TREC studies were designed to investigate the interaction between exercise training and cancer risk, prognosis and treatment. Their study designs are consistent in that they all include metabolic measurements, and those investigating the role of insulin are designed to primarily reflect insulin supply and demand in the fasted state. **The relationship between fasting and postmeal insulin supply and demand and biomarkers of cancer recurrence are unclear, and therefore study 2 will investigate the relationship using information from the pre-intervention glucose tolerance testing and fasting biomarkers of cancer risk taken at the same timepoint.** Lack of information regarding the effect of exercise training on postprandial hyperinsulinemia could lead to a significant underestimation of the role of exercise training in modifying cancer risk in this population. **To address this potential confounding relationship between fasting and postmeal insulin concentrations with cancer risk, study 3 will examine the change in insulin supply and demand following exercise training and how that relates to the change in cardiometabolic health in breast cancer survivors**. Results from this study should aide in the understanding of treatment-specific mechanisms, potentially identifying situations in which reducing postmeal insulin concentrations though exercise may be more critical than reducing fasting insulin concentrations via pharmacology, such as metformin. Evaluating postprandial insulin within this population takes additional importance from the recent results from our lab that suggest that adding metformin to exercise training blunts the effects of the exercise (Malin et al. 2012). Should changes to postprandial rather than fasting insulin concentrations drive the beneficial adaptations to biomarkers of breast cancer risk, breast cancer survivors who both exercise and take metformin may actually be increasing their risk of recurrence. This well-controlled, albeit relatively small, prospective study into the interaction between postprandial hyperinsulinemia, exercise and breast cancer recurrence may not be enough to alter the conclusions derived from these large generously funded multi-site RCTs, however it could potentially serve as the basis for larger proposals investigating this relationship in the future.

CHAPTER III

EXERCISE TRAINING AND METFORMIN DIFFERENTIALLY IMPACT COUPLING OF INSULIN SUPPLY AND DEMAND

Introduction

A key aspect of type 2 diabetes (T2D) prevention is the ability to appropriately match the production of insulin with the demand of whole-body insulin resistance. Up to 70% of individuals with prediabetes (fasting and/or post-challenge glucose concentrations above normal but below the range of frank T2D) are characterized by an inappropriate matching of insulin secretion and sensitivity (DeFronzo & Abdul-Ghani 2011), and without lifestyle or pharmacological intervention the transition from prediabetes to T2D is highly likely (Nijpels et al. 1996). Results from the United States Diabetes Prevention Program suggest that both lifestyle intervention (comprised of weight loss and increased physical activity) and the anti-hyperglycemia medication metformin delay the transition from prediabetes to T2D (Kitabchi et al. 2005). Despite a plausible expectation of additivity, recent evidence suggests that combining metformin and exercise training confers no added benefit to whole-body insulin sensitivity and markers of cardiovascular risk when compared to exercise training alone (Jenkins et al. 2012, Malin et al. 2012, Malin et al. 2013). Lifestyle interventions increase whole-body insulin sensitivity and reduce circulating insulin concentrations, which is primarily a result lower insulin secretion (Kahn et al. 1992). However, circulating insulin concentrations may also be modified by insulin synthesis in the beta cells (Polonsky et al. 1994) or altered rates of insulin extraction and clearance by the liver (Escobar et al. 1999). These effects likely lie outside the closed-loop relationship between whole-body insulin sensitivity and compensatory insulin secretion, and may not be effectively captured with standard tools (e.g. euglycemic clamp) and metrics (e.g. HOMA-IR) used to evaluate changes to insulin sensitivity and secretion. Understanding the difference between tissue-specific and whole body insulin supply and demand regulation has more than academic importance given that preservation of beta-cell capacity is a key to diabetes prevention. Metformin influences glycemic control through inhibition of hepatic glucose production (Madiraju et al. 2014), and it is likely that there are other tissue-specific responses (e.g. hepatic insulin clearance) through which metformin may influence glycemic control. Without evaluating the combined effects of exercise training and metformin on tissue-specific markers of glycemic control (e.g. beta cell proinsulin processing, insulin clearance) that lie outside the insulin secretion-sensitivity feedback loop, it is possible that the utility and drawbacks of combining lifestyle interventions and metformin to prevent or delay T2D are not fully understood.

Proinsulin, the precursor prohormone to insulin and a marker of insulin synthesis, is primarily contained within the beta cells of the pancreas, where it is cleaved into insulin and C-peptide prior to release into the general circulation. Elevated circulating proinsulin concentrations represent a decoupling between glucose variations and the synthesis and release of insulin, as proinsulin that could otherwise be used for insulin production 'leaks out' into the general circulation. It is therefore not surprising that total proinsulin concentrations, as well as the ratio between proinsulin and both insulin (PI/I) and C-peptide (PI/C), are elevated in adults with prediabetes (Larrson et al. 1999, Warcham et al. 1999). Additionally, hyperproinsulinemia is associated with both the development and severity of T2D (Loopstra et al. 2011, Roder et al. 1999), and may serve as a link between impaired glycemic control and cardiovascular disease (Choi et al. 1999, Zethelius et al. 2002). Circulating proinsulin concentrations are reduced following intervention with lifestyle or metformin (Kitabchi et al. 2005), but the effects of combining the two interventions are unknown. First pass hepatic insulin extraction (HIE) involves the degradation of insulin by the liver after secretion from beta cells but prior to reaching the general circulation (Toffolo et al. 2006). Obese individuals and individuals with hyperinsulinemia often display reduced HIE (Kim et al. 2007, Mittelman et al. 2000), which may partly contribute to their elevated circulating insulin concentrations. HIE may be higher following interventions that reduce hyperinsulinemia or improve glucose tolerance (Krogh-Madsen et al. 2014), however the impact of exercise training and/or pharmacological interventions is unclear. As a result little is known about how HIE changes with respect to alterations to insulin secretion and sensitivity, or the relationship between HIE and changes in glycemic control (Krogh-Madsen et al. 2013, Mittelman et al. 2000). Insulin clearance (IC), or the systemic breakdown of insulin, occurs primarily in the liver (~80%) and kidney (~20%) and is also reduced in obesity (Ader et al. 2014, Valera Mora et al. 2003) and prediabetes (Castel-Auvi et al. 2012, Marini et al. 2014). IC may respond to pharmacologically augmented weight loss (Kim et al. 2014) but the effects of lifestyle interventions on IC are unknown.

There is a pressing public health need to prevent T2D, and maximizing the efficacy/precision of prevention will require evaluating the independent and interactive effects of lifestyle and pharmacological interventions. Thus the purpose of this study was to evaluate the effects of a 12-week exercise training and/or metformin intervention on insulin synthesis, clearance and extraction. Given our previous work suggesting metformin and exercise training in combination is not more beneficial than exercise alone with respect to cardiometabolic health (Malin et al. 2012, Malin et al. 2013), we hypothesized that metformin would not confer any added benefit, and may even blunt, the beneficial tissue-specific effects of exercise training on IC, HIE and proinsulin processing.

Methods

Overview

The protocol has been previously described in detail elsewhere (Malin et al. 2012, Malin et al. 2013). Briefly, overweight to obese sedentary women and men (Table 1) with impaired glucose tolerance, as determined by a 75 g oral glucose tolerance test, were randomly assigned to one of four groups: Placebo (P, n=8), Metformin (M, n=9), exercise training plus placebo (E+P, n=9), and exercise training plus metformin (E+M, n=10). Exclusion criteria were smoking, weight instability (> 5 kg change over previous 6 months), regular physical activity (> 60 min/wk), or contraindications to metformin. Peak aerobic capacity (VO_2peak, cycle ergometer), maximal strength (1 repetition max for key muscle groups), and body composition (dual X-ray absorptiometry, DEXA, Lunar Technologies, Chicago IL) were tested before and after the intervention. All participants were verbally briefed about the study and signed informed consent documents approved by the University of Massachusetts Amherst Institutional Review Board.

Intervention protocol

All participants were instructed to maintain baseline diet and physical activity levels throughout the 12-week intervention, and no change in diet (3-day diet records) or habitual ambulation (pedometer) was observed (18). Participants were randomly assigned to receive metformin (1000 mg twice per day separated by 8-12 hours) or an identical placebo and further subdivided into exercise training (3 days/week, 225 total minutes of supervised aerobic and resistance exercise) or non-training groups.

Blood collection and hyperinsulinemic-euglycemic clamp

Following 24-hours of dietary and physical activity control (meals provided to ensure caloric and macronutrient balance) and a 10-12 hour overnight fast, blood samples were taken from an indwelling catheter placed in an antecubital vein. Blood samples were collected and plasma was separated in tubes containing EDTA (proinsulin, insulin and C-peptide) and NaF (glucose) and stored at -80° for subsequent analysis. Following the fasting blood draw a 120-minute hyperinsulinemic-euglycemic clamp (5mmol, 80 $mU/m^2/min$) was used to determine peripheral (skeletal muscle) insulin sensitivity.

Biochemical analysis

Fasting blood glucose was determined using the glucose oxidase method (GM7 analyzer, Analox Instruments, Lunenberg MA). Fasting plasma proinsulin, insulin and C-peptide concentrations were determined using a commercial radioimmunoassay (Millipore, Billerica MA). The cross-reactivity of the human proinsulin RIA with insulin and C-Peptide is <0.1%, and the cross-reactivity of the human C-Peptide RIA for total proinsulin is <4%. The intra-assay coefficient of variation was 4.7%, and the interassay coefficient of variation was <10%.

Proinsulin processing, hepatic insulin extraction and insulin clearance

Total fasting proinsulin, proinsulin to insulin (PI/I) and proinsulin to C-peptide (PI/C) ratios were calculated to depict proinsulin secretion. Hepatic insulin extraction (HIE) was assessed using the volume-adjusted insulin to C-Peptide ratio as defined by Cobelli and colleagues (Cobelli et al. 2007). Insulin clearance was determined during the last 30 minutes of the clamp by dividing the insulin infusion rate by the circulating steady-state plasma insulin concentration (SSPI), as previously used by Marini and colleagues (Marini et al. 2014, Marini et al. 2013). Whole-body insulin sensitivity was defined as the glucose infusion rate (M) during the last 30 minutes of the clamp divided by the SSPI.

Statistics

Data were analyzed using R statistical software (Vienna AU 2010, http://www.R-project.org). Baseline and group differences were evaluated using a one-way ANOVA. Pre to post differences within groups were determined using paired t-tests. Pearson product moment correlation coefficients were used to determine associations between changes in proinsulin, HIE and IC as well as changes in insulin action and markers of cardiometabolic health. Statistical significance was accepted as $p<0.05$.

Results

Baseline characteristics and effects of training

Baseline body weight, fitness, insulin sensitivity, and physical activity levels were similar among groups (Table 1). As reported previously (Malin et al. 2012, Malin et al. 2013), VO_2peak increased in both E+P (+18%) and E+M (+10%), and weight loss was greater after M (-4%) and E+M (-7%) compared with P (0%) and E+P (-0.2%). Insulin sensitivity also higher following all treatments, and the rise in sensitivity was 25-30% greater in E+P compared with E+M (Malin et al. 2012). While there were no changes to fasting glucose, insulin or C-peptide in the P and M groups, there were significant reductions in fasting plasma insulin and C-peptide following E+P and significant decreases in fasting plasma glucose and C-Peptide in the E+M group (Table 2).

Proinsulin and proinsulin ratios

Baseline proinsulin concentrations did not differ among groups. Compared to baseline, fasting plasma proinsulin was not different in P, M, or E+P, however proinsulin concentrations were significantly lower following E+M (Figure 1). There were no significant differences in the PI/I ratio or PI/C ratio across the interventions (Table 2). There was also no significant correlation between the change in fasting proinsulin and change in insulin sensitivity ($r=-0.127$, $p=0.46$), fasting plasma glucose ($r=0.199$, $p=0.25$), or body fat percentage ($r=0.323$, $p=0.64$).

Hepatic extraction and insulin clearance

There were no significant changes to first pass HIE in the control group or any of the intervention groups (Table 2). There was also no significant correlation between the change in HIE and the change in fasting proinsulin ($r=0.053$, $p=0.76$), insulin sensitivity ($r=-0.064$, $p=0.96$), body fat percentage($r=0.139$, $p=0.88$) or body weight ($r=-0.208$, $p=0.43$). Steady state plasma insulin was significantly lower in M and E+M, but there was no difference in P or E+P (Table 2). Similarly, insulin clearance was also significantly increased in M and E+M, and unchanged in P and E+P (Figure 2). There was a significant association between the change in insulin clearance and change in insulin sensitivity (r=0.344, p=0.014), however there was no association between insulin clearance and fitness (r=0.291, p=0.15) or body fat (r=-0.143, p=0.42).

Discussion

In this study, fasting proinsulin was significantly reduced (-24%) only after exercise training was combined with metformin. Although not significant, the decrease with metformin alone (-20%) was comparable in magnitude to the combined intervention. Additionally, rates of steady state insulin clearance during a hyperinsulinemic-euglycemic clamp were also significantly greater following 12 weeks of metformin, with or without exercise training, but not with placebo or exercise training alone. These results surprised us because we expected insulin clearance to follow a similar pattern to that of whole-body glucoregulatory responses, such as insulin sensitivity, which were most strongly impacted by exercise training alone (Malin et al. 2012, Malin et al. 2013). The decoupling of changes to proinsulin and insulin clearance from insulin sensitivity and fasting insulin implies that metformin and exercise differentially impact the relationship between insulin demand (tissue sensitivity) and supply (secretion).

There are several potential explanations for these results. In the current study, only the two groups that received metformin lost weight. Weight loss drives many of the beneficial outcomes of lifestyle or pharmacologic interventions on cardiometabolic health/disease risk. However the driving force behind these positive changes is generally considered to be loss of fat, particularly loss of abdominal fat (Malin et al. 2012). In our study it was only the two exercise groups, not the metformin-only group, who lost total and central adiposity. Therefore, a causal relationship between weight/fat loss and both lower proinsulin concentrations and greater steady state insulin clearance in our study is not supported. Similarly, although whole-body insulin sensitivity was strongly associated with higher cardiorespiratory fitness (CRF), there was no change to fasting proinsulin or insulin clearance in the exercise-only group, which showed the largest rise in CRF. In contrast, metformin alone caused a 20% reduction in proinsulin and a 20% increase in insulin clearance despite no change in CRF. These results imply that changes in CRF are not necessary to alter fasting proinsulin or insulin clearance.

It is also possible that in men and women with prediabetes, fasting hyperproinsulinemia and reduced insulin clearance are more closely associated with fasting hyperglycemia than hepatic or peripheral insulin resistance. If so, fasting proinsulin may only decline in response to lowering of fasting glucose. We observed no association between change in proinsulin and changes in fasting glucose, however it is conceivable that any relationship between the two was obscured by the relatively modest fasting hyperglycemia at baseline, restricting the magnitude of any declines in fasting glycemia and, therefore, proinsulin. The only group that exhibited a statistically significant decline in proinsulin was the E+M group, which also had a statistically significant reduction in fasting glucose concentration.

