

Evaluation of glucose metabolism in breast cancer patients following a combined aerobic
and resistance exercise intervention

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

BACKGROUND: Breast cancer patients typically present with impaired glucose metabolism at diagnosis. Given that increased fat mass and decreased lean mass develop throughout the treatment trajectory and into survivorship, these impairments in glucose metabolism may worsen and may increase the risk of breast cancer patients developing secondary diseases such as diabetes in survivorship. Aerobic and resistance exercise training are known to improve glucose regulation in various clinical populations, but this has not been comprehensively explored in breast cancer patients.

OBJECTIVES AND HYPOTHESES: The primary objectives of this study were to: 1) examine the differences in glucose and related markers, as well as potential explanatory measures such as body composition, inflammatory markers, aerobic capacity, muscular strength, and dietary intake between a heterogeneous group of breast cancer patients and a group of young healthy females, and 2) evaluate the potential changes in these collective measures in breast cancer participants following 24 one hour sessions of a combined cardiovascular and strength training program. Secondary objectives were to: 1) examine potential effects of the time to complete 24 one hour sessions on metabolic parameters and 2) evaluate baseline measures of the breast cancer group that dropped out to those that completed the exercise program to identify potential bias in the group that completed the exercise program. We hypothesized that breast cancer participants, prior to exercise training, would present with poorer glucose concentrations and related markers, body composition, inflammatory markers, aerobic capacity, muscular strength, and dietary intake in comparison to the healthy young reference group. We also hypothesized that all measures would improve with the exercise program and that dietary intake would

remain the same. Additionally, we anticipated that patients who completed the 24 exercise sessions in a shorter time-frame would exhibit greater improvements in glucose-related parameters compared with those who took longer to complete the program. Baseline measures between breast cancer exercise completers were expected to be similar to those that dropped out of the program.

METHODS: Breast cancer patients, at any stage of disease, any phase of treatment and any treatment time, were recruited to this study. Participants underwent a supervised exercise program consisting of 24 1-hour sessions of combined aerobic and resistance exercise (usually aerobic training to start followed by resistance training). Attendance of two exercise sessions per week for one hour was prescribed. Assessments were done at baseline and following the exercise program for the breast cancer patients. Baseline assessments from the patients were compared with similar measures collected, at a single time point, in the young healthy female reference group. We evaluated glucose and related markers by biochemical analysis on fasting blood. Body composition was assessed by dual energy x-ray absorptiometry (DXA) scans, body mass index (BMI) and waist circumference (WC). Cardiovascular capacity in breast cancer patients was measured by a graded exercise test and strength was assessed using a predictive one repetition maximum (1RM) test on the upper and lower limbs. Dietary intake was assessed using 3-day food diaries.

RESULTS: Prior to exercise training, breast cancer patients presented with higher measures of fasting plasma glucose, glycated hemoglobin, IGF-1, insulin and a tendency for higher c-peptide when compared to the young healthy reference group; however, there were no differences in inflammatory markers between groups. Furthermore,

percentage of fat, total fat mass, BMI and WC were elevated in breast cancer patients compared with the reference group. Aerobic exercise tests, resting heart rate and muscular strength demonstrated poorer results relative to the reference group. Daily caloric intake was higher in the reference group but macronutrient distribution was not significantly different. Following 24 sessions of combined aerobic and resistance training, there were significant reductions in fasting plasma glucose (5.4 ± 0.7 vs. 5.0 ± 0.4 mM, $p=0.008$) and IGF-1:IGFBP-3 (0.33 ± 0.12 vs 0.27 ± 0.09 ng/mL, $p= 0.018$). Homeostatic model assessment for insulin resistance (HOMA-IR) (2.9 ± 2.4 vs. 2.1 ± 1.7 ng/mL, $p= 0.06$) tended to improve. IGF-1 and lactate tended to decrease following the exercise program as well. Inflammatory markers primarily remained unchanged but a significant increase in TNF- α as well as a tendency for increased IL-1 β were observed. Increasing delta TNF- α (from baseline to post-exercise program) was correlated with increasing delta of total fat mass ($r= 0.908$, $p=0.0124$, $n= 6$). Cardiovascular capacity, resting blood pressure as well as lower limb muscular strength improved following the exercise program. There were no changes in dietary intake during the exercise program. No differences were identified between the breast cancer dropout group and the breast cancer group that completed the exercise program.

DISCUSSION AND CONCLUSIONS: Breast cancer patients presented with various metabolic, body composition, and fitness parameters that were poorer than the young healthy reference group. The exercise intervention was associated with improved plasma glucose, HOMA-IR and IGF-1:IGFBP-3, suggesting improvements in glucose metabolism. Future work needs to further evaluate these improvements and compare them with a usual care group that is undergoing treatment but no exercise intervention.

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List of Abbreviations

1RM	One repetition maximum
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CRP	C-reactive protein
CT	Computed tomography
DXA	Dual energy x-ray absorptiometry
GLUT1	Glucose transporter type 1
GLUT4	Glucose transporter type 4
HbA1c	Glycated hemoglobin
HOMA-IR	Homeostatic model assessment of insulin resistance
IDF	International Diabetes Federation
IGF-1	Insulin-like growth factor- 1
IGFBP-3	Insulin-like growth factor binding protein- 3
IL-1 β	Interleukin- 1 β
IL-4	Interleukin- 4
IL-6	Interleukin- 6
IL-8	Interleukin- 8
IL-10	Interleukin- 10
mRNA	messenger ribonucleic acid
OGTT	Oral glucose tolerance test
RPE	Rate of perceived exertion
rpm	Revolutions per minute
SD	Standard deviation
TNF- α	Tumour necrosis factor- α

WC Waist circumference

1.0 Overview

Although breast cancer is the most common type of cancer, the success of diagnostic tools and treatment regimens have led to an impressive five-year survival rate of 88% (Canadian Cancer Society, 2015). Despite these advances in detection and treatment, knowledge about metabolic changes (i.e. development of glucose intolerance, insulin resistance) that accompany patients throughout the treatment trajectory is limited. Though literature to date primarily focuses on insulin resistance and risk of diabetes development in breast cancer survivors (Bordeleau et al., 2011; Ligibel et al., 2008; Lipscombe et al., 2013), signs of insulin resistance and impairments in glucose metabolism have also been shown in patients near diagnosis and during treatment (Bell et al., 2013; Can et al., 2013; Goodwin et al., 2009; Lu et al., 2014). Impairments in glucose metabolism increase the risk, not only for development of secondary diseases such as diabetes and cardiovascular disease, but also increase the risk for breast cancer recurrence and death (Erickson et al., 2011; Goodwin et al., 2002; Yerrabothala et al., 2014).

Breast cancer patients undergoing treatment often experience gains in fat mass during this period and into survivorship (Aslani et al., 1999; Campbell et al., 2007; Demark-Wahnefried et al., 2001; Demark-Wahnefried et al., 2004; Harvie et al., 2004). Obesity in non-malignant populations is related to glucose dysregulation (Amati et al., 2009). Uniquely, breast cancer patients have also shown decreases in lean mass in addition to the gain in fat mass (Cheney et al., 1997; Demark-Wahnefried et al., 2001; Demark-Wahnefried et al., 2002; Freedman et al., 2004; Harvie et al., 2004), which has important implications since skeletal muscle is responsible for ~85% of whole body glucose disposal (DeFronzo et al., 1981). Collectively, body composition features and changes in these compartments may relate to deleterious changes in glucose handling during the treatment, and overall, disease trajectory.

Several factors need to be evaluated that affect glucose handling that alter during the cancer trajectory: inflammation, diet and physical activity. Pro-inflammatory cytokines are generally elevated in breast cancer patients (Kozłowski et al., 2003; Lee et al., 2003; Pierce et al., 2009; Plomgaard et al., 2005; Rivas et al., 2008; Salgado et al., 2003), and this has been linked to tumour proliferation, metastasis, insulin resistance and decreased rates of survival (Cole, 2009; Lee et al., 2003; Pierce et al., 2009; Plomgaard et al., 2005; Rivas et al., 2008). In contrast, other studies have shown no significant increase in pro-inflammatory cytokines in breast cancer patients (Bell et al., 2013; Pusztai et al., 2004). Caloric intake has been shown to be reduced in breast cancer patients during chemotherapy (Demark-Wahnefried et al., 2001); in contrast, increased caloric intake mid-way through chemotherapy as well as 3 months following completion of treatment have also been found (Harvie et al., 2004). It is likely that changes in diet are related to the time in the disease trajectory that diet is recorded during. The methods used to record changes in diet are also likely to influence interpretations from study to study. In addition, caloric intake needs to be balanced with physical activity. Indeed, breast cancer patients with any stage of breast cancer and any type of treatment have higher sedentary behaviour post-diagnosis (Irwin et al., 2003). This decrease in physical activity has been suggested to promote loss of muscle and fat gain (the development of sarcopenic obesity) in patients (Demark-Wahnefried et al., 2001), and this may occur in absence of changes in caloric intake.

Taken together, glucose related markers, body composition, inflammation, and energy balance are interconnected and all play a role in breast cancer and the occurrence of glucose metabolism impairments (Figure 1.1 and 1.2). By targeting energy expenditure through fitness, perhaps some of the deleterious changes shown by previous studies that occur during the treatment trajectory can be avoided. To date, no studies have investigated glucose metabolism

and factors that may affect it in a comprehensive manner in breast cancer patients that are undergoing treatment.

A balanced diet and physical activity have shown to improve glucose metabolism in non-malignant populations (Eriksson et al., 1997; Schmitz et al., 2010). To date, studies investigating exercise interventions mainly focused on breast cancer survivors and aerobic exercise training (Fairey et al., 2003; Guinan et al., 2013; Thomas, et al., 2013). Fewer studies have investigated resistance exercise but those that have, found improvements in lean mass which were not observed in studies investigating aerobic exercise (Courneya et al., 2007; Schmitz et al., 2005). A combination of aerobic and resistance exercise have shown improvements in glucose clearance in non-malignant diabetic populations (Cuff et al., 2003). There is only one study that has used combined aerobic and resistance exercise in breast cancer survivors but they were able to demonstrate improvements in fasting insulin (Ligibel et al, 2008). However, the effects of combined aerobic and resistance exercise on glucose metabolism in breast cancer patients who are undergoing anti-neoplastic treatment are unknown.

This thesis examines the effects of a 24-session, mixed aerobic and resistance training program on glucose metabolism in breast cancer patients and recent survivors. Measures including body composition, diet and inflammatory mediators were examined to understand their role on potential changes in glucose metabolism with exercise training in this population. This thesis will advance our understanding of potential metabolic changes that may associate with exercise training and will provide the basis for designing future larger-scale, exercise training studies.

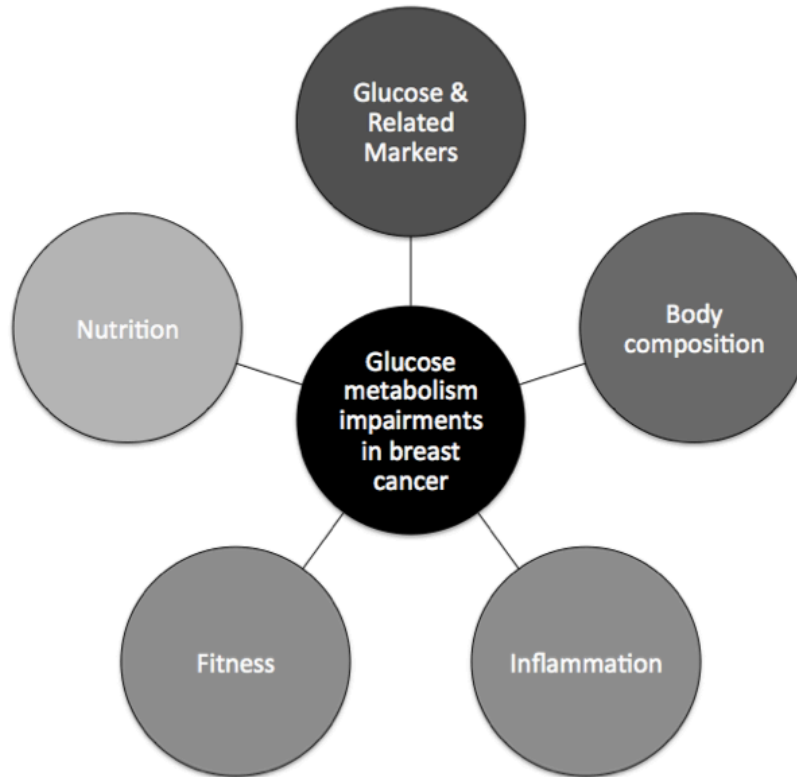


Figure 1.1 Key factors related to impairments in glucose metabolism in breast cancer. All measures in the grey circles are factors that may contribute to impairments in glucose metabolism. Glucose and related markers are used to measure impairments in glucose metabolism.

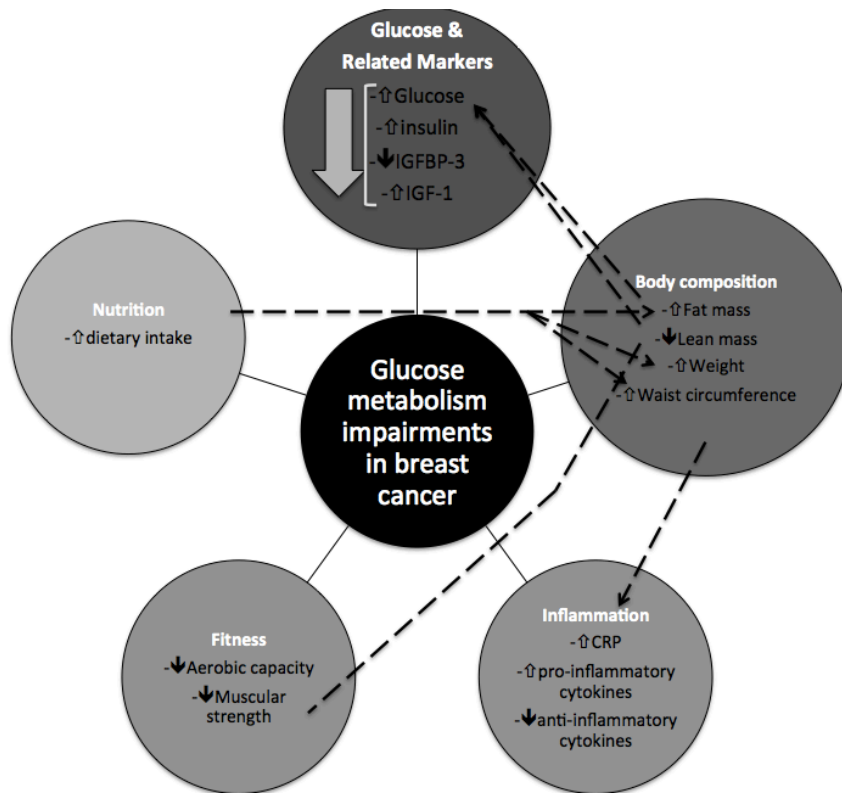


Figure 1.2 Interconnections between different factors contributing to impairments in glucose metabolism in breast cancer. The various factors that contribute to impairments in glucose metabolism are interrelated (interrelationships are indicated by the dashed lines). An increase in circulating glucose concentrations will increase insulin concentrations, which lower IGFBP-3 concentrations and thereby increase the amount of bioactive IGF-1. Changes in body composition, particularly increases in adiposity, increase various pro-inflammatory markers. Decreases in muscular strength can be related to decreases in lean mass and vice versa. Increases in caloric intake as well as dietary fat intake can lead to increases in fat mass. All of these factors can lead to impairments in glucose metabolism.

2.0 Literature Review

2.1 *Systemic glucose metabolism impairments in non-malignant populations*

One in four people in Canada are diabetics, prediabetics or have undiagnosed diabetes (Canadian Diabetes Association, 2005). Additionally, 90% of diabetics have type 2 diabetes (Canadian Diabetes Association, 2005). This type of diabetes is characterized by a decrease in glucose uptake due to insulin resistance, at tissues such as skeletal muscle, and eventually leads to elevated circulating glucose concentrations and a state of hyperglycemia (Petersen & Shulman, 2006). To compensate for the increase in circulating glucose, the pancreas releases more insulin ultimately leading to a hyperinsulinemic state (Petersen & Shulman, 2006). Eventually the pancreas can no longer counter hyperglycemia with increasing insulin, resulting in progressive beta cell function declines (Kahn, 2001) and reduced numbers of beta cells (Butler et al., 2003); with these degradative changes, the pancreas may no longer be able to produce adequate supplies of insulin (Kahn, 2001). Consequently, insulin-mediated glucose uptake is decreased, raising blood glucose levels and resulting in a state of type 2 diabetes. This may not be the only mechanism of diabetes. The pancreas could continue to produce insulin, leading to increases in circulating insulin as well as increased glucose concentrations due to impaired clearance by the muscle.

Skeletal muscle constitutes the most important part of glucose clearance seeing as it is the largest site of insulin sensitive tissue (Wasserman et al., 2011). Normally, glucose is transported via the bloodstream to the muscle. There, insulin binds to an insulin receptor on the muscle membrane, initiating a signaling cascade that leads to GLUT4 translocation to the membrane and ultimately glucose clearance from the bloodstream (Saltiel & Kahn, 2001). In an insulin resistant or diabetic state, insulin signaling is impaired and therefore, increases in circulating glucose

concentrations are seen due to the inability of glucose uptake by the muscle (Saltiel & Kahn, 2001). Another signaling cascade which activates translocation of GLUT 4 occurs through muscle contraction, thereby also allowing for muscle glucose uptake without requiring insulin signaling (Kennedy et al., 1999; Wasserman et al., 2011). Though skeletal muscle is the largest site of glucose disposal, other organs such as the liver are involved as well. Accumulation of fat in the liver has been shown to result in hepatic insulin resistance (Samuel et al., 2004). In addition to this, during an insulin resistant state, the liver is unable to properly convert glucose to glycogen during the postprandial state and increases gluconeogenesis, thereby leading to a hyperglycemic state (Savage et al., 2007). Based on this information, it can be presumed that liver insulin resistance may precede systemic insulin resistance which also affects glucose uptake at skeletal muscles.

