

The effect of exercise intensity on insulin levels in postmenopausal breast cancer survivors

by

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Abstract

Higher serum levels of glucose, C-peptide and insulin have been linked to poorer breast cancer prognosis and fluctuations of these substances resulting from acute bouts of exercise in breast cancer survivors have not been studied. In this pilot study, 28 postmenopausal women were in 3 groups: controls (n = 10), aromatase inhibitor users (AI, n = 8) and breast cancer survivors not using aromatase inhibitors (BCS, n = 10). Fasting glucose, C-peptide and insulin levels were compared and changes in these substances with acute exercise of different intensities were explored. VO_2peak values were determined and fasting blood samples were obtained. Blood was collected before, immediately after, and 45 minutes after 2 exercise bouts: moderate (45 minutes at 60% VO_2peak) and intense (10 minutes at 85% VO_2peak). Fasting glucose, C-peptide and insulin values, plus HOMA-IR values, were homogeneous between groups. Exercise intensity affected glucose, C-peptide and insulin levels similarly in all 3 groups. For the breast cancer survivor groups combined (n = 19), the pre-post changes and post-recovery changes of glucose and C-peptide were significantly different between the moderate and intense bouts (glucose: p = 0.01 and 0.01; C-peptide: p = 0.04 and 0.04, respectively) showing greater increases in glucose and C-peptide during intense exercise. The pre-post change in insulin approached a significant difference between intensities (p = 0.09) showing a greater increase during intense exercise. Of importance, glucose, C-peptide and insulin levels all transiently increased with intense exercise in breast cancer survivors. In conclusion, more research is warranted on the possible detrimental effects of transiently high glucose, C-peptide and insulin levels induced by intense exercise on breast cancer etiology and prognosis.

Preface

Sherry Hunt created the research proposal and protocol, recruited subjects and scheduled research sessions, led the research sessions with subjects, assisted with blood collection and analysis, compiled results, performed some statistical analyses, interpreted results and wrote the consent forms and thesis.

Don McKenzie obtained ethical approval for the research, provided the facilities and equipment for the research sessions, supervised the research sessions and collected blood samples, and provided guidance and editing in writing the proposal, protocol and thesis.

Daniel Holmes provided his expertise in measuring blood components. Serum C-peptide and insulin levels were measured in his laboratory by professional technicians under his supervision.

Ethical approval was provided by the Clinical Research Ethics Board of the University of British Columbia, approval ID H09-02619.

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List of Abbreviations

AI: Aromatase inhibitors, also refers to the survivor group taking this medication

BCS: Breast cancer survivor group (not taking aromatase inhibitors)

VO₂peak : peak oxygen consumption, a measure of cardiovascular fitness

BMI: Body mass index

PAR-Q: Physical Activity Readiness Questionnaire

HRmax: maximum heart rate obtained during the VO₂peak test

HOMA-IR: Homeostatic Model Assessment, a measure of insulin resistance

Pre, pre-exercise: refers to measurements collected before exercise has begun.

Post, post-exercise; refers to measurements collected immediately after an exercise session has ceased

45 post, 45post-exercise, post-recovery: refers to measurements collected 45 minutes after an exercise session has ceased

GLM: General Linear Model

RR: Relative risk

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Chapter 1: Introduction

Introduction

This review focuses on how exercise intensity may affect glucose and insulin levels, why this might be relevant to breast cancer survivors, and why it is justified to explore aromatase inhibitor use and insulin resistance. Literature has been separated into sections: the glucose and insulin system; hyperinsulinemia; mechanisms linking cancer with insulin/glucose; clinical studies linking cancer and glucose/insulin (risk, prognosis and treatment); exercise and insulin, exercise and cancer; breast cancer survivors, exercise and insulin; estrogen and insulin; estrogen and exercise; and, aromatase inhibitors for cancer therapy, insulin and exercise. Thoughtful analysis of scientific literature allows researchers to find inspiration in existing knowledge; interdisciplinary research requires an understanding of multiple topics, and the ability to unite meaningful results into paths for future research.

Glucose and Insulin System Overview

The pancreatic β -cells produce insulin, a hypoglycemic hormone. Primarily, beta cells are stimulated to release insulin upon elevated blood sugar levels, although increased plasma levels of amino acids and fatty acids also trigger its release, as does the release of acetylcholine by parasympathetic nerve fibers. As blood levels of nutrients drop insulin secretion is suppressed. Also, hyperglycemic hormones which increase blood glucose levels indirectly stimulate insulin release (1).

In normal individuals, glucose levels remain between 4-7 mM due to the regulation of intestinal glucose absorption, hepatic production, and metabolic uptake by peripheral tissues. Insulin primarily regulates blood glucose levels by increasing glucose uptake in muscle and fat and inhibiting hepatic glucose production. Also, insulin stimulates cell growth and differentiation, plus promotes substrate storage within fat, muscle and liver by instigating the synthesis of fat, glycogen and protein (2). Glucose uptake in cells is increased by insulin stimulated translocation of the glucose transporter GLUT4 from within the cell to the cell surface. Insulin binding to its receptor on the cell surface signals a chain of events leading

to the vesicles containing GLUT 4 transporters to fuse with the cell membrane, allowing GLUT4 to usher glucose into the cells. Skeletal muscle receives up to 75% of the insulin-dependent glucose disposed of from the blood, whereas fat receives only a small amount, and the liver receives none. Yet, in the liver insulin blocks glycogenolysis and gluconeogenesis, and also stimulates glycogen synthesis thereby regulating fasting glucose levels (2).

In the fasted state the main glucoregulatory action of insulin is restraining hepatic glucose production. Postprandial glucose levels stimulate glucose utilization; carbohydrate ingestion raises insulin levels while decreasing levels of glucagon, the primary hyperglycemic hormone. Interestingly, insulin levels are signaled to rise from hormones in the intestinal tract, and the disposal of glucose during its absorption prevents hyperglycemia (3). After an overnight fast, the rates of glucose production and utilization are equal, averaging 2.2 mg/kg/min, allowing plasma glucose concentrations to remain stable. After a meal the rate of glucose being delivered into the circulation can be more than double the rate of post-absorptive endogenously produced glucose. Endogenous glucose production is suppressed during exogenous glucose absorption, and glucose use by the liver, muscle and fat is increased thereby assimilating exogenous glucose and returning plasma glucose levels to the approximate fasting state (3). Postprandial hyperglycemia is determined by factors such as meal timing, quantity, composition and carbohydrate content, plus the resulting secretion of insulin and suppression of glucagon (3). Mixed meals containing amino acids provoke greater insulin secretion than glucose alone. Normal fasting plasma glucose level is 5.1 mmol/L, and 2-h plasma glucose concentration after drinking a 75 gram glucose load is 5.4 mmol/L (compared to >7 and > 11.1 respectively for those diagnosed with glucose intolerance and diabetes, respectively).

Hyperinsulinemia

Insulin resistance occurs when cells do not respond well to insulin, thus glucose cannot effectively enter muscle and liver cells. In response to low levels of glucose in the cells, the liver releases more stored

glucose into the blood, and increased blood glucose levels stimulate the pancreas to secrete more insulin. This results in hyperglycemia and hyperinsulinemia (4). Insulin resistance is linked to obesity, inactivity, and diet. Hyperglycemia creates an environment in which uncontrolled glucose oxidation creates free radicals, which damage proteins, fats and DNA. These destructive actions are thought to impede insulin action. Although there is a continuum for insulin resistance severity, those affected show elevated fasting glucose, hyperglycemia and a reduction of insulin action. Insulin resistance is a precursor of type 2 diabetes(5)and is linked to cardiovascular disease (6). It is also linked to some cancers, including breast cancer(7). Insulin resistance is also one of several components of the metabolic syndrome, which predisposes individuals to type 2 diabetes, cardiovascular disease, and some cancers(7).