Metformin also affects signaling pathways in the beta cell (Leclerq et al. 2004), and can alter insulin production within the beta cells through AMPK-dependent changes to insulin synthesis (Kefas et al. 2004, Masini et al. 2014). It is therefore possible that metformin has a direct impact on insulin production in humans but exercise does not. Similar reductions in both insulin and proinsulin in the metformin groups suggests that metformin may act directly on the beta cell to lower the output of proinsulin into the portal vein rather than altering hepatic extraction. It is also possible that metformin influences hepatic and renal regulation of insulin kinetics via upregulation of key insulin degradation enzymes and possibly improvement of hepatic and renal function (Cao et al. 2014, Foretz et al. 2010) but that exercise training has a minimal effect on these processes. Directly testing how combining exercise training and metformin impact beta-cell function will require animal models and cell culture work in follow-up studies.

In contrast to the effects of metformin, discord between changes in circulating insulin, proinsulin, insulin sensitivity and insulin clearance in the exercise groups suggests that exercise training may modify glycemic control primarily by enhancing peripheral insulin sensitivity with consequent reductions in circulating insulin (Kahn et al. 1992). While insulin secretion represents the primary mechanism by which circulating insulin can be adjusted, first-pass hepatic insulin extraction may also play a significant role (Kim et al. 2007). We attempted to evaluate the effects of exercise and metformin on this tissue-specific moderator of insulin supply using the relationship between circulating insulin and C-peptide to estimate hepatic extraction. There were no differences in fasting hepatic extraction attributable to any of the 3 interventions. The calculation of hepatic insulin extraction using fasting C-peptide and insulin kinetics relies on several assumptions, and without a pre- and post-intervention glucose challenge it is hard to argue that hepatic extraction plays no role in the interaction between exercise training and metformin. A better understanding of how exercise training or metformin alter beta cell secretion and circulating insulin metabolism/kinetics will require cleverly designed studies to tease apart several interrelated processes.

Understanding the effects of exercise or metformin on measures of glycemic control requires considering the larger context. The hyperbolic law of insulin kinetics suggests that coupling between insulin demand (sensitivity or resistance) and supply (secretion and clearance) is coupled such that increasing sensitivity leads to lower circulating insulin concentrations (Stumvoll et al. 2005). The current study suggests that metformin, but not exercise, can change insulin synthesis/clearance without necessity for upstream changes to insulin sensitivity. If true, there are potentially important clinical ramifications. For example, one of the oft-cited benefits to improving insulin sensitivity is reducing hyperinsulinemia and "resting" the pancreas to preserve beta cell function. If lower circulating insulin is a result of changes in post-secretion insulin clearance instead of reductions in first and/or second phase insulin secretion, there may be little or no reduction in beta cell "stress" following exercise training. Additionally, if there is recognized value in reducing insulin synthesis by the islets regardless of the degree of insulin reaching the general circulation, there may be practical reasons to choose between metformin or exercise for patients who are still able to compensate for insulin resistance with hyperinsulinemia. The divergent impact of exercise and/or metformin on tissue-specific (e.g. proinsulin and insulin clearance) compared to whole-body (e.g. insulin action) metrics of glycemic control suggests that the utility of selecting one treatment versus the other, or combining both treatments, is outcome-specific.

Scaling up to the critical public health issue of preventing diabetes and cardiovascular disease in humans, the independent and combined actions of physical activity and/or metformin on the transition from prediabetes to T2D is difficult to predict from studies of insulin sensitivity alone. To understand the comparative efficacy of physical activity, metformin or both on T2D and cardiovascular disease prevention, studies will need to be conducted in the target population with the development of frank T2D or cardiovascular disease as the primary outcome.

Tables

Table 3.1: Participant characteristics

	P	**M**	**E+P**	**E+M**
N (male)	8(2)	9(4)	9(4)	10(4)
Age (years)	49.75 ± 3.87	46.33 ± 2.57	46.22 ± 2.60	49.50 ± 1.77
Weight (kg)	94.78 ± 5.28	96.47 ± 5.26	97.3 ± 4.74	93.44 ± 4.77
Body Fat (%)	45.96 ± 3.05	42.47 ± 2.45	42.16 ± 2.31	41.43 ± 2.21
VO_2Peak (mlkg^{-1}min^{-1})	21.44 ± 2.32	24.18 ± 2.75	25.28 ± 2.21	27.25 ± 1.76
Fasting Glucose (mmol)	5.34 ± 0.18	5.23 ± 0.24	5.45 ± 0.25	5.86 ± 0.21
2-h Glucose (mmol)	9.36 ± 0.47	9.23 ± 0.47	10.37 ± 0.34	9.81 ± 0.49

Data presented as mean ± SEM. There were no significant differences at baseline across the four conditions

Table 3.2: Changes to glycemic control after intervention period

		P	M	E+P	E+M
Glucose (mmol/L)					
	Pre	5.34 ± 0.18	5.23 ± 0.24	5.45 ± 0.25	5.86 ± 0.21
	Post	5.29 ± 0.12	5.28 ± 0.25	5.41 ± 0.21	5.49 ± 0.13*
Insulin (pmol/L)					
	Pre	139.9 ± 28.9	152.9 ± 27.5	94.9 ± 11.5	95.6 ± 20.8
	Post	149.9 ± 34.0	119.7 ± 26.3	84.5 ± 9.7*	80.3 ± 13.9
C-Peptide (pmol/L)					
	Pre	874.1 ± 151.0	1375.1 ± 142.8	1154.9 ± 195.8	1052.2 ± 96.4
	Post	969.8 ± 142.3	1226.4 ± 150.7	983.2 ± 169.3*	765.8 ± 76.4*
PI/I (pmol)					
	Pre	0.15 ± 0.02	0.16 ± 0.03	0.27 ± 0.11	0.24 ± 0.04
	Post	0.19 ± 0.04	0.18 ± 0.04	0.27 ± 0.08	0.19 ± 0.02
PI/C (nmol/pmol)					
	Pre	0.25 ± 0.04	0.21 ± 0.03	0.19 ± 0.03	0.17 ± 0.03
	Post	0.23 ± 0.04	0.17 ± 0.04	0.21 ± 0.05	0.20 ± 0.05
HIE (%)					
	Pre	71.2 ± 3.8	74.4 ± 4.0	84.4 ± 1.7	84.2 ± 3.1
	Post	75.8 ± 3.2	79.5 ± 3.2	83.3 ± 2.6	79.8 ± 5.1
SSPI (pmol/L)					
	Pre	1462.5 ± 75.4	1474.4 ± 74.8	1494.4 ± 118.9	1449.0 ± 86.4
	Post	1456.3 ± 58.6	1247.2 ± 102.7*	1295.5 ± 57.3	1199.0 ± 77.5*

Data presented as mean ± SEM. PI/I- Fasting proinsulin/insulin ratio. PI/C- Fasting proinsulin/C-peptide ratio. HIE-Hepatic insulin extraction. (*) Indicates significant change from baseline ($p<0.05$).

Figures

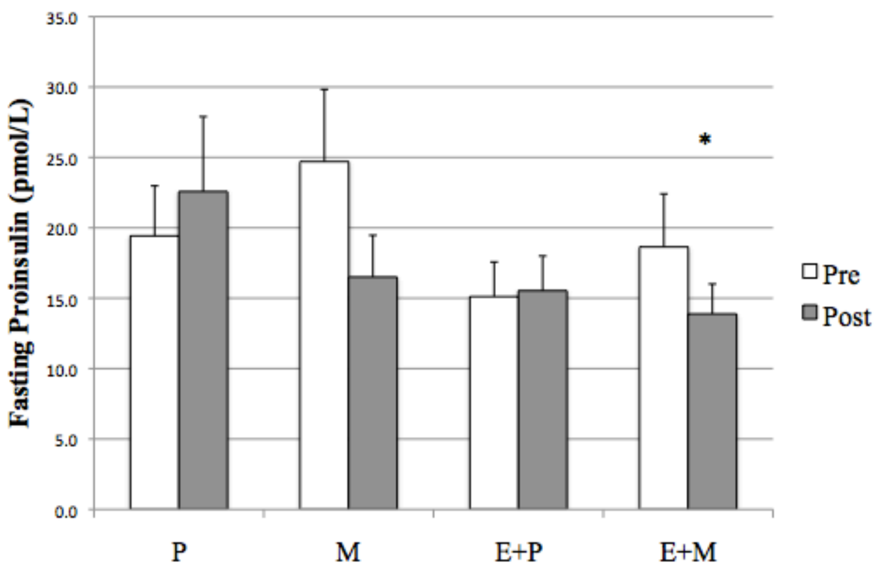

Figure 3.1: Fasting proinsulin concentrations

Data presented as mean±SEM. *Indicates significant difference from baseline ($p<0.05$)

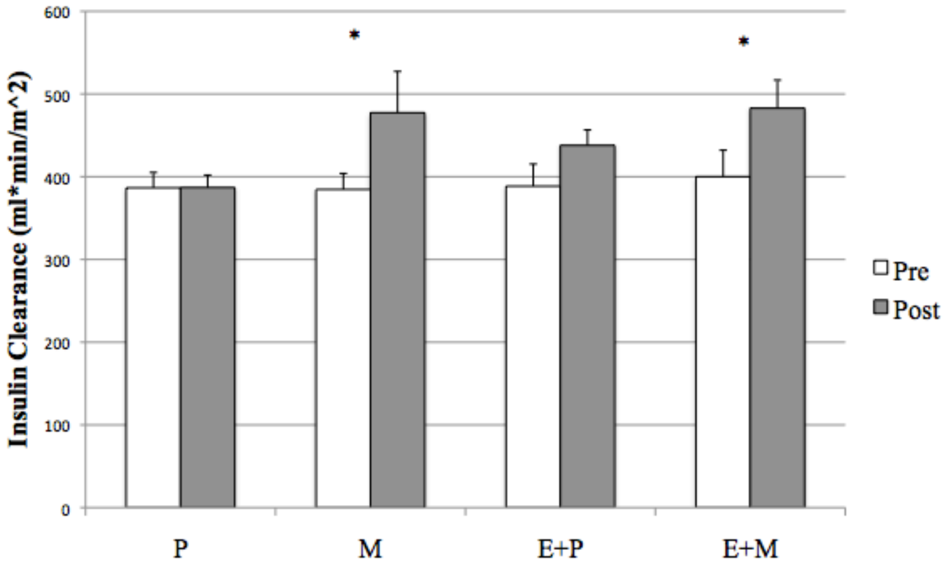

Figure 3.2: Clamp-derived insulin clearance

Data presented as mean±SEM. *Indicates significantly different from baseline ($p<0.05$)

Prologue to chapters IV and V

Studies 2 and 3 (chapters IV and V) of this dissertation differ from study 1 in terms of the population (men and women with prediabetes vs. postmenopausal breast cancer survivors) and research techniques (euglycemic clamp vs. oral glucose tolerance test). While this gives the appearance of two disconnected and unrelated projects, there are several areas of overlap between the studies that are worth mentioning.

The benefits of metformin as means to restrain blood glucose concentrations have been studied for decades, however the potential utility of metformin as a mediator/moderator of cancer risk is a relatively novel and unexpected finding (Bodmer et al. 2010). As with any unexpectedly fortuitous result, research has been directed towards identifying the mechanisms behind metformin-induced reductions in cancer risk (Chae et al. 2016) and optimally incorporating metformin into existing oncotherapy (Grossmann et al. 2015). Metformin appears to lower blood glucose concentrations by reducing hepatic glucose production (Madiraju et al. 2014), which leads to lower insulin concentrations in both the fasting and the postmeal state. Exercise training primarily regulates blood glucose through reductions in skeletal muscle insulin demand in the postmeal state, leading to a compensatory reduction in insulin supply. It is therefore reasonable to suspect that metformin and exercise training would have additive effects on glycemic control, as was the hypothesis for the series of experiments by Malin et al. (Malin et al. 2012, Malin et al. 2013). This was not the case however, as combining metformin with exercise training did not enhance, and may have even attenuated, the beneficial adaptations to systemic insulin supply and demand that occurred as a result of exercise training alone. The results from study 1 support the hypothesis that exercise training acts to reduce diabetes risk through systemic modification of insulin supply and demand (e.g. increased insulin sensitivity), whereas metformin may accomplish this same task (albeit to a lesser degree) through specific modifications of insulin-sensitive tissue. While it is tempting to expand this conclusion to encompass both exercise and metformin-induced reductions in carcingenesis, the lack of information regarding the relationship between changes to insulin supply and demand and cancer risk following lifestyle or pharmacological interventions prevents such interpretation from being made.

In 2011 The National Cancer Institute, in conjunction with several research institutions across the country, began a series of research studies known as the Translational Research in Energetics of Cancer (TREC) project (Patterson et al. 2011). One such study mimics the four-armed intervention used in study 1 to evaluate the benefits of exercise training and metformin in combination on cancer recurrence. While this may shed light on the efficacy and utility of the combined and independent effects of each intervention as oncotherapy, the lack of information regarding the tissue vs. systemic effects of exercise training on insulin supply and demand in both the fasted and fed state in cancer survivors may influence the interpretation of the benefits of each treatment on metabolic health. The purpose of studies 2 and 3 of this dissertation project were therefore to develop a greater understanding of changes to postmeal insulin supply and demand and the physiological mechanisms responsible for them as a result of exercise training in cancer survivors. These small, highly controlled research studies could therefore ‘fill in the gaps’ of large clinical trials, and by doing so guide future clinical trials and enhance the efficacy of personalized targeted therapy to reduce the risk of cancer and diabetes.

CHAPTER IV

EXERCISE TRAINING LOWERS POSTMEAL, BUT NOT FASTING, INSULIN CONCENTRATIONS IN BREAST CANCER SURVIVORS

Introduction

Women who regularly engage in physical activity have significantly lower rates of cancer development and recurrence (Moore et al. 2016), as well as reduced rates of cancer-specific and all-cause mortality (Ballard-Barbash et al 2012). Although the precise mechanisms are unclear, lower circulating insulin concentrations in active women may play a role. Insulin appears to have mitogenic effects on cancer cells, and results from cell culture, animal model and epidemiological studies support a role for elevated insulin concentrations (hyperinsulinemia) in the development of breast cancer (Gallagher et al. 2013) and breast cancer-specific mortality (Irwin et al. 2011). Despite a mechanistically plausible hypothesis that lower insulin concentrations following exercise training contribute to reduced cancer risk and improved cancer prognosis, this relationship has not been consistently observed in breast cancer survivors. Fairey et al. first evaluated the effects of exercise training on circulating insulin concentrations in breast cancer survivors, and their results suggested that 24 weeks of aerobic exercise training did not lower insulin concentrations (Fairey et al. 2003). Several subsequent exercise training studies in breast cancer survivors also failed to observe a significant reduction in circulating insulin concentrations or decrease in insulin resistance following 12-, 16- or 26-week exercise training interventions (Ligibel et al. 2008, Irwin et al. 2009, Campbell et al. 2012, Guinan et al. 2013). These results are surprising, as reductions in circulating insulin concentrations have been consistently observed following exercise training in other populations, including postmenopausal women (Friedenreich et al. 2011). It is unclear why exercise training interventions in breast cancer survivors do not elicit significant changes in circulating insulin concentrations. One plausible explanation is that the relationship between insulin and cancer risk is obscured by the use of fasting insulin, and metrics of insulin resistance derived from fasting insulin levels (e.g. HOMA), as the sole representation of exercise-induced improvements in insulin action and regulation.