2.2 Impairments in glucose metabolism across the breast cancer treatment trajectory

Breast cancer patients have shown impairments in glucose metabolism near diagnosis (Can et al., 2013; Goodwin et al., 2009; Lu et al., 2014), throughout treatment (Bell et al., 2013; E. M. Guinan et al., 2014) and into survivorship (Bordeleau et al., 2011; Ligibel et al., 2008; Lipscombe et al., 2013). These impairments can lead to secondary diseases such as diabetes and increased risk of cancer recurrence in survivorship (Bordeleau et al., 2011; Erickson et al., 2011; Lipscombe et al., 2013; Yerrabothala et al., 2014). Furthermore, impairments in glucose metabolism and related markers have been found to increase breast cancer recurrence and decrease survival rates (Erickson et al., 2011; Goodwin et al., 2002; Yerrabothala et al., 2014). Assessing glucose metabolism in breast cancer patients across the treatment trajectory is an

important step in preventing the development of unfavourable changes in glucose or at least managing glucose and glucose-related parameters.

There is limited research evaluating glucose metabolism in newly diagnosed breast cancer patients. Hyperinsulinemia was found in newly diagnosed breast cancer patients and correlated to insulin resistance as measured by a number of calculations including homeostatic model assessment (HOMA) (Goodwin et al., 2009). This aligns with a study that showed a pre-diabetic state in newly diagnosed patients, confirmed by an oral glucose tolerance test (OGTT), in a Chinese breast cancer population, whereby glucose handling was impaired (Lu et al., 2014). HOMA also demonstrated insulin resistance in another sample of breast cancer patients, but it was not associated with prognostic factors such as tumour size (Can et al., 2013). Conversely, this pre-diabetic state could progress into diabetes as patients endure treatment.

Impairments in glucose metabolism have been examined during treatment as well. Even at the beginning stages of chemotherapy treatment (1-2 cycles), where some would argue that treatment effects would have potentially not taken place yet, basal concentrations of c-peptide have been shown to be elevated compared with non-malignant females who were age- and body mass index (BMI)- matched as well as a young, healthy, non-malignant group of females (Bell et al., 2013). During an OGTT, these patients reached higher concentrations of glucose which, along with c-peptide and insulin, remained elevated for a greater period of time in comparison to non-malignant matched and young, healthy females (Bell et al., 2013). It can be presumed that insulin was continually released from the pancreas in an effort to clear the elevated glucose from the system; implying that glucose clearance may be impaired early in the disease trajectory, when treatment-induced metabolic changes were less likely to have occurred (Bell et al., 2013). Another cross-sectional study supported these findings by demonstrating that breast cancer

patients at diagnosis were pre-diabetic, while those who were undergoing chemotherapy treatment (before the 5th or 6th cycle) were found to be diabetic (Lu et al., 2014). Since treatment was the only difference between the two groups, it was assumed that chemotherapy was associated with this impairment in glucose metabolism. Another group performed a similar study that examined glycated hemoglobin (HbA1c), fasting insulin and insulin resistance (as demonstrated with the HOMA-IR) in breast cancer patients prior to and following adjuvant treatment (including chemotherapy and radiation treatment). With adjuvant treatment, patients showed elevated concentrations of fasting insulin, HbA1c as well as insulin resistance (Guinan et al., 2014). While there are a few studies that have examined glucose metabolism during the treatment trajectory, a large gap remains in terms of understanding the impairments in glucose handling that arise during anti-neoplastic treatment.

Even after completion of treatment, breast cancer patients have been shown to be at an increased risk of developing secondary diseases (such as cardiovascular disease and diabetes) in survivorship (Bordeleau et al., 2011; Lipscombe et al., 2013; Juanjuan et al., 2015). The onset of diabetes may vary depending on the treatment provided. With adjuvant chemotherapy, risk of diabetes has been shown to be increased within the first two years post-diagnosis (Lipscombe et al., 2013). Interestingly, mutations in Breast Cancer gene 1 and 2 (BRCA 1 and 2), which increase the risk of developing breast cancer, may also have a role in contributing to the development of diabetes. Women who test positive for gene mutations in BRCA1 or BRCA2 gene are also likely to develop diabetes within 15 years from diagnosis than those who did not develop breast cancer (relative risk, 2.0) (Bordeleau et al., 2011). This further demonstrates that breast cancer itself may potentially lead to the development of diabetes. Breast cancer survivors still present with elevated insulin levels and insulin resistance as measured by HOMA (Ligibel et

al., 2008). Hyperinsulinemia has been shown to increase risk for breast cancer recurrence as well as death in both pre- and post-menopausal women with breast cancer (Erickson et al., 2011; Goodwin et al., 2002; Yerrabothala et al., 2014). Examining potential contributors to deteriorating glucose management during treatment and beyond, including body composition, nutrition, inflammation and physical activity, is important.

2.3 Body composition as a potential contributor to glucose metabolism impairments

In non-malignant populations, obesity, often attributed to an energy imbalance with greater energy intake than output, leads to fat accumulation and eventually insulin resistance in most individuals. Obese individuals are typically less insulin sensitive than normal weight sedentary individuals who are less insulin sensitive than normal weight active individuals (Amati et al., 2009). Obese type 2 diabetics show more fatty acid transporter FAT/CD36 translocation to the cell membrane and therefore, allow more fatty acids to enter the muscle and remain in storage in the form of triglycerides (Bonen et al., 2004). This increased uptake of triglycerides may, ultimately, lead to the formation of lipotoxic intermediates (such as ceramides and diacylglycerols) (Goodpaster et al., 2000; Pan et al., 1997). These lipotoxic intermediates are believed to block the insulin signaling pathway, thereby contributing to a state of insulin resistance.

On the other hand, skeletal muscle is the main site for glucose disposal and therefore, a decline in muscle quantity would be expected to impair glucose clearance. Even if one is obese, low muscularity may have profound effects on glucose management. Sarcopenic obese individuals (individuals who were obese and had lower than normal muscle mass) were insulin resistant (as measured by HOMA) and had higher percentages of HbA1c compared with non-

sarcopenic obese individuals (Srikanthan et al., 2010). Interestingly, Srikanthan et al (2010) also found sarcopenic, non-obese individuals exhibited insulin resistance.

These findings in non-malignant populations have interesting implications in breast cancer. Weight gain in patients has been shown across the treatment trajectory and into survivorship (Harvie et al., 2004) with an average of 1.5kg gained one year after the end of treatment, 2.7kg after two years, and 2.8kg three years following completion of treatment (Makari-Judson et al., 2007). Though hyperinsulinemia and insulin resistance (measured by HOMA) have been correlated with body mass index (BMI) in breast cancer patients (Goodwin et al., 2009), it is important to specifically evaluate changes in fat and lean mass since weight and BMI measures cannot distinguish changes in particular compartments of body composition. For example, the loss of muscle in a patient might be masked by weight gain, and consequently, fat gains may be underestimated in the circumstances where muscle is lost. An increase in fat mass and, a concomitant decrease in lean mass, is seemingly characteristic in breast cancer patients and can increase the risk for developing chronic diseases in survivorship (Harvie et al., 2004; Mourtzakis & Bedbrook, 2009).

Unique to breast cancer patients in comparison to non-malignant individuals is that their increase in fat mass is accompanied by a decrease in lean mass. This decrease in lean mass has been shown within 6 months of diagnosis as well as 12 months post-diagnosis (Cheney et al., 1997; Demark-Wahnefried et al., 2001; Demark-Wahnefried et al., 2002; Freedman et al., 2004; Harvie et al., 2004). Opposing results, with no change in lean mass following adjuvant chemotherapy, was found by one group, although a sample size of only 8 breast cancer patients was used to come to this conclusion (Campbell et al., 2007). The majority of studies have investigated the effects of chemotherapy on body composition but few have looked at the effects

of radiation therapy. Demark-Wahnefried et al (2001), found that chemotherapy was associated with reduced lean mass while radiation therapy was not. In contrast, Kutynec et al (1999) found that radiation therapy also resulted in a decrease in lean mass, although the loss was not as profound as chemotherapy-treated patients (Kutynec et al., 1999). Therefore, it is generally agreed that chemotherapy treatment has specific effects on decreasing lean mass in breast cancer patients.

Recently diagnosed breast cancer patients showed elevated visceral adipose tissue, measured by computed tomography (CT) scans, in comparison to a matched reference group (Schapira et al., 1994). Increases in total fat mass are also evident with treatment in both pre- and post-menopausal women with breast cancer (Aslani et al., 1999; Campbell et al., 2007; Demark-Wahnefried et al., 2002). Interestingly, Cheney et al (1997) showed increased visceral adiposity following treatment even in the absence of weight gain. When investigating changes in fat mass from treatment into survivorship, the gain found during treatment continued to increase 6 months and 1 year post-diagnosis (Demark-Wahnefried et al., 2001; Harvie et al., 2004). Similarly, Freedman et al found an increase in total body fat along with a decrease in fat free mass 6 months following chemotherapy completion in absence of any significant weight change in these patients (Freedman et al., 2004). Additionally, a trend of increasing body fat with a decrease in BMI has been shown not just one year, but up to 3 years post-diagnosis (Irwin et al., 2005). This increase in fat mass was not related to caloric intake, menopausal status, stage of cancer or treatment (Irwin et al., 2005).

2.4 *High caloric intake as a potential contributor to glucose metabolism impairments*

Since caloric intake, if greater than energy output, leads to adiposity, diet plays a crucial role in development of impairments in glucose metabolism. Breast cancer patients have shown a range of results when it comes to dietary changes during treatment. Some have found decreases in caloric consumption during chemotherapy (Demark-Wahnefried et al., 1997; Goodwin et al., 1999). Others have found no difference between energy and macronutrient intake in breast cancer patients in the early stages of treatment in comparison to age- and BMI- matched non-malignant as well as young healthy reference groups (Bell et al., 2013). Using 2-day dietary recalls and food frequency questionnaires, no differences were found in caloric intake or percentage of fat from total caloric intake over a one year period, in breast cancer patients receiving chemotherapy versus those receiving radiation therapy (Demark-Wahnefried et al., 2001). This finding indicates that treatment type may not influence changes in dietary intake. Additionally, increases in fat mass seen in breast cancer patients was not related to changes in caloric or fat intake (since they remained the same over a one year period compared with baseline, which was close to diagnosis) (Demark-Wahnefried et al., 2001), suggesting that treatment or symptoms related to treatment (like cancer-related fatigue) may have led to these changes in body composition. Conversely, others have shown increased dietary consumption of fat following completion of chemotherapy or radiation treatment (Rockenbach et al., 2011). As such, data on the influence of diet in body composition and metabolic changes is inconsistent, and this may be related to the timeframe in which data were collected or the method used to obtain dietary information.

It is important to consider the challenges in tracking nutrient intake acutely and over a lengthy period of time. There are many difficulties and imprecisions to this type of data

collection, making it difficult to discover small changes without a large sample population. Most large-scale studies use more crude measures of assessing dietary intake (such as 24- hour dietary recalls or food frequency questionnaires) due to the large cost and burden associated with more comprehensive methods (such as 3-or 7- day food diaries). The more comprehensive measures are still faced with challenges such as burden on the patient which may lead to poor documentation of dietary intake over 3 or 7 days. Patients may also unintentionally alter their diet. Additionally, patients undergoing treatment may have high variability across several assessments during their treatment trajectory due to treatment-induced symptoms. However, dietary analysis can provide supplementary information to better understand the changes in glucose metabolism in cancer patients.

2.5 Inflammatory mediators as potential contributors to glucose metabolism impairments

There are two types of inflammation: acute and chronic. An acute inflammatory response will occur following a form of trauma to the body (Scott et al., 2004). This is a positive response, meant to aid in the healing process; however, chronic inflammation is usually negative.

Cytokines, which by definition act on other cells, are secreted by a variety of cell types including adipose tissue (Clément et al., 2004; Xu et al., 2003). In a state of obesity, pro-inflammatory cytokines are often elevated, leading to issues such as insulin resistance (Xu et al., 2003).

In non-malignant populations, chronic low-grade inflammation has been well documented and is correlated with glucose impairment and type 2 diabetes (Donath & Shoelson, 2011; Temelkova-Kurktschiev et al., 2002; Xu et al., 2003). Increasing fat mass, especially central obesity, has been proposed to provide a hypoxic environment which leads to infiltration of macrophages (Donath & Shoelson, 2011; Xu et al., 2003). These macrophages then release

pro-inflammatory cytokines such as tumour necrosis factor- α (TNF), Interleukin (IL) -1 β , IL-6 and IL-8, ultimately aiding in the development of impairments in glucose metabolism (Donath & Shoelson, 2011; Xu et al., 2003). Xu et al (2003) demonstrated that macrophage and inflammatory gene expression were upregulated prior to increased concentrations of circulating insulin; therefore suggesting that obesity-related inflammation may be a precursor to insulin resistance (Xu et al., 2003).

Inflammatory mediators has been an area of interest for research in breast cancer patients as well. Despite that some studies have not found significant increases in pro-inflammatory cytokines (Bell et al., 2013; Pusztai et al., 2004), there are several studies that have conversely demonstrated increased inflammation in breast cancer patients, which may be associated with increased risk for developing impaired glucose metabolism in survivorship. Breast cancer patients were found to have elevated pro-inflammatory cytokine concentrations in comparison to a healthy female reference group, especially as cancer stage increased (stage III vs II) (Kozłowski et al., 2003). In line with this association between pro-inflammation and disease stage, tumour production of inflammatory cytokines is related to increased risk of tumour metastasis as well as cancer recurrence (Cole, 2009). TNF α , a pro-inflammatory marker, which has been shown to be elevated in breast cancer patients, is also linked to increasing tumour growth and proliferation as well as impaired insulin signaling at the muscle (Lee et al., 2003; Plomgaard et al., 2005; Rivas et al., 2008). C-reactive protein (CRP), another marker of inflammation, has been shown to be elevated in breast cancer patients and was associated with decreased survival rates in this patient population (Pierce et al., 2009). However, not all existing literature has revealed increases in pro-inflammatory cytokines in breast cancer patients. Our lab compared a small group of Stage I-IIIa breast cancer patients (n=8) to an age- and BMI- matched

non-malignant female group as well as a healthy young female group found no significant differences in serum pro-inflammatory and anti-inflammatory cytokines between breast cancer patients and either reference group (Bell et al., 2013). Similar findings were found by Pusztai et al (2004). However, given the complexity in studying cytokines and the variable, and relatively short half-lives that characterize many cytokines, small sample sizes may not be adequate for understanding the role of cytokines in glucose regulation in breast cancer. It is possible that inflammation may be a common, but complex, link between breast cancer, obesity, and diabetes in breast cancer and, may be related to different stages of the disease.

2.6 The role of physical inactivity in glucose regulation

Physical inactivity has been associated with a number of health conditions, including at least 10% of breast cancer cases in the world (Lee et al., 2012). Furthermore, inactivity has been shown to result in 9% of premature deaths which equates to over 5.3 million individuals in 2008 alone (Lee et al., 2012). Although physical activity is immensely important, it is a simple modifiable lifestyle factor that remains difficult to implement and manage in daily routines.

One common model that has been used to study the metabolic effects of prolonged periods of physical inactivity is through bed rest. Oral glucose tolerance tests revealed that 5 days of bed rest in healthy participants significantly increased the insulin and glucose responses (Hamburg et al., 2007). Additionally, fasting glucose and insulin concentrations were elevated as well (Hamburg et al., 2007), suggesting that less than a week of inactivity can adversely affect overall glucose handling. It is possible that impaired vascular function, which was shown in bed-ridden participants (Hamburg et al., 2007), played a part in reducing insulin sensitivity by hindering delivery of glucose to the muscles for uptake. A sedentary lifestyle can be expected to

ultimately lead to impaired glucose metabolism. This is in line with work by Amati et al (2009), where chronically sedentary individuals were less insulin sensitive than regularly active individuals.

Although few studies have focused investigations on changes in physical activity post-diagnosis, decreases in physical activity have been reported in breast cancer patients (Irwin et al., 2003; Irwin et al., 2004). Moderate- and vigorous- intensity, as well as sports and recreational activity, are significantly reduced following breast cancer diagnosis compared to pre-diagnosis (Irwin et al., 2003). This decreasing time spent in an active state is associated with increasing categories of BMI (Irwin et al., 2004), and increasing age was also significantly related with decreased the time spent in vigorous-intensity physical activity (Irwin et al., 2004). Although decreases in physical activity were more prevalent in breast cancer patients with a greater BMI and age (Irwin et al., 2004), increased sedentary behavior has been found post-breast cancer diagnosis compared with prior to diagnosis in patients with any BMI, age, stage of breast cancer or treatment type (Irwin et al., 2003). Total physical activity declined by the greatest magnitude in patients that received chemotherapy (Irwin et al., 2003). It is possible that this is the case due to chemotherapy related side effects such as nausea and fatigue. Chemotherapy-related fatigue has been reported beyond one year of treatment (Goodwin et al., 2002). Breast cancer survivors, at least 3 years post-diagnosis, also showed increased sedentary behaviour in comparison to non-malignant individuals (Kim et al., 2013). It is possible that this is at least partially to blame for the long term increase in fat mass and decrease in lean mass; both of which play a role in insulin resistance. A decrease of physical activity in breast cancer patients has shown to lead to sarcopenic obesity (Demark-Wahnefried et al., 2001), further demonstrating the need for interventions that promote physical activity in these patients.

2.7 *Effects of exercise interventions on glucose and related markers*

In non-malignant populations, a balanced diet and physical activity have shown to improve glucose metabolism (Eriksson et al., 1997; Schmitz et al., 2010). Individuals who present with insulin resistance or diabetes have demonstrated improvements in insulin and glucose metabolism with aerobic exercise, resistance training and a combination of the two exercise modalities (Cuff et al., 2003; Maiorana et al., 2002).