Possible Mechanisms Linking Cancer with Insulin and Glucose

Insulin resistance increases risk for cancers of the breast and moderately increased glucose levels have been indicated as a risk of breast cancer (8). During uncompensated hyperinsulinemia there is also hyperglycemia. Elevated serum glucose helps increase DNA synthesis of tumour cells and provokes free radical production disrupting DNA and its repair mechanisms. Hyperglycemia also leads to glycated protein structures that enhance production of free radicals, cytokines and growth factors (9). As reviewed by Dang and Semenza (10), the hypoxic environment in solid tumours has allowed for the adaptation of enhanced glucose use via glycolysis and high rates of cellular glucose uptake. This glucose uptake correlates with tumour aggressiveness and prognosis. In addition, GLUT1 expression is increased in cancer cells. Insulin independently stimulates a protein associated with hypoxia (HIF-1) which elevates the expression of genes involved with angiogenesis and glucose transport (VEGF and GLUT-1)(11). This promotes survival of cancer cells in their hypoxic environment. It seems that hyperglycemia helps cancer cells thrive by fueling glycolysis; hyperinsulinemia and hypoxia independently invoke a pathway leading to angiogenesis and increased glucose transport into cells. If this is the case, the high levels of glucose

and insulin in pre-diabetes provide a perfect microenvironment for cancer progression. Importantly, glucose deprivation causes apoptosis in cancerous cells relying on glucose for energy. Together, this implies that starving cancer of glucose kills cancer cells while limiting insulin reduces the ability of a tumour to recruit new blood cells, so reducing cancer exposure of glucose and insulin may limit cancer progression. Yet, studies correlating hyperglycemia with cancer might be masking hyperinsulinemia as a main factor. Of relevance, tumour growth is reduced in the insulin deficient setting of type 1 diabetes model regardless of hyperglycemia (12). Despite the glucose requirements of cancer cells, meta-analyses of related studies do not consistently support that reducing glucose levels in those with hyperglycemia reduces cancer risk (13). In diabetics, lifestyle or pharmacological interventions increasing insulin sensitivity may influence cancer risk, independently of their effect on hyperglycemia (13).

Still, current research is revealing gamma-glutamyltransferase (GGT) as an indicator of cancer risk. GGT is a predictor of type 2 diabetes, cardiovascular disease and chronic kidney disease, and is an enzyme involved in cellular redox state (14). Since hyperglycemia can lead to overproduction of reactive oxygen species (ROS) by the mitochondrial electron transport chain (and may explain increased malignancies in diabetics), this pathway is thought to link GGT and tumour development. The association between GGT, hyperglycemia and cancer incidence was examined in a Swedish prospective cohort study with 37 809 primary cancers. Results showed that for those with glucose levels above 6.11 mmol/L, the association between GGT and risk of prostate, breast and liver cancer became stronger (for pancreatic cancer the association with GGT was weaker for those with elevated glucose levels). The researchers concluded that the strength of association between GGT levels and developing cancers may vary by glucose levels because hyperglycemia can result in oxidative stress initiating damaging pathways of carcinogenesis (15). It is worth considering that hyperinsulinemia and hyperglycemia often arise together with insulin

resistance, so preventing insulin resistance might be key in reducing cancer risk regardless of whether hyperinsulinemia or hyperglycemia have more of an independent link to cancer.

Post-menopausal breast cancer has been associated with elevated fasting insulin levels (16). Aside from insulin's metabolic role, it is a growth factor that enhances the production and mitogenic properties of insulin-like growth factors leading to cell proliferation (9). Insulin receptor (IR) activation may be more important than hyperglycemia regarding cancer growth, and the effects of IR activation in cancer cells may relate more to cell survival and mitogenesis than to enhanced glucose uptake since glucose uptake in cancer cells is generally high and independent of insulin (12).

The importance of the insulin receptor in cancer etiology was stated succinctly by Dossus and Kaaks in a 2008 review (17):

“The insulin receptor is a tyrosine kinase that transactivates the ras/raf/MAP-kinase and phosphatidyl-inositol/Akt (protein-kinase B) pathways, which are central in the control of cellular growth, proliferation and apoptosis. The central role of these in tumour development is illustrated by the presence, in these pathways, of numerous proto-oncogenes that are frequently mutated and constitutively activated in tumours, providing cells with a strong selective growth advantage, as well as tumour suppressor genes, such as PTEN, that in tumours are frequently inactivated(18) Second,(...) hyperinsulinaemia can increase the synthesis and/or biological availability of other endogenous hormones such as IGF-I or sex-steroid hormones (androgens, oestrogens and progesterone) that in turn can enhance tumour development (19).”

The insulin receptor activates pathways needed by cancer for cellular growth, proliferation and apoptosis. Insulin reduces levels of the binding protein IGFBP-1 (20), allowing for more circulating free IGF-1, which is potent as a mitogen and antiapoptotic (21). IGF-1 binds to IGF-1, insulin and hybrid

receptors, which are commonly expressed in all subtypes of breast cancer cells (22). Although the insulin receptor has been found on breast cancer cells, the relation between serum insulin levels and the regulation of insulin receptor levels on cancer cells is unknown, and research is conflicting on the prognostic value of insulin receptor level (12). The IGF-1 receptor, as with the insulin receptor, activates the Ras/Raf/MAP-kinase and PI3/AKT pathways, stimulating mitogenic and metabolic responses; yet, insulin's pathways are predominantly metabolic, stimulating cell growth, and IGF-1's pathways are mainly mitogenic (23). Insulin and IGF-1 pathways stimulate proliferation, invasion, metastasis and protection from apoptosis, possibly aiding cancer promotion and progression. Hyperinsulinemia might also affect cancer indirectly through its effects on IGF-1 (24).

It is well known that estrogen is associated with many breast cancers (25). For postmenopausal women, the enzyme aromatase converts androgens originating from the adrenals and adipose into estrogen. Because of this, adiposity is linked to estrogen levels in postmenopausal women; adiposity often correlates with hyperinsulinemia. In the blood, a large proportion of estrogen binds to sex hormone binding globulin (SHBG) causing it to be biologically inactive. Increased serum insulin reduces hepatic synthesis and serum levels of SHBG, increasing bioavailable estrogen (12,17). In addition, the mitogenic effects of insulin are potentially mediated via suppression of sex-hormone binding globulin (SHBG) production (26).

Breast cancer patients can likely benefit from metabolic rehabilitation treatments that aim to reduce hormonal-metabolic disturbances including excess body fat, glucose intolerance and insulin resistance. Treatments can be used separately or in combination, and include nonpharmacological (nutrition, moderate physical activity) or pharmacological (antidiabetic biguanides such as metformin) interventions (27). Metformin treatment reduces levels of circulating glucose and insulin in patients with insulin resistance and hyperinsulinemia, possibly through reduced hepatic glucose output. Metformin has inhibited cell proliferation of breast cancer cell lines likely through activation of AMPK (28).

Interestingly, metformin has more of an anticancer effect on mice eating a high energy diet associated with hyperinsulinemia and tumour growth, suggesting metformin's insulin lowering effect leads its anti-cancer effect (29). Human metformin treatment reduces risk of cancer compared to other glucose lowering therapies (12).

On a related note, caloric restriction is linked to longevity, and a mutation disrupting the insulin and IGF-1 pathways slows ageing and extends lifespan in *C.elegans* (30). Interestingly, these mutations, which reduce insulin and IGF-1 signaling, raise blood glucose levels, and are thought to extend lifespan by creating a danger signal that shifts physiology towards cell protection and maintenance (30). In humans, a PTEN mutation stimulates the creation of cancer by elevating insulin and IGF-1 signaling, which inhibits the FOXO3a, preventing apoptosis and promoting cell proliferation. FOXO3a is an ortholog to the *C.elegans* gene DAF-16 which extend lifespan by disrupting insulin and IGF-1 signaling (31). Interestingly, by increasing DAF-16 activity and thereby decreasing insulin and IGF-1 signaling a tumour suppressor effect was found in *C.elegans*, suggesting that reduced insulin and IGF-1 signaling may be linked to cancer resistance in mammals mediated by FOXO (31). Novel concepts are being explored to metabolically starve or reprogram cancer cells and antineoplastic therapies that reduce nutritional intake require personalized nutrition support (32).