While the relationship between insulin and cancer is often predicated on its anabolic and mitogenic nature, the role of insulin as a glucoregulatory hormone makes it unique among biomarkers of cancer risk. Insulin levels rise and fall several times throughout the day in response to meals, restraining blood glucose concentrations via induction of skeletal muscle glucose uptake and suppression of liver glucose production. The volume of insulin required for postmeal glycemic control can account for 50-80% of daily insulin exposure (Basu et al. 2003), and often shows a high degree of discordance with fasting insulin concentrations (Varghese et al. 2016). Additionally, interventions that improve glycemic control do not do so equally across different insulin-sensitive tissues and metabolic states. Exercise training is a potent skeletal muscle insulin sensitizer, and is more effective at reducing postmeal than fasting insulin concentrations (Jenkins and Hagberg 2011). In fact, while fasting insulin resistance metrics have a moderate correlation with gold standard measures of insulin resistance, it is unclear what specific physiological components of glucose metabolism they reflect (Reaven 2013). This potentially makes fasting insulin and associated metrics of insulin resistance inappropriate tools for evaluating the effects of exercise training, in which changes to glycemic control primarily manifest through changes to postmeal insulin action and volume.

It is possible that studies investigating the links between exercise training and cancer risk/prognosis underestimate the role of insulin by failing to assess changes in postmeal insulin concentrations. To date, no study has evaluated the effects of an exercise training program on postmeal insulin concentrations or metrics of insulin resistance that incorporate both fasting and postmeal glucose and insulin concentrations in breast cancer survivors. Therefore, the purpose of this study was to evaluate the effects of 12-weeks of supervised exercise training on postmeal insulin concentrations in breast cancer survivors, and identify any significant associations between changes in postmeal insulin concentrations and changes in biomarkers of cancer recurrence. We expect that exercise training will minimally change fasting insulin levels but will cause a significant reduction in postmeal insulin concentrations. Additionally, we expect that changes in cancer biomarkers (e.g. IGF-1, leptin, SHBG) will be more closely associated with changes to postprandial insulin concentrations than with changes in fasting insulin concentrations.

Methods

Recruitment and participants

Seventeen breast cancer survivors were recruited from the western Massachusetts area. Participants were between the ages of 35 and 70, and were either postmenopausal (or had oophrectomy) as determined by questionnaire. Participants were in overall good physical health, and were free from diabetes, heart disease and any injury that would prevent them from engaging in exercise training. Participants reported not meeting the current physical activity guidelines of 150 minutes/week of moderate to vigorous physical activity. Several participants were taking endocrine therapy for ER+ breast cancer, including Arimidex (n=5), Tamoxifen (n=2) and Aromasin (n=1). All medication use remained stable throughout the intervention period. All participants completed an informed consent document approved by the University of Massachusetts Institutional Review Board prior to starting the study.

Baseline fitness and anthropometric testing

Prior to metabolic testing, participants reported to the Energy Metabolism Lab in the Department of Kinesiology on the University of Massachusetts campus for determination of health history, body composition and fitness. Participants filled out a baseline health history and fitness questionnaire, followed by measurement of their height and weight (stadiometer and physicians scale, Detecto, Webb City, MO), blood pressure (manual sphygmomanometer, Santa medical inc., Tustin, CA) and waist circumference (tape measurer).

Baseline fitness levels were determined using a submaximal exercise test on a cycle ergomter (ACSM guidelines for exercise testing and prescription, 9th ed.). After a two minute resting period, participants were instructed to begin pedaling at an initial resistance of 25 watts, maintaining a cadence of >60 revolutions per minute. Resistance was increased in 25-watt increments every two minutes, and heart rate and rating of perceived exertion (RPE) were recorded at the end of each two minute stage. Additionally, expired gasses were continuously collected using a metabolic cart (Parvomedics, Sandy, UT) for the determination of volume of oxygen consumption (VO_2) and respiratory exchange ratio (RER). Stages were increased until participants reached 80% of age-predicted maximal heart rate (HR_{max}). Upon completion of the test, heart rate and VO_2 responses from each stage were plotted and a linear line-of-best-fit was used to estimate VO_2peak at age-predicted HR_{max}. To determine body composition, participants underwent a DEXA scan (Lunar technologies, Chicago IL) at the University of Massachusetts Health Services Center. This test was used to determine body fat percentage, bone mineral density as well as android (central) obesity and fat free mass (FFM).

Fasting blood sample and oral glucose tolerance test (OGTT)

Participants entered the lab following an overnight fast for assessment of fasting and postmeal glycemic control, as well as determination of cancer biomarkers. Participants were asked to refrain from physical activity and maintain habitual dietary patterns 24 hours prior to the glucose challenge. An indwelling catheter was placed in an antecubital vein by a trained research technician, and fasting blood samples were collected in tubes containing Sodium Fluoride (Glucose), Potassium EDTA (Insulin) and Serum Separator (cancer biomarkers). Following the baseline blood collection, participants consumed a 75 g oral glucose test beverage (Sundex, ThermoFisher, Waltham MA), and additional blood samples were taken at 30, 60, 90 and 120 minutes following glucose consumption for determination of glucose and insulin concentrations. Blood plasma/serum was centrifuged at 3000xg, aliquoted into polypropylene cryotubes and stored at -80 degrees for future analysis.

Supervised exercise training intervention

All participants were required to exercise at least once a week and at most four times a week under the supervision of study personnel at the University of Massachusetts Department of Kinesiology exercise facility (The Body Shop, Amherst, MA). Supervising members of the research team were responsible for recording results of the exercise training session (e.g. heart rate, METs, RPE), as well as ensuring appropriate exercise intensity and safety. The exercise training protocol consisted of aerobic exercise for 45-60 minutes per session at an intensity of 65-90% HR_{max}. Participants were allowed to choose between a cycle ergometer, elliptical machine and/or treadmill, and exercise sessions on a specific machine were required to last at least 20 minutes. As fitness improved over the course of the 12-week intervention the intensity of exercise was increased to avoid a plateau effect, including the introduction of higher intensity intervals. The overall goals of the exercise training program were to: 1) provide sufficient exercise stimulus to increase in aerobic fitness, insulin sensitivity, and meet the physical activity guidelines and induce the health benefits of exercise training, as well as 2) mimic real-world exercise training programs most commonly used in non-laboratory situations.

Post-intervention testing

Upon completion of the 12-week exercise training period participants returned to the lab for post-intervention assessment of glycemic control, aerobic fitness and body composition. In order to avoid confounding the exercise training effect with acute changes to glycemic control due to diet and/or physical activity, participants were asked to refrain from physical activity and match (as accurately as possible) their food intake from the 24h period prior to their baseline glucose tolerance test. All participants completed their final exercise training session 24-36 hours prior to post-intervention glucose tolerance testing.

Hormone, biomarker and metabolite analysis

Several different analytical techniques were used to determine the circulating concentrations of hormones, metabolites and biomarkers, and pre- and post-intervention samples from each participant were assayed in duplicate on the same assay in order to reduce inter- and intra-assay variability, respectively. Circulating glucose concentrations were determined using the glucose oxidase method (Analox instruments, Atlanta, GA) and an inter-assay coefficient of variability (CV) of <5%. Concentrations of insulin, leptin and adiponectin were determined using a commercially available radioimmunoassay (RIA, Millipore, Billerica, MA) and an interassay CV of <10%. Concentrations of 17b Estradiol (E2), IGF-1, IGFBP3 and SHBG were determined using high-sensitivity enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis MN) and an interassay CV of <10%.

Postmeal insulin concentrations and insulin sensitivity

In addition to the insulin concentrations at each timepoint of the oral glucose challenge (30, 60, 90 and 120 minutes), total postmeal insulin was quantified as 1) the highest measured insulin concentration during the OGTT (peak insulin) and 2) insulin area under the curve (AUC) using the trapezoidal method. To estimate changes to insulin sensitivity due to the exercise training, we used the glucose and insulin values from the oral glucose tolerance test to determine the Composite Insulin Sensitivity Index (C-ISI) based on the formula developed by Matsuda et al. (Matsuda & DeFronzo, 1999).

This metric is a reflection of fasting glucose and insulin homeostasis AND the responsiveness to a glucose load (insulin action) and has a high degree of correlation (0.78) with the euglycemic clamp (the gold standard measure of insulin sensitivity). The formula for the determination of C-ISI is (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]).

Statistical analysis

All statistical analyses were performed using the R statistics package and computing language using an a priori alpha of <0.05. Paired t-tests were used to evaluate pre- to post-intervention changes in fitness, body composition, fasting hormones, metabolites and biomarkers as well as pre- to post- intervention differences between single timepoints of the oral glucose tolerance test. Linear mixed models were used to evaluate the relationship between the intervention and oral glucose tolerance over the sequential timepoints of the test, as well as the effect of any mediators of the relationship between exercise training and change in glycemic control (e.g. the presence or absence of Aromatase Inhibitor). Finally, associations between biomarkers and variables of interest were determined using Pearson product-moment correlation coefficients.

Results

Participant characteristics

Of the 17 participants enrolled in this study, two were unable to complete the fasting and glucose challenge blood draw and were thus excluded from the results. Additionally, one participant completed the fasting blood draw but was unable to complete the oral glucose challenge and was eliminated from any post-challenge comparisons (but was included for fasting metabolite/biomarker analysis). One participant declined the DEXA scan, and her data was therefore eliminated from body composition analysis. A flowchart of participant recruitment and inclusion is presented in figure 4.1, and participant characteristics are presented in table 4.1. Data are presented as mean ± SD.

Exercise training

Participants attended an average of 34.4 ± 7.7 training sessions over the 12-week intervention. Due to the progressive nature of the exercise training program, the average number of training sessions attended by participants increased throughout the intervention, with participants averaging 2.7 ± 1.1 training sessions per week during weeks 1-4 and 3.3 ± 0.8 training sessions per week during weeks 9-12. Exercise volume increased throughout the supervised exercise training sessions, from 141.3 ± 32.9 min/wk during weeks 1-4 to 166.8 ± 30.6 min/week during weeks 9-12. Additionally, exercise intensity increased over the duration of the study, from 77.4 ± 5.2% HR_{max} during weeks 1-4 to 85.7 ± 5.7% HR_{max} during weeks 9-12, for an average of 81.8 ± 5.5% HR_{max} over the entire 12-week protocol. As this protocol was personalized to better encourage attendance/compliance and mimic non-laboratory exercise prescriptions, participants were allowed to engage in several bouts of High Intensity Interval Training (HIIT) per week at the discretion of the trainer. Each session involved 24-30 total minutes of HIIT activity as a component of the 45-60 minute training session, and included 4 or 5 1-minute periods of high intensity (>90% Age-predicted HR_{max}) cycling followed by 3-5 minutes of moderate intensity (<60% Age-predicted HR_{max}) cycling. Participants engaged in a total of 11.1 ± 7.8 HIIT sessions over the course of the intervention, with the majority occurring later in the intervention (1.6 ± 0.8 HITT sessions/week over weeks 9-12). A full summary of the dose of exercise (e.g. volume, intensity, duration) for the exercise training intervention by individual response is provided in Appendix A.

Fitness, body composition and ancillary health outcomes

Participants experienced a significant increase in aerobic fitness and a significant decrease in body weight following the intervention (Table 4.2), however this reduction in body weight was not a result of significant reductions in body fat or fat free mass. There was no significant association between baseline fitness and change in fitness ($r=0.13$, $p=0.74$), nor were there any significant associations between volume of exercise performed over the 12-week intervention period and change in fitness ($r=0.18$, $p=0.52$) or body weight ($r=0.08$, $p=0.94$). In addition to the small but significant change in body weight there were significant reductions in waist circumference and systolic blood pressure, but no significant changes in fasting or postmeal blood glucose concentrations (Table 4.2).

Cancer biomarkers

One participant had E2 concentrations below the detectable limit of the assay (5pg/ml) and was excluded from analysis. There were no significant pre- to post-intervention differences in IGF-1, IGBP3, Adiponectin or SHBG levels as a result of the exercise training (Table 4.2), however leptin and E2 levels were significantly lower following exercise training (Table 4.2). Surprisingly, this change in circulating leptin concentrations was not significantly associated with change in aerobic fitness ($r=0.06$, $p=0.92$), body composition ($r=0.18$, $p=0.48$) or any of the variables associated with insulin concentrations. The change in circulating E2 concentrations was not associated with changes to body fat or other adipocyte-derived biomarkers, however there was a significant inverse association between the change in E2 and participants average exercise intensity ($r=-0.55$, $p=0.04$) during the 12-week intervention period (Figure 4.2).

Fasting and postmeal insulin concentrations

There were no significant differences in fasting insulin concentrations (11.4 ± 5.2 vs. 11.6 ± 5.1 uU/ml) or at timepoints 30 minutes (74.4 ± 19.8 vs. 83.2 ± 37.6 uU/ml), 60 minutes (95.8 ± 30.4 vs. 98.4 ± 23.3 uU/ml) and 90 minutes (91.3 ± 32.8 vs. 88.3 ± 29.5 uU/ml) of the glucose challenge test (Figure 4.3 A). Insulin concentrations were significantly lower 120 minutes (2h) following glucose ingestion (68.8 ± 34.5 vs. 56.2 ± 31.9 uU/ml, $p<0.05$, Figure 4.3 A). There were no significant differences in insulin AUC (301.6 ± 82.2 vs. 301.9 ± 93.6 uU/ml, Figure 4.3 B) or peak insulin concentrations during the OGTT (107.4 ± 27.8 vs. 106.5 ± 26.5, Figure 4.3 C), nor was insulin sensitivity (3.29 ± 1.76 vs. 3.27 ± 1.27, Figure 4.3 D) different following the exercise training intervention.

Factors influencing the insulin response to exercise

There were no differences in the training response when participants were stratified based on age, stage of cancer diagnosis or cancer receptor type. However, women who were currently taking or had taken Aromatase Inhibitors (AIs) within the last five years (n=6) had significantly different and opposing responses in peak insulin (-11.99 (non-AI) vs +13.91 (AI) uU/mL) and insulin AUC (-24.03 (non-AI) vs +32.73 (AI) uU/mL) compared to those women who did not take AI (N=8, Figure 4.4 A and B).

Women who were currently or had taken AIs also displayed an opposing response in C-ISI (+0.39 (non-AI) vs. -1.12 (AI) Figure 4.4 C) following exercise training and a blunted reduction in 2h insulin concentrations (-20.2 ± 15.8 vs. -2.7 ± 22.7 uU/ml, Figure 4.4 D). This result occurred despite no significant differences in exercise training volume as well as no differences in baseline and/or change in body weight, body composition or aerobic fitness between the two groups. Given the role of AIs are to reduce circulating estrogen concentrations, we then evaluated the relationship between changes to peak insulin, insulin AUC and C-ISI and changes to basal, raw and percent change in E2. We observed a significant negative relationship between the change in peak insulin concentrations and the change in E2 as a result of the exercise training (r=-0.57, p=0.04, Figure 4.4).