In diabetic patients, aerobic exercise on a cycle ergometer has been shown to induce GLUT4 vesicle translocation to the plasma membrane of their skeletal muscle, ultimately enhancing their glucose clearance (Kennedy et al., 1999). Additionally, endurance exercise in older women (age 60-84 years) has been associated with decreased fat mass, increased lean mass in the legs and improved glucose clearance as measured by an OGTT (Evans et al., 2001). Significant decreases in IL-6, CRP and TNF- α have also been exhibited in obese women undergoing an aerobic exercise intervention (12 weeks and 6 months in duration) (Straczkowski et al., 2001; You et al., 2004), where decreased pro-inflammatory markers occurred independently of adipose tissue loss (You et al., 2004). Thus, it is plausible glucose metabolism may improve independently as well.

Studies investigating resistance training in non-malignant individuals also showed improvements in glucose related markers. Resistance exercise in 30-50 year old women showed decreases in fasting glucose, insulin and IGF-1 along with decreases in percentage of body fat following 15 weeks of training (Schmitz et al., 2002). Additionally, one year of resistance training in overweight women was associated with decreased CRP concentrations (Olson et al., 2007). Thus, if improvements in glucose metabolism and related markers are independently observed in both aerobic and resistance exercise modalities, then it would be anticipated that

improvements may be of greater magnitude if aerobic and resistance exercise training was combined in a given exercise program.

A 16 week, combined resistance and aerobic training program performed by non-malignant women with type 2 diabetes resulted in improved glucose clearance (measured by a euglycemic hyperinsulinemic clamp), which was correlated with decreased subcutaneous fat and increased muscle cross sectional area (Cuff et al., 2003). Muscle cross-sectional area was measured by computed tomography scans, which also demonstrated improved density, indicating potential reduction in fatty infiltration in muscle (Cuff et al., 2003). These women were also compared to a group who underwent aerobic training alone. The combined resistance and aerobic exercise training group showed significant improvements in insulin sensitivity compared with the group that underwent aerobic training only, suggesting that a combination of aerobic and resistance exercise are optimal in enhancing the ability to clear glucose from the circulation (Cuff et al., 2003). Another group studying aerobic and resistance exercise in an 8 week circuit training program in diabetic patients found improvements in fasting blood glucose as well as body fat and physical fitness parameters (Maiorana et al., 2002), further showing the benefits of combined exercise. Of course there is also an opposing area of research which states that aerobic exercise interferes with anabolic effects normally exhibited from resistance exercise (Coffey et al., 2009). However, the goal of the exercise interventions in this study were to improve glucose metabolism; thus, while interference in anabolic processes may have occurred in this study, participants were still able to improve glucose parameters.

The majority of exercise training research performed in breast cancer patients focuses only on aerobic exercise interventions as well as breast cancer survivors rather than patients actively undergoing treatment. Aerobic exercise done for 120-150 minutes per week (with 3 days

supervised and 2 days unsupervised per week) showed improvements in parameters of metabolic syndrome in breast cancer survivors as well as decreases in fasting glucose 6 months after starting the program (Thomas et al., 2013). While others found no changes in insulin resistance, insulin or glucose concentrations, however IGFBP-3 increased and IGF-1 as well as IGF-1:IGFBP-3 molar ratio decreased following 15 weeks of aerobic training (3 times per week for 15 to 35 minutes on a cycle ergometer) in breast cancer survivors (Fairey et al., 2003). It is possible that the duration of the study may have had an influence on these results. However, the findings regarding IGF-1 and IGFBP-3 are important because bioactive IGF-1 acts as a tumour proliferative and antiapoptotic agent, and aerobic exercise improved these markers in breast cancer survivors.

Related measures such as body composition and inflammatory markers have been assessed in aerobic exercise studies done on breast cancer patients as well. A decrease in percentage of body fat has been observed in patients undergoing chemotherapy undertaking an aerobic exercise program on a cycle ergometer 3 times per week for a mean of 17 weeks in total (Courneya et al., 2007). Furthermore, decreases in waist circumference were demonstrated in breast cancer patients who adhered to the prescription of exercising 2 times per week on a treadmill, stationary bike or rowing ergometer (Guinan et al., 2013). Studies assessing inflammatory markers found that 3, 3.5 and 6 months of aerobic exercise training decreased CRP levels in breast cancer survivors (Fairey et al., 2005; Guinan et al., 2013; Jones et al., 2013). Interestingly, Guinan et al (2013) found greater decreases in CRP during the 8 week supervised portion of the exercise intervention. Despite decreases in CRP, the 6 month aerobic exercise intervention (consisting of brisk walking or, less commonly, training on a stationary bike or elliptical) and also reported increases in IL-6 and TNF- α (Jones et al., 2013).

Research on resistance exercise in breast cancer patients is more sparse. Six months of partially supervised (supervised small groups by fitness professional for first 3 months) resistance training performed 2 times per week has shown decreases in percentage of body fat and increases in lean in breast cancer survivors (Schmitz et al., 2005). Other metabolic markers such as insulin and glucose were unchanged (Schmitz et al., 2005). In these patients, as well as breast cancer patients performing resistance exercise during treatment (one of the few papers that examined exercise training during treatment), lean body mass increased with training, and percentage of body fat decreased (Courneya et al., 2007; Schmitz et al., 2005), without any diet intervention strategies. Upper and lower body strength improved in patients performing resistance exercise (Courneya et al., 2007). Evidently, there are independent benefits from both aerobic and resistance training, and so, combining the two exercise modalities may lead to greater metabolic benefits.

Even fewer studies have investigated the effects of a combination of aerobic and resistance exercise training on glucose metabolism in breast cancer patients. Sixteen weeks of combined, partially supervised (with the resistance training sessions supervised and the home-based aerobic training portion unsupervised) exercise has shown to decrease fasting insulin as well as tending to decrease HOMA-IR in breast cancer survivors (Ligibel et al., 2008). Following the exercise intervention, no changes were reported in fasting glucose or features of body composition (such as fat mass or lean mass); however, waist circumference tended to decrease (Ligibel et al., 2008). Another study investigating measures related to glucose metabolism impairments used group exercise training and found that 16 weeks of aerobic and resistance exercise training 3 times per week improved aerobic capacity, strength and resting systolic blood pressure (Kolden et al., 2002). Inflammatory markers assessed in breast cancer

survivors showed improvements in IL-8 and CRP in a reference group completing 6 months of aerobic, strength and flexibility exercises (Parma et al., 2015). Conversely, results from another group showed unexpected increases in IL-6 and decreases in IL-10 following 3 months of unsupervised aerobic and resistance band exercises (Rogers et al., 2013). There is limited work that examines the effects of supervised, combined aerobic and resistance exercise training on glucose and related measures.

3.0 Rationale

Breast cancer patients have been shown to develop poor glucose clearance and related markers with treatment and into survivorship (Bell et al., 2013; Can et al., 2013; Goodwin et al., 2009; Guinan et al., 2013; Ligibel et al., 2008; Lu et al., 2014). Additionally, body composition changes of increasing fat mass and decreasing lean mass are exhibited by breast cancer patients following treatment (Aslani et al., 1999; Campbell et al., 2007; Cheney et al., 1997; Demark-Wahnefried et al., 2001; Demark-Wahnefried et al., 2002; Freedman et al., 2004; Harvie et al., 2004). This increases their risk of developing secondary diseases such as type 2 diabetes in survivorship (Bordeleau et al., 2011; Juanjuan et al., 2015; Lipscombe et al., 2013; Patnaik et al., 2011; Ravasco et al., 2005). With 5-year survival rates being 88% (Canadian Cancer Society, 2015), the proportion of survivors is escalating; thus, understanding changes in glucose metabolism is important to help prevent the development of diabetes in survivors.

It has been stated that exercise rehabilitation is not generally included in patient care (Jones & Courneya, 2002). Additionally, physical activity levels typically decrease in breast cancer patients (Irwin et al., 2003; Irwin et al., 2004). This is largely due to treatment-related fatigue, which has been reported to be present past 1 year of treatment (Goodwin et al., 2002). It is likely that decreased physical activity contributes to the development of impaired glucose metabolism and unfavourable changes in body composition in breast cancer patients, and that exercise training might prevent or offset these negative changes. The majority of studies investigating the effects of exercise in this patient population have looked at breast cancer survivors rather than patients undergoing treatment. Based on the deleterious changes that occur with treatment, it would make sense to study the effects of exercise during breast cancer treatment as a form of prevention. Furthermore, the majority of exercise studies in breast cancer

survivors have investigated aerobic exercise with few investigating resistance training or a combination of the two. Aerobic exercise and resistance training have each shown distinct benefits. For instance, a study directly comparing the two types of interventions in breast cancer patients found that aerobic exercise improved aerobic capacity and decreased percentage of body fat while resistance training increased muscular strength and lean mass (Courneya et al., 2007). A combined aerobic and resistance training program may capture a combination of these improvements and provide the most benefits towards improving glucose regulation.

Here, we examined the effects of a combined, supervised aerobic and resistance training program on glucose and related markers in breast cancer patients who were actively undergoing anti-neoplastic treatment. We used highly precise modalities for body composition measures. Fitness outcomes (strength, cardiovascular capacity, resting heart rate, and systolic and diastolic blood pressure) were measured and nutrition was assessed to better understand and interpret findings related to potential longitudinal changes in glucose and related markers. This is the first comprehensive and integrative examination of the effects of combined aerobic and strength training that has been performed, to our knowledge, in a breast cancer patients in active treatment. Previous literature have assess glucose metabolism, body composition, inflammatory markers, nutrition and fitness but never all together. Furthermore, measures such as c-peptide, lactate and IGF-1:IGFBP-3 are novel measures in the field of exercise and breast cancer as well.

4.0 Objectives

Primary Objectives

In a heterogeneous group of breast cancer participants, our primary objectives were to:

1. a. Evaluate glucose and related parameters and compare these to a reference group of young, apparently healthy females.
- b. Compare body composition (i.e. waist circumference, lean mass and adiposity), inflammatory markers, aerobic capacity and strength, and dietary intake with a young, healthy female reference group to explain potential deviations in glucose and related parameters in the breast cancer participants.
2. a. Examine potential changes in glucose and related parameters following 24 sessions of a combined cardiovascular and strength training program.
- b. Examine potential changes in body composition (i.e. waist circumference, lean mass, and adiposity), inflammatory markers, aerobic and strength assessments, and dietary intakes following 24 sessions of a combined cardiovascular and strength training program.

Secondary Objectives

In a heterogeneous group of breast cancer participants, our secondary objectives were to:

1. a. Examine the number of sessions that participants completed. Of those who completed the full 24 sessions of exercise training, we will examine the time to completion of this program. Also, to assess whether time to completion of the 24 exercise sessions had potential effects on metabolic parameters.

b. Assess potential differences on baseline metabolic and body composition parameters between all participants who dropped out of the study and those that completed the program.

5.0 Hypotheses

Primary Hypotheses

1. Compared with the young, healthy reference group, breast cancer participants we hypothesized:
 - a. Worse fasting glucose concentrations and related metabolic parameters.
 - b. Higher waist circumference, fat mass and lower in lean mass.
 - c. Higher concentrations of pro-inflammatory cytokines and lower concentration of anti-inflammatory cytokines.
 - d. Lower aerobic capacities and muscular strength.
 - e. Similar caloric intake.
2. Compared with pre-exercise assessments, breast cancer participants that have undergone 24 exercise sessions would present with:
 - a. Maintained or improved fasting glucose and related parameters
 - b. Maintained or lowered waist circumference, fat mass and maintained or increased lean mass.
 - c. Maintained or lowered concentrations of pro-inflammatory cytokines and maintained or increased concentrations of anti-inflammatory cytokines.
 - d. Increased aerobic capacities and muscular strength.
 - e. Maintained caloric intake.

Secondary Hypotheses

1. a. All participants were expected to complete at least 12 sessions of exercise.
Furthermore, participants that complete the full 24 exercise sessions were expected to take longer than 12 weeks to complete the program. Of those who completed the exercise

program, time to completion of the 24 exercise sessions was expected to affect metabolic parameters (i.e. those who took less time to complete the program showed greater improvements).

b. Metabolic and body composition parameters at baseline would not differ between breast cancer participants that completed the exercise program and the group that dropped out.

6.0 Evaluation of glucose metabolism in breast cancer patients following a combined aerobic and resistance exercise intervention

The work presented in this chapter will be submitted to *Clinical Nutrition* as:

Milburn A G, Di Sebastiano K, Bos L, Schmidt S, Russell C, Noble M, Thayer S, Rellinger Smith E, Kraemer L, Dubin J, Hanning R, and Mourtzakis M. Evaluation of glucose metabolism in breast cancer patients following a combined aerobic and resistance exercise intervention

6.1 Introduction

Breast cancer is the most common cancer in women (Canadian Cancer Society, 2015). With emerging successes in detection and treatment, 5-year survival rates have reached 88%, thereby rapidly increasing the population of breast cancer survivors (Canadian Cancer Society, 2015). Impairments in glucose metabolism have been found throughout the treatment trajectory (Bell et al., 2013; Can et al., 2013; Goodwin et al., 2009; Guinan et al., 2014), increasing the risk of developing secondary diseases such as diabetes in survivorship. Understanding the development of impaired glucose metabolism during the disease trajectory and identifying ways to mitigate these impairments are becoming increasingly important areas of research.

Obesity, muscle atrophy, pro-inflammation, increased caloric intake as well as reduced physical activity contribute to insulin resistance in non-malignant populations (Amati et al., 2009; Donath & Shoelson, 2011; Hamburg et al., 2007; Spiegelman & Flier, 2001). Breast cancer patients have demonstrated increases in fat mass and decreases in lean mass during treatment and into survivorship (Aslani et al., 1999; Campbell et al., 2007; Cheney et al., 1997; Demark-Wahnefried et al., 2001; Demark-Wahnefried et al., 2002; Freedman et al., 2004; Harvie et al., 2004). Multiple studies have demonstrated increased inflammatory markers in breast cancer patients (Kozłowski et al., 2003; Lee et al., 2003; Pierce et al., 2009; Plomgaard et al., 2005; Rivas et al., 2008). Though caloric intake may not change significantly in breast cancer

patients undergoing treatment (Bell et al., 2013; Demark-Wahnefried et al., 2001), physical activity has been shown to decrease (Irwin et al., 2003); ultimately, this would lead to a positive net energy balance resulting in weight gain (Spiegelman & Flier, 2001). Much of this weight gain is likely increased adiposity masking a loss of lean tissue mass. Adiposity is a known contributor to impaired glucose metabolism (Amati et al., 2009). Given that skeletal muscle contributes ~85% of whole body glucose disposal (DeFronzo et al., 1981), loss of lean tissue may also contribute to glucose dysregulation.

Exercise interventions have been evaluated with the aim of alleviating negative side effects from treatment (Courneya et al., 2014; Courneya et al., 2007); however, metabolic effects of exercise in breast cancer patients are unclear. Few studies have investigated the effects of exercise on glucose metabolism, and those that have are focused only on breast cancer survivors (Courneya et al., 2007; Fairey et al., 2003; Guinan et al., 2013; Segal et al., 2001; Thomas et al., 2013). Despite the profound improvements in glucose disposal that result from combined aerobic and resistance exercise in non-malignant populations (Cuff et al., 2003), few studies in breast cancer survivors and no studies in breast cancer patients undergoing treatment have used this approach to improve metabolic outcomes. In breast cancer survivors, Ligibel et al. (2008) showed improvements in fasting insulin following a 16 week combined aerobic and resistance training program (Ligibel et al., 2008). Another study used group exercise training and found that 16 weeks of aerobic and resistance exercise training 3 times per week improved aerobic capacity, strength and resting systolic blood pressure (Kolden et al., 2002).

The aim of this study was to investigate the effects of a 24-session, mixed aerobic and resistance exercise program on glucose metabolism. Body composition, diet and inflammatory markers were measured to support our understanding of the potential changes in glucose-related

parameters during the exercise program. Baseline and post-training measures were additionally compared to a group of young, healthy females. We hypothesized that breast cancer patients would demonstrate improvements in all glucose-related parameters, and these changes may be related to reduced body fat, pro-inflammatory mediators and increased lean mass.

6.2 *Methods*

6.2.1. *General study design*

This study involved 25 participants: 15 female breast cancer patients (≥ 18 years old) and 10 healthy, young females (18-25 years old with normal fasting blood glucose). Following medical clearance, all participants were assessed for: 1) fitness (including cardiovascular and muscular strength), 2) glucose-related measures and inflammatory markers from fasted blood sample, 3) body composition using body mass index (BMI), dual energy X-ray absorptiometry (DXA), and waist circumference (WC), and 4) dietary intake using 3-day food diaries. All of these baseline assessments were completed within 1 week and compared with a young, healthy reference group. Following the baseline assessment, breast cancer participants underwent a 24-session exercise intervention including a combination of strength and aerobic training. The assessments performed at baseline were then repeated at cessation of the exercise program for longitudinal comparisons. This study was reviewed and received ethics clearance by the University of Waterloo Office of Research Ethics Board for both the breast cancer and young healthy female participants and by the Tri-Hospital Research Ethics Board for the breast cancer participants.

6.2.2 *Participants*

Breast cancer patients and survivors (survivors being participants that have recently finished treatment) were recruited who: were physically able to exercise, were at any stage of the disease, received any treatment, and were at any stage of treatment (even if recently completed). Patients were referred by oncologists from a local community cancer centre to participate in exercise training at the University of Waterloo WELL-FIT Centre.

The young, healthy female reference group was recruited from the Kitchener-Waterloo region and included recreationally active women who had fasting blood glucose concentrations ≤ 5.6 mM and who did not have any adverse medical conditions or history of medical conditions. Participants completed all tests within day 1-7 of their menstrual cycle.