Studies Linking Cancer and Glucose/Insulin

Risk

Although epidemiological studies support the association of insulin and glucose with post-menopausal breast cancer risk (8), it is possible insulin and glucose are more involved with cancer progression. In an Italian nested case-control study, 10633 healthy women age 35-69 years gave blood samples and were followed for a median of 13.5 years (8). During this time, 356 cases of breast cancer were identified. These cases were each matched to up to 4 healthy controls. Women in the highest quartile of fasting

glucose had a significantly greater risk of developing breast cancer than those in the lowest quartile (RR 1.63; 95% CI: 1.14-2.32, p for trend 0.003). The positive association of fasting glucose with breast cancer remained significant for both pre-and post-menopausal women at baseline, and for women over 55 at diagnosis. Women in the highest quartile for HOMA-IR, a measurement of insulin resistance, had a significantly greater risk of developing breast cancer than those in the lowest quartile (RR 1.44; 95% CI: 1.02-2.03, p for trend 0.029). In the subgroup of women diagnosed after the age of 55, breast cancer risk was significantly associated with high fasting glucose, high HOMA-IR, and low SHBG. When baseline BMI was considered, RRs for all associations attenuated slightly (8). This study suggests that reducing hyperglycemia and insulin resistance may reduce incidence of breast cancer. In a prospective case-cohort study in postmenopausal women (835 case subjects and 816 controls), fasting insulin levels were associated with breast cancer risk (highest vs. lowest quartile of insulin level, HR = 1.46, 95% CI = 1.00 to 2.13, trend P = 0.02), yet the association varied by hormone therapy (HT) use (interaction P = 0.01)(16). In non-HT users, after controlling for multiple breast cancer risk factors including estradiol level, insulin levels were significantly associated with breast cancer risk, at 2.4 fold greater risk for those in the highest quartile of insulin levels compared to those in the lowest quartile (highest vs. lowest quartile of insulin level, HR = 2.40, 95% CI = 1.30 to 4.41, trend P < 0.001). In this subgroup obesity was also associated with breast cancer risk, but this was attenuated by adjustment for insulin; the association between BMI and breast cancer risk was decreased more by controlling for insulin than for estrogen (16). Of importance, the breast cancer risk imparted by insulin levels was not decreased when estrogen levels were considered; also, the breast cancer risk imparted by estrogen levels was not decreased when insulin levels were considered, showing insulin and estrogen levels to be independent risk factors. The authors concluded that interventions aiming to reduce fasting insulin and circulating estrogen levels may reduce breast cancer risk in postmenopausal women; suggested interventions included weight loss, physical activity and pharmacologic approaches. In a published response to “Insulin, Insulin-like growth

factor-I, and risk of breast cancer in postmenopausal women” (16), an Italian cancer research group (33) agreed with the authors conclusions yet rationalized that reducing insulin levels in postmenopausal women may not adequately reduce their risk of breast cancer due to the over-expression of the insulin receptor and IR-A in the cancerous tissue, noting that in 159 breast cancer samples insulin receptor expression was 6 times greater than in normal breast tissue (34) and in 584 node-negative breast cancers insulin resistance level was the strongest independent predictor of disease-free survival (35). This Italian research group believes that hyperinsulinemia likely promotes the growth and progression of subclinical breast cancers but does not likely initiate cancer. Still, diabetic therapies leading to elevated exogenous or endogenous insulin levels (insulin or sulfonylureas) appear to be related to increased cancer risk, whereas treatments that decrease insulin levels (metformin or thiazolidinediones) reduce cancer risk (36), supporting the link between elevated insulin and cancer risk.

Those with the metabolic syndrome show elevated levels of insulin and glucose, and the syndrome poses increased risk of breast cancer. In an Italian nested case-control study 777 healthy women and women with breast cancer were recruited to evaluate the association between postmenopausal breast cancer and the metabolic syndrome (central adiposity, insulin resistance, low serum high density lipoprotein cholesterol, high serum triglyceride and high blood pressure). A higher prevalence of the metabolic syndrome was found in postmenopausal breast cancer patients (30%) compared to healthy women (19%). The activation of multiple pathways underlying the metabolic syndrome might contribute to carcinogenesis, and the metabolic syndrome may be an indicator of breast cancer risk in postmenopausal women (37).

An indirect way to assess how glucose and insulin affect cancer risk may be to look at how dietary patterns that are expected to increase glucose and insulin levels affect cancer risk. The effect of dietary patterns on breast cancer risk has been examined with mixed results. In one study, dietary glycemic

index (GI) and load were not associated with increased risk of postmenopausal breast cancer (n = 63307, 1142 cases) (relative risk = 1.03; 95% confidence interval, 0.87-1.22 and relative risk = 0.90; 95% confidence interval, 0.76-1.08, respectively) after adjustment for multiple breast cancer risk factors (38). Yet, the association between the intake of sweets and breast cancer risk was assessed with 1434 cases and 1440 controls and consumption of desserts, sweet drinks, and added sugars were positively associated with breast cancer risk (39). In a study that assessed 49613 Canadian women with 1461 breast cancer cases which developed over 16.6 years, consumption of diets with high glycemic index values may be associated with an increased risk of breast cancer among postmenopausal women, perhaps more in those who participated in vigorous physical activity, have used hormone replacement therapy and who are not overweight. In this study there was evidence of effect modification of the association between GI and breast cancer risk by menopausal status (p = 0.01), the hazard ratio for the highest versus the lowest quintile level of GI being 0.78 (95% CI = 0.52-1.16; p = 0.12) in premenopausal women and 1.87 (95% CI = 1.18-2.97; p = 0.01) in postmenopausal women (40). In a case control study with 2569 breast cancer patients and 2588 controls, average daily glycemic index and glycemic load were calculated from a 78-item food frequency questionnaire. Direct associations with breast cancer were found for glycemic index (OR for highest vs lowest quintile = 1.4, p for trend <0.01) and glycemic load (OR 1.3, p <0.01). These moderate direct associations between glycemic index and or load with cancer risk suggest a possible role for hyperinsulinemia /insulin resistance in breast cancer development (57).

Prognosis

The internal milieu of breast cancer survivors has at one time supported cancer, and survivors have a higher likelihood of having cancer again than individuals who have never had cancer. Regardless of the cause of this risk difference, survivors may have detectable and modifiable differences in their internal environments than those who have not had cancer, mediating cancer risk.

Increased levels of both glucose and insulin following diagnosis have been associated with worse cancer prognoses. Hyperglycemia and insulin resistance may be high risk conditions for cancer mortality.

Markers of glucose and insulin metabolism were associated with cancer mortality in the Third National Health and Nutrition Examination Survey (NHANES III; 1988 – 1994) prospective cohort study with an average follow up of 8.5 years to death. After adjusting for age, race, sex, smoking status, physical activity and BMI, for every 50 mg/dl increase in plasma glucose there was a 22% increased risk of overall cancer mortality. Insulin resistance was associated with a 41% increased risk of overall cancer mortality (95% CI 1.07-1.87, $p = 0.01$). Associations were stronger after removing deaths from lung cancer for insulin resistant individuals, particularly among those with lower levels of physical activity (41).

In the article, “Controlling hyperglycemia as an adjunct to cancer therapy,” Krone and Ely (42) review links between glucose and cancer. Hyperglycemia is common in cancer patients. High intake of sugar and refined carbohydrates plus high blood sugar is strongly associated with cancer risk, and high carbohydrate intake has been linked with poorer survival after early breast cancer diagnosis. Glycated hemoglobin (indicates average blood glucose over 2-3 months) was measured in a group of cancer patients, some with active cancer and some in remission. Significantly lower average blood glucose levels were found in patients in remission. Countries with the highest intakes of sugar also have the highest incidence of breast cancer mortality independent of other variables (42). Plus, there is a link between lowering blood glucose levels and remission of malignancy; in a murine model of breast cancer mortality correlated with average blood glucose (measured using glycated hemoglobin), the highest blood glucose levels resulted in the highest mortality(42). In addition to low calorie, low GI diets and exercise, the authors suggested ascorbic acid supplementation as an antioxidant and to improve the immune response and modulate the negative effects of hyperglycemia (42).