Discussion

The mitogenic role of insulin in carcinogenesis is well established, and it is plausible that lower insulin concentrations are one of the primary benefits of physical activity interventions on cancer risk. Several previous exercise training interventions in cancer survivors have failed to observe any significant differences in insulin concentrations, however these studies were limited in scope by their use of fasting insulin (and fasting metrics of insulin resistance) as the sole representation of insulin supply and demand. Given the large contribution of postmeal insulin to 24-hour insulin exposure and the relationship between exercise and postmeal insulin action, it is possible that a comprehensive relationship between exercise training and insulin supply and demand in breast cancer survivors has yet to be established. This goal of this study was to use the oral glucose tolerance test, a common tool for evaluating postmeal insulin supply and demand, to evaluate the impact of exercise training on postmeal insulin responses in breast cancer survivors.

Results from this study demonstrate that insulin concentrations two hours after a glucose challenge are significantly lower following exercise training, despite no significant change in fasting insulin concentrations. This result partially supports our hypothesis and suggests that the relationship between cancer risk/prognosis, insulin and/or exercise training cannot be fully determined through the use of fasting insulin concentrations alone. While insulin may contribute to carcinogenesis, the lack of association between fasting insulin and more established cancer biomarkers (e.g. estrogen) and minimal or absent reductions in insulin following exercise have led to a general consensus that insulin is a minimal contributor to the improved prognosis of cancer survivors following lifestyle interventions (McTiernan et al. 2010). The results of this study suggest that interventions that alter postmeal insulin concentrations (e.g. physical activity) may have a greater impact on cancer risk reduction than previously estimated. Additionally, comparative efficacy studies on cancer risk/prognosis (e.g. exercise training vs. metformin) may be underestimating the potential benefits of exercise training by selecting a primary outcome (i.e. fasting insulin) that fails to capture the full extent of the benefits of exercise training on insulin supply and demand.

In addition to lower postmeal insulin concentrations, there were several other areas of improved cardiometabolic health following this exercise training program that may influence prognosis in cancer survivors. Jones et al. have suggested that low aerobic fitness following cancer treatment contributes to the elevated risk of cardiovascular disease observed in cancer survivors, and increasing cardiorespiratory fitness through exercise training may significantly reduce all-cause mortality in the years following a cancer diagnosis (Jones et al. 2015). Our personalized and progressive exercise program was sufficient to significantly increase cardiorespiratory fitness, while still providing the flexibility inherent in recreational and non-laboratory based exercise programs. It is possible that by increasing and/or varying the exercise training volume and intensity during the intervention, participants experienced greater improvements in fitness and enjoyment than they would have in an intervention based on fixed volume and/or intensity. Future studies that combine mixed doses (volume, intensity, duration and mode) of exercise in ways that mimic real-world exercise accrual are needed in order to establish the most effective dose for both cancer prevention and the litany of detrimental health outcomes associated with cancer treatment.

In addition to increases in cardiorespiratory fitness, we also observed significant decreases in circulating leptin and estrogen concentrations following exercise training. While many of the health risks associated with leptin are linked to obesity, leptin also contributes to cancer risk by enhancing adipocyte-derived estrogen activity (Vona-Davis & Rose 2007) and increasing cancer cell motility and invasiveness (Ando et al. 2014). We did not observe any significant associations between the changes in leptin and body composition or fitness. This was surprising, as lower leptin concentrations after exercise training are often the result of exercise-induced reductions in body fat (Friedenreich et al. 2011, Abbenhardt et al. 2013). However, high intensity exercise training can reduce circulating leptin concentrations independent of weight loss in adults with type 2 diabetes (Balducci et al. 2010), and it is possible that the relatively high intensity of the exercise training in the current study (85% HRmax) was enough to induce a similar beneficial leptin response. Given the notorious difficulty of initiating and sustaining weight loss in older individuals (Kassier et al. 1998), identifying potential ways interventions can reduce leptin concentrations without the requirement for weight loss may have important clinical applicability. We also observed a small but significant reduction in circulating estrogen concentrations, which was associated with intensity, but not volume, of exercise training. Given the extremely low concentrations of estrogen at baseline in this population as well as the confounding factor of AI use, it is difficult to determine whether the minor reduction in estrogen as a result of the exercise training intervention has clinical relevance. There is a significant relationship between elevated estrogen concentrations and increased risk of cancer development and recurrence in postmenopausal women (Zhang et al. 2013), and reductions in circulating estrogens have often been cited as a major contributor to lower cancer risk (Cummings et al. 2009). Future exercise studies in breast cancer survivors that are large enough in size to account for the low concentrations of estrogen (especially in those taking AIs) are required before the relationship between physical activity and estrogen in this population can be fully understood.

Despite significant reductions in insulin concentrations 120 minutes after oral glucose consumption, we did not observe any significant differences in insulin concentrations at 30, 60 or 90 minutes during the OGTT, nor did we observe any change in insulin sensitivity, insulin peak concentration or area under the insulin curve. This is surprising, as an increase in insulin sensitivity and reduction in insulin AUC are commonly observed following exercise training interventions in many different populations. There are several potential reasons for this outcome that are worth exploration. First, most participants in this study had normal glucose tolerance and therefore had relatively low fasting and postmeal insulin concentrations prior to beginning the exercise training program. Given the participants were normoglycemic, it is possible that the dose of exercise was not sufficient to reduce insulin resistance or insulin AUC from this already low baseline. While the significant increase in fitness in the current study suggests that the exercise dose was sufficient, we did not observe any significant associations between change in fitness and subsequent metabolic responses. We therefore cannot rule out the possibility that our exercise dose was sufficient to induce physiological improvements, but insufficient to alter glucoregulatory metabolism.

A second possibility for the null effect of the insulin action response is the potential role that endocrine therapy may play in the metabolic adaptations to exercise training. Past/present AI users had a blunted, and in some cases opposing, response to exercise training compared to those women not taking any endocrine therapy or taking tamoxifen. Given the role of AI to reduce estrogen production and concentrations in circulation, we attempted to identify any potential relationships between change in estrogen and change in postmeal insulin in both AI and non-AI participants. We observed a significant inverse relationship between change in estrogen concentrations and change in peak insulin, which suggests that low or significantly reduced estrogen concentrations may negatively impact postmeal glycemic control. This supports findings by Evans et al. that estrogen plays a role in metabolic adaptations to exercise training in postmenopausal women (Evans et al. 2001) and warrants further investigation into the role of estrogen concentrations as a mediator of changes to cardiometabolic health in breast cancer survivors following exercise.

Exercise training represents a potent tool to improve prognosis and reduce cancer recurrence in breast cancer survivors, however the mechanisms that explain this relationship are unclear. As we move towards the idea of personalized oncotherapy and precision medicine, it is important to develop a greater understanding of the relationship between exercise training and its metabolic effects in different populations. Results from this study suggest that while exercise training may have little impact on fasting insulin in breast cancer survivors, it has significant effects on postmeal insulin concentrations, albeit blunted compared to those seen in other populations. Future studies designed to link changing insulin concentrations with cancer risk and prognosis should incorporate measures of fasting and postmeal insulin whenever possible. It is also important for larger studies to identify whether breast cancer survivors have a diminished postmeal insulin response compared to non-cancer survivors. As the population of cancer survivors grows, so too does the population of cancer survivors at risk for diabetes and cardiovascular disease. Understanding the metabolic regulation of glycemic control and its response to exercise in cancer survivors is necessary in order to deliver personalized lifestyle and pharmacological interventions to this already large and growing segment of the population.

Tables

Table 4.1: Participant characteristics

N=15	**Mean ± SD**
Age *(yrs)*	59.9 ± 9.2
Years post diagnosis	4.0 ± 3.5
Stage (1-3)	1.67 ± 0.62
ER+ (%)	11 (73%)
Endocrine Therapy (%)	7 (47%)
Weight *(kg)*	75.5 ± 16.4
Body Mass Index *(kg/m²)*	27.6 ± 5.4
Body Fat *(%)*	43.1 ± 9.9
Estimated VO_{2peak} *(ml/kg/min)*	25.2 ± 5.4
Blood Pressure *(mmHg)*	124.5/75.5 ± 10.7/5.2
Fasting Blood Glucose (*mg/dL)*	102.0 ± 13.7
2 hour Blood Glucose *(mg/dL)*	120.6 ± 21.6

Data reported as mean ± SEM.

Table 4.2: Changes in fitness, body composition, health and biomarkers

	Baseline	Post-training	Change	p-value
Fitness, body composition and cardiometabolic health				
Weight (kg)	75.5 ± 16.4	74.5 ± 15.8*	-1.0	0.04
BMI (kg/m^2)	27.6 ± 5.4	26.9 ± 5.2*	-0.9	0.03
Body fat (%)	43.1 ± 9.9	42.6 ± 9.7	-0.5	0.31
WC (cm)	91.2 ± 11.9	88.6 ± 10.1*	-2.6	0.02
Android obesity (%)	48.1 ± 11.9	47.2 ± 11.1	-0.9	0.76
BMD (g/cm^2)	1.16 ± 0.11	1.16 ± 0.11	-0.0	0.94
FFM (kg)	42.8 ± 4.9	42.5 ± 4.5	-0.3	0.88
Est. VO_{2peak} (ml/kg/min)	25.2 ± 5.4	27.7 ± 5.0*	+2.5	<0.01
Fasting glucose (mg/dl)	102.0 ± 13.7	99.1 ± 11.8	-2.9	0.19
2h glucose (mg/dl)	120.6 ± 21.6	115.5 ± 20.4	-5.1	0.54
Systolic BP (mmHg)	124.5 ± 10.7	120.2 ± 13.1*	-4.3	0.03
Diastolic BP (mmHg)	75.5 ± 5.2	73.8 ± 4.9	-1.7	0.10
Cancer-relevant biomarkers				
Insulin (uU/mL)	11.4 ± 5.4	11.6 ± 5.1	+0.2	0.79
Leptin (ng/mL)	30.8 ± 19.3	23.8 ± 13.0*	-7.0	0.03
Adiponectin (ng/mL)	649.2 ± 208.1	622.8 ± 241.9	-26.4	0.63
E2 (pg/mL)	12.9 ± 6.3	10.2 ± 3.4*	-2.7	0.04
SHBG (nmol)	66.2 ± 47.4	64.4 ± 41.5	-1.8	0.70
IGF-1 (ng/mL)	31.6 ± 8.4	33.1 ± 9.8	+1.5	0.45
IGFBP3 (nmol)	70.3 ± 6.7	70.1 ± 8.2	-0.2	0.95

Data reported as mean ± SD. BMI= Body Mass Index, WC= Waist circumference, BP=Blood pressure, E2= 17b-estradiol, SHBG= Sex hormone binding globulin
*$p<0.05$

Figures

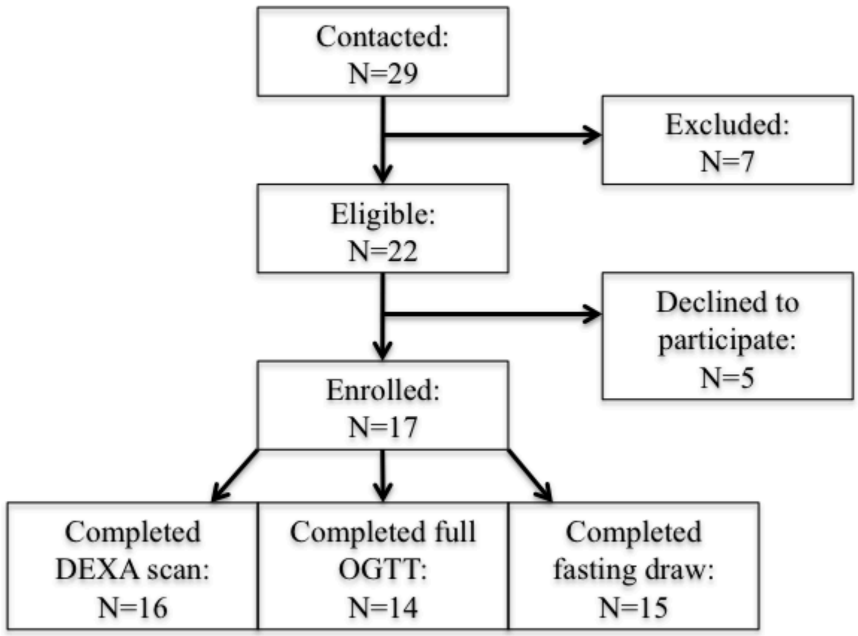

Figure 4.1: Participant enrollment and intervention completion

Data from participants who did not complete the fasting blood draw were excluded from analysis. Data from participants who completed the fasting blood draw but not the OGTT were excluded from all comparisons between intervention outcomes and OGTT-derived metrics (e.g. postmeal insulin)

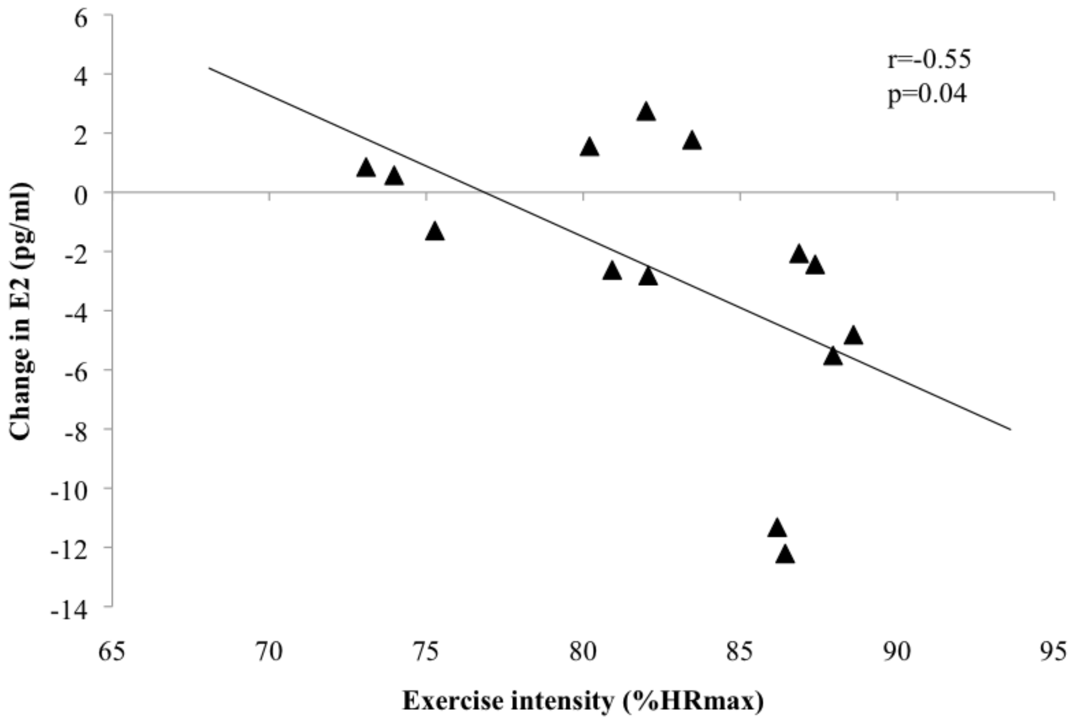

Figure 4.2: Association between estradiol and exercise intensity and change in Estradiol

There was a significant negative association between the exercise intensity (% age-predicted HR_{max}) throughout the 12 week exercise training intervention and the change in circulating 17-B estradiol (E2) concentrations.