6.2.3 *Blood sampling and biochemical analysis*

Participants arrived in the morning, following an 8-hour overnight fast. A sterile catheter was inserted into an antecubital vein for the collection of 40 mL of blood. Less than 1 mL of whole blood was used to measure glycated albumin (HbA1c) (using A1c now+, Bayer, Mississauga, ON) as well as fasting blood glucose (using accu-check aviva glucose meter, Roche Diagnostics, Mississauga, ON). Remaining blood was allowed to clot for 30 minutes at room temperature before being centrifuged. Serum was stored at -80°C and used to assess glucose, insulin, c-peptide, IGF-1, IGFBP-3, lactate, TNF- α , IL-6, IL-4, IL-8, IL-10, and CRP.

Radioimmunoassays were used to measure insulin (Insulin Specific Radioimmunoassay kit, Millipore; Etobicoke, ON), and c-peptide (Human C-Peptide Radioimmunoassay kit, Millipore; Etobicoke, ON). ELISA kits were used to measure IGF-1 (Human IGF-1 Quantikine ELISA kit, R&D Systems; Minneapolis, MN), and IGFBP-3 (IGFBP-3 Quantikine ELISA kit, R&D Systems; Minneapolis, MN). Lactate was measured by spectrofluorometric assay

(method modified from Marbach & Weil, 1967). Inflammatory mediators, TNF- α , IL-6, IL-8, IL-10 and IL-4 were measured using a BD Biosciences FACSCalibur flow cytometer and the BD Cytometric Bead Array (Human Soluble Protein Master Buffer Kit, BD Biosciences; Mississauga, ON). Lastly, ELISA kits were used to measure CRP (Human C-Reactive Protein/CRP Quantikine ELISA kit, R&D Systems; Minneapolis, MN).

6.2.4 Body Composition

Body composition measures including DXA, BMI and WC were performed for all healthy reference participants and at baseline and post-training in the breast cancer patients. Using DXA (Hologic Discovery, Hologic, Toronto, ON), fat mass and lean mass for the whole body and for specific regions of the body were evaluated for the identification of sarcopenic individuals. Sarcopenic individuals were categorized by using the cut off of $< 5.45 \text{ kg/m}^2$ of appendicular lean mass (Baumgartner et al., 1998). BMI (kg/m^2) was assessed using their weight (kg) and height (m). WC, which is a criteria for assessing metabolic syndrome (if $> 88 \text{ cm}$) (Alberti et al, 2006), was measured at the top of the iliac crests (American College of Sports Medicine, 2009) with a cotton measuring tape. DXA, BMI and WC measures were also performed in the young, healthy reference group but only at one time point.

6.2.5 Nutrition Analysis

Quality and quantity of nutrients were analyzed using 3-day food diaries. Participants recorded everything they ate and drank for two week days and one weekend day as previously described (Thompson & Byers, 1994). ESHA food processor software (ESHA research, Salem, OR) was used to measure daily caloric intake and macronutrient breakdown.

6.2.6 Cardiovascular and muscular strength assessments

Breast cancer participants underwent a graded exercise test on a cycle ergometer. The test was stopped when 50rpm could no longer be maintained for a minimum of 30 seconds or a Rate of Perceived Exertion (RPE; using the Borg Scale) of 15 was reached. The reference group underwent a similar test on a cycle ergometer (ergoselect 100, ergoline; Bitz, Germany) but were brought to their VO_2 peak utilizing a Vmax breath-by-breath system (Care Fusion; San Diego, CA). The test was stopped when 50rpm could no longer be maintained for a minimum of 30 seconds. Heart rate and blood pressure and RPE were assessed for both groups throughout the test (every minute for HR and every 2 minutes for BP and RPE).

A predictive one repetition maximum (1RM) test was performed to measure muscular strength in the upper and lower limbs. Maximal isometric force was measured by a force transducer in forearm flexion as well as leg extension; contralateral sides were tested independently. Isometric contractions were held for 2 seconds and repeated 3 times for each test.

6.2.7 Exercise training program

Combined moderate cardiovascular exercise and resistance training was designed with prescribed heart rate range of 40-60% Heart Rate Reserve. Target heart rate was prescribed based on the heart rate achieved during 11-13 RPE of the cardiovascular test from the baseline fitness assessment. Heart rate bands (Polar; Lachine, QC) were worn for the full duration of each session of exercise. Resistance exercise was performed for all major muscle groups of the body. Participants were instructed to perform 10-20 repetitions for each exercise and progressed by increasing intensity to the next weight when 20 repetitions were reached at least two times with proper form for the entire 20 repetitions. It was the aim of the study to complete 24 sessions within 12 weeks (i.e. exercise 2 times per week for approximately one hour per session). Number

of exercise sessions completed as well as the time to complete all 24 sessions was recorded and assessed. All research participants were supervised during each exercise session.

6.2.8 Calculations

Insulin resistance was evaluated by HOMA-IR (Bonora et al., 2000):

$$\text{HOMA-IR} = (\text{fasting glucose} \times \text{fasting insulin}) / 22.5$$

To identify participants with sarcopenia (less than normal muscularity; with values $< 5.45 \text{ kg/m}^2$), sarcopenic index was calculated using (Baumgartner et al., 1998):

$$\text{sarcopenic index} = \text{sum of appendicular lean mass (kg)} / \text{height}^2 (\text{m}^2)$$

1RM prediction was calculated by:

$$1\text{RM (Nm)} = \text{moment arm (m)} \times \text{highest force trial (N)}$$

BMI was measured by:

$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / \text{height (m}^2\text{)}.$$

6.2.9 Statistics

Results are presented as mean \pm standard deviation. Significance was reported as a $p < 0.05$ and a trend was defined as $0.05 \leq p \leq 0.10$. All statistical analyses were performed on

SigmaPlot Version 11.0 (Systat Software Inc., San Jose, CA). Independent-samples t-tests were used to compare baseline blood parameters and body composition measures of breast cancer exercise completers with the young, healthy female reference group. Paired t-tests were used to compare baseline parameters of the breast cancer group with the measures at cessation of the exercise program. Independent t-tests were used to compare the breast cancer dropouts to the breast cancer exercise completers to show no bias in the group of participants that completed the program. Linear regression analysis was used to determine whether time to completion as well as days since treatment initiation had an effect on changes seen in measured parameters.

6.3 Results

6.3.1 Participant Characteristics

Out of 105 breast cancer patients referred to the University of Waterloo WELL-FIT program, 96 breast cancer patients started the WELL-FIT program, 36 were interested in participating in this study and 22 breast cancer patients consented to participation in the study and completed all baseline assessments including the blood draw. A final sample of 15 participants completed the post assessment with the blood draw following completion of the exercise program (Figure 6.1).

The breast cancer patient group (n=15) ranged from 34-75 years old (55 ± 11 years old; Table 6.1). Treatment consisted of a combination of surgery, chemotherapy, radiation and hormonal treatments, and ranged early to late treatment with one of the dropouts having recently completed treatment (Table 6.1).

Overall, breast cancer participants had an average BMI in the overweight category (26.2 ± 4.0 kg/m², n=15; Table 6.2). There were 9 participants (60%) that were overweight or obese, while none were underweight. In the reference group, the average BMI was in the normal range (23.3 ± 1.9 kg/m²; Table 6.2). Average resting heart rate, systolic and diastolic blood pressure were normal for the breast cancer patients as well as the young reference group (Table 6.2).

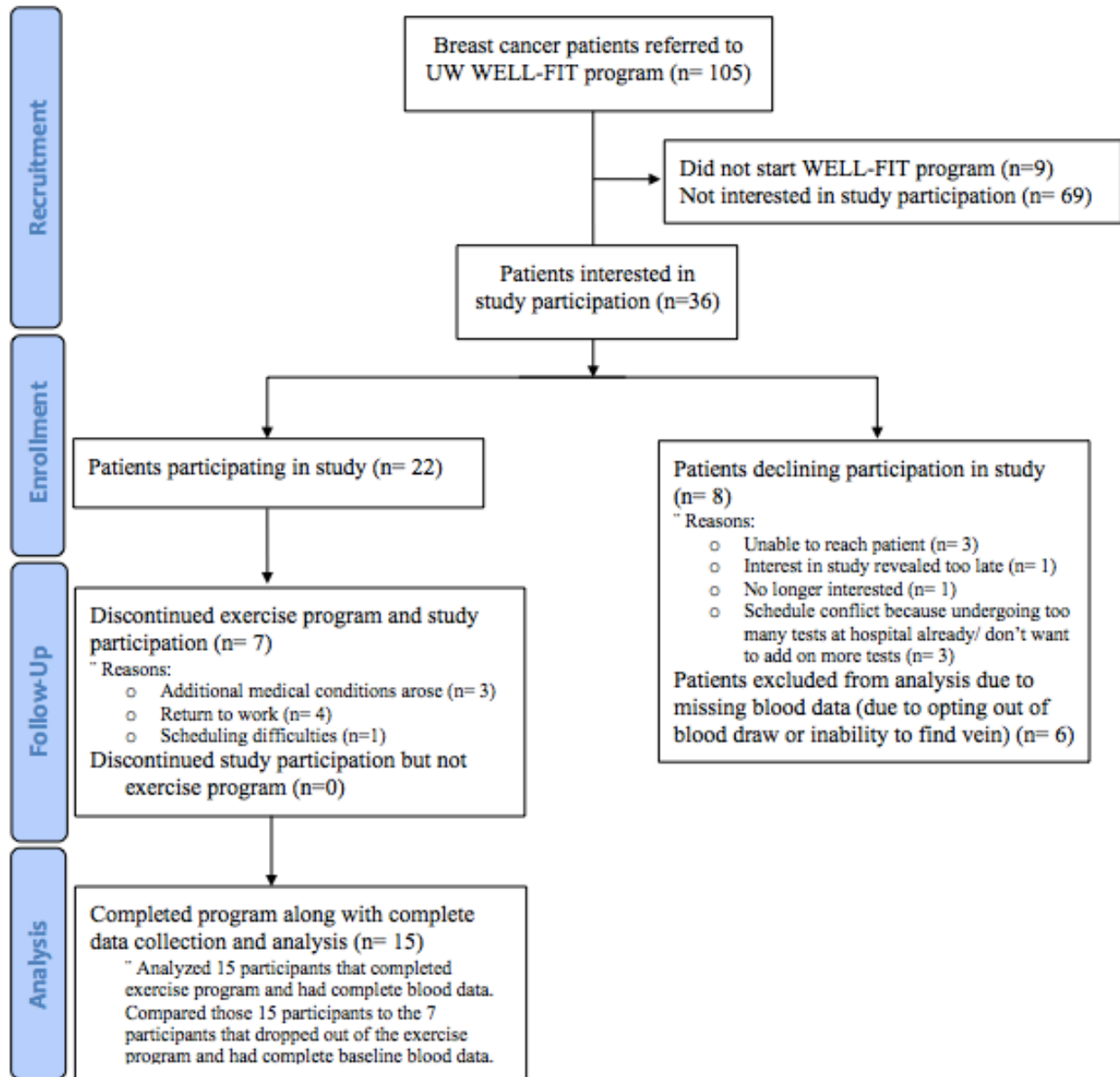


Figure 6.1 Breast cancer patient flow numbers. This consort diagram explains patient recruitment, enrollment, follow-up (explaining participants that dropped out) and the final sample analyzed.

Table 6.1 Treatment characteristics of all breast cancer participants at baseline

Type of treatments undergone	Baseline of breast cancer dropouts (n=7)	Baseline of breast cancer exercise completers (n=15)	P-values
Chemotherapy	4	12	
Chemotherapy Stage:			
1 cycle	1	2	
2 cycles	1	3	
3 cycles		1	
>3 cycles	2	6	
Days since chemotherapy initiation (mean days \pm SD)	83 \pm 79	85 \pm 66	0.966
Radiation	3	5	
Days since radiation treatment initiation (mean days \pm SD)	22 \pm 22	25 \pm 16	0.835
Recently completed radiation treatment with no treatment to follow	1	0	
Hormone Treatment	2	6	
Days since hormone treatment initiation (mean days \pm SD)	201 \pm 204	54 \pm 49	0.286
Types of surgery undergone			
Mastectomy	3	8	
Lumpectomy	2	6	

All values in the table represent n-values unless otherwise stated.

* Indicates $p < 0.05$

Table 6.2 Physical characteristics of all participants

	Baseline of breast cancer dropouts	Baseline of breast cancer exercise completers	Post-assessment of breast cancer exercise completers	Young, Healthy Females
Sample size	7	15	15	10
Age	52 ± 11	54 ± 12	53 ± 11	23 ± 2
BMI (kg/m ²)	27.0 ± 5.3	26.2 ± 4.0 †	26.4 ± 4.2	23.3 ± 1.9
Normal (n)	3	6	5	7
Overweight (n)	2	7	7	3
Obese (n)	2	2	3	0
Waist circumference (cm)	93.4 ± 13.7 ≥88cm: 5 (~71%)	93.5 ± 13.3 † ≥88cm: 8 (~53%)	92.4 ± 13.5 ≥88cm: 9 (~60%)	72.6 ± 5.0 ≥88cm: 0 (0%)
Weight (kg)	72.1 ± 11.9	70.1 ± 11.2	70.8 ± 12.2	65.6 ± 8.0
Height (m)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
Resting Heart Rate (bpm)	70 ± 12	72 ± 11 †	71 ± 10	84 ± 13
Resting Systolic Blood Pressure (mmHg)	117 ± 16	119 ± 12 *	113 ± 10	114 ± 5
Resting Diastolic Blood Pressure (mmHg)	74 ± 11	78 ± 11 #	73 ± 6	77 ± 5

* indicates significant differences between the baseline and post-assessment of breast cancer exercise completers

indicates a trend between the baseline and post-assessment of breast cancer exercise completers

† indicates significant differences between baseline of breast cancer exercise completers and the young healthy reference group

‡ indicates a trend between baseline of breast cancer exercise completers and the young healthy reference group

Note: no statistical differences were seen between the breast cancer dropout group and the breast cancer exercise completers at baseline.

BMI ranges: Normal= 18.5-24.9, Overweight= 25-29.9, Obese ≥ 30 kg/m² (World Health Organization, 2000)

6.3.2. Glucose and related markers in breast cancer participants exercise completers at baseline compared to young, healthy females

Fasting blood samples revealed that HbA1c levels and plasma glucose were significantly higher in breast cancer participants than in the reference group (HbA1c= 5.8 vs 5.0 %, $p < 0.001$; plasma glucose= 5.3 vs 4.6 mM, $p = 0.001$; Table 6.3 and Figure 6.2, respectively). Fasting insulin was significantly higher and c-peptide tended to be higher in breast cancer participants compared with the reference group (Table 6.3). HOMA-IR was significantly higher in breast cancer patients as well (Figure 6.3). Insulin-like growth factor (IGF) -1 as well as concentrations of IGF binding protein- 3 (IGFBP-3) were lower in breast cancer participants versus young healthy females (Table 6.3). However, the ratio of IGF-1:IGFBP-3 were not different between groups (Table 6.3).

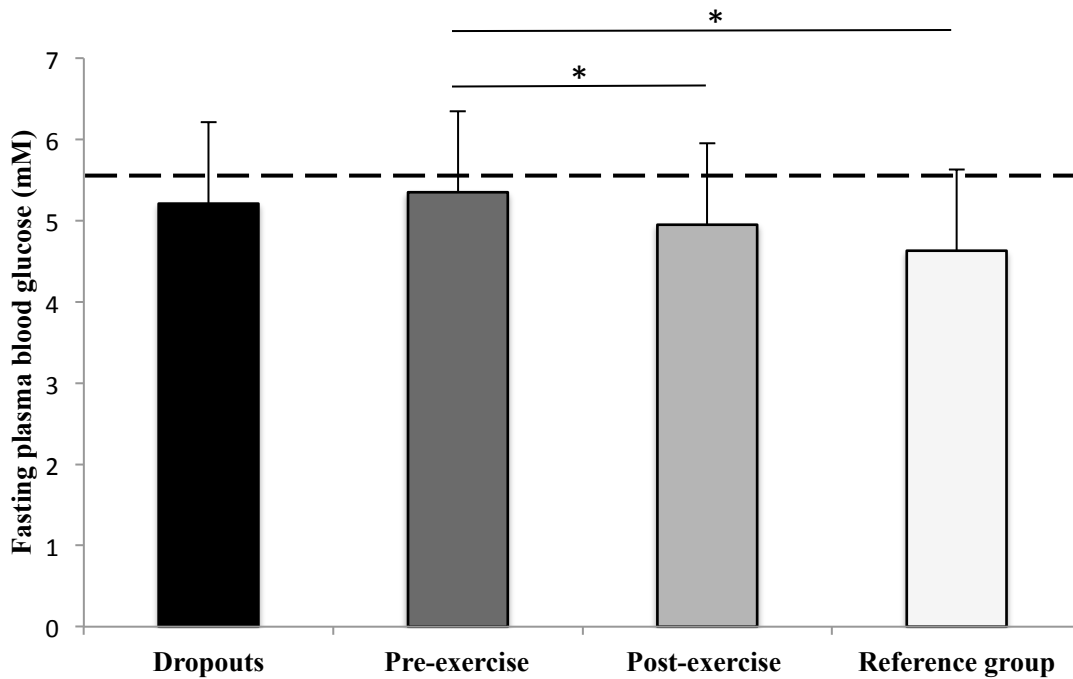


Figure 6.2 Fasting plasma blood glucose concentrations in breast cancer participants pre- and post- exercise program in comparison to young, healthy females. International Diabetes Federation (IDF) cut-point (represented as the dashed line) indicates fasting glucose concentrations above 5.6mM to be a risk factor for metabolic syndrome (IDF, 2006). Pre-exercise values were higher than the reference group but decreased post-exercise intervention. 4 participants were above the IDF cut-point at baseline and only 1 was above post-exercise program.