The adverse affect of insulin and c-peptide on breast cancer outcome was also noted by the Health, Eating, Activity and Lifestyle (HEAL) prospective cohort study, in which higher BMI and lower levels of physical activity measured following diagnosis were associated with higher insulin levels in breast cancer survivors. Women with invasive disease and high insulin levels had 3 times the risk of dying compared to women with the lowest insulin levels (43). Serum C-peptide, a marker of insulin secretion, was also measured in the HEAL cohort. Increased fasting C-peptide levels were associated with an increased risk of death from all causes and breast cancer in women without type 2 diabetes (44). A 1-ng/mL increase in C-peptide was associated with a 31% increased risk of any death (HR = 1.31; 95% CI, 1.06 to 1.63; $P = .013$) and a 35% increased risk of death as a result of breast cancer (HR = 1.35; 95% CI, 1.02 to 1.87, $P = .048$). Women with ER+ tumours, higher stage disease, BMI under 25 and type 2 diabetes showed greater associations between C-peptide levels and breast cancer death (43). In a separate study supporting insulin's link to cancer prognosis, 603 breast cancer patients were followed for 10 years in which time there were 112 breast cancer deaths (45). High levels of insulin were associated with poorer survival for postmenopausal women [odds ratio, 1.9; 95% confidence interval (CI), 0.7-6.6, comparing highest to lowest tertile, P trend = 0.10], while high dietary fat intake was associated with poorer survival for premenopausal women (relative risk, 4.8; 95% CI, 1.3-18.1, comparing highest to lowest quartile). Higher dietary protein intake was associated with better survival for all women (relative risk, 0.4; 95% CI, 0.2-0.8, comparing highest to lowest quartile) (45). The relationship between insulin resistance and breast carcinoma has also been explored in Iranian women. HOMA-IR values were calculated for 82 patients with malignant breast tumours, 48 subjects with benign breast mass, and 838 healthy women; 130 women were evaluated preoperatively (46). After adjustments for age and central obesity, HOMA-IR values were 3.6 (95% CI, 2.8-4.4), 2.3 (1.7-2.9) and 1.7 (1.6-1.8) for subjects with breast malignancies, benign masses, or healthy subjects, respectively. The interactions of age on breast mass with HOMA-IR was significant ($F(54) = 10$, $p < 0.001$, partial eta squared = 0.03). The interaction of

central obesity on this association was also significant ($F(54) = 37$, $p < 0.001$, partial eta squared = 0.11).

This link between insulin resistance and breast cancer supports directs attention to the pathology of breast carcinogenesis (46).

Treatment

Hyperinsulinemia in newly diagnosed breast cancer patients is from insulin resistance. Hyperinsulinemia may impact breast cancer prognosis by the interaction of insulin with the insulin/IGF receptors to stimulate proliferation, the effect of insulin on circulating levels of IGFs and their binding proteins, and the effect of insulin of free circulating estrogen, partially by reducing SHBG. Cross talk between insulin and estrogen pathways may also be relevant. The prognostic effect of insulin is important because insulin levels are modifiable using metformin or lifestyle interventions focusing on weight loss or physical activity. Beyond cancer, hyperinsulinemia in breast cancer patients also increases their risks of diabetes and cardiovascular diseases thus deserving attention (47).

An interesting scientific editorial discussed expert opinions on reducing insulin levels as a means to treat breast cancer (48). Increased insulin levels pose a moderate risk of breast cancer and increase risks of breast cancer recurrence and death. Metformin, a drug prescribed for Type 2 diabetes (49), has also been linked to lower cancer rates(50) and reduced risk of cancer death (51). Medical oncologist Pamela Goodwin and her colleagues report that for nondiabetic women with early staged cancer, women in the highest proportion quartile of insulin levels have a triple risk of death; if insulin levels could be reduced by 25%, and if those reductions reverse the adverse prognostic effect of insulin, an improvement of 5-6% might be obtained for 5 year disease free survival (52). Goodwin *et al.* assert that the adverse effect of high insulin levels on postmenopausal breast cancer outcome is of the same magnitude as the benefit of adjuvant chemotherapy. Michael Pollak agrees that therapies reducing insulin levels can dramatically reduce cancer related deaths. Pollak and his research group found that metformin reduced breast cancer cell growth in-vitro, and activated AMPK which reduced cell growth and division (53). Yet, Kevin

Claffey found that metformin also stimulates VEGF induced angiogenesis in one breast cancer cell line. His work reaffirms that breast cancers are very diverse and suggests that metformin may produce distinct positive or negative effects in different breast cancers (54).

Aside from pharmaceuticals, physical activity decreases insulin levels and has few side effects (48). Ligibel *et al.* (55) demonstrated that a mixed exercise intervention focusing on strength and endurance resulted in a significant decrease in circulating insulin levels and non-significant decrease in insulin resistance in sedentary, obese breast cancer survivors (previous studies did not focus on obese sedentary survivors). Metformin may also act on cancer by inhibiting mTOR (56). Goodwin found metformin to reduce insulin levels in nondiabetic breast cancer patients by 22%, similar to the reductions found by Ligibel *et al.* with an exercise intervention (47). Interestingly, metformin is used to treat the insulin resistance related to polycystic ovarian syndrome, and has been shown to inhibit aromatase in human ovary cells (57).

On a different note, insulin treatment seems to have a positive impact on those with cancer cachexia. Cancer patients (mainly gastrointestinal) with cachexia treated with insulin showed increased survival ($p=0.03$). Insulin treatments stimulated carbohydrate ingestion, decreased serum free fatty acids, increased whole body fat without affecting lean mass and improved metabolic efficiency during exercise (58).

Exercise and Insulin

The effect of exercise training on reducing blood glucose levels and improving insulin sensitivity is well known (59). In fact, there is enough scientific literature on this topic to support exercise as a means to prevent and treat diabetes (60). Exercise induced improvements in insulin sensitivity depend on the type, intensity, duration, and frequency of exercise (61). Interventions of more intense exercise or longer duration moderate exercise affect insulin sensitivity for up to 1 or 2 days as blood glucose is being

used to replenish glycogen stores used by the activity. Lower intensity exercise uses more fat than glycogen stores, and has less of an effect on insulin sensitization. In healthy women over 60, participation in 9 months of low, moderate or high intensity exercise of equal energy expenditure resulted in better improvement in insulin sensitivity in the high intensity exercisers (80% VO₂ peak). This is thought to be due to longer lasting transient effects (GLUT4 translocation, oxidative enzymes) for high intensity exercise bouts rather than exercise adaptations (mitochondrial concentration, muscle changes, etc.). Seventy-two hours after the last exercise bout those who exercised at higher intensities still had a 21% improvement in insulin action. An interesting study found that aerobic exercise training (25-60 min running at 60-85% max HR 3 days/week for 6 months) improves insulin sensitivity in both younger and older women, but that the insulin sensitizing effect of exercise does not last for as long after the final bout in older women. This implicates the acute effects of the last training bout in older women and the chronic adaptation of the intervention in younger women for improving insulin sensitivity (62). The authors suggested that older women must partake in aerobic exercise daily to gain its benefits on insulin sensitivity, whereas younger women could exercise 3 times per week. In another study, moderate intensity walking interventions for middle aged, sedentary, insulin resistant adults have also shown improvements in insulin sensitivity, showing that exercise training does not necessarily need to be composed of high intensity exercise to affect insulin sensitivity (63). A recent study has shown that, in addition to the well known ability for exercise training to improve peripheral insulin sensitivity, endurance training also alters pancreatic beta cell function. In rats performing endurance training one, three or five times per week over eight weeks, glucose induced insulin secretion was dose dependently reduced proportional to exercise frequency, probably by activating the AMPK pathway (64). Also, isolated pancreatic islets from endurance trained rats secreted almost 50% less insulin when incubated with glucose compared to islets from sedentary control rats. The enhanced glucose uptake of exercise training has been attributed to increased activity of proteins involved with glucose uptake and

metabolism in skeletal muscle, plus changes in the activity of proteins involved with insulin signaling in skeletal muscle (AMPK, Act substrate AS160) and increased lipid turnover (59).