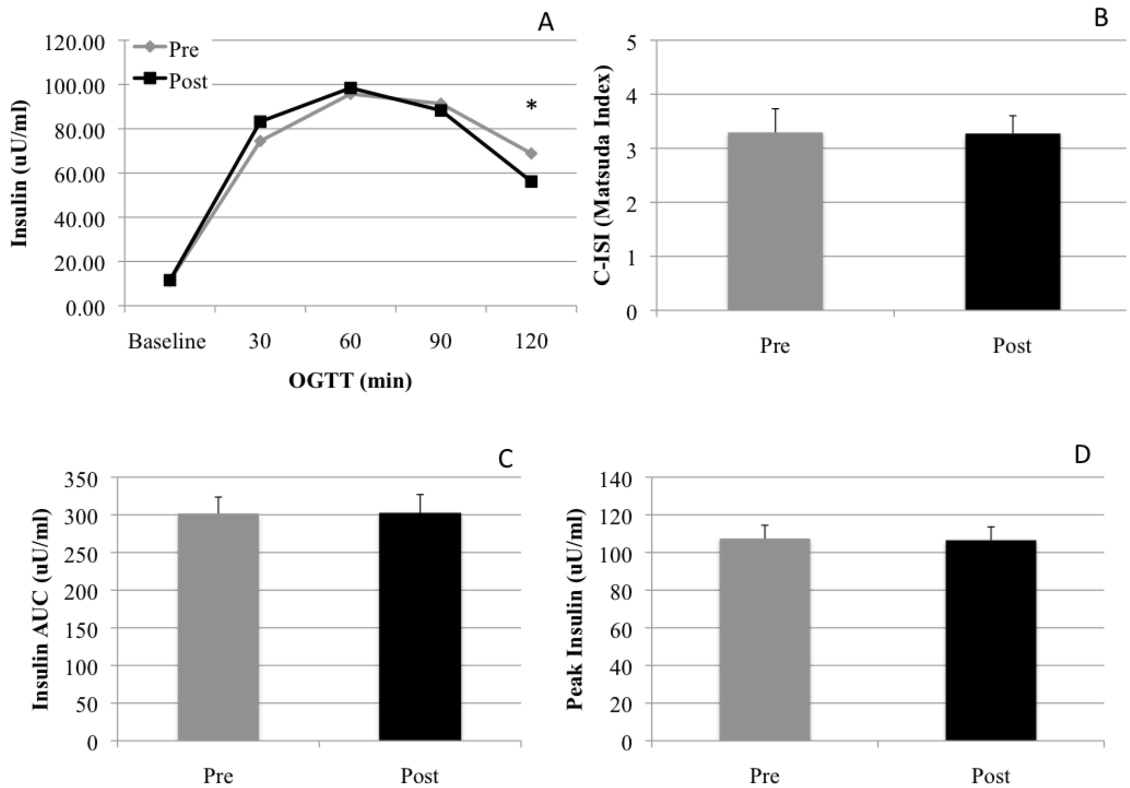

Figure 4.3: Postmeal insulin responses

(A)- Insulin concentrations at each timepoint of the glucose challenge. (B)- Composite insulin sensitivity index (C-ISI). (C) Insulin area under the curve (AUC). (D) Peak insulin concentrations. Data presented as Mean ± SEM. *$p<0.05$

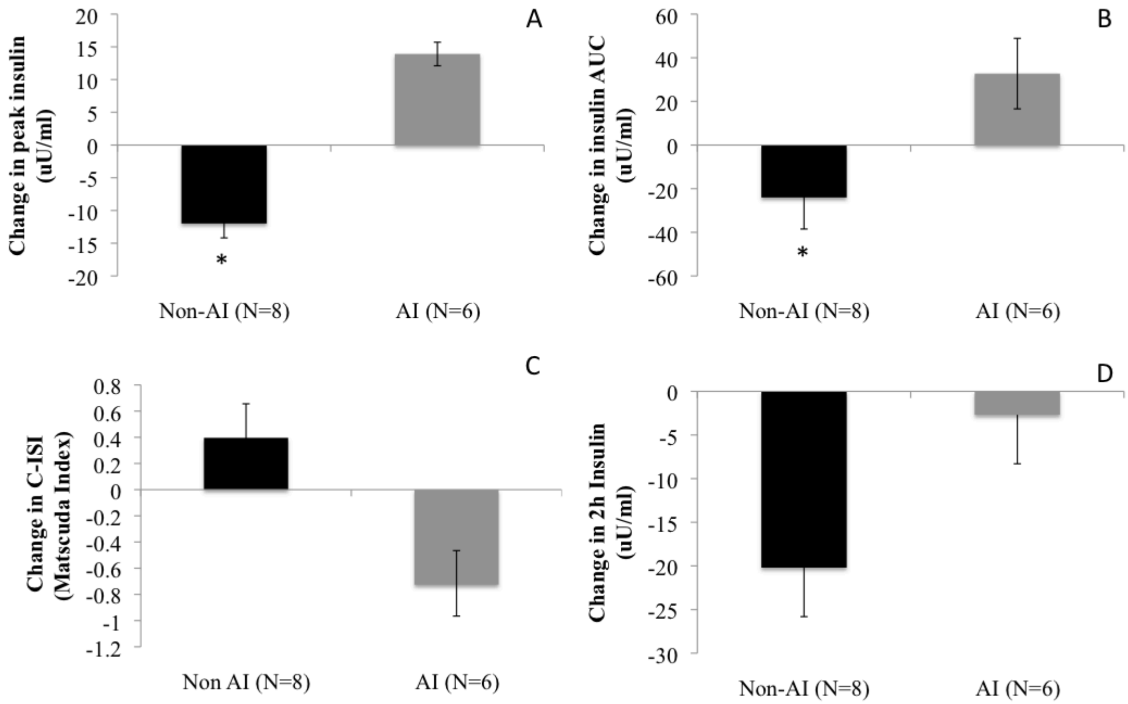

Figure 4.4: Postmeal insulin responses in women with a history of aromatase inhibitor (AI) use

(A)- Change in peak insulin concentration recorded during OGTT. (B)- Change in insulin area under the curve (AUC). (C)- Change in insulin sensitivity (C-ISI). (D)- Change in 120 min (2h) insulin concentrations. Data presented as mean ± SEM. (*) denotes significant difference between AI and Non-AI response ($p<0.05$)

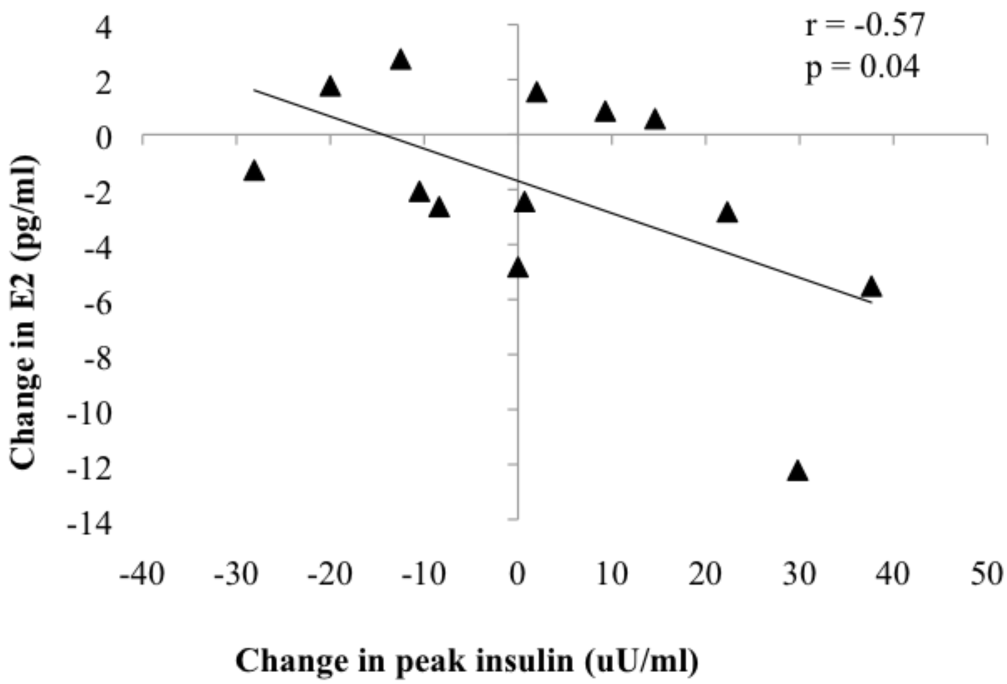

Figure 4.5: Relationship between change in estrogen and change in peak insulin

Relationship between change in 17-b estradiol and change in peak insulin following exercise training

CHAPTER V

CHANGES TO INSULIN SUPPLY AND DEMAND MAY BE BLUNTED FOLLOWING EXERCISE TRAINING IN BREAST CANCER SURVIVORS

Introduction

As the population of the United States increases in age, so too does the number of women diagnosed with breast cancer. In addition to the increase in breast cancer prevalence, better screening and treatment methods have greatly increased the survival rate and longevity of breast cancer survivors (Bray et al. 2004). Interventions designed to improve prognosis in cancer survivors primarily focus on preventing cancer recurrence, however breast cancer survivors also face higher rates of diabetes and heart disease compared to age and BMI-matched non-cancer survivors (Hooning et al. 2007). While cancer-specific mortality is the leading cause of death in the decade following a cancer diagnosis, all-cause mortality (primarily as a result of cardiometabolic disease) eventually surpasses cancer-specific mortality as the leading cause of death in cancer survivors (Bardia et al. 2012). As the number of breast cancer survivors increases, precision interventions designed to prevent cardiometabolic disease in cancer survivors may be just as important as interventions to prevent cancer recurrence.

One common physiological component that may link cancer and cardiometabolic disease is insulin resistance and the subsequent increase in insulin supply required to maintain glycemic control (Belardi et al. 2013). Insulin has anabolic and mitogenic properties, and hyperinsulinemia may enhance carcinogenesis (Gallagher et al. 2013) and contribute to poor prognosis (Irwin et al. 2011) in breast cancer survivors. Prior studies investigating the role of insulin and insulin resistance as a mediator of breast cancer risk and prognosis have primarily measured insulin in the fasted state, or used metrics of insulin resistance that are derived from fasting glucose and insulin concentrations. The mechanisms behind insulin resistance and hyperinsulinemia are complex however, and represent a delicate balance of tissue-specific insulin demand (e.g. skeletal muscle insulin sensitivity) matched by the appropriate volume of insulin supply (e.g. insulin secretion) that will maintain blood glucose concentrations within a relatively tight physiological range. Tissue-specific insulin demand changes throughout the day in response to meals, and there can be a high degree of discordance between insulin demand and supply in the fasted state compared to the fed state, both within (Faerch et al. 2008) and between (Varghese et al. 2016) different populations.

Exercise training is often recommended to cancer survivors in order to counter the detrimental effects of cancer treatment, prevent cancer recurrence and improve cardiometabolic health (Lahart et al. 2015). One of the oft-cited benefits of exercise training is reduced insulin resistance (Colberg et al. 2010), and this lower insulin demand may be beneficial for cancer survivors by reducing insulin supply and thus lowering mitogenic load. Several previous exercise training studies in breast cancer survivors have evaluated insulin resistance through the use of fasting glucose and insulin homeostasis modeling (HOMA), and found no significant changes to insulin sensitivity (Ligibel et al. 2008, Guinan et al. 2013). While HOMA scores effectively characterize insulin resistance in large populations, the failure of HOMA scores to account for changes to postmeal glycemic control makes them inadequate tools for evaluating changes to insulin supply and demand following interventions that significantly affect postmeal insulin resistance, such as exercise training (Wallace et al. 2004). Given that 50-80% of total daily insulin exposure (and thus mitogenic load) can occur in response to meals (Reaven 1979), a more comprehensive evaluation of the changes to insulin resistance and insulin secretion in breast cancer survivors may have significant implications for public health and cancer prognosis.

Compared to other populations, it is possible that chemotherapy-induced impairments to aerobic fitness and glycemic control (Jones et al. 2015), increased sedentary behavior (Sabiston et al. 2015), and the use of endocrine therapies designed to lower estrogen concentrations all contribute to fundamentally different adaptations to insulin supply and demand in breast cancer survivors following exercise training. The purpose of this study was therefore to use an oral glucose challenge to evaluate the effects of a personalized 12-week exercise training program on systemic and tissue-specific metrics of insulin resistance, insulin secretion and other aspects cardiometabolic health in postmenopausal breast cancer survivors. Additionally, exercise training can reduce fatigue and enhance quality of life (Demark-Wahnefried et al. 2015), however the relationship between these subjective measures of health and well-being and changes to glucoregulatory control are unknown. We expect that exercise training will increase insulin sensitivity, decrease insulin secretion and improve both beta cell insulin processing and function in a dose-dependent fashion, however this response may be blunted in those breast cancer survivors with low circulating estrogen concentrations. These improvements in cardiometabolic health would also be significantly associated with reductions in fatigue and increases in quality of life, suggesting a potential link between the physiological and subjective/psychological benefits of exercise training in breast cancer survivors.

Methods

NOTE: Participants in this study were the same as those in study 2 (Chapter IV), and underwent similar testing and exercise training protocol. Several aspects of the methods as well as some results (e.g. estimated VO_{2peak}, E2 concentrations) are integral to both studies and are reported in both Chapter IV and V in order to facilitate understanding and enhance continuity.

Participants and recruitment

Seventeen breast cancer survivors were recruited from the Western Massachusetts area. In order to qualify for the study participants had to be postmenopausal (or have an oophrectomy) as determined by questionnaire, and completed primary breast cancer treatment greater than 6 months but less than 10 years ago. Additionally, participants were free from any diagnosed cardiometabolic disease or medications that would interfere with carbohydrate metabolism. Participants taking anti-depressants (n=3), cholesterol- (n=1) or blood pressure lowering medications (n=2) remained on a stable dose throughout the intervention period. Participants who were prescribed aromatase inhibitors (n=6) or selective estrogen receptor modulators (n=2) were asked to remain at a similar dose and regimen throughout the duration of the study.

Baseline fitness and body composition

Prior to metabolic testing, participants reported to the Energy Metabolism Lab in the Department of Kinesiology on the University of Massachusetts campus for determination of health history, body composition and fitness. Participants filled out a baseline health history and fitness questionnaire, followed by measurement of height and weight (stadiometer and physicians scale, Detecto, Webb City, MO), blood pressure (manual sphygmomanometer, Santa medical inc., Tustin, CA) and waist circumference (tape measurer).

Baseline fitness levels were determined using a submaximal exercise test on a cycle ergomter (ACSM guidelines for exercise testing and prescription, 9th ed.). After a two minute resting period, participants were instructed to begin pedaling at an initial resistance of 25 watts, maintaining a cadence of >60 revolutions per minute. Resistance was increased in 25-watt increments every two minutes, and heart rate and rating of perceived exertion (RPE) were recorded at the end of each two minute stage. Additionally, expired gasses were continuously collected using a metabolic cart (Parvomedics, Sandy, UT) for the determination of volume of oxygen consumption (VO_2) and respiratory exchange ratio (RER). Stages were increased until participants reached 80% of age-predicted maximal heart rate (HR_{max}). Upon completion of the test, heart rate and VO_2 responses from each stage were plotted and a linear line-of-best-fit was used to estimate VO_2peak at age-predicted HR_{max}. To determine body composition, participants underwent a DEXA scan (Lunar technologies, Chicago IL) at the University of Massachusetts Health Services Center. This test was used to determine body fat percentage, bone mineral density as well as android (central) obesity and fat free mass (FFM).