Legend:

- Baseline of breast cancer dropouts (n=7)
- Pre-exercise= Baseline of breast cancer exercise completers (n=15)
- Post-exercise= Post-assessment of breast cancer exercise completers (n=15)
- Reference group of healthy young females (n=10)

* Indicates $p < 0.05$

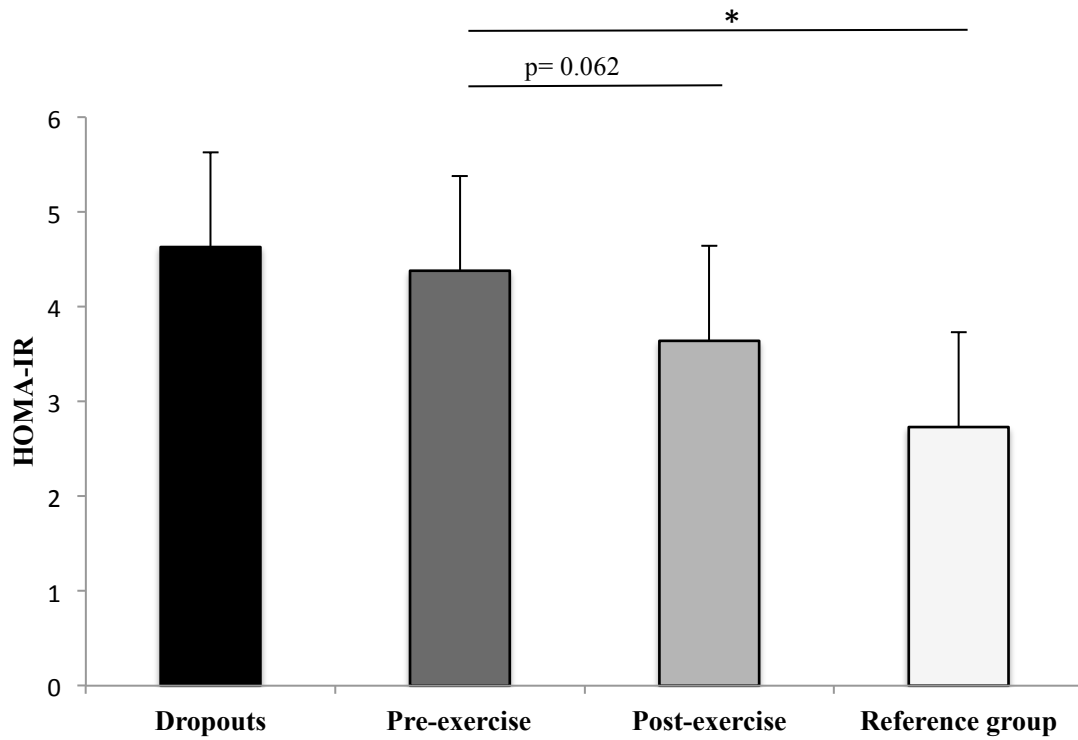


Figure 6.3 HOMA-IR results in breast cancer participants pre- and post- exercise program in comparison to young, healthy females. HOMA-IR was higher at pre-exercise than the reference group but tended to decrease post-exercise program.

Legend:

- Baseline of breast cancer dropouts (n=7)
- Pre-exercise= Baseline of breast cancer exercise completers (n=15)
- Post-exercise= Post-assessment of breast cancer exercise completers (n=15)
- Reference group of healthy young females (n=10)

* Indicates $p < 0.05$

Table 6.3 Glucose related markers in breast cancer participants and in young, healthy females

	Baseline of breast cancer dropouts (n=7)	Baseline of breast cancer exercise completers (n=15)	Post-assessment of breast cancer exercise completers (n=15)	Young, Healthy Females (n=10)
HbA1c (%)	6.2 ± 1.5	5.8 ± 0.7 †	-	5.0 ± 0.4
Range 5.7-6.4 ¹ (n)	4	6		1
Range 6.0-6.4 ² (n)	2	2		0
≥ 6.5 ² (n)	1	2		0
Insulin (µU/mL)	19.7 ± 7.0	18.3 ± 6.1 †	16.2 ± 6.4	13.2 ± 4.7
C-peptide (ng/ml)	6.5 ± 2.3	5.3 ± 2.3 ‡	4.9 ± 2.9	3.7 ± 1.1
IGF-1 (ng/mL)	133.2 ± 54.4	108.9 ± 45.2 †#	87.3 ± 26.9 †	168.2 ± 41.1
IGFBP-3 (ng/mL)	355.7 ± 66.2	331.4 ± 81.1 †	340.1 ± 77.7 †	453.7 ± 65.9
IGF-1:IGFBP-3	0.38 ± 0.13	0.33 ± 0.11 *	0.27 ± 0.09	0.37 ± 0.09
Lactate (mM)	2.5 ± 0.8	2.9 ± 1.3 #	2.2 ± 0.7	2.3 ± 0.7

* indicates significant differences between the baseline and post-assessment of breast cancer exercise completers

indicates a trend between the baseline and post-assessment of breast cancer exercise completers

† indicates significant differences between baseline of breast cancer exercise completers and the young healthy reference group

‡ indicates a trend between baseline of breast cancer exercise completers and the young healthy reference group

Note: no statistical differences were seen between the breast cancer dropout group and the breast cancer exercise completers at baseline.

¹ (American Diabetes Association, 2015)

² (Canadian Diabetes Association, 2013)

6.3.3 Comparison of body composition, inflammatory markers, physical fitness, and dietary intake between breast cancer participants and young, healthy females

WC was also significantly higher for breast cancer participants than the reference group (94 vs 73 cm, $p < 0.001$; Table 6.2). There were 8 of 15 breast cancer participants that were abdominally obese (defined as $WC \geq 88\text{cm}$). In line with this finding, body fat % measured by DXA was significantly higher for breast cancer participants than the reference group (38.2 vs 31.2 %, $p = 0.002$; Table 6.4). Total lean mass in the limbs was significantly lower in the breast cancer participants compared with the reference group (Table 6.4). Interestingly, 5 breast cancer participants and none of the reference group were sarcopenic ($< 5.45 \text{ kg/m}^2$).

Pro-inflammatory mediators (TNF- α , IL-6, IL-8, IL-1 β and CRP) and anti-inflammatory cytokines (IL-4 and IL-10) showed no significant differences between the breast cancer participants and the young healthy female reference group (Table 6.5). Predicted $VO_{2\text{peak}}$ was lower in breast cancer participants as compared with $VO_{2\text{peak}}$ measured in the young female reference group (29.0 vs 42.0 mL/kg/min, $p = 0.008$; Table 6.4). Additionally, muscular strength of all four limbs was lower in the breast cancer group (Table 6.4).

Total caloric intake was significantly lower in the breast cancer participants than the young healthy reference group (1886 ± 328 vs 2262 ± 578 kcal, $p = 0.008$). When investigating percentage of macronutrient intake, no differences were seen between the two groups (Appendix IV).

Table 6.4 Body composition & fitness measures in breast cancer participants and in young, healthy females

	Baseline of breast cancer dropouts (n=7)	Baseline of breast cancer exercise completers (n=14)	Post-assessment of breast cancer exercise completers (n=14)	Young, Healthy Females (n=10)
Body Composition				
% body fat	39.9 ± 5.9	38.2 ± 5.3 †*	39.9 ± 5.0	31.2 ± 3.8
Total fat mass (kg)	28.6 ± 8.4	25.8 ± 6.0 †*	27.3 ± 7.3	20.3 ± 4.5
Total lean mass (kg)	39.6 ± 4.2	39.2 ± 6.2	38.3 ± 5.3	41.8 ± 3.8
Total lean mass in the limbs (kg)	16.8 ± 1.9	16.3 ± 3.0	16.2 ± 2.6	18.4 ± 1.8
Appendicular Skeletal Muscle Mass (kg/m ²)	6.3 ± 0.8	6.1 ± 0.9	6.00 ± 0.7	6.6 ± 0.5
Fitness Measures				
Isometric Right Arm Flexion (Nm)	34.7 ± 5.7	30.2 ± 10.9 †	32.5 ± 11.5	41.3 ± 10.5
Isometric Left Arm Flexion (Nm)	29.2 ± 8.3	28.2 ± 10.2 † (n=13)	30.4 ± 10.6 (n=13)	41.9 ± 12.8
Isometric Right & Left Arm Flexion (Nm)	63.9 ± 10.6	56.9 ± 19.6 † (n=13)	61.4 ± 20.5 (n=13)	83.2 ± 23.1
Isometric Right Leg Extension (Nm)	99.3 ± 27.1	88.8 ± 28.5 †#	96.0 ± 33.5	168.4 ± 28.9
Isometric Left Leg Extension (Nm)	101.6 ± 22.1	84.1 ± 30.1 †*	98.7 ± 33.4	166.9 ± 30.0
Isometric Right & Left Leg Extension (Nm)	200.9 ± 47.7	172.9 ± 56.3 †*	194.8 ± 66.3	335.3 ± 57.2
VO ₂ peak (mL/kg/min)	32.7 ± 7.8	29.0 ± 8.6 †* (n=13)	31.3 ± 9.4 (n=13)	42.0 ± 2.7

* indicates significant differences between the baseline and post-assessment of breast cancer exercise completers

indicates a trend between the baseline and post-assessment of breast cancer exercise completers

† indicates significant differences between baseline of breast cancer exercise completers and the young healthy reference group

‡ indicates a trend between baseline of breast cancer exercise completers and the young healthy reference group

Note 1: no statistical differences were seen between the breast cancer dropout group and the breast cancer exercise completers at baseline.

Note 2: n=14 resulted due to scheduling difficulties for either baseline DXA scan or fitness post-assessment.

Note 3: n=13 because a participant was restricted to using only her right arm or because there was an outlier (defined as more than 2 SD from the mean) in the VO_2 peak data.

Table 6.5 Concentrations of inflammatory mediators in breast cancer participants and in young, healthy females

	Baseline of breast cancer dropouts	Baseline of breast cancer exercise completers	Post-assessment of breast cancer exercise completers	Young, Healthy Females
Pro-inflammatory cytokines				
TNF- α (pg/mL)	6.7 \pm 0.7 (n=6)	5.9 \pm 0.7 * (n=7)	7.2 \pm 1.2 (n=7)	6.7 \pm 1.2 (n=7)
IL-6 (pg/mL)	7.6 \pm 1.7 (n=4)	6.7 \pm 2.5 (n=7)	8.4 \pm 4.8 (n=7)	5.4 \pm 0.4 (n=4)
IL-8 (pg/mL)	14.8 \pm 2.7 (n=6)	12.4 \pm 3.4 (n=13)	14.5 \pm 4.4 (n=13)	13.5 \pm 5.1 (n=10)
IL-1 β (pg/mL)	6.5 \pm 0.3 (n=4)	6.0 \pm 0.6 # (n=8)	6.9 \pm 1.2 (n=8)	6.4 \pm 0.8 (n=7)
CRP (mg/L)	0.2 \pm 0.1 (n=15)	0.3 \pm 0.2 (n=15)	0.3 \pm 0.2 (n=15)	0.2 \pm 0.1 (n=15)
Anti-inflammatory cytokines				
IL-4 (pg/mL)	5.2 \pm 0.2 (n=3)	6.0 \pm 0.7 (n=7)	6.5 \pm 1.0 (n=7)	5.9 \pm 0.6 (n=3)
IL-10 (pg/mL)	6.2 \pm 0.7 (n=7)	6.5 \pm 1.6 (n=13)	6.4 \pm 1.1 (n=13)	6.4 \pm 1.3 (n=10)

* indicates significant differences between the baseline and post-assessment of breast cancer exercise completers

indicates a trend between the baseline and post-assessment of breast cancer exercise completers

† indicates significant differences between baseline of breast cancer exercise completers and the young healthy reference group

‡ indicates a trend between baseline of breast cancer exercise completers and the young healthy reference group

Note 1: no statistical differences were seen between the breast cancer dropout group and the breast cancer exercise completers at baseline.

Note 2: n values vary based on detection rates of cytokine analysis.

6.3.4. Effect of 24 exercise sessions on glucose and related markers in breast cancer participants (n=15)

Following the exercise program, breast cancer participants presented with fasting plasma glucose lower than baseline (5.4 ± 0.7 vs. 5.0 ± 0.4 mM, $p=0.008$; Figure 6.2). Fasting insulin and c-peptide concentrations did not change significantly following the exercise program (Table 6.3). HOMA-IR scores tended to improve with exercise (4.4 ± 1.6 vs. 3.6 ± 1.7 , $p= 0.062$; Table 6.3). IGF-1 concentrations tended to decrease in participants that completed the exercise program (108.9 ± 45.2 vs 87.3 ± 26.9 ng/mL, $p= 0.061$; Table 6.3). IGF-1:IGFBP-3 ratio significantly improved (0.33 ± 0.12 ng/mL vs 0.27 ± 0.09 , $p= 0.018$; Table 6.3), despite the lack of change in IGFBP-3 (Table 6.3). Lactate concentrations tended to decrease following the exercise program (Table 6.3).

6.3.5 Comparison of body composition, inflammatory markers, physical fitness, and dietary intake between pre- and post-exercise training in breast cancer participants

Weight, BMI, and WC did not change with exercise training (Table 6.2). Surprisingly, body fat % increased following exercise program (38.2 ± 5.3 vs. $39.9 \pm 5.0\%$, $p= 0.038$; Table 6.4). Total fat mass also showed significant increases (25.8 ± 6.0 vs. 27.3 ± 7.3 kg, $p= 0.038$; Table 6.4). Total lean mass as well as total appendicular lean mass did not change (Table 6.4). Appendicular skeletal muscle mass index (normalized by height) also remained similar after the exercise program (6.0 ± 0.9 vs 5.9 ± 0.8 kg/m², $p= 0.182$; Table 6.4). Interestingly, only 3 participants were in the sarcopenic range following the program compared with 5 sarcopenic participants at baseline.

Pro-inflammatory cytokine TNF- α increased significantly post-exercise intervention (5.9 ± 0.7 vs 7.2 ± 1.2 pg/mL, $p=0.011$; Table 6.5) and IL-1 β tended to increase (6.0 ± 0.6 vs 6.9 ± 1.2 pg/mL, $p=0.095$; Table 6.5). Delta TNF- α (which showed increases from baseline to post-exercise program) was correlated with increasing delta of percentage of fat ($r= 0.873$, $p=0.0230$) and total fat mass ($r= 0.908$, $p=0.0124$, $n= 6$). Pro-inflammatory cytokines IL-6, IL-8 and CRP as well as anti-inflammatory cytokines, IL-4 and IL-10, remained unchanged with exercise training (Table 6.5).

Predicted VO_{2peak} in breast cancer participants significantly increased following the exercise program (29.0 ± 8.6 vs 31.3 ± 9.4 mL/kg/min, $p= 0.026$; Table 6.4). Additionally, systolic blood pressure significantly decreased and diastolic blood pressure tended to decrease following the exercise program (Table 6.2). While isometric right leg extension strength only tended to improve with the exercise program, left isometric leg extension as well as combined right and left isometric leg extension strength increased following completion of the exercise program (Table 6.4).

Total caloric intake remained the same between baseline and cessation of the exercise program (1718 ± 358 kcal vs 1729 ± 410 kcal, $p=0.881$). Percentage of macronutrient intake revealed that there were no changes in protein, carbohydrate nor fat intake (Appendix IV). No differences in any of the blood, body composition, inflammatory, fitness or dietary measures were found between the breast cancer dropout group and the breast cancer exercise program completers.

6.3.6 Time until program completion and its effects on metabolic parameters

Participants took an average of 132 ± 45 days (range: 84 to 218 days) to complete the exercise program (defined as completion of 23-24 sessions). One participant only completed 23 sessions prior to her post-assessment because her surgery date interfered with the scheduled 24th session. Interestingly, 21 of the 22 participants who had initially enrolled in the study, were able to adhere to the program until approximately the 12th session (Figure 6.4). Days until program completion was correlated with change in fasting plasma glucose measures. Reductions in plasma glucose were greater when the program duration was shorter ($r^2 = 0.357$, $p=0.019$), suggesting that those who followed the exercise prescription exhibited the greatest benefits in blood glucose. Similarly, delta TNF- α tended to increase for those that took longer to complete the exercise program ($r^2 = 0.523$, $p=0.066$).

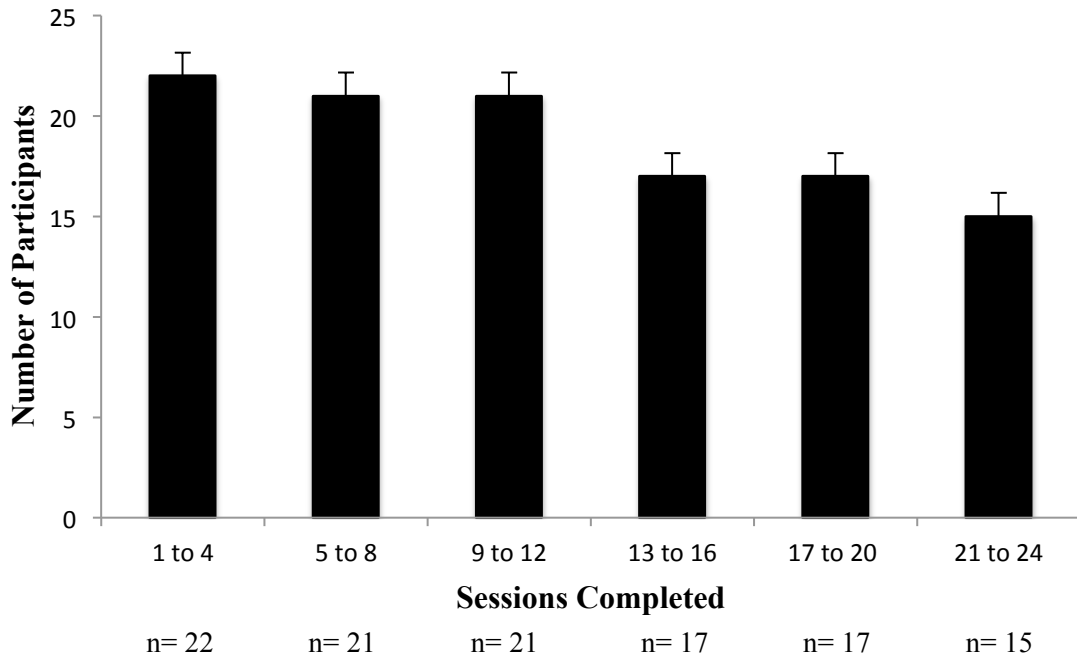


Figure 6.4 Number of exercise sessions completed by participants

6.4 Discussion

This is the first study, to our knowledge, that examined changes in glucose and related parameters following 24 sessions of supervised, combined aerobic and resistance exercise intervention in breast cancer patients who were concurrently receiving treatment. Compared with a healthy, young female reference group, breast cancer patients presented with higher fasting plasma glucose, HbA1c, insulin, c-peptide, HOMA-IR and lower IGF-1, and IGFBP-3. These differences are potentially attributed to a body composition phenotype that is higher in fat and lower in lean mass, greater overall age as well as lower fitness levels in the breast cancer patients. In contrast, dietary intake and inflammatory markers were relatively similar between the 2 groups. We have previously shown that in a small group of BMI- and age-matched non-malignant women, Stage I-II breast cancer patients tended to have poorer glucose handling than the matched reference group (Bell et al., 2013). Here, breast cancer patients that completed the exercise program showed significant improvements in fasting plasma glucose and IGF-1:IGFBP-3 as well as a tendency towards improved HOMA-IR, IGF-1 and lactate concentrations. The exercise program led to improved physical strength and aerobic capacity suggesting that the integrity and metabolic function of the muscle was improved and these metabolic improvements may have explained the reduced plasma glucose concentrations following exercise. Lean mass, most inflammatory markers and dietary intake remained similar to baseline, but improvements in fasting glucose were associated with reduced time to complete 24 sessions of combined aerobic and resistance exercise training. In contrast, fat mass and TNF- α were higher than baseline measurements, and changes in these features were related to each other. In other words, those who gained fat, were likely to exhibit increases in TNF- α .