Conversely, inactivity has been shown to have a negative effect on insulin sensitivity. In healthy men, the effect on insulin sensitivity of lowering daily activity from 10,000 to 1,300 steps/day has been assessed (65). After 2 weeks of reduced activity, body weight increased with lean mass reductions in the trunk and legs; cardiovascular fitness decreased by 6-7%, and peripheral insulin sensitivity decreased by 17% as reflected in reduced insulin-stimulated Akt phosphorylation in skeletal muscle. Reduced Akt phosphorylation from reduced activity suggests reduced insulin signaling and is consistent with decreased insulin mediated glucose uptake. As noted in an accompanying commentary (66), similar effects have been noted after bed rest, and this reduced activity level corresponds with the inactivity of many individuals. The speed at which these metabolic changes can be reversed after resuming active habits is unknown. This data is relevant considering how cancer and its treatments affect the activity levels of cancer patients, and how these metabolic changes might still affect cancer survivors after they have completed their treatments.

A single exercise bout increases skeletal muscle glucose uptake via an insulin-independent mechanism, but this insulin independence disappears after about 48 hours (59). In one study, an acute bout of endurance exercise, but not sprint interval exercise, has been shown to increase insulin sensitivity (67). Young males and females exercised either for 45 minutes at 75% VO₂ peak or did five 30 second sprints at 125% VO₂ peak. Indices of insulin sensitivity were composite insulin sensitivity index (ISI-COMP) and ISI-hepatic insulin sensitivity (HOMA). Relative to baseline, both indices of insulin sensitivity were increased by endurance exercise by 70% - 100% independent of VO₂ peak whereas neither index of insulin sensitivity increased with sprint interval exercise. The authors cite Schenk and Horowitz (68) for their recent study showing endurance exercise (1.5 h at 65% VO₂ peak) protected humans from free

fatty acid induced insulin resistance and raised insulin sensitivity 25 % from baseline, noting the possible mechanism of lipogenesis for increasing exercise induced insulin sensitivity. AMPK activation, cytokines and intramuscular triacylglycerol were also suggested as possible mechanisms. In 8 healthy untrained men of approximately 25 years old, a single bout of 60 minute cycling (50% VO₂ peak) resulted in lower levels of glucose and insulin than the resting condition, with epinephrine and norepinephrine also decreasing and free fatty acids increasing (69). A bout of aerobic exercise (3 times 10 minutes at 60%, 5 minute rests) decreased insulin levels in obese young women but resistance exercise did not (70). Still, a single bout of resistance exercise reduces postprandial insulin levels by 30% compared to aerobic exercise in overweight and obese individuals (71).

The current guideline for diabetes prevention through glycemic control is at least 150 minutes of moderate exercise or at least 90 minutes of vigorous exercise per week distributed over at least 3 days with no more than 2 consecutive days without activity (72), although this recommendation has been questioned. In young men, two weeks of extremely low volume, high intensity interval training (total of 6 session, 4-6 30s cycling sprints per session) reduced the area under the curve for plasma glucose and insulin 12% and 37%, respectively ($p < 0.001$ for both) although fasting glucose and insulin levels remained unchanged. Insulin sensitivity was improved by 23% ($p < 0.01$), and remained improved 3 days after the last session (73). The authors suggest that this high intensity exercise uses more glycogen stores than moderate intensity interventions, thus having a greater effect on insulin sensitivity. They assert that the current guidelines of longer duration, less intense exercise might not be ideal for improving glycemic control.

When considering the effect of acute exercise on serum insulin, intensity is a critical factor. At intensities above 80% of maximum oxygen consumption (VO₂max) catecholamine levels rise, which suppress insulin levels. During recovery, however, catecholamine levels decrease and insulin levels rise

substantially. At intensities <60% VO₂max, insulin levels remain constant, or may even decrease slightly (74). It appears that high intensity exercise may cause insulin spikes during recovery and low intensity exercise may keep insulin levels stable.

Exercise at intensities greater than 80% VO₂max relies solely on glucose for fuel, and this glucose originates in muscle and liver glycogen stores. Glucose production rises 7-8 fold and glucose utilization increases 3-4 fold allowing for increased glucose levels. Catecholamines rise 14-18 fold; due to suppression by catecholamines, plasma insulin levels remain constant or decrease slightly despite huge increase in glucose production. It is thought that catecholamines are responsible for glucose control during intense exercise, and insulin resumes its glucoregulatory role during recovery from intense exercise presumably to restore glycogen stores. At exhaustion, glucose utilization begins to decrease more than glucose production decreases leading to increased hyperglycemia requiring a considerable rise in insulin for up to one hour to restore pre-exercise glucose levels (type 1 diabetics experience hyperglycemia after intense exercise because they lack this insulin spike). Increased catecholamine levels stimulated by intense exercise are credited for increasing glucose production and restraining the increase of glucose utilization; at exhaustion catecholamines decrease and insulin levels spike to restore high levels of circulating glucose into glycogen (74). In a study demonstrating this effect, 18 fit men cycled at approximately 100% VO₂max to exhaustion (75). Plasma glucose was 4.90 ± 0.08 mM/L at rest, increased during exercise, declined to 6.91 ± 0.40 mM/l at 4 min then continued to decline. Insulin remained constant during exercise, then doubled to 162 ± 28 pmol/l until 20 min recovery then declined. Glucagon increased by 71 ± 11 pg/mL. Norepinephrine increased 18-fold and epinephrine increased 14-fold, and both declined by 20 minutes recovery. Glucose production increased 7-fold by exhaustion to 13.0 ± 1.18 mg/kg/min then decreased to 2.43 ± 0.24 mg/kg/min by 9 minutes and 2 mg/kg/min for the rest of recovery. Glucose utilization increased 3-fold to 6.61 ± 0.70 mg/kg/min and remained lower than glucose production to 7 minutes recovery then declined more slowly.

At intensities below 60% VO₂max, insulin secretion is suppressed by β -cell α -adrenergic receptor activation. This alteration in the glucagon/insulin ratio stimulates glucose production, and glucose utilization increases to match glucose production. The increased use of glycogen in muscles causes translocation of GLUT4 from a different pool than insulin. Hence, during moderate exercise, plasma glucose levels remain constant (postabsorptive) or decline (postprandial) even though glucose production may double due to muscle demands (74).

Exercise interventions are thought to normalize insulin levels by increasing glucose transporter proteins, increasing post-receptor insulin signaling, increasing glucose delivery to muscles and improving clearance of free fatty acids. In 1996 healthy postmenopausal women from the prospective cohort of the Women's Health Initiative, higher levels of physical activity, lower BMI and lower caloric intake were related to lower fasting insulin levels (76). Regular exercise makes sense for postmenopausal women wishing to maintain or improve insulin sensitivity and maintain or reduce insulin levels. Since insulin is linked to breast cancer recurrence and mortality, breast cancer survivors may wish to partake in regular exercise for this reason.

Exercise and Cancer

Physical activity reduces the risk of breast cancer by 20-30% (77), may reduce mortality from breast cancer and can be used as a treatment for the negative effects of cancer. Research on the ideal exercise intensities for reducing cancer occurrence and morbidity is ongoing. The mechanisms underlying the impact of exercise on cancer outcomes are being explored.

Currently, there are exercise prescription recommendations relating to cancer survivorship from the American College of Sports Medicine (ACSM) and prevention in a joint recommendation from the American Institute for Cancer Research (AICR) and the World Cancer Research Fund (WCRF), respectively. ACSM prescribes a minimum of 30-min moderate-intensity exercise 5 days/week for cancer

survivors and healthy adults (78) whereas AICR/WCRF prescribe 60 min of moderate-intensity or 30 min of vigorous-intensity exercise daily to reduce the risk of cancer (79).

There is probable evidence that physical activity beneficially affects the risks of colon, breast and endometrial cancers. The proposed mechanisms include the effect of physical activity on insulin resistance, body composition, sex steroid hormones and perhaps vitamin D, adipokines, inflammation and immune function. It has been estimated that between 165 000 and 330 000 (between 9 and 19%) cases of breast, colon, lung, prostate, endometrium and ovarian cancer could have been prevented in Europe if the population had had adequate physical activity (80). In a systematic review assessing epidemiologic evidence on the association between physical activity and breast cancer risk, there was a 25% average risk reduction among physically active women as compared to the least active women. The associations were strongest for recreational activity, activity sustained over the lifetime or done after menopause, and for activity of moderate to vigorous intensity performed regularly. There were stronger associations for postmenopausal women, women who are normal weight, have no family history of cancer and are parous (81). For example, the relationships between light and moderate to vigorous intensity and physical activity during 4 periods of life to postmenopausal breast cancer risk were examined in a prospective cohort of 118 899 post-menopausal women in which 4 387 incident breast cancers arose over 6.6 years(82). Women who participated in moderate to vigorous activity more than 7 hours/week during the past 10 years had 16% reduced risk of postmenopausal breast cancer (RR:0.84; 95%CI:0.76,0.93) compared with inactive women. The association remained statistically significant after adjustment for BMI (RR:0.87; 95%CI:0.78,0.96) (82). Recently, physical activity has been shown to reduce the risk of triple negative breast cancer to the same extent as ER positive breast cancer (83). Studies suggest that lifestyle interventions can improve insulin sensitivity, alter the balance of circulating sex steroid hormones and IGF-1 proteins and change the functioning of immune cells in peripheral

blood. More research needed to establish which combination of diet and physical activity will best reduce cancer risk (84).