Fatigue, self-efficacy and quality of life

Prior to metabolic testing, participants filled out several questionnaires to determine their current subjective levels of fatigue, general and exercise self-efficacy and quality of life (QoL). Total of fatigue, self-efficacy and QoL, as well as specific components of fatigue and QoL, were determined by evaluating the responses to the entire questionnaire as well as subscore analysis.

*Piper fatigue scale (PFS):*The piper fatigue scale is a series of 22 validated (Piper et al. 1998) questions in which a seven point likert scale is used to evaluate current fatigue levels over the span of days, weeks and months. In addition to overall fatigue, the PFS has four subscales comprised of groupings of the questions to address specific components of subjective fatigue. This includes: behavioral impact/severity of fatigue, affective meanings of fatigue, sensory, and the impact of fatigue on cognition/mood.

General Self-Efficacy: General self-efficacy was determined using a 10-question test with four point likert scale responses validated by Luszczynska et al. (2005).

Barriers to Self-Efficacy (BARSE): The barriers to self efficacy scale is a series questions validated in breast cancer survivors (Awick et al. 2016) in which a five- or seven-point likert scale is used to determine the barriers that are keeping individuals from engaging in regular physical activity.

Exercise Self-Efficacy: Exercise self-efficacy was determined using an 8-question test developed by McCauley (1993). Participants answered all questions on a 10 point likert scale and both total and single questions were used.

European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30: Health and QoL was determined using the EORTC QLQ-C30, a quality of questionnaire designed for cancer patients and survivors. This questionnaire asks a series of 28 specific cancer-related questions on weekly QoL on a 4-point likert scale, and two questions regarding overall health and QoL on a seven point likert scale.

Oral glucose tolerance test (OGTT)

Participants entered the lab following an overnight fast for assessment of fasting and postmeal glucose homeostasis. Participants were asked to refrain from physical activity and maintain habitual dietary patterns in the 24 hours prior to the glucose challenge. An indwelling catheter was placed in an antecubital vein by a trained research technician, and fasting blood samples were collected in tubes containing Sodium Fluoride (Glucose), Potassium EDTA (Insulin, C-Peptide, Proinsulin, Triglyceride and Free Fatty Acid) and Serum Separator (E2). Following the baseline blood collection, participants consumed a 75 g oral glucose test beverage (Sundex, ThermoFisher, Waltham MA), and additional blood samples were taken at 30, 60, 90 and 120 minutes following glucose consumption for determination of glucose and insulin concentrations. Blood samples were centrifuged at 3000xg, aliquoted into polypropylene cryotubes and the plasma/serum was stored at -80 degrees for future analysis.

Exercise training

All participants were required to exercise at least once a week and at most four times a week under the supervision of study personnel at the University of Massachusetts Department of Kinesiology exercise facility (The Body Shop, Amherst, MA).

Supervising members of the research team were responsible for recording components of the exercise training session (e.g. heart rate, METs, Rating of Perceived Exertion), as well as ensuring appropriate exercise intensity and safety. The exercise training protocol consisted of aerobic exercise for 45-60 minutes per session at an intensity that would elicit a heart rate between 60-80% of maximal heart rate. Specific details of the exercise training intervention are provided in the methods section of chapter IV as well as the appendix.

Hormone and metabolite analysis

Several different analytic techniques were used to determine the circulating concentrations of hormones, metabolites and biomarkers, and pre- and post-intervention samples from each participant were assayed in duplicate on the same assay in order to reduce inter- and intra-assay variability. Circulating glucose and triglyceride concentrations were determined using the glucose and triglyceride oxidase method (Analox instruments, Atlanta, GA) and an inter-assay coefficient of variability (CV) of <5%. Concentrations of insulin, C-Peptide, proinsulin and leptin were determined using a commercially available radioimmunoassay (RIA, Millipore, Billerica, MA) and an interassay CV of <10%. Concentrations of Free Fatty Acids were determined using an Enzyme linked colormetric assay (Sigma Aldrich, St. Louis MO) and Estradiol concentrations were determined using high-sensitivity enzyme-linked immunosorbent assay (ELISA, R&D Systems, Minneapolis MN) and an interassay CV of <10%.

Systemic and tissue-specific components of insulin supply and demand

Beta-cell insulin processing: Beta cell insulin processing was evaluated using pre- and post-intervention determination of fasting proinsulin as well as proinsulin area under the curve (AUC). Additionally, the ratio of Proinsulin to C-Peptide (PI/C) normalized to C-Peptide was used as a reflection of insulin processing. This ratio has been associated with diabetes risk (Halfner et al. 2008).

Skeletal muscle metabolic (glucose) clearance rate: Skeletal muscle glucose uptake was estimated using the Metabolic Glucose Clearance Rate (MCR) derived by Stumvoll et al. (Stumvoll et al. 2000). This metric estimates the degree by which insulin can induce skeletal muscle uptake, and is therefore primarily a reflection of peripheral insulin sensitivity without the confounding effects of basal insulin supply and demand. The MCR has a high correlation with the glucose disposal rate determined by the euglycemic clamp (0.80) and is derived from the equation (18.8 - (0.271 x BMI) – (0.0052 x Ins120) – (0.27 x Glu90)).

Insulin supply: Insulin supply was comprised of three specific components. First, the Insulinogenic $\text{Index}_{0\text{-}30}$ ($\text{IGI}_{0\text{-}30}$), which represents first phase insulin secretion and is determined by dividing the increase in C-Peptide over the first 30 minutes of the OGTT by the ambient glucose concentration over that same timeframe. In addition to the commonly used $\text{IGI}_{0\text{-}30}$, we also determined $\text{IGI}_{60\text{-}120}$ in a similar fashion in order to evaluate second phase insulin secretion. Finally, the percent first pass hepatic insulin extraction (HIE) was evaluated at each timepoint and over the entire OGTT through the use of insulin and C-Peptide area under the curve ($(1-\text{AUC}_I/\text{AUC}_C)*100$) and adjusted for body volume based on the calculations developed by Cobelli and colleagues (Cobelli)

Insulin demand: Whole body insulin sensitivity was estimated using the Composite insulin sensitivity index (C-ISI) developed by Matsuda and DeFronzo (Matsuda & DeFronzo 1999). This metric is a reflection of both hepatic and peripheral insulin demand, and is derived from measures in both the basal state and after a carbohydrate load. C-ISI has a high correlation (0.68) with the gold standard measure of insulin sensitivity (euglycemic clamp) and is derived from the equation (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]).

Beta-cell function/matching of supply and demand: Beta cell function was determined using the Disposition index (DI), or the product of whole body insulin sensitivity (C-ISI) and both first- (IGI_{0-30}) and second- (IGI_{60-120}) phase insulin secretion (IGI0-30). This metric is based on the hyperbolic relationship between insulin sensitivity and secretion (Kahn et al. 1992), and is typically used to identify situations where an individual risks the development of diabetes by failing to match a reduction in insulin sensitivity with a change in secretion. As a change in sensitivity should be matched with an change in secretion of the opposite magnitude, the product of insulin supply and demand should be similar regardless of the effects of an intervention, and a change in the DI represents a change in the appropriate matching of insulin supply and demand.

Statistical analysis

All statistical analyses were performed using the R statistics package and computing language and an a priori alpha of <0.05. Paired t-tests were used to evaluate pre- to post-intervention changes in fitness, body composition, fasting hormones, metabolites and biomarkers as well as pre- to post- intervention differences between single timepoints of the oral glucose tolerance test. Linear mixed models were used to evaluate the relationship between the intervention and oral glucose tolerance over the sequential timepoints of the test, as well as the effect of any mediators of the relationship between exercise training and change in glycemic control (e.g. the presence or absence of Aromatase Inhibitor). Finally, associations between biomarkers and variables of interest were determined using Pearson product-moment correlation coefficients.

Results

Participant characteristics

Fifteen participants completed the study protocol, including fitness and anthropometric testing, subjective fatigue, self efficacy, QoL, blood analysis and exercise training. Indwelling catheter failure led to an incomplete OGTT for one participant, and thus this participant was included for the exercise training and basal blood parameters but not for the metrics of insulin supply and demand. Participant characteristics are included in table 5.1.

Exercise training

Participants attended 34.4 ± 7.7 training sessions, or 2.8 sessions/wk, and averaged 156.9 ± 30.6 minutes/wk of monitored exercise over the course of the 12-week intervention period. Data from the exercise training intervention can be found in the results section of chapter IV as well as the appendix.

Fitness and body composition

Aerobic fitness significantly increased as a result of the exercise training intervention (25.2 ± 5.4 vs. 27.7 ± 5.0 ml/kg/min, $p<0.05$). This represents a percent change of +12.9 ± 3.2% over baseline. While participants experienced a significant reduction in weight (74.3 ± 15.5 vs. 73.2 ± 15.3 kg, $p<0.05$) and BMI (27.2 ± 5.2 vs. 26.8 ± 5.1 kg/m^2, $p<0.05$), this was not a result of a significant change in body fat. Fitness and body composition results are presented in Table 5.2.

Markers of cardiometabolic health

Hormones and metabolites that both reflect and regulate cardiometabolic health are included in Table 5.2. There were no significant changes in fasting glucose or glycemic responses to a glucose load. While there were no significant changes in circulating triglycerides or free fatty acids, there were significant reductions in leptin and 17-b estradiol (E2) concentrations as a result of the exercise training intervention (Table 5.2).

Metrics of insulin supply and demand

There were no significant differences in metrics of beta-cell processing (Proinsulin, PI/C), however there was a significant increase in estimated skeletal muscle glucose clearance rate (5.7 ± 1.8 vs. 7.2 ± 1.8, mmol*pmol*kg/m^2 $p<0.05$) as a result of the exercise training intervention (figure 5.1). There were no significant differences in insulin secretion/supply ($IGI_{0\text{-}30}$, $IGI_{60\text{-}120}$, HIE) or insulin sensitivity (C-ISIS) or beta cell function/matching of insulin demand and supply (DI, table 5.3).

Self-Efficacy, fatigue and quality of life questionnaires

There were no significant differences in general, exercise or barriers of self-efficacy as a result of the intervention. Subscore analysis revealed significant reductions in fatigue severity (18.5 ±13.5 vs. 11.2 ±8.8, Figure 5.2 A) as well as the impact of fatigue on cognition and mood (23.5 ± 10.1 vs. 19.0 ± 8.7, Figure 5.2 B). Additionally question 1 of the PFS, an independent question not included in subscore and total analyses regarding duration of fatigue on a six point likert scale (Months, Weeks, Days, Hours, Minutes, and Seconds) was significantly lower following exercise training (3.2 ± 2.3 vs. 1.9 ± 1.6 Figure 5.3 C). There were no significant differences in cancer-specific symptoms or fatigue as determined by the EORTC- QLQ C-30, however participants had a significant improvement in the QoL subscale (5.3 ± 1.0 vs. 5.9 ± 0.9 Figure 5.3 D) as a result of the exercise training. Neither the reductions in fatigue nor the improvements in quality of life were significantly associated with any changes in the primary physiological (e.g. estimated VO_{2peak}) or metabolic (e.g. C-ISI) outcomes.

Discussion

The goal of the current study was to investigate the effects of 12 weeks of personalized exercise training on the components of fasting and postprandial insulin secretion and sensitivity in breast cancer survivors. The exercise training program used in the current study was effective at increasing physical activity levels, which lead to a modest but significant reduction in weight and a significant increase in aerobic fitness. We also observed significant reductions in leptin and estrogen concentrations and an increase in skeletal muscle glucose clearance rate, which suggests that the exercise training in this study was of sufficient dose to induce peripheral adaptations in adipocytes and skeletal muscle. We also observed a significant reduction in fatigue and increase in quality of life. Despite improvements to fitness, fatigue, adipokine function and skeletal muscle, the intervention had a minimal effect on many of the systemic components of insulin supply and demand, such as insulin resistance and beta cell function. This result is surprising, given that an increase in skeletal muscle insulin sensitivity and compensatory reduction in insulin secretion to maintain glucose homeostasis is commonly observed in exercise training interventions (Malin et al. 2012, Kirwan & Jing, 2002).

There are several possible explanations for the lack of changes to insulin supply and demand following exercise training, which may have both scientific and clinical relevance. First, it is possible that the exercise dose applied in this current intervention was not sufficient to induce changes to liver and pancreatic function that contribute to overall regulation of insulin supply and demand. Participants in this study exercised at a relatively high intensity and volume compared to other studies within this population, and therefore the most likely contributor to a dose-dependent lack of effect may be the duration of exercise training. While unlikely, it is possible that metabolic adaptations are delayed in breast cancer survivors, and a 12-week exercise intervention that would be effective in many other populations is not of sufficient duration to induce changes to glycemic control.

A second potential explanation for the lack of observed changes to insulin secretion, insulin sensitivity and beta cell function in the current study is that baseline fasting and postmeal glucose concentrations were not impaired. Most of the OGTT-derived metrics for evaluating insulin supply and demand incorporate changes to blood glucose as a component, and it is possible that the lack of change in glucose concentrations may have obscured some of the underlying mechanisms responsible for regulating glycemia. Future studies in breast cancer survivors that include women with prediabetes and/or evaluate insulin supply and demand via gold-standard techniques (e.g.

euglycemic clamp) are needed to help address these potential confounding effects of exercise training on cancer and cardiometabolic disease prognosis in this population.

Finally, it is possible that menopause induces physiological changes that blunt or abrogate many of the metabolic adaptations to exercise training, and this effect is exacerbated in postmenopausal breast cancer survivors. Cardiometabolic health declines across the stages of the menopause (El Khoudary et al. 2012), and postmenopausal women have significantly impaired cardiovascular adaptations to both acute (Serviente et al. 2016) and chronic exercise training (Tenzi et al. 2013) compared to premenopausal controls. This cardiovascular response may be partially explained by the requirement of estrogen for exercise-induced improvements in endothelial function (Moreau et al. 2013), however the role of estrogen as a mediator/moderator of both diabetes pathophysiology and changes to glucoregulatory metabolism following exercise training is unclear. Postmenopausal women taking exogenous estrogen appear to have significantly reduced risk of diabetes and prediabetes development (van Genugten et al. 2006), and combining exogenous estrogen with exercise training appears to improve insulin sensitivity to a greater degree than exercise alone in postmenopausal women (Evans et al. 2001). It is therefore possible that the low circulating estrogen concentrations in postmenopausal breast cancer survivors, especially those on estrogen-lowering therapy (e.g. aromatase inhibitors), are directly increasing the risk of diabetes and blunting the improvements to insulin supply and demand following exercise training. While we observed a significant reduction in estrogen concentrations as a result of the exercise training, there were no significant associations between baseline or change in estrogen and any of the metrics of insulin supply and demand. Estrogen concentrations were very low (<15 pg/ml) however, and OGTT-derived glucoregulatory metrics may not be precise enough to accurately reflect these relationships.