In the present study, we demonstrated that 24 sessions of combined moderate intensity aerobic and resistance exercise was associated with improved fasting plasma glucose. The supervised exercise program was prescribed with a frequency of 2 times per week, however, only 8 out of 15 participants of participants were able to complete 23-24 sessions in the prescribed 12 week range given that factors related to the disease, treatment, family etc. may prohibit adherence to the prescribed frequency. Although it took an average of ~ 132 days to complete the exercise program, fasting plasma glucose significantly decreased. A study investigating a program of supervised resistance training and unsupervised aerobic training in breast cancer survivors reported no significant changes in fasting blood glucose (Ligibel et al., 2008). In a previous study from our lab, no significant differences in fasting blood glucose were found between breast cancer patients and a reference group of young healthy females as well as another reference group of age and BMI matched females (Bell et al., 2013); only an oral glucose tolerance test (OGTT) was sensitive enough to depict impaired glucose clearance in the breast cancer group (Bell et al., 2013). Clearly, these reductions in fasting blood glucose are impressive but need to be interpreted with some caution as treatment itself may, independently (Guinan et al., 2014) or in conjunction with exercise, have contributed to this reduction.

Previous studies on non-malignant individuals have shown increased GLUT4 protein content in individuals undergoing a resistance training program (Holten et al., 2004) as well as an aerobic training program (Hughes et al., 1993). Despite the low volume of exercise in this pool of participants, we predict that GLUT4 content was increased in our participants and that this allowed for greater clearance of plasma glucose during exercise sessions. During basal states, however, GLUT1 would have been responsible for glucose clearance (Kennedy et al., 1999). Although muscle biopsies were not taken to confirm whether increased GLUT proteins or

GLUT translocation played a role in decreased fasting glucose, the finding of unchanged concentrations of circulating insulin and c-peptide imply that increased glucose clearance was not due to insulin-stimulated uptake. Blood draws were conducted at a minimum of 48 hours after exercise to ensure any acute effects of exercise were negligible. In basal conditions where insulin or exercise stimulated glucose uptake is not at play, it is possible that the liver has also decreased insulin resistance, with proper glycogen synthesis in the postprandial state and gluconeogenesis in the fasted state (Savage et al., 2007). Although the exercise program may have improved fasting plasma glucose, it is also possible that treatment could have lowered its concentrations as well. Literature in pancreatic cancer patients has demonstrated the ability of the tumour to induce insulin resistance (Permert et al., 1993; Saruc et al., 2009). It is possible that treatment, by eliminating the tumour and its potentially lingering effects, may have improved fasting glucose concentrations. However, literature in breast cancer patients has shown increases in glucose, insulin and c-peptide (Guinan et al., 2014; Ligibel et al., 2008) supporting the effectiveness of the exercise program seeing as glucose concentrations improved and insulin and c-peptide remained unchanged.

IGF-1:IGFBP-3 showed significant decreases in a group of breast cancer survivors undergoing an aerobic exercise training regime for 15 weeks (Fairey et al., 2003). To our knowledge, our study is the first to show a decrease in IGF-1:IGFBP-3 in a group of breast cancer patients primarily still undergoing treatment or recently completed treatment following a combined aerobic and resistance training program. IGF-1 resembles the molecular structure of insulin and has also been shown to be related to insulin resistance (Friedrich et al., 2012). The decreased bioavailability of IGF-1 implicated by a lower molar ratio of IGF-1:IGFBP-3 indicate positive effects on insulin sensitivity.

Although we expected muscular strength to improve significantly in the upper and lower body as in the study by Kolden et al (2002), we only saw improvements in the lower body. The improvements that were observed in strength and metabolic parameters, including fasting glucose, were nonetheless impressive given that the exercise protocol was based on moderate intensity and low frequency exercise. Additionally, an increase in aerobic capacity may have some implications for improved concentrations of glucose and related markers since higher insulin sensitivity is exhibited by aerobically active individuals (Amati et al., 2009). A number of measures, including body composition, which is normally related to metabolic outcomes, did not improve. However, breast cancer patients and survivors typically experience increasing fat mass and decreasing lean mass during the disease and survivorship trajectory (Aslani et al., 1999; Campbell et al., 2007; Cheney et al., 1997; Demark-Wahnefried et al., 2001; Demark-Wahnefried, et al., 2002; Freedman et al., 2004; Harvie et al., 2004). However, if we had included a usual care group that did not undergo the exercise program, we may have found significant differences between the usual care and exercise intervention group with attenuated increases in fat mass and attenuated decreases in lean mass in the exercise intervention group.

Based on previous studies (Bell et al., 2013; Demark-Wahnefried et al., 2001), we did not expect to find caloric changes as well as macronutrient intake. However, it is possible that participants did not expend enough energy during the exercise program to counter-balance their energy intakes, and this may have led to increased fat mass. Although the majority of pro- and anti-inflammatory mediators (CRP, IL-6, IL-8, IL-4 and IL-10) remained the same, pro-inflammatory TNF- α increased significantly and IL-1 β tended to increase following the exercise intervention. However, increased TNF- α may be explained by its positive correlation with the increase in fat mass demonstrated in this study. This relationship is supported by previous

literature that reported TNF- α release by macrophages in adipose tissue (Suganami & Ogawa, 2010). Of course, cytokines such as TNF- α are released by several tissues, and may therefore have increased independently of increases in fat mass.

In summary, this is the first study to assess metabolic features during supervised exercise in breast cancer patients during treatment. Our results show that breast cancer patients present with poor metabolic markers, body composition and fitness compared to a group of healthy young females at baseline. Furthermore, these patients significantly improved their plasma glucose and IGF-1:IGFBP-3 concentrations following 24 sessions of combined aerobic and resistance training. Despite the relatively small group of participants that were evaluated in this study, our comprehensive and integrative measurements in this population, that is challenged by treatment-related issues (i.e. symptoms, scheduling etc), demonstrated improvements in various glucose parameters. Taken together, these parameters were likely related to the 24 sessions of combined aerobic and strength exercise program. These findings lay the foundation for future, larger-scale studies that may combined this type of exercise program with an appropriate nutrition intervention to prevent glucose dysregulation and potentially enhance clinical and metabolic outcomes for breast cancer survivors.

7.0 General Discussion

7.1 Improvements in breast cancer participant glucose concentrations following the exercise program are likely related to improved glucose clearance

Exercise is known to improve glucose clearance by a number of mechanisms including increased GLUT4 protein content and translocation (Holten et al., 2004; Hughes et al., 1993), increased activity of glycogen synthase (Perseghin et al., 1996), and increased muscle capillary density (Gavin et al., 2007) that lead to greater glucose uptake. In our study, another variable to consider is the fact that the majority of patients underwent treatment as well. It could therefore be possible that treatment may have contributed to improvements in plasma glucose. Since the tumour primarily requires energy through glycolysis (Gillies et al., 2008), known as the Warburg effect, it is a potential mechanism for glucose clearance. Treatment is a method for eradicating the tumour and so, it would be expected that glucose in the circulation would rise since that glucose clearance mechanism has been removed. In that case, it would be the exercise then that improved the plasma glucose concentrations in our breast cancer patients. In contrast, studies in pancreatic cancer patients have shown that glucose clearance improved up to 6 weeks post-tumour extraction (Fogar et al., 1994). Further work is required to determine the roles of the tumour, treatment and exercise in glucose clearance, specifically in breast cancer patients. Moreover, there is limited, if any, data that exist on type or regimen of treatment and effects of glucose metabolism, also meriting future study.

One methodological approach that would have aided in distinguishing the effects of treatment from the effects of exercise in this population, would be the use of a usual care group. Previous literature has demonstrated increased concentrations of insulin as well as insulin resistance (HOMA-IR) following adjuvant treatment (Guinan et al., 2014). Our study showed no

changes in insulin and a tendency to improved insulin resistance, suggesting that if a usual care group was included in our work, we may have been able to show that exercise mitigated the rise in insulin in the intended usual care group. Additionally, a retrospective study also found increased incidence of type 2 diabetes following treatment (Juanjuan et al., 2015). Taken together, it is possible that our exercise intervention not only improved plasma glucose concentrations but potentially prevented worsening of the metabolic profile that has been shown in previous studies.

We found that participants with greater adherence to the program (i.e. exercising 2 times per week for one hour and therefore, completing the program in 12 weeks), had greater improvements in plasma glucose concentrations. The days until program completion varied substantially based on patient circumstances, such as conflicts with participant medical appointments related to treatment and participants experiencing treatment-related side effects that prevented them from feeling well enough to exercise in some cases. Despite the lengthened timeframe and relatively low volume of exercise, it was still impressive to observe significant improvements in fasting glucose.

7.2 Lack of change or tendency for improvement in glucose related measures may also be a positive result

Also novel to studies examining exercise in breast cancer patients and survivors is our measure of c-peptide. As expected, we obtained similar results in both insulin and c-peptide. The measurement of c-peptide is important as it reflects insulin secretion and has a greater half-life than insulin, making it a reliable measure of insulin secretion (Bonser & Garcia-Webb, 1984).

Previous studies have also found no changes in insulin concentrations following an exercise intervention in breast cancer survivors (Fairey et al., 2003; Guinan et al., 2013; Schmitz et al., 2005). Insulin and c-peptide concentrations remaining unchanged, however, may still be positive result seeing as these measures often increase during the breast cancer trajectory (Guinan et al., 2014; Ligibel et al., 2008). Still, HOMA-IR showed improvements, suggesting that insulin resistance was lowered with exercise training. Additionally, our results show that IGF-1 decreased and IGFBP-3 did not change. Since IGF-1 is a known tumour proliferator and has been shown to increase the risk of breast cancer (Bruning et al., 1995; Peyrat et al., 1988), the decrease in IGF-1:IGFBP-3 ratio in our breast cancer participants post exercise training may be indicative of potentially preventing cancer recurrence.

Lactate, which showed a trend for decreasing following the exercise program, could also be related to improvements in glucose and related markers. Diabetics have shown higher concentrations of lactate, potentially due to adipose tissue hypoxia and decreased skeletal muscle oxidative capacity (Crawford et al., 2010). This would therefore imply that improvements seen in aerobic capacity and presumably capillary density, both helped lower lactate production. On the other hand, lactate has been reviewed and described as being used for fuel and also produced by anaerobic glycolytic tumours (Feron, 2009). Treatment reduction in tumour size or existence would therefore lead to less or no anaerobic glycolysis by the tumour and therefore could be another reason for decreases observed in lactate concentrations. A usual care group would help determine whether exercise or treatment contributed to decreases in lactate.

7.3 Body composition, inflammatory markers, physical fitness, and dietary intake

A previous study that investigated a combination of unsupervised aerobic and supervised resistance training in breast cancer survivors also found no changes in weight or body composition (Ligibel et al., 2008). However, bioelectrical impedance analysis (BIA) was used and precision with this measure is dependent on proper preparation by the participant in terms of hydration and identifying the most appropriate predictive equations; although BIA may be practical, it may not be as reliable in cancer populations compared with other healthy populations (Di Sebastiano & Mourtzakis, 2012). A study comparing aerobic training to resistance training found that aerobic training prevented a gain of fat mass and resistance training increased the amount of lean mass in breast cancer survivors (Courneya et al., 2007), as identified by DXA. Since decreases in fat mass were not seen in other study of breast cancer survivors, it is possible that the increases in fat mass in our participants is due to treatment, since the majority were still undergoing treatment, or perhaps our training protocol was not vigorous enough to elicit any changes.

Previous studies have shown that longer durations of exercise interventions result in greater weight loss and decreased adiposity (Neilson et al., 2009). Perhaps a longer duration of our exercise intervention, although potentially leading to greater challenges of participant retention in the program, would have yielded improvements in body composition. Additionally, volume, as investigated by days until program completion, did not show any significant effects on changes in body composition. It did, however, have an impact on fasting plasma glucose concentrations, showing that increased frequency of exercise sessions completed by breast cancer participants yielded greater improvements in fasting plasma glucose. The intensity of the training protocol may have shown more benefits if amplified, but then other issues such as

injuries may have become a problem. It is possible that we may have seen larger benefits with greater duration, frequency and intensity of exercise. However, our study was able to show improvements in blood measures and fitness even with a moderate intensity exercise protocol that included variable time to completion, showing that future studies should investigate varying exercise protocol durations, frequency and intensities that will provide optimal benefit to breast cancer patients. Based on our findings, even exercising sparsely (as little as one time per week based on the average days until program completion) is superior to not exercising at all.

CRP, an acute-phase inflammatory marker, has been shown to decrease the chance of survival in breast cancer patients (Pierce et al., 2009). Previous studies have found significant decreases in CRP with aerobic exercise in breast cancer survivors (Fairey et al., 2005; Guinan et al., 2013), although these differences were not significant when compared to a healthy young reference group (Guinan et al., 2013). In our case no changes were seen with exercise or between the breast cancer and healthy young reference groups. No differences were seen in IL-6, IL-8, IL-4 or IL-10 either. Perhaps no effect was seen due to a small sample size and low detection rate. Despite this, significant increases were seen in TNF- α and a trend for increases in IL-1 β . High adiposity has been shown to result in elevated TNF- α through macrophages (Suganami & Ogawa, 2010). A study examining TNF- α mRNA in obese and normal weight women found higher levels of TNF- α mRNA in the obese women than the normal weight women and this was correlated with the level of hyperinsulinemia (Hotamisligil et al., 1995). Reductions in weight of the obese females resulted in decreased TNF- α mRNA expression and improved insulin sensitivity, suggesting that TNF- α concentrations have an effect on insulin resistance (Hotamisligil et al., 1995). Others, however, have argued that TNF- α is not related to insulin resistance but rather, just fat mass (Carey et al., 2004). In our study, the change in TNF- α was

also shown to be related to changes in fat mass but not glucose, insulin, or HOMA-IR. Again, a usual care group would be beneficial in order to see what changes would have occurred without exercise. Since cytokines are produced by various cell types, cytokines can have diverse effects on metabolism – some may be beneficial and others may have detrimental effects. Thus, interpreting our results must be done with caution. Increases in pro-inflammatory cytokines have been shown in the tumour microenvironment and may increase as a result of chemotherapy treatment (Balkwill & Mantovani, 2001; Mantovani et al., 2008).

As previously found in aerobic training interventions (Courneya et al., 2007; Segal et al., 2001), our study participants showed improved aerobic capacity. A study by Kolden et al (2002) also found improvements in aerobic capacity with mixed aerobic and resistance exercise in a group-training format for breast cancer patients (where 83% were within 1 year of diagnosis). Additionally, the drop in resting systolic and diastolic blood pressure implies that there is less pressure on the arteries and that there were strength adaptations in the heart, potentially improving stroke volume and reducing heart rate. Improvements in strength were not observed consistently throughout all limbs. All limbs showed increases in strength, but significance and a trend was only found for the lower limbs. Though others utilizing purely strength training (Courneya et al., 2007; Schmitz et al., 2005) or a mixture of strength and aerobic exercise (Kolden et al., 2002) have found improvements in strength in breast cancer patients and survivors, those employing only aerobic training have not (Courneya et al., 2007). It is possible that for the limbs that did show improvements in strength, neural adaptations have taken place, allowing for increased strength without increased lean mass (Narici et al., 1989). For the limbs that did not improve in strength, neural changes may still be in progress or the intensity of the resistance training may not have been high enough to elicit significant increases in upper body

muscular strength. Based on a study by Coffey et al (2009), it is also possible that the anabolic response of resistance training was cancelled out by the aerobic training component. They suggest that performing both types of exercise concurrently is not optimal for allowing anabolic responses to take place (Coffey et al., 2009). Although all participants initiated training sessions with aerobic exercise, it is also possible that lean tissue did not increase due to the strength training focusing on muscular endurance rather than strength. Additionally, in a non-malignant population, anabolic goals may be in the forefront. However, in this clinical population of breast cancer patients, goals may not be to build muscle but rather, improve glucose metabolism. With the prescribed exercise program, that is exactly what was shown with decreases in fasting glucose concentrations and other related markers.