In a systematic review of physical activity and cancer prevention, clinical trials that assessed the effect of physical activity on cancer related biomarkers were examined from studies involving a minimum of 4 week interventions for cancer survivors or people showing one or more cancer risk factors(77). It was concluded that exercise had a small to moderate effect on improving concentrations of blood biomarkers implicated in breast and colon cancer pathways including insulin, leptin, estrogens, and apoptosis regulation. For breast cancer survivors, exercise has a small to moderate effect on improving levels of biomarkers related to prognosis such as insulin-like growth factor axis proteins, insulin, and inflammation, and a large effect on enhancing immune function. Of note, two recent randomized controlled trials of exercise in sedentary, overweight postmenopausal women assessed reductions in biomarkers with exercise (85,86). Both trials involved post menopausal women working up to performing aerobic exercise for 45 minutes 5 times per week for 12 months. In the study by Friedenreich et al. there were significant reductions in estrogen after 12 months, and in the McTiernan study the estrogen reductions were only in women who had also lost body fat. McTiernan et al. (87) trial also showed decreased levels of insulin, insulin resistance and leptin in inactive overweight postmenopausal women. Friedenreich et al. also report reductions in insulin, insulin resistance and leptin, and an increase in the adiponectin/leptin ratio, without changes in glucose, adiponectin or IGFBP-3 (88). Other trials involving breast cancer survivors showed improvements in breast cancer biomarkers with exercise interventions. Ligibel *et al.* (55) report a 20-25% decrease in insulin concentration, Irwin et al. (89) report reductions in insulin as well as IGF-1 (77).

Although many studies find independent effects of exercise on insulin levels and other markers of breast cancer risk, some do not. For example, a cross sectional study assessed physical activity and

sedentary time with biomarkers of breast cancer risk in healthy postmenopausal women (n=1024, with 443 fasting). Accelerometer data was divided and associated with biomarker data. Moderate- to vigorous-intensity activity had significant, inverse associations with body mass index, waist circumference, C-reactive protein, fasting plasma glucose, fasting insulin and homeostasis model assessment of insulin resistance. Yet, significant associations relating to insulin resistance and inflammation were not present after adjustment for waist circumference, which supports adiposity as an independent risk factor and a mediator of other biological pathways related to cancer risk (90). It is well known that obesity is linked to post-menopausal breast cancer, and the endocrine effect of adipose has been a focus; adipose is linked to insulin resistance and increased availability of estrogen. In addition, a bout of heavy exercise induces a temporary suppression of the gonadal axis, decreasing plasma testosterone and estrogen levels in men and women, respectively (91). Gonadal hormones promote the growth and development of reproductive tissues. Exercise induced reductions in gonadal hormones might reduce carcinogenesis. Exercise training suppresses estrogen production. Exercise training affects body fat levels, modifying estradiol metabolism, estradiol binding, and the synthesis of estrogens from androstenedione (91). Normally 2-3% of estradiol circulates in an unbound, biologically active state, but in menopause (more so in the obese) estradiol bioavailability increases because of the decrease in SHBG, the main carrier of estradiol. Increased triglyceride levels compete with SHBG to bind with estradiol; estradiol binds with albumin, which is less secure. Adipose tissue is also a major site of aromatization of androstenedione into estrone (91).

As discussed by Peters (82) the ideal exercise intensities required to reduce the risks of breast cancer occurrence are unknown. Vigorous intensity exercise has been linked to greater reductions in breast cancer risk compared to low intensity exercise (92–95). Also, breast cancer risk has been reported to be reduced by 26% from vigorous exercise compared to 22% by moderate exercise; physical activity was associated with a 25 to 30% decreased risk of breast cancer in 62 studies with 85% of the positive studies

reporting a dose response (96). Another review reported 15-20% reduced risk of breast cancer with higher physical activity and risk reduction of 6% per hour of physical activity per week. The association was stronger for postmenopausal women than for premenopausal, and the association between physical activity and breast cancer was consistent regardless of dietary intake, BMI, race, tumour stage or histological subtypes (97). However, at least 8 other studies have not found differences in breast cancer risk with exercise intensity (98–106).

Exercise therapy is a validated treatment for a variety of diseases including metabolic syndrome related disorders, heart and pulmonary diseases, muscle, bone and joint diseases, cancer, depression, asthma and diabetes. A common symptom of cancer is reduction in muscle mass, fatigue and reduced physical functioning resulting from reduced fitness and muscle atrophy. Physical inactivity results from feeling unwell, disrupted appetite, demanding treatment regimens and difficulties in daily life. As much as 1/3 of the poor physical condition of cancer patients can be attributed to inactivity. Some of the aims of training cancer patients relate to positive effects on fitness, muscle strength, physical well being anxiety, depression and quality of life. Mixed aerobic and strength programs beginning at low intensity have been recommended for cancer patients who have completed treatment (107).

Research also supports physical activity as a means to reduce the risk of dying from breast cancer in 3 large prospective cohort studies (93,108,109), but no relationship was noted in 2 other large prospective cohort studies (110,111). In the California Teachers Study participants provided information on long term (high school to age 54) and recent (past 3 years) participation in moderate and strenuous physical activities. Of these women, 3539 were diagnosed with breast cancer; 460 of these women died and 221 deaths were from breast cancer (93). Physical activities were divided into low, intermediate or high depending on frequency and intensity. Women with high or intermediate levels of long-term physical activity had lower risk of breast cancer death (RR, 0.53; 95% CI, 0.35-0.80; and RR 0.65; 95% CI, 0.45-

0.93, respectively) than women with low activity levels. These associations were consistent across estrogen receptor status and disease stage, but were confined to overweight women (93,108). In the Nurses Health Study there were 208 breast cancer deaths of 2987 participants after 8 years follow up; those who exercised equivalent of 3-5 hrs per week of walking had half the risk of dying of breast cancer (Relative Risk 0.5, 95% CI 0.38 to 0.84) with no increased benefit for more exercise. Also, a reduced risk of recurrence and total mortality was found with physical activity, especially in those with hormone responsive tumours (109). In the Collaborative Women's Longevity Study there were 109 deaths of 4482 women with breast cancer after 6 years follow up which reported comparable findings to the previous study for the association between physical activity and decreased risk for breast cancer death and total death (112). In Life After Cancer Epidemiology (LACE) (n = 1970)(110) and Health, Eating, Activity and Lifestyle (HEAL) (n=933) (111) prospective cohort studies there was a decreased risk for total but not breast cancer deaths with greater physical activity. The HEAL study reported 67% reduction in mortality with physical activity after diagnosis compared to 31% reduction in mortality from physical activity before diagnosis and 45% decreased risk of death for women who increased their physical activity after diagnosis. In a recent longitudinal study of 4643 women diagnosed with invasive breast cancer, associations among pre- and postdiagnosis physical activity, change in postdiagnosis physical activity, and all cause and breast cancer specific mortality in postmenopausal women were assessed (44). Women participating in 9 MET-h/wk or more (~3 h/wk of fast walking) of physical activity before diagnosis had a lower all-cause mortality (HR = 0.61; 95% CI, 0.44–0.87; $P = 0.01$) compared with inactive women in multivariable adjusted analyses. Women participating in ≥ 9 or more MET-h/wk of physical activity after diagnosis had lower breast cancer mortality (HR = 0.61; 95% CI, 0.35–0.99; $P = 0.049$) and lower all-cause mortality (HR = 0.54; 95% CI, 0.38–0.79; $P < 0.01$). Women who increased or maintained physical activity of 9 or more MET-h/wk after diagnosis had lower all-cause mortality (HR = 0.67; 95% CI, 0.46–0.96) even if they were inactive before diagnosis. It was concluded that high levels of physical

activity may improve survival in women with breast cancer, even in women reporting low activity levels before diagnosis and that women diagnosed with breast cancer should be encouraged to participate in regular physical activity (44). As discussed in a commentary by Ogunleye and Holmes (113), physical activity has a stronger association with decreasing breast cancer risk and death in postmenopausal women compared to premenopausal women. Regular physical activity may reduce breast cancer risk and morbidity by decreasing circulating levels of insulin and estrogen. This may be from the exercise alone or from the ability of exercise to reduce adiposity.