As the number and longevity of breast cancer survivors increases, the potential role of estrogen to both increase the risk of cancer recurrence and decrease the risk of cardiometabolic disease may have wide-ranging clinical implications. Exercise training reduces the risk of both cancer recurrence and cardiometabolic disease, which may be of great importance in breast cancer survivors with high diabetes risk taking estrogen lowering medications. In order to develop personalized interventions for breast cancer survivors at risk for cardiometabolic disease, there is a pressing need to identify potential areas of overlap between cancer and cardiometabolic disease and their systematic and tissue-specific response to lifestyle interventions.

Tables

Table 5.1: Participant characteristics

N=15	**Mean ± SD**
Age *(yrs)*	59.9 ± 9.2
Years post diagnosis	4.0 ± 3.5
Stage (1-3)	1.67 ± 0.62
ER+ (%)	11 (73%)
Endocrine Therapy (%)	7 (47%)
Weight *(kg)*	75.5 ± 16.4
Body Mass Index *(kg/m^2)*	27.6 ± 5.4
Body Fat *(%)*	43.1 ± 9.9
Estimated VO_{2peak} *(ml/kg/min)*	25.2 ± 5.4
Blood Pressure *(mmHg)*	124.5/75.5 ± 10.7/5.2
Fasting Blood Glucose (*mg/dL)*	102.0 ± 13.7
2 hour Blood Glucose *(mg/dL)*	120.6 ± 21.6

Data presented as mean ± SEM

Table 5.2: Changes in fitness, body composition and cardiometabolic health

	Baseline	Post-training	% Change	Significance
Fitness and body composition				
Weight (kg)	75.5 ± 16.4	74.5 ± 15.8*	-1.4	p=0.04
BMI (kg/m^2)	27.6 ± 5.4	26.9 ± 5.2*	-2.3	p=0.03
Body fat (%)	43.1 ± 9.9	42.6 ± 9.7	-1.3	NS
WC (cm)	91.2 ± 11.9	88.6 ± 10.1*	-2.0	p=0.02
Est. VO_{2peak} (ml/kg/min)	25.2 ± 5.4	27.7 ± 5.0*	+12.9	p<0.01
Cardiometabolic health				
Fasting glucose (mg/dl)	102.0 ± 13.7	99.1 ± 11.8	-2.8	NS
2h glucose (mg/dl)	120.6 ± 21.6	115.5 ± 20.4	-4.2	NS
Glucose AUC (mg/dl)	599.2 ± 134.5	569.0 ± 76.8	-7.9	NS
Leptin (ng/mL)	30.8 ± 19.3	23.8 ± 13.0*	-22.7	p=0.03
E2 (pg/mL)	12.9 ± 6.3	10.2 ± 3.4*	-20.9	p=0.04
Free Fatty Acid (mmol)	0.92 ± 0.37	0.77 ± 0.40	-16.5	NS
Triglycerides (mg/dl)	105.4 ± 34.9	98.5 ± 29.8	-6.6	NS
Systolic BP (mmHg)	124.5 ± 10.7	120.2 ± 13.1*	-3.6	p=0.03
Diastolic BP (mmHg)	75.5 ± 5.2	73.8 ± 4.9	-3.0	NS

BMI= Body Mass Index, WC=Waist Circumference, BMD=Bone Mineral Density, FFM=Fat Free Mass, E2= 17b Estradiol, BP=Blood Pressure, Data presented as Mean ± SD (*) p<0.05

Table 5.3: Changes in metrics of glycemic control

	Baseline	Post-Intervention	Change	p-value
Fasting PI (pmol)	11.3 ± 4.6	10.7 ± 3.6	-0.6	0.4
PI AUC (pmol)	221.1 ± 93.8	210.6 ± 25.7	-10.5	0.4
PI/C	2.2 ± 1.1	2.0 ± 0.9	-0.2	0.3
$IGI_{0\text{-}30}$	0.22 ± 0.4	0.21 ± 0.3	-0.1	0.9
$IGI_{60\text{-}120}$	0.37 ± 0.6	0.35 ± 0.7	-0.2	0.8
HIE (%)	19.9 ± 4.1	20.3 ± 5.6	+0.4	0.7
C-ISI	3.3 ± 1.8	3.4 ± 1.3	+0.1	0.8
$DI_{0\text{-}30 \text{ x C-ISI}}$	0.73 ± 0.17	0.71 ± 0.15	-0.01	0.7
$DI_{60\text{-}120 \text{ x C-ISI}}$	1.16 ± 0.24	1.19 ± 0.27	+0.03	0.6

PI=Proinsulin, AUC=Area under the curve, IGI=Insulinogenic Index, HIE=Hepatic Insulin Extraction, DI=Disposition Index Data presented as Mean ± SD

Figures

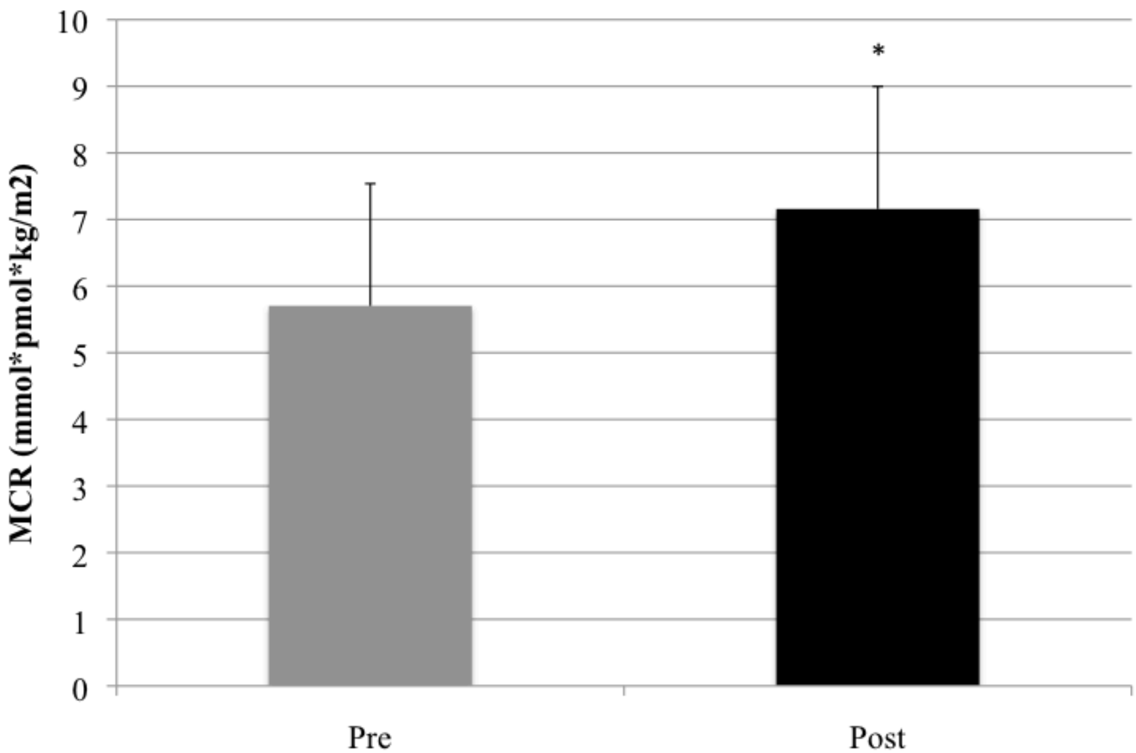

Figure 5.1: Metabolic glucose clearance rate (MCR)

Skeletal glucose clearance rate based on five sample oral glucose tolerance test from the equation established by Stumvoll et al. Data presented as Mean ± SD (*) $p<0.05$

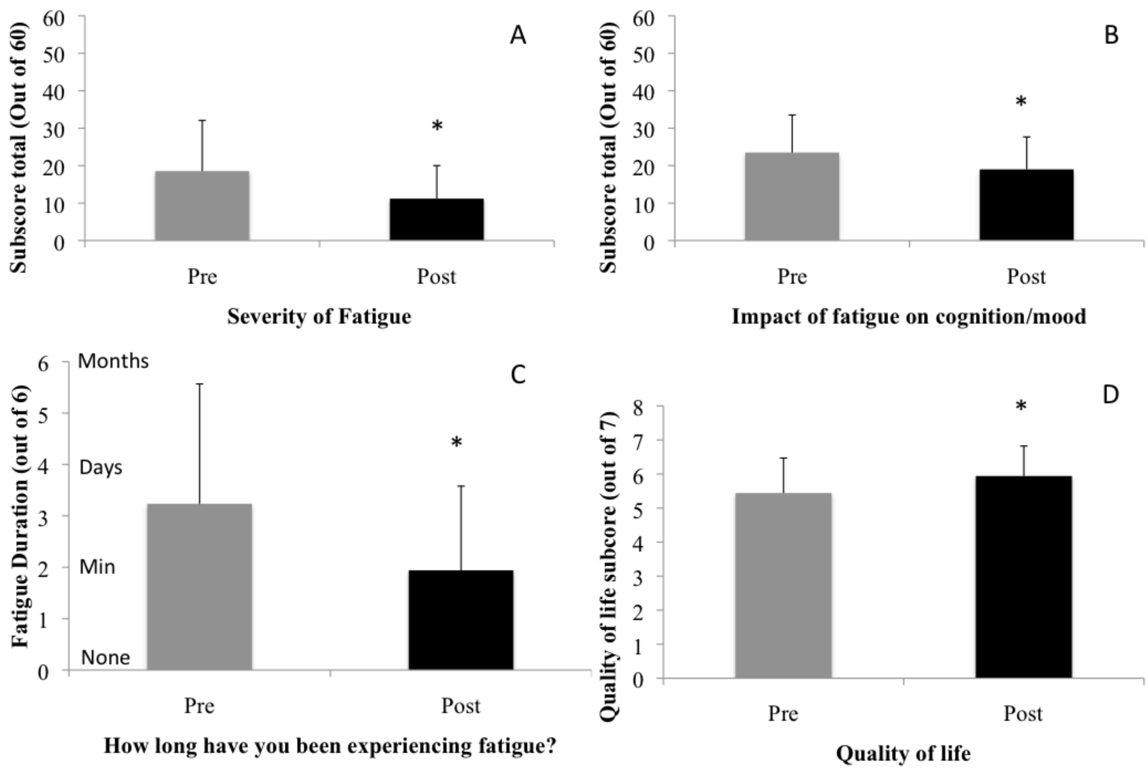

Figure 5.2: Subjective fatigue and quality of life

Subjective fatigue (Piper Fatigue Scale) and quality of life (EORTC QLQ-C30) determined by subscale analysis. (A)- Fatigue severity subscale, (B) Subjective assessment of fatigue on cognition/mood, (C) Fatigue Duration and (D) overall health and quality of life. Data presented as mean ± SD. (*) $p<0.05$

CHAPTER VI

SUMMARY AND CONCLUSIONS

The incidence and prevalence of both cancer and type 2 diabetes (T2D) are increasing at alarming rates in the United States as well as other developed countries.

Physical activity decreases the risk of developing both diabetes and cancer (Kahn et al. 1993, Moore et al. 2016) and improves prognosis for those individuals who have already received a diagnosis (Ballard-Barbash et al. 2012), however the exact mechanisms are still unclear. While the changes to insulin supply (i.e. insulin secretion) and demand (i.e. sensitivity) following lifestyle interventions or pharmacology in adults at risk for diabetes and cancer have been investigated independently, relatively little is understood regarding the effects of combined exercise training and pharmacology interventions. A recent study from our lab suggested that adding metformin (a common anti-diabetes medication) to exercise training did not enhance, and may have even blunted, the improvements to insulin sensitivity derived from exercise training alone (Malin et al. 2012). While insulin sensitivity and secretion represent key contributors to glycemic control, several other factors that lie outside this systemic closed loop may play a role in the regulation of diabetes risk. These tissue-specific responses may be more responsive to medication than to exercise, and while the systemic response to exercise training and metformin may be non-additive, that may not be the case within specific tissues. The purpose of study one was therefore to investigate the independent and combined effects of exercise training and metformin on aspects of insulin supply and demand that exist outside the typical insulin supply and demand relationship.

While this study was underway the consortium for the Transdisciplinary Research into the Energetics of Cancer (TREC) began a study that also investigated the effects of exercise training and/or metformin (Patterson et al. 2011). However instead of adults at risk for diabetes, this project was evaluating the impact of exercise training and/or metformin on cancer risk in a group of breast cancer survivors, in a four-arm approach similar to study one. While this study will likely contribute significantly towards the understanding of combined interventions on cancer recurrence, it may be limited by the use of fasting insulin as the primary measure of insulin supply and demand. Given that postmeal insulin exposure can be 50-80% of daily insulin exposure (Reaven, 1979) and exercise training primarily reduces postmeal (not fasting) insulin concentrations in non-diabetic adults (Jenkins & Hagberg, 2011), evaluating an exercise training intervention on the responses of fasting insulin alone may significantly underestimate the role of exercise-induced reductions in insulin. The purpose of study two was to evaluate the effects of exercise training on postmeal insulin concentrations in breast cancer survivors, and determine if there was any relationship between the changes in postmeal insulin concentrations and cancer-relevant biomarkers.

Finally, several aspects of breast cancer treatment may influence the mechanisms behind fasting and postmeal insulin concentrations, such as insulin sensitivity, secretion and beta cell function. As the number and longevity of breast cancer survivors increases, more and more postmenopausal breast cancer survivors are at risk for cardiometabolic disease. While significant contributions have been made to the understanding of exercise induced cardioprevention and recovery of cardiovascular function in cancer survivors following treatment, much less is understood regarding the recovery of metabolic function, such as insulin supply and demand. The purpose of study three was therefore to evaluate the systemic and tissue-specific aspects of insulin supply and demand in breast cancer survivors, and evaluate their response to exercise training.

Summary of study 1

Study 1 used a combination of a fasting blood draw and hyperinsulinemic-euglycemic clamp to evaluate several metrics of insulin supply and demand that lie outside the closed loop of insulin secretion and sensitivity prior to and following an exercise training and/or metformin intervention in adults with prediabetes. The three specific outcomes were proinsulin processing, hepatic first pass insulin extraction, and insulin clearance. Proinsulin concentrations are a reflection of how hard the beta cells of the pancreas are working to produce insulin. We found that circulating proinsulin concentrations were significantly reduced following combined exercise training and metformin intervention, but were not different in exercise alone or placebo. Metformin alone led to a decrease in proinsulin of approximately the same magnitude, however this did not reach significance. We found no influence of any treatment on hepatic insulin extraction, however there was a significant increase in the rate of insulin clearance during the hyperinsulinemic euglycemic clamp in both metformin groups (metformin alone and exercise plus metformin).

Taken together, these results suggest that while exercise training may have wide-ranging systemic effects on insulin supply and demand, metformin may work in a tissue-specific manner to regulate glycemic control. This result has significant implications for researchers designing comparative efficacy studies as well as implications for clinicians attempting to personalize lifestyle and pharmacological interventions to adults at risk for diabetes development. For example, it is possible that individuals with poor insulin production and/or clearance but normal insulin sensitivity would derive more benefit from metformin than they would from exercise training, whereas those adults with insulin resistance would not only respond more favorably to exercise training than metformin, but should possibly avoid the attenuating effects of metformin on exercise adaptations should they begin an exercise training program while on metformin.