It is acknowledged that the study is not without limitations. First, the sample size for the study is fairly small. However, it is not unusual to have small sample sizes in cancer population studies especially ones undergoing exercise. Our data is important for setting the foundation for future, more comprehensive, work. Based on our finding, future studies will know what measures to focus. Secondly, the breast cancer patients in our study were heterogeneous in terms of age, treatment type and stage. Despite the use of a heterogeneous group and the lack of a usual care group or an age and BMI matched non-malignant group, we were still able to see improvements in a number of metabolic measures. Third, exercisers on average had a long time to completion along with low to moderate intensity and low frequency of exercise. Despite this and increases in fat mass (which may mean that energy expenditure did not change much since dietary intake remained the same), improvements in lower body muscular strength as well as resting blood pressure were seen.

There are many challenges in performing an exercise program in breast cancer patients that are undergoing treatment. For instance, the potential presence of lymphedema requires precautions to be taken in designing the exercise program. Additionally, different treatments may have diverse effects on metabolism, fatigue, pain, and appetite, all of which can affect patient motivation. It is important to ensure that the patients are exercising at a level that allows them to see metabolic benefit while also maintaining an adequate white blood cell count to avoid treatment delays. Last, but certainly not least, scheduling can be a major challenge in this patient population. Numerous medical appointments as well as acute effects of treatment felt by the participants can result in difficulties of exercise class attendance or scheduling of assessments. All of these challenges should be considered in the creation of future study designs.

8.0 Future Directions

First and foremost, the next step to this study is to recruit a group of breast cancer patients undergoing usual care and no exercise program. This will distinguish whether the results observed in this study were positively affected by exercise or by other factors. Additionally, larger scale, multicenter, studies should be conducted in the future. These studies will be expected to collect a large sample of breast cancer patients with either completely homogeneous groups or subgroups of patients in terms of age, stage of cancer and type and stage of treatment. This will help tease out potential roles of the tumour as well as treatment, while removing bias for culture, hospital policy etc.

We know that glucose clearance in breast cancer patients at the early stages of treatment, as measured by OGTT, is impaired (Bell et al., 2013). In order to get a deeper understanding of the metabolic changes that occurred with mixed aerobic and resistance training, an OGTT or, ideally, a euglycemic hyperinsulinemic clamp, should be performed. A euglycemic hyperinsulinemic clamp would be an ideal measure to include as it is the gold standard for measurement of insulin resistance (DeFronzo et al., 1979). However, an OGTT may be a more feasible start for this clinical population since this will provide us with information about glucose clearance in these patients without being a relatively large burden as compared with clamp studies.

Based on previous literature, breast cancer patients are known to develop impairments in glucose metabolism (Bell et al., 2013; Can et al., 2013; Goodwin et al., 2009; Guinan et al., 2014; Juanjuan et al., 2015; Lu et al., 2014). Literature on exact mechanisms of impaired glucose metabolism in the insulin signaling pathway is lacking in breast cancer patients. Identifying these

mechanisms as well as potential changes that happen with exercise in breast cancer patients would help our understanding of glucose metabolism impairments in this patient population. As an alternative to muscle biopsies, which are difficult to obtain, examining human muscle cells *in vitro* incubated with the blood of the participants pre and post exercise program can be used for analysis. Various insulin signaling protein expressions can be measured via western blotting. These measures will give a more comprehensive understanding of where impairments in glucose metabolism are taking place in breast cancer patients and how exercise may prevent these changes from occurring.

9.0 Conclusion

We found that breast cancer patients presented with poorer blood measures, body composition and fitness when compared to a young, healthy reference group. The exercise program completed by the breast cancer patients showed a number of positive changes including improvements in fasting plasma glucose and IGF-1:IGFBP-3 as well as HOMA-IR. Since glucose and related markers normally worsen with breast cancer treatment (Guinan et al., 2014), the findings in the present study may mean that the exercise program was able to prevent these negative outcomes. Although increases in body fat were observed, it is possible that the amount of fat gained was less than what would have been seen if we had a usual care group. The increase in fat mass was also correlated with an increase in TNF- α . Additionally, aerobic fitness improved as did lower body muscular strength despite no changes in lean mass. Dietary intake as well as macronutrient distribution remained unchanged.

To our knowledge, this is the first study to investigate the effects of a combined aerobic and resistance training program on glucose and related markers as well as body composition, inflammatory markers, fitness and nutrition in a group of breast cancer patients who primarily were still undergoing treatment. Our results show promise that the exercise program, even though only moderate in intensity and frequency, may lead to improvements in patients that may aid in preventing risk of secondary diseases.

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Appendix I Ethics clearance forms

Caryl Russell

From: ORE Ethics Application System <OHRAC@uwaterloo.ca>
Sent: Thursday, November 07, 2013 10:39 AM
To: sharratt@healthy.uwaterloo.ca; Caryl Russell; fitness@healthy.uwaterloo.ca; mnoble@healthy.uwaterloo.ca; Erin Rellinger Smith; Stephanie Thayer
Subject: Progress Report on Continuing Human Research Project Received (ORE # 15766)

Dear Researcher:

Title: A Phase III Study of the impact of a Physical Activity Program on Disease -Free Survival in Patients with High Risk Stage II or Stage III Colon Cancer: A Randomized Control Trial (CHALLENGE) ORE #: 15766
Principal/Co-Investigator: Michael Sharratt (sharratt@healthy.uwaterloo.ca)
Principal/Co-Investigator: Caryl Russell (crussell@uwaterloo.ca)
Principal/Co-Investigator: Dr. Stacey Hubay ()
Principal/Co-Investigator: Dr. Christopher Booth ()
Collaborator: Lori Kraemer (fitness@healthy.uwaterloo.ca)
Collaborator: Madeleine Noble (mnoble@healthy.uwaterloo.ca)
Collaborator: Erin Smith (ersmith@uwaterloo.ca)
Collaborator: Stephanie Thayer (s2thayer@uwaterloo.ca)

The annual progress report on Continuing Human Research for this project was received in the Office of Research Ethics in September 2013. Based on this, full ethics clearance of the corresponding continuing project is extended for another twelve-month period.

The information provided in the Progress Report has undergone ethics review through the Office of Research Ethics and is considered acceptable.

Note that submission of an annual progress report form is required for each year of the project.

Alexander Rich
Research Ethics Assistant
Office of Research Ethics

UNIVERSITY OF WATERLOO
OFFICE OF RESEARCH ETHICS

Notification of Ethics Clearance of Application to Conduct Research with Human Participants

Principal/Co-Investigator: Caryl Russell, MSc.	Department: Kinesiology
Principal/Co-Investigator: Marina Mourtzakis, PhD.	Department: Kinesiology
Principal/Co-Investigator: Michael Sharratt, PhD.	Department: Kinesiology
Collaborator: Dr. John Moule; Ms. M. Burnett, Ms. Jing Ouyang	Department: Kinesiology
Collaborator: Madeleine Noble, Lori Kraemer, Erin Smith, Stephanie Thayer	Department: Kinesiology

ORE File #: 18987

Project Title: UW WELL-FIT Exercise Program for Cancer Patients

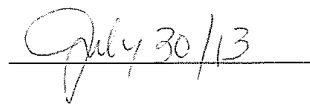
This certificate provides confirmation the above project has been reviewed and are considered acceptable in accordance with the University of Waterloo's Guidelines for Research with Human Participants and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. Thus, the project now has received ethics clearance through a University of Waterloo Research Ethics Committee.

Note 1: *This ethics clearance is valid for one year from the date shown on the certificate and is renewable annually. Renewal is through completion and ethics clearance of the Annual Progress Report for Continuing Research (ORE Form 105).*

Note 2: *This project must be conducted according to the application description and revised materials for which ethics clearance has been granted. All subsequent modifications to the project also must receive prior ethics clearance (i.e., Request for Ethics Clearance of a Modification, ORE Form 104) through a University of Waterloo Research Ethics Committee and must not begin until notification has been received by the investigators.*

Note 3: *Researchers must submit a Progress Report on Continuing Human Research Projects (ORE Form 105) annually for all ongoing research projects or on the completion of the project. The Office of Research Ethics sends the ORE Form 105 for a project to the Principal Investigator or Faculty Supervisor for completion. If ethics clearance of an ongoing project is not renewed and consequently expires, the Office of Research Ethics may be obliged to notify Research Finance for their action in accordance with university and funding agency regulations.*

Note 4: *Any unanticipated event involving a participant that adversely affected the participant(s) must be reported immediately (i.e., within 1 business day of becoming aware of the event) to the ORE using ORE Form 106. Any unanticipated or unintentional changes which may impact the research protocol must be reported within seven days of the deviation to the ORE using ORE form 107.*

<http://iris.uwaterloo.ca/ethics/form101/ad/reports/certificateB1.asp?id=24176>

7/30/2013

Maureen Nummelin, PhD
Director, Office of Research Ethics

Date

OR
Susanne Santi, MMath
Senior Manager, Research Ethics

OR
Julie Joza, MPH
Manager, Research Ethics

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**JOINT RESEARCH ETHICS COMMITTEE
 CERTIFICATE OF APPROVAL TO CONDUCT PROPOSED STUDY AT
 GRAND RIVER HOSPITAL
 AND ST. MARY'S GENERAL HOSPITAL**

A Joint Research Ethics Committee composed of:

VOTING MEMBERS	OCCUPATION
MEETING of September 5th, 2001	
Nancy Martin, Ph.D.	Chair, Joint Research Ethics Committee
Joy Bent	Community Member
Marion Bramwell	Vice president Patient Services/Chief Nursing Officer
Carolyn Campbell	Oncologist
Michael Coughlin	Bioethicist
Glen Kearns	Chief Information Officer
Greg Knight	Oncologist
Paul Motz	Community Member
Vicki Sills	Pharmacist
Erin Tjam	Director Research

has reviewed the application for research study **Exercise and Wellness Program for Cancer Patients – A Pilot Project – University of Waterloo with Principal Investigator as Dr. Mike Sharratt** and is considered acceptable by the Joint Research Ethics Committee of Grand River Hospital and St. Mary's General Hospital. This approval is valid for one year.

Approval is granted to conduct the research project in accordance with the protocol specified in the application.

We wish to advise the Joint Research Ethics Committee operates in compliance with ICH Good Clinical Practice Guidelines and the Tri-Council Policy Statement.

Requirements for ongoing approval:

- a) Annual review of the submission will be undertaken by the JREC
- b) All significant adverse events experienced by subjects enrolled in the trial must be reported to the JREC as outlined in the letter to the Investigator.
- c) Any changes in the protocol, information sheets, questionnaires, or informed consent documents must be reported to the Chair, JREC immediately.
- d) Upon completion of the study, the JREC must be notified in writing, given a short summary of the progress of the trial (e.g. number of patients enrolled, problems encountered, etc.) and a full report of study results.
- e) The final report on the study is to be provided to the JREC within three months of study completion.


 Nancy Martin, Ph.D.
 Chair, Joint Research Ethics Committee
 c/o Research, Grand River Hospital

Date Oct 31, 2001

ORE Ethics Application System

To: Marina Mourtzakis, lbos@uwaterloo.ca
Cc: a2milburn@uwaterloo.ca, s9schmidt@uwaterloo.ca
Ethics Clearance of Modifications, no comments (ORE # 20518)

May 4, 2015 at 10:36 AM

[Hide Details](#)

[Inbox - connect.uwaterloo.ca](#)

Dear Researcher:

A Request for ethics review of a modification or amendment (ORE 104) to your ORE application:

Title: Evaluation of body composition & metabolism of healthy females
ORE #: 20518
Principal/Co-Investigator: Marina Mourtzakis, PhD (mmourtzakis@uwaterloo.ca)
Collaborator: Lisa Bos (lbos@uwaterloo.ca)
Student Investigator: Amanda Milburn (a2milburn@uwaterloo.ca)
Student Investigator: Schuyler Schmidt (s9schmidt@uwaterloo.ca)

together with a copy of relevant materials, was received in the Office of Research Ethics on:
04/30/2015 - CIHR Funding Period 2010-2016

The proposed modification request has been reviewed and has received full ethics clearance.

Note 1: This project must be conducted in accordance with the description in the application and modification for which ethics clearance has been granted. All subsequent modifications to the protocol must receive prior ethics clearance through the Office of Research Ethics.

Note 2: Researchers must submit a Progress Report on Continuing Human Research Projects (ORE Form 105) annually for all ongoing research projects. In addition, researchers must submit a Form 105 at the conclusion of the project if it continues for less than a year.

Note 3: Any events related to the procedures used that adversely affect participants must be reported immediately to the ORE using ORE Form 106.

Nick Caric
Research Ethics Administrative Coordinator
Office of Research Ethics
Needles Hall 1024
519.888.4567 ext. 35217
ncaric@uwaterloo.ca

Appendix II Detailed methodological descriptions of blood analysis

Table 1 Fasting metabolic measures assessed

Glucose Metabolism	Inflammatory Markers
Glucose	TNF- α
Insulin	IL-6
C-peptide	IL-4
HbA1c	IL-8
IGF-1	IL-10
IGFBP-3	IL-1 β
Lactate	CRP

Biochemical Analysis

Whole blood was given 30 minutes to clot at room temperature. The blood was then rimmed with a plain wood applicator and centrifuged at room temperature for 15 minutes at 2500 rpm. Following centrifugation, the serum was extracted, aliquoted into separate eppendorf tubes to avoid multiple thaw/re-freeze cycles, and stored at -80° C until the day of further analysis. Serum was collected for insulin, c-peptide, HbA1c, IGF-1, IGFBP-3, lactate, TNF- α , IL-6, IL-4, IL-8, IL-10, IL-1 β and CRP.

Plasma glucose

Using an accu-check aviva glucose meter (Roche Diagnostics, Mississauga, ON), plasma glucose was measured from fresh whole blood samples. The kit used electrochemical techniques to determine the amount of plasma glucose. To do this, a drop of fresh whole blood collected by finger prick was applied to the sampling area of the test strip which was inserted in the glucose meter. From there, the blood moved up the tube via capillary action and then underwent chemical reactions with the enzyme on the strip (Mut. Q-GDH from *Acinetobacter calcoaceticus*,

recombinant in *E. coli*) to convert glucose to gluconolactone. The chemical reaction creates an electrical current that runs up the electrodes in the strip to the glucose meter where the amount of blood glucose is calculated based on the strength of the current (Evaluation Report, Roche Diagnostics, 2011). Values are calculated and converted to plasma glucose and readings of this value are given on the glucose meter display.

Serum insulin

Using an Insulin Specific Radioimmunoassay kit (Millipore; Etobicoke, ON), serum insulin was measured in duplicate. 100 μL of standard, quality controls or sample was added to 12x75mm glass tubes. 100 μL of ^{125}I -labeled insulin was added to all tubes followed by 100 μL of human insulin antibody. The tubes were vortexed and incubated for 20-24 hours at room temperature. During the 24 hour incubation, ^{125}I -labeled insulin competed with insulin in the standard or sample for binding sites on the insulin antibody molecules. Following the incubation period, 1.0 mL of cold (4°C) precipitating reagent was added to each tube. Tubes were then centrifuged for 20 minutes at 2,000-3,000 xg in order to separate the antibody-bound tracers from the free tracers and supernatant was aspirated from the tubes. The tubes were left with a small pellet, which was counted for radioactivity for 1 minute using a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences; Woodbridge, ON). A high concentration of insulin in the sample or standard is less radioactive as it will bind less ^{125}I -labeled insulin.

Serum c-peptide

Using a Human C-Peptide Radioimmunoassay kit (Millipore; Etobicoke, ON), serum c-peptide was assessed in duplicate. 100 μL of standard, quality controls or sample was added to 12x75mm glass tubes. 100 μL of ^{125}I -labeled c-peptide was added to all tubes followed by 100 μL of human c-peptide antibody. The tubes were vortexed and incubated for 20-24 hours at 4°C . During the 24 hour incubation, ^{125}I -labeled c-peptide competed with c-peptide in the standard or sample for binding sites on the c-peptide antibody molecules. Following the incubation period, 1.0 mL of cold (4°C) precipitating reagent was added to each tube. Tubes were then centrifuged for 20 minutes at 2,000-3,000 xg in order to separate the antibody-bound tracers from the free tracers and supernatant was aspirated from the tubes. The tubes were left with a small pellet, which was counted for radioactivity for 1 minute using a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences; Woodbridge, ON). A high concentration of c-peptide in the sample or standard is less radioactive as it will bind less ^{125}I -labeled c-peptide.

Glycated Hemoglobin (HbA1c)

Using an A1CNow⁺ kit (Bayer Healthcare LLC, Sunnyvale, CA), glycated hemoglobin was measured from fresh whole blood samples. The kit used immunological reactions to determine the amount of glycated hemoglobin and a chemical reaction to determine total hemoglobin. Using these values, the percentage of glycated hemoglobin was calculated. For the assay, the blood collector provided with the kit used 5 μL of whole blood. Once blood was in the line, the blood collector was placed together with the manufacturer sampler body and shaken in order to dilute the sample properly. The diluted sample was then combined with the test cartridge where blue microparticles bound to the anti-HbA1c antibodies and moved along

reagent strips until they reached the detectors. The concentration of blue microparticles present on the strip is equal to the total HbA1C in the sample. The sample diluent underwent a reaction with the hemoglobin present in the sample. This converted hemoglobin to methemoglobin, which was red-brown in colour. The concentration of the red-brown colour was measured on the reagent strips and was representative of the concentration of hemoglobin. Finally, the monitoring device provided a reading of the glycosylated hemoglobin percentage.

Serum IGF-1

Using a commercially available human IGF-1 Quantikine ELISA kit (R&D Systems, Minneapolis, MN), IGF-1 was measured. Prior to starting the assay, samples were pre-treated for release of IGF-1 from its binding protein and diluted 100-fold. The assay required 150 μ L of Assay Diluent RD1-53 to be added to each well followed by 50 μ L of sample, standard and control to be added to the wells coated with a monoclonal antibody specific for IGF-1. The plate was then incubated for 2 hours at 2-8° C. To remove all unbound substances from the plate, the plate was washed. Next, 200 μ L of cold IGF-1 Conjugate was added to each well and the plate was incubated for 1 hour at 2-8° C. Again, the plate was washed and then, 200 μ L of substrate solution, which bound to each IGF-1 polyclonal, was added to each well. The plate was then incubated for another 30 minutes at room temperature, protected from light. 50 μ L of stop solution was added to stop the substrate reaction in each well. The end result was the formation of a yellow colour, which could be read at 450 nm, 540 nm or 570 nm. The concentration of IGF-1 in the sample was directly proportional to the total signal.