There have been at least two reviews assessing the possible mechanisms linking physical activity and cancer inhibition. In a 2009 review by Thompson et al. (114), three hypotheses were considered regarding candidate mechanisms that may account for the effect of physical activity on tumour burden. The first hypothesis explains that physical activity may inhibit cancer development by suppressing mTOR activation in mammary carcinomas. Physical activity affects levels of circulating hormones that regulate mTOR (IGF-1, AMPK, Act), and in cancer this results in reduced cell proliferation, a pro-apoptotic environment, suppression angiogenesis. The mTOR system is misregulated in most breast cancers, and mTOR is downstream of IGF-1 and its receptor which are centrally involved in cell growth and division. Physical activity activates AMPK, resulting in mTOR downregulation, and physical activity may decrease IGF-1, which would decrease Act activation. IGF-1 affects mTOR, and is linked to breast cancer; physical activity has been shown to reduce its level and signaling in mammary carcinoma. The second hypothesis explains that the carcinogenic response to physical activity might be nonlinear and be accounted for by a physiological cellular stress response. In this hypothesis the carcinogenic response to physical activity is hermetic (U or J shaped), and lower intensity/frequency/duration activity increases cancer risk, moderate activity is preventative, and higher activity increases risk. Molecules that may play a role in this are Sirtuins, FoxO transcription factors, NF-kappaB and Nrf-2/ARE and cytokines. The third hypothesis is that physical activity limits the amount of glucose and glutamine available to mammary

carcinomas inducing apoptosis because tumour associated metabolic programming is reversed. Physical activity takes fuel and precursor molecules (glucose, amino acids) that cancer might use for its own development, hence limiting tumour growth. Genes known to be involved in breast cancer regulate metabolism, and alterations in metabolism have been linked to the hallmarks of cancer. The intensity and duration of exercise affect major metabolic shifts that may affect cancer metabolism supporting tumour growth and proliferation. The authors posed questions regarding how the systemic effects of physical activity may affect breast epithelia: 1) what does contracting muscle release into or remove from circulation that would modify epithelial carcinogenesis? 2) are the effects of contraction on cancer endocrine, paracrine, autocrine, or intracrine? 3) does muscle contraction affect cancer cells differently than normal cells? (114)

In another review Dossus and Kaaks (17) outlined biological mechanisms that might provide metabolic links between nutrition, physical activity and cancer such as insulin resistance, reduced glucose tolerance, increased IGF-1 receptor activation, sex steroid availability and low grade chronic inflammation. Suspicions of the link between metabolic factors and some cancers were originally based on the observations that exercise and diet had been linked to insulin resistance, and insulin favors cancer development directly and indirectly by affecting other growth factors and hormones such as IGF-1 and estrogen. Physical activity helps prevent insulin resistance; exercise training generally leads to improvements in insulin sensitivity in previously sedentary people and acute exercise aids glucose uptake and insulin sensitivity in skeletal muscles. Although changes in IGF-1 concentration with exercise are controversial, it is possible that they rise slightly and transiently with acute exercise, and exercise training but are reduced after prolonged exhaustive exercise over a period of days. In postmenopausal women, cross sectional studies show reduced levels of estrogens and androgens for higher intensity exercise after adjustment for BMI (17).

Rodent studies have been able to link the effect of physical activity on cancer at a cellular level. Rats (n = 120) were injected with a carcinogen and randomized to a physically active or sedentary group. The active group was given free access to an activity wheel, and running was reinforced with a food reward. Physical activity reduced mammary cancer incidence (p = 0.015) and cancer multiplicity (p = 0.01) and induced changes in plasma insulin, IGF-1 and corticosterone suggesting altered glucose metabolism. Proteins involved with cell proliferation were reduced (p <0.001), and those involved in apoptosis were increased (p <0.001) by physical activity. It was hypothesized that these effects were mediated by AMPK activation and protein kinase B down-regulation, which together would down-regulate mTOR, and evidence of this hypothesis was supported in western blots (115). In rats, a moderate intensity training program showed a 17.4% decrease in H₂O₂ production in macrophages, 25% decrease in glucose production and 47.1% decrease in lactate production, and an increased CO₂ production from the oxidation of labeled glucose in tumour bearing rats. Levels of enzymes relating to metabolism were also altered: hexokinase activity reduced 68.8% and citrate synthase activity increased 10.1%. These metabolic changes are linked to increased survival in the rats (116). In a separate study, high intensity exercise training in tumour bearing rats extended lifespan, reduced tumour mass and prevented indicators of cachexia. The reduction in tumour growth was associated with an impairment of tumour cell glucose and glutamine metabolism (117). Also, a study in mice demonstrated that different genes in mammary tissue are independently activated by either caloric restriction or exercise suggesting that caloric restriction and exercise exert their effects on mammary carcinogenesis by separate pathways (118).

Breast Cancer Survivors, Exercise and Insulin

Cancer survivors are at a higher risk of new malignancies, and account for 16% (1 in 6) of the new cancers diagnosed annually in America, although cancer survivors account for only 3.5 % of the population (119). A variety of factors may contribute to this heightened risk, including past cancer

treatments, shared environmental situations, and genetic predisposition (120). Among several suggestions regarding minimizing the risk of new cancers for survivors is the promotion of healthy lifestyles (121). Physical exercise has been widely accepted as a mode of breast cancer prevention (122); however, the mechanisms behind how exercise affects cancer etiology are poorly understood, making it difficult to prescribe particular exercise regimes for cancer prevention. Breast cancer survivors often change lifestyle habits after diagnosis with the aims of increasing well being, maintaining health and preventing recurrence. As reviewed by Ingram and Visovsky (123), exercise has many benefits for breast cancer survivors in the areas of psychology and quality of life, cancer related fatigue, physical functioning, body weight and composition, muscle strength and endurance and immune function.

A recent systemic review identified 21 randomized clinical trials in the past 3 years examining diet and exercise interventions in cancer survivors. Results suggest that physical activity interventions for survivors are safe and improve fitness, strength, physical function and cancer-related psychosocial variables. Dietary interventions improve diet quality, nutrition related biomarkers and weight.

Preliminary evidence suggests that diet and exercise may positively influence biomarkers associated with progressive disease and overall survival such as insulin levels, oxidative DNA damage and tumour proliferation rates. Aside from their risk of recurrence, survivors are at greater risk of developing second malignancies, CVD, diabetes, osteoporosis and functional decline than age and race matched populations. These comorbidities are likely from cancer treatment, genetic predisposition and/or common lifestyle factors among survivors. Organizations such as the American Cancer Society, World Cancer Research Fund, American Institute for Cancer Research and American College of Sports Medicine have guidelines for diet and/or physical activity choices for survivors (124).

Due to the growing understanding of insulin and cancer etiology, and the impact of exercise on insulin levels, several studies have assessed the impact of exercise interventions on breast cancer survivors.

Although a 12 month strength training intervention for 85 breast cancer survivors (125) and a 15 week aerobic program for 53 postmenopausal breast cancer survivors (126) showed no changes in insulin levels, several other studies have shown physical exercise to decrease insulin levels in breast cancer survivors. For example, 101 sedentary overweight breast cancer survivors were randomized to a 16 week cardiovascular or strength training intervention or usual care. Results were collected from 82 patients. Fasting insulin levels decreased by 2.86 $\mu\text{U}/\text{ml}$ in the exercise group ($p = 0.03$), with no significant change in the usual care group ($-0.27 \mu\text{U}/\text{ml}$, $p = 0.65$). In the exercise group there was a trend towards an improvement in insulin resistance ($p = 0.09$) but no change in fasting glucose levels. The exercise group had reduced hip measurements but no change in weight or body composition (55).