Summary of studies 2 and 3

Studies 2 and 3 investigated the impact of 12- weeks of personalized aerobic exercise training on postmeal insulin concentrations (Study 2) and metrics of insulin supply and demand (study 3) in postmenopausal breast cancer survivors. These two studies were designed to be viewed in conjunction with each other, with study two focused on the role of exercise-induced changes to insulin concentrations and their relationship with cancer risk and recurrence, while study 3 was focused on the cardiometabolic health of postmenopausal breast cancer survivors and the mechanisms by which cancer treatment may influence diabetes risk through the mechanisms that regulate insulin supply and demand. Participants significantly increased their physical activity levels as a result of the exercise training intervention, attending an average of 34 sessions over the 12 week period and engaging in 156 minutes/wk of supervised exercise. The progressive nature of the exercise program was adhered to by the majority of the participants, and both volume and intensity of exercise increased over the 12 week period as participants fitness improved. Aerobic fitness significantly increased and weight significantly decreased as a result of the exercise training program, suggesting that the dose of exercise was sufficient to induce beneficial physiological adaptations.

Additionally, participants had reduced levels of fatigue and improved quality of life. The exercise training program could therefore be considered successful for the participants based on those metrics alone, as quality of life and aerobic fitness represent areas where cancer treatment has large detrimental effects.

While the exercise training intervention led to significant reductions in several cancer biomarkers (e.g. leptin, 17b-estradiol) and a reduction in insulin concentrations 2 hours following the administration of an oral glucose challenge, exercise training did not lower overall postmeal insulin concentrations or metrics of insulin supply and demand. This result was surprising as a reduction in postmeal insulin concentrations and increase in insulin sensitivity is often observed in exercise training interventions. A secondary analysis of contributing factors to this lack of effect of exercise training suggests that there was a significantly opposing effect of past/present aromatase inhibitor use on metabolic adaptations to exercise training. Given the primary role of aromatase inhibitors to reduce circulating estrogen concentrations in postmenopausal women, it is likely that estrogen plays a role in the metabolic adaptations to exercise training in breast cancer survivors. We observed a significant negative association between the change in peak estrogen concentrations and change in peak insulin concentrations following exercise training in women with past/present AI use, but not in those women who were not taking or who had never taken AIs. Additionally, the change in insulin sensitivity (C-ISI) in women who were not taking aromatase inhibitors was negatively correlated with percent change in estrogen concentrations, but this was not the case for women taking AIs. While this data presents a somewhat conflicting view of the role of estrogen in metabolic adaptations to exercise training, it is clear that estrogen concentrations, and by proxy the use of estrogen lowering medications, may play a significant role in both cancer and cardiometabolic disease risk in breast cancer survivors.

Conclusion

When taken together, these studies appear to shed some light on the nature of exercise training as a means to modify two hormones that play opposing roles in the pathophysiology of diabetes and cancer. While insulin and estrogen contribute to better blood glucose control, the mitogenic properties inherent in both hormones likely make them detrimental towards the prevention of cancer recurrence. Results from this dissertation suggest that exercise training can reduce circulating concentrations of insulin, however this is specific to the postmeal state. This response appears to be blunted in individuals with a past or present history of aromatase inhibitor use. We also observed a significant reduction in estrogen as a result of exercise, however the clinical implications are unclear based on the relatively low levels at baseline. Exercise training may therefore represent an option for managing post-treatment concentrations of hormones that have a significant influence on cancer risk as well as diabetes development. This may go a long way towards the idea of precision medicine, where the use of exercise, diet, and pharmacolology (e.g. metformin) would be optimally integrated and combined with the appropriate dose and duration of endocrine therapy in order to maximally reduce the risk of cancer recurrence with minimal impact on diabetes risk. While this dissertation sheds some light on the potential mechanisms behind the relationship between cancer recurrence and diabetes risk following exercise training, it raises far more questions than it answers. Future exercise training studies in postmenopausal breast cancer survivors that focus on populations at high risk for diabetes, use gold-standard measures of insulin supply and demand and are designed to directly evaluate the role of estrogen and/or aromatase inhibitor use are required before the dream of personalized precision post-treatment oncotherapy can become a reality.

APPENDIX

INDIVIDUAL RESPONSES EXERCISE TRAINING IN BREAST CANCER SURVIVORS

One of the strengths of studies 2 and 3 is the personal supervision of all exercise training sessions. Because data was continuously collected throughout the exercise training intevention on each participant, we are able to examine the progression of fitness and evaluate whether these individual responses may have contributed to the overall changes observed in fitness, body composition and cardiometabolic health. Presented below are individual changes to exercise volume (Figure A1) and intensity (Figure A2) broken into weeks 1-4, 5-8 and 9-12, along with the total number of sessions (Figure A3) and the number of high intensity interval sessions (Figure A4).

Figures

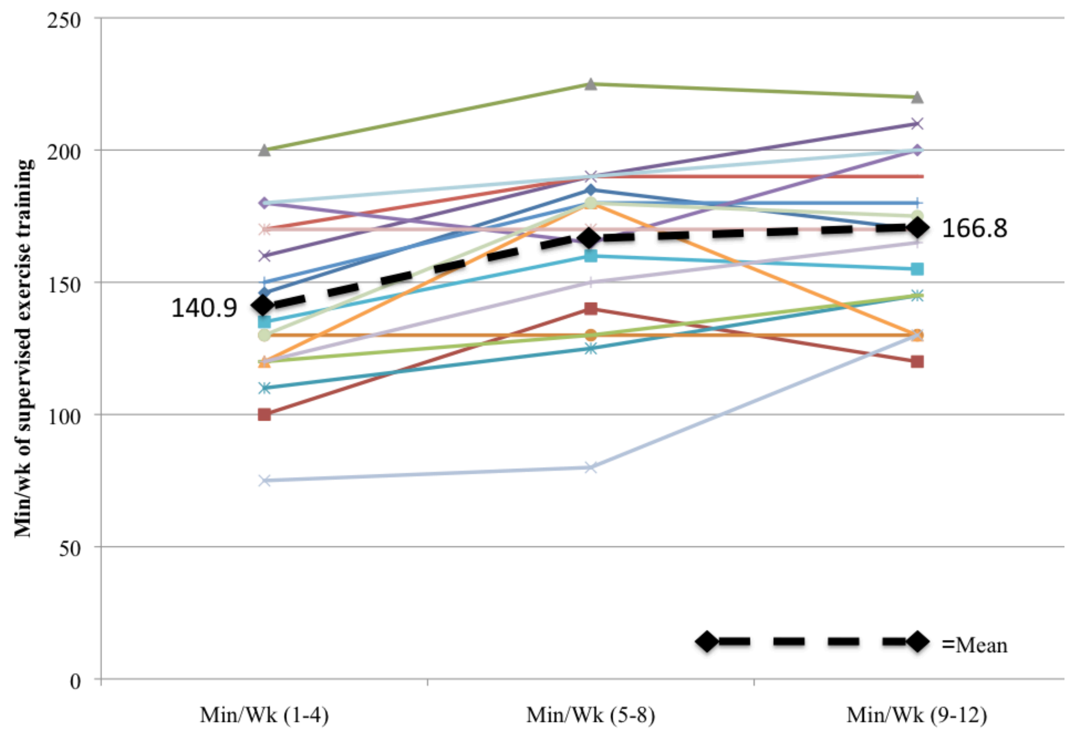

Figure A1: Exercise training volume

Individual changes to supervised exercise volume over the course of the 12 week intervention period.

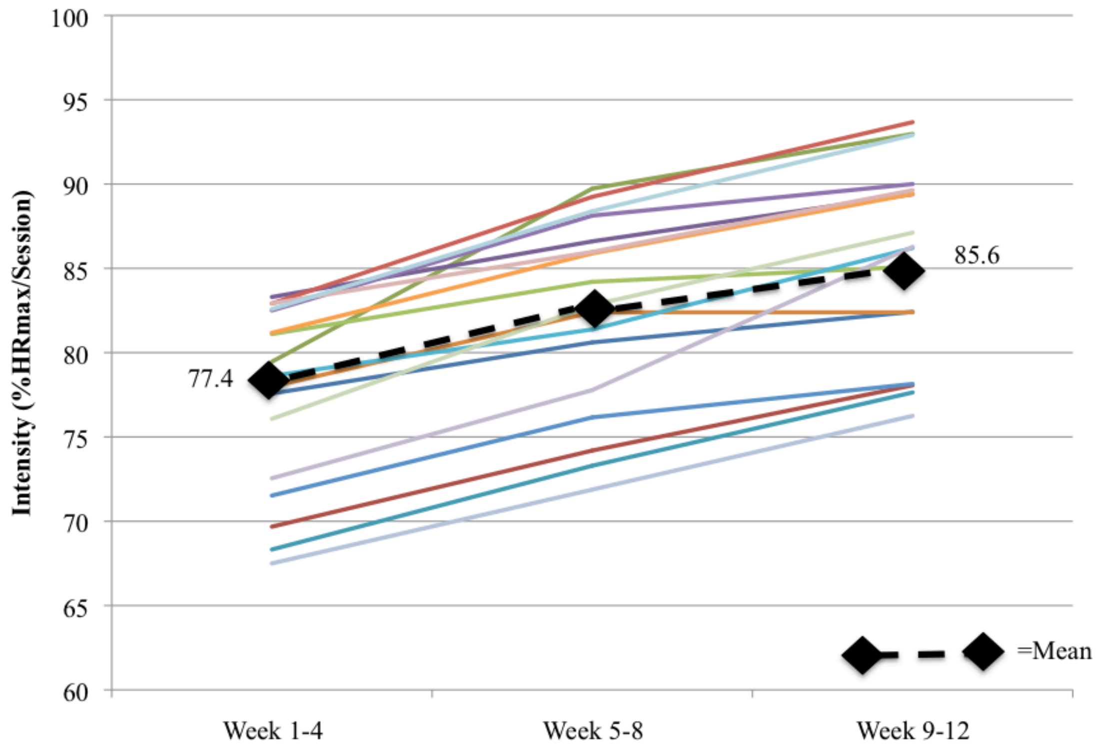

Figure A2: Exercise training intensity

Individual changes to supervised exercise training intensity, reported as percent of age-predicted maximum heart rate (%HRmax) per session during the exercise training intervention

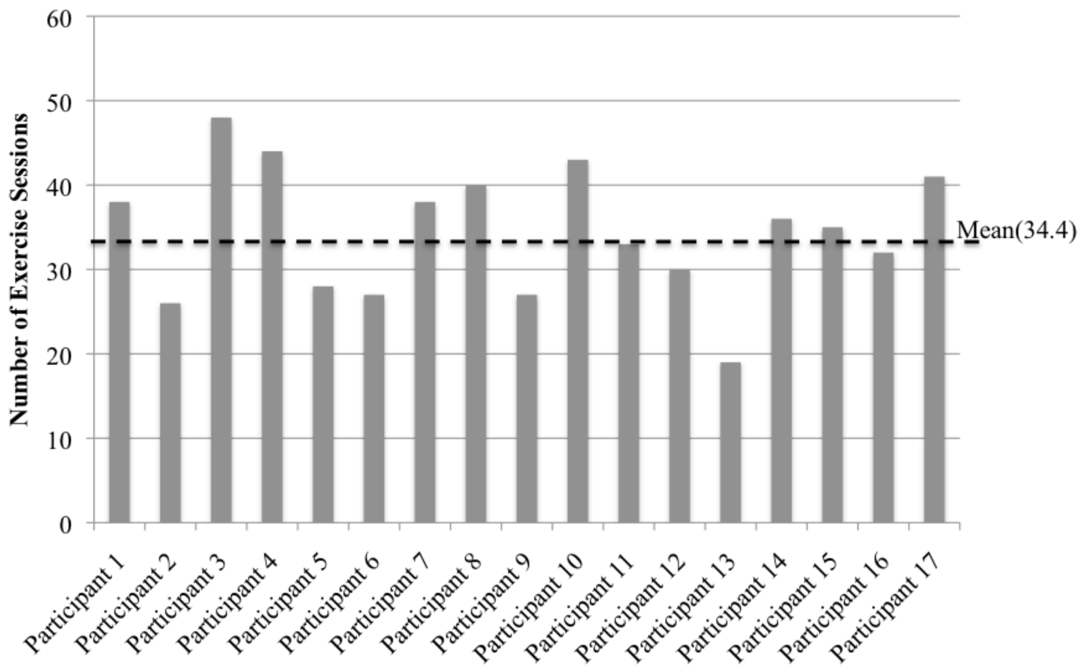

Figure A3: Exercise sessions

Total number of exercise sessions over the 12-week intervention period for each participant enrolled in the study.

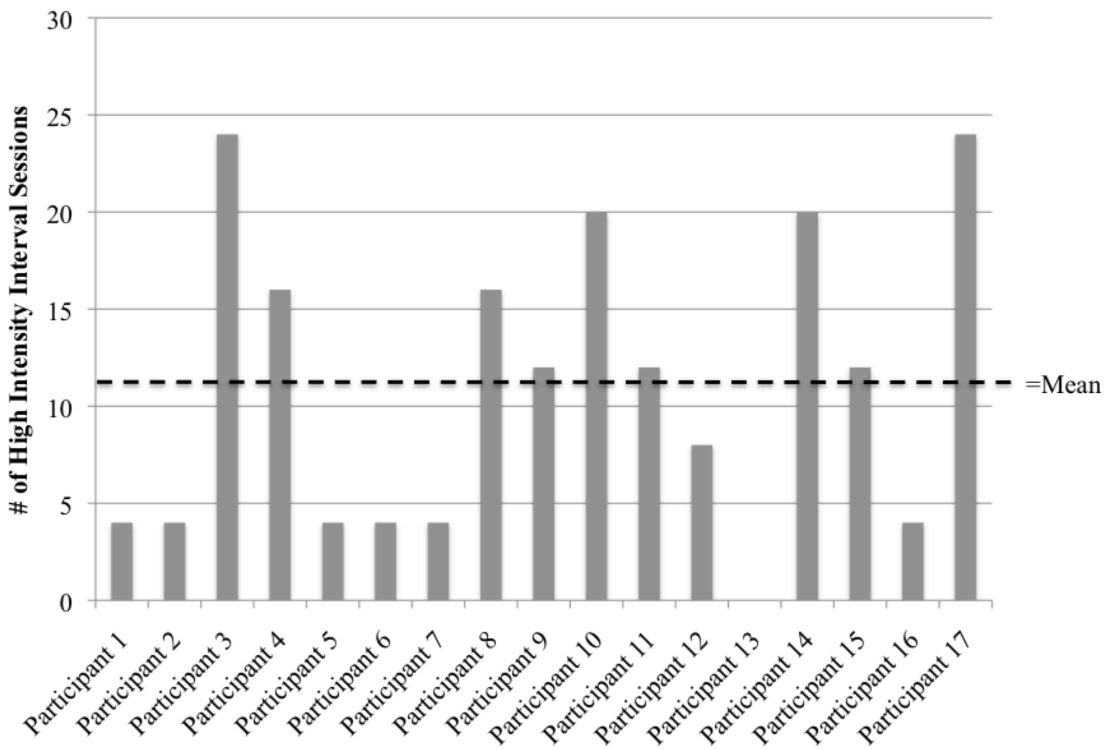

Figure A4: High intensity interval sessions

Total number of high intensity interval training (HIIT) sessions by each participant over the course of the 12-week intervention period

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