IGFBP-3

IGFBP-3 was measured using a commercially available human IGFBP-3 Quantikine ELISA kit (R&D Systems, Minneapolis, MN). To start, samples were diluted 100-fold. 100 μ L of Assay Diluent RD1-62 was added to each well followed by 100 μ L of sample, standard and control to be added to the wells coated with a monoclonal antibody specific for IGFBP-3. The plate was then be incubated for 2 hours at 2-8 $^{\circ}$ C. To remove all unbound substances from the plate, the plate was washed. Next, 200 μ L of cold IGFBP-3 Conjugate was added to each well and the plate was incubated for 2 hours at 2-8 $^{\circ}$ C. Again, the plate was washed and then, 200 μ L of substrate solution, which bound to each IGFBP-3 polyclonal, was added to each well. The plate was then incubated for another 30 minutes at room temperature, protected from light. 50 μ L of stop solution was added to stop the substrate reaction in each well. The end result was the formation of a yellow colour, which could be read at 450 nm, 540 nm or 570 nm. The concentration of IGFBP-3 in the sample was directly proportional to the total signal.

Lactate

Prior to performing the lactate assay, samples needed to undergo Perchloric Acid (PCA) Extraction. Extraneous proteins that may inhibit the detection of metabolites such as lactate, are found in serum. In order to eliminate these extra proteins, a solution of 0.6 M perchloric acid was be made by mixing perchloric acid stock solution and water together. Following this, 100 μ L of serum was combined with 500 μ L of 0.6M perchloric acid in an eppendorf tube.

Throughout the procedure, all tubes and solutions were kept on ice. The tubes were vortexed and then centrifuged for 2 minutes at 4 $^{\circ}$ C at 15 000g. Following this, 250 μ L of 1.25 M potassium bicarbonate was added to each tube which were then incubated on ice for 10 minutes and centrifuged once again. The resulting supernatant was transferred to new eppendorf tubes and

stored in the -80 °C freezer. With the PCA extraction, a dilution factor of 8.5 was introduced and this was taken into account when performing calculations of final lactate concentrations.

Following the PCA extraction, serum lactate was measured using a spectrofluorometric assay. To start, a reagent solution was made by combining 15 mL of hydrazine, 15 mL glycine, and 1500 μL of NAD^+ , which was then brought to a volume of 150 mL with distilled water and a pH of 10.0 was achieved. Next, 250 μL of lactate dehydrogenase (Sigma-Aldrich, St. Louis, MO) was combined with 1 mL of the reagent in order to dilute the 5200 U/mL lactate dehydrogenase. Subsequently, 25 μL of dilute blank, standard, and sample was added to the glass test tubes in triplicate. 1 mL of dilute reagent was added to each test tube and was vortexed. With the absorbance set between 365 nm and 455 nm on the spectrofluorometer (RF-1501; Shimadzu, Columbia, MD), baseline readings were taken. Following baseline readings, 25 μL of dilute lactate dehydrogenase was added to each test tube. Next, all tubes were vortexed and incubated for 120 minutes in the dark. A final reading was completed of each tube and then, the baseline readings were subtracted from the final reading in order to determine the final absorbency. Lactate present in the tubes during incubation reacted with NAD^+ to form NADH and pyruvate by the lactate dehydrogenase enzyme. Pyruvate then reacted with the hydrazine found in the reagent which lead to the end of the reaction.. The reduction of NAD^+ caused NADH to fluoresce and this is directly proportional to the concentration of lactate in each sample.

Serum TNF- α , IL-1 β , IL-6, IL-4, IL-8, IL-10

Using the BD Cytometric Bead Array Human Soluble Protein Master Buffer Kit and BD FACSCalibur flow cytometer (BD Biosciences; Mississauga, ON), serum cytokines were

analyzed. Five types of capture beads coated with an antibody specific to TNF- α , IL-6, IL-1 β , IL-4, IL-8 or IL-10 were provided in a kit. Every bead had a corresponding detection reagent and this fluoresced at a specific activity. Before starting the assay, all beads were put into one tube labelled 'Mixed Capture Beads' and then vortexed. Detection reagents were all put into a single tube labelled 'Mixed Detection Reagents'. Lastly, the serum samples were diluted to a 1:4 ratio with serum diluent (from the manufacturer).

For the assay, 50 μ L of each standard or diluted sample was added to their corresponding tubes. A volume of 50 μ L of the Mixed Capture Beads was added to each tube and they were then vortexed and incubated for one hour at room temperature. While incubating, the beads matched the cytokine antibodies and formed. Following incubation, 50 μ L of Mixed Detection Reagents was added to each tube, followed by vortexing each tube and then incubating them at room temperature for two hours. During incubation, a sandwich structure was formed by the detection reagent specific to each bead connecting with the bead/cytokine complex. Following incubation, each tube had 1 mL of wash buffer added to it, was vortexed and centrifuged for five minutes at 200g. Next, the supernatant was aspirated and the remaining pellet was re-suspended with 300 μ L wash buffer and finally, attained on the flow cytometer. Each of the bead/cytokine/detection reagent complexes had its own fluorescence. Concentrations of each cytokine was determined by comparing the mean fluorescence of the populace to the standard curve for each cytokine.

Serum C-Reactive Protein (CRP)

Using an enzyme-linked immunoassay (ELISA) (R&D Systems, Minneapolis, MN), serum CRP was measured. The ELISA plate wells were pre-coated with monoclonal antibody

specific for human CRP. Following addition of 100 μ L of Assay Diluent RD1F to each well, 100 μ L of standard, control or sample (diluted 100-fold) was added and the plate was incubated for 2 hours at room temperature. In this time, CRP in the standards or samples is bound to the antibody-coated wells. The plate was then washed to remove all unbound substances and each well had 200 μ L of CRP Conjugate added to it. The plate was then be incubated for 2 more hours during which time, the immunoconjugate was bound to the CRP-antibody complex attached to the wells. Following this incubation, the plate was washed 200 μ L of Substrate Solution was added to each well. In the dark, the plate underwent a final incubation at room temperature for 30 minutes. 50 μ L of stop solution was added to stop the substrate reaction in each well. The end result was the formation of a yellow colour which could be read at 450 nm. The concentration of CRP in the sample was directly proportional to the amount of colour produced.

Appendix III 3-day food diary

3-Day Food Diary

Patient ID: _____

Record Dates: DAY 1: _____ (DD/MM/YY)

DAY 2 : _____ (DD/MM/YY)

DAY 3 : _____ (DD/MM/YY)

University of Waterloo

Department of Kinesiology

Instructions for Recording Daily Food Intake

Your food diary will provide us with information on what you eat and drink during a 3-day period –specifically, it will help us determine the types of food and drinks, number of calories, amount of protein, carbohydrates, fats, and other nutrients that you consume.


Instructions on how to record your information are provided below. A ‘**Sample Day: Evening Meal**’ sheet is also provided for your reference.

A) FOOD AND BEVERAGE ITEMS

- List **all** the foods and beverages you consumed
- Record the specific type of food (i.e. **Whole grain** bread, **Shredded Wheat** cereal)
- Record any items that were added in the same column (i.e. butter, jam, sugar, gravy, milk, etc.)

B) DESCRIPTION OF ITEM

For each food or beverage item, include the following where applicable:

- BRAND: **Heinz** ketchup, **Chips Ahoy** cookie,  **McCain’s** French fries
- TYPE OF FLAVOUR: **Vanilla** ice cream, **Banana** muffin
- METHOD OF COOKING: **Fried** rice, **Baked** fish, **BBQ’d** chicken
- ALL RELEVANT INFORMATION ON FOOD LABEL: **Low fat** mayonnaise, **28% M.F.** marble cheese, **Lean** ground beef

C) UNIT OF MEASURE/ NUMBER OF UNITS

- For each food or beverage item (including toppings and/or items added), record the unit of measure & the number of units consumed:



D) Examples: **1 cup** of milk, **2 tablespoons** of garlic, **1 ½ ounces** of chicken, **1 piece** of cake, **1 teaspoon** of sugar

FILL IN BLANKS

Please also fill in the blanks at the bottom of the record. You will need to indicate:

- Time of your meal or snack
- Where your meal or snack was eaten (i.e. a restaurant, at home)
- If you did not eat a meal or snack, please acknowledge this by placing a check mark (✓) in the appropriate blank.

RECORD ALL FOODS!

ALL foods (including toppings) and beverages you consume are important, whether it be a glass of water or a full course meal!



PLEASE REMEMBER:

1. At the end of each ‘Day’ section, please remember to complete the questions that request you to compare that given day with your normal diet.
2. Also, do not forget to fill out the last 2 pages on supplements that you are currently taking and the ‘Patient Generated Subjective Global Assessment’ questionnaire.
2. Be as accurate as possible. **Please do not change your normal eating habits** for the 3 days that you are recording your food intake. Your **honesty is crucial to the success of this research study.**

SAMPLE DAY: EVENING MEAL (Dinner)

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<i>For every item, please indicate the unit of measure used and the number of units consumed.</i>
Spaghetti with tomato sauce and meatballs		
Pasta	Barilla Pasta Plus spaghetti, cooked	2 cups
Tomato Sauce	Organic Ragu sauce (Traditional flavour)	1 cup
Meat Balls	Made with President's Choice extra lean ground beef	5 (1oz/ball)
Parmesan cheese	Kraft, 30% Milk Fat (M.F.)	1 tablespoon
Cheesy Garlic Bread	McCain's garlic fingers (with cheese)	2 pieces
Caesar Salad		
Lettuce	Romaine	1 cup
Croutons	Pepperidge Farm classic cut seasoned croutons	2 tablespoons
Salad Dressing	Kraft, Ranch, Fat Free	2 tablespoons
Orange Juice	Tropicana, No Pulp, 100% Vitamin C	1 cup
Cheesecake	President's Choice Red Velvet cheesecake	1/10 th of cake
Coffee	Gevalia Black Iced Coffee	1 cup

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 1: MID-MORNING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i>	<i>Remember to include details such as:</i> <ul style="list-style-type: none">▪ Brand Name▪ Flavour▪ Method of Cooking▪ All other relevant information on food/ drink label	<i>For every item, please indicate the unit of measure used and the number of units consumed.</i>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 1: MID-DAY MEAL (Lunch)

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES	
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>	

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 1: MID-AFTERNOON SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 1: EVENING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (√) if you did not eat or drink at this meal or snack time: _____

DAY 1 MEALS

Compared to my normal diet, I ate:

- The same amount as I would usually eat
- More than I would usually eat
- Less than I would usually eat



Please circle how you felt today for each of the symptoms below:

0 1 2 3 4 5 6 7 8 9 10

Best Appetite

Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10

Not Nauseated

Worst Possible Nausea

0 1 2 3 4 5 6 7 8 9 10

Not Tired

Worst Possible Tiredness

DAY 2: MORNING MEAL (Breakfast)

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____ Location meal/snack was consumed:

Please CHECK (✓) if you did not eat or drink at this meal or snack time: ____

DAY 2: MID-MORNING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed.</i> <i>For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 2: MID-AFTERNOON SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____ Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 2: EVENING MEAL (Dinner)

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed.</i> <i>For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (√) if you did not eat or drink at this meal or snack time: _____

DAY 2: EVENING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 2 MEALS

Compared to my normal diet, I ate:

- The same amount as I would usually eat
- More than I would usually eat
- Less than I would usually eat



Please circle how you felt today for each of the symptoms below:

0 1 2 3 4 5 6 7 8 9 10

Best Appetite

Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10

Not Nauseated

Worst Possible Nausea

0 1 2 3 4 5 6 7 8 9 10

Not Tired

Worst Possible Tiredness

DAY 3: MID-MORNING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (✓) if you did not eat or drink at this meal or snack time: _____

DAY 3: EVENING MEAL (Dinner)

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i>	<i>Remember to include details such as:</i> <ul style="list-style-type: none"> ▪ <i>Brand Name</i> ▪ <i>Flavour</i> ▪ <i>Method of Cooking</i> ▪ <i>All other relevant information on food/ drink label</i> 	<i>For every item, please indicate the unit of measure used and the number of units consumed.</i>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed: _____

Please CHECK (√) if you did not eat or drink at this meal or snack time: _____

DAY 3: EVENING SNACK

FOOD AND BEVERAGE ITEMS	DESCRIPTION OF ITEM	NUMBER OF UNIT MEASURES
<p><i>Include ALL food and beverage items consumed. For combination foods, please remember to list each item.</i></p>	<p><i>Remember to include details such as:</i></p> <ul style="list-style-type: none"> ▪ Brand Name ▪ Flavour ▪ Method of Cooking ▪ All other relevant information on food/ drink label 	<p><i>For every item, please indicate the unit of measure used and the number of units consumed.</i></p>

FILL IN THE BLANKS:



Time of meal/snack: _____

Location meal/snack was consumed:

Please CHECK (✓) if you did not eat or drink at this meal or snack time: ____

DAY 3 MEALS

Compared to my normal diet, I ate:

- The same amount as I would usually eat
- More than I would usually eat
- Less than I would usually eat



Please circle how you felt today for each of the symptoms below:

0 1 2 3 4 5 6 7 8 9 10

Best Appetite

Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10

Not Nauseated

Worst Possible Nausea

0 1 2 3 4 5 6 7 8 9 10

Not Tired

Worst Possible Tiredness

Appendix IV 3-day food diary results

Table 1 Dietary intake

	Baseline of breast cancer dropouts (n=7)	Baseline of breast cancer exercise completers (n=15)	Post-assessment of breast cancer exercise completers (n=15)	Young, Healthy Females (n=10)
Caloric Intake (kcal)	1885 ± 328	1718 ± 358	1729 ± 410	2262 ± 578
Protein (%)	18 ± 2	17 ± 3	17 ± 4	20 ± 6
Fat (%)	35 ± 4	34 ± 6	34 ± 8	31 ± 9
Carbohydrate (%)	49 ± 3	51 ± 7	50 ± 9	52 ± 8

Appendix V Power calculations

Table 1 Power and sample size calculations for changes seen in glucose and related measures

Measure	Mean change from baseline to post-exercise program \pm SD (n=15)	Power	Sample size required to achieve 80% power
Plasma glucose (mM)	-0.4 \pm 0.5	0.822	15
Insulin (uIU/mL)	-2.1 \pm 5.2	0.306	51
C-peptide (ng/mL)	-0.3 \pm 2.2	0.087	324
HOMA-IR	-0.7 \pm 1.4	0.472	31
IGF-1 (ng/mL)	-21.6 \pm 41.0	0.475	31
IGFBP-3 (ng/mL)	8.8 \pm 61	0.082	381
IGF-1:IGFBP-3	-0.61 \pm 0.9	0.704	19
Lactate (mM)	-0.8 \pm 1.5	0.460	32

Table 2 Power and sample size calculations for changes seen in body composition measures

Measure	Mean change from baseline to post-exercise program \pm SD	Power	Sample size required to achieve 80% power
Weight (kg)	0.7 \pm 2.6 (n=15)	0.162	113
BMI (kg/m ²)	0.2 \pm 0.9 (n=15)	0.122	159
WC (cm)	-1.1 \pm -4.5 (n=15)	0.146	130
Appendicular lean muscle mass (kg/m ²)	-0.1 \pm 0.4 (n=14)	0.149	117
% fat	1.7 \pm 2.7 (n=14)	0.570	23
Total fat mass (kg)	1.5 \pm 2.6 (n=14)	0.529	25
Total lean mass (kg)	-0.9 \pm 2.0 (n=14)	0.351	40
Total lean mass in limbs (kg)	-0.2 \pm 1.0 (n=14)	0.081	356

Table 3 Power and sample size calculations for changes seen in fitness measures

Measure	Mean change from baseline to post-exercise program \pm SD (n=14)	Power	Sample size required to achieve 80% power
Right leg extension (Nm)	7.2 \pm 14.2	0.422	33
Left leg extension (Nm)	14.7 \pm 22.4	0.620	21
Right & left leg extension (Nm)	24.1 \pm 34.9	0.666	19
Right arm flexion (Nm)	2.3 \pm 5.0	0.349	41
Left arm flexion (Nm)	2.3 \pm 6.6 (n=13)	0.280	69
Right and left arm flexion (Nm)	4.5 \pm 10.4 (n=13)	0.303	44
Predictive VO ₂ (mL/kg/min)	2.4 \pm 3.3 (n=13)	0.654	18
Resting heart rate (bpm)	-1.4 \pm 9.4	0.083	340
Systolic blood pressure (mmHg)	-6.0 \pm 6.6	0.867	12
Diastolic blood pressure (mmHg)	-4.3 \pm 8.7	0.401	35

Table 4 Power and sample size calculations for changes seen in inflammatory markers

Measure	Mean change from baseline to post-exercise program \pm SD	Power	Sample size required to achieve 80% power
CRP (mg/L)	0.01 \pm 0.19 (n=15)	0.051	12099
TNF- α (pg/mL)	1.2 \pm 0.9 (n=7)	0.853	7
IL-8 (pg/mL)	2.1 \pm 4.4 (n=13)	0.361	36
IL-6 (pg/mL)	1.8 \pm 5.3 (n=7)	0.117	73
IL-1 β (pg/mL)	0.9 \pm 1.3 (n=8)	0.385	19
IL-4 (pg/mL)	0.5 \pm 1.3 (n=7)	0.156	47
IL-10 (pg/mL)	-0.1 \pm 1.4 (n=13)	0.058	1197

Table 5 Power and sample size calculations for changes in dietary measures

Measure	Mean change from baseline to post-exercise program \pm SD (n=15)	Power	Sample size required to achieve 80% power
Caloric intake (kcal)	11 \pm 265	0.052	5027
Protein (%)	-0.6 \pm 5.3	0.070	596
Fat (%)	-0.5 \pm 7.4	0.058	1419
Carbohydrate (%)	-0.2 \pm 9.0	0.051	17287