In a randomized controlled trial, 75 postmenopausal breast cancer survivors were randomly assigned to either an exercise ($n = 37$) or usual care ($n = 38$) group. The exercise group performed aerobic exercise moderately 150 minutes per week for 6 months, whereas the usual care group maintained their current exercise levels. Fasting blood samples were collected at baseline and 6 months. Average exercise in the exercise group was 129 minutes weekly compared to 45 minutes in the usual care group. Women in the exercise group showed decreases in insulin, IGF-1 and IGFBP-3, whereas women in the usual care had increases in these hormones. Exercisers had 20.7% less insulin ($P = 0.089$), 8.9% less IGF-1 ($P = 0.026$) and 7.9% IGFBP-3 ($P = 0.006$) in their circulation after the intervention than controls. Women in the exercising group showed a 1.75 $\mu\text{U}/\text{ml}$ (7.1%) reduction in insulin while controls showed a 3.49 $\mu\text{U}/\text{ml}$ (13.6%) increase (89). It is relevant to note that there were a greater proportion of high grade cancers in the control group, which may have affected their higher baseline insulin and IGF levels and increased levels of Insulin and IGF over 6 months (127).

No known studies have assessed the impact of exercise intensity on insulin levels in breast cancer survivors.

At least two studies have explored how exercise affects breast cancer survivors differently than people who have not had cancer. To compare exercise energy metabolism in breast cancer survivors and controls at different exercise intensities, survivors were matched with controls and exercised at 3 intensities: 40%, 60% and 70% VO_2 max. Oxygen consumption and RER were used to determine substrate oxidation rates for CHO and fat. Blood lactate and glucose were measured before and after each session. Survivors had significantly lower CHO oxidation rate and higher fat oxidation rate at all intensities compared to controls. Survivors had significantly lower blood lactate responses to exercise across all intensities compared with controls. Glucose responses tended to be more elevated ($p < 0.08$) in survivors before and after exercising compared with controls. This suggests that survivors have augmented fat metabolism and reduced CHO-based energy metabolism during submaximal exercise, perhaps from cancer or its treatments (128). In a study assessing cardiovascular function of breast cancer, postmenopausal women treated with endocrine therapy for hormone receptor positive cancer, 47 patients and 11 controls were studied(129). Breast cancer patients had significantly lower peak exercise stroke volume, cardiac output, and VO_2 peak than controls. Patients with the most impaired VO_2 peak had the worse cardiovascular risk profiles. Outcomes differed for those taking Tamoxifen versus aromatase inhibitors, leading to the conclusion that aromatase inhibitor (AI) therapy may be associated with a less favourable cardiovascular risk profile than Tamoxifen therapy. AI users had higher BMI, waist girth, triglyceride level, fasting insulin level, fasting glucose level and CRP level, and a lower peak heart rate and HDL level. It is unknown if these differences are from the absence of the Tamoxifen protective effect, or if AIs are independently affecting cardiovascular risk. Radiation and chemotherapy are associated with cardiotoxicity. Tamoxifen has beneficial estrogenic agonist effects on lipid profiles leading to fewer cardiac events for breast cancer patients. AIs deplete estrogen, raising concerns for their effects on the cardiovascular system. Compared to Tamoxifen users, AI users have been linked to more cardiovascular events and more hypercholesterolemia. This is relevant because of the risk to

benefit ratio of cancer treatment since cardiovascular disease is a competing cause of mortality in breast cancer survivors (129).

Estrogen and Insulin

Because estrogen supports many breast cancers, breast cancer treatments often reduce estrogen levels selectively or systematically. This alteration in estrogen levels may affect other aspects of physiology, including metabolism.

As reviewed thoroughly by Faulds et al. (130), estrogen affects metabolism. Premenopausal women are more insulin sensitive and less prone to insulin resistance than men, and have increased GLUT4 expression (131,132). Also, hormone replacement therapy can improve insulin sensitivity and lower blood glucose levels in healthy postmenopausal women and reduce the incidence of diabetes in postmenopausal women with heart disease (133,134). Estrogen scarcity is strongly linked to the development of insulin resistance. Estrogen deficiency or declines in estrogen from menopause often lead to disregulated metabolism, including insulin resistance, impaired glucose disposal, increased hepatic gluconeogenesis with ensuing glucose secretion and increased levels of inflammatory markers. The reduced level of estrogen in postmenopausal women increases their risk of insulin resistance, the metabolic syndrome and type 2 diabetes. In general, estrogen receptors are thought to control energy intake and expenditure, suppress lipogenesis in white adipose and liver tissues, improve insulin sensitivity, and favor pancreatic β function and survival(135). At physiological concentrations, estradiol increases insulin production and protects the pancreatic β cells against injuries encountered in diabetes such as lipotoxicity, oxidative stress and apoptosis. Also, excess androgens in women are thought to provoke insulin resistance. Estrogen therapy has been considered to treat metabolic disorders, but this would have an obvious adverse impact on the risk of hormone dependent cancers (135).

The metabolic syndrome has been implicated as a long term cancer risk, but also, hormone modifying cancer therapies may predispose cancer survivors to the metabolic syndrome. Abnormalities in sex hormone levels have been linked to metabolic irregularities that are symptomatic of the metabolic syndrome, and anti-estrogen therapies for breast cancer alter sex hormone levels. Aromatase inhibitors stop estrogen from being produced in postmenopausal women, and this anti-estrogen therapy for breast cancer has been linked to cardiovascular risk factors, adverse lipid profiles, and liver disease. Interestingly, Tamoxifen has an estrogen agonist effect on many tissues outside of the breast, and it is cardioprotective, reducing incidence of myocardial infarction, and improves lipid profiles in women receiving it. Clinical suggestions include monitoring lipid profiles, liver enzymes, body composition and markers of glycemic function to prevent the metabolic syndrome in cancer survivors who have had alterations in sex-hormone levels, and to suggest lifestyle interventions of physical activity and dietary modifications (136).

Because aromatase inhibitors used for breast cancer therapy greatly reduce the function of aromatase, situations also impeding aromatase function in humans have been briefly reviewed, focusing on metabolic consequences. A young girl (8-12 years) with aromatase deficiency showed severe insulin resistance (HOMA 5.6) and glucose intolerance, plus high levels of testosterone (2.28 nmol/l), androstenedione (4.92 nmol/l) and FSH (13.4 mIU/ml). Estrogen replacement did not have an effect on these hormone levels or insulin resistance. She developed diabetes while under Metformin therapy. Although GnRHa therapy improved gonadotropin and androgen levels, severe insulin resistance continued (137). At least 19 cases of aromatase gene (CYP19) deficiency have been reported, and although the phenotype differs depending on sex and age, insulin sensitivity may be abnormal in both men and women. In girls, high levels of gonadotropins and androgens facilitate the formation of ovarian cysts (138).

Males with aromatase deficiency or loss of ER α function have impaired glucose metabolism, insulin resistance and hyperinsulinemia (139), plus, in at least one aromatase deficient man, altered lipid profile, impaired liver function and hepatic steatosis (140). Estrogen treatment has improved the metabolic abnormalities in aromatase deficient men, but not in a man without ER α function, suggesting the importance of ER α in metabolism (140). Rodent studies support the importance of ER α in metabolism. Both male and female mice without ER α function have diabetes, obesity and severe hepatic insulin resistance. Ovariectomy in these mice reduces obesity and leads to normal glucose and insulin levels suggesting that ER β activity may lead to diabetes and obesity. Mice without ER β function have improved insulin sensitivity and glucose tolerance and normal body fat levels suggesting ER α helps maintain metabolism (141). Although aromatase deficient mice are insulin resistant, mice with aromatase and intact ovaries are protected from hyperglycemia (142). Estrogens are known to control pancreatic β cell function via ER α . Long term estrogen exposure impacts insulin levels, insulin target gene expression and insulin release in mice without changing β cell mass. In β cells derived from ER α deficient mice, the estrogen dependent insulin release was reduced; this was not the case in cells derived from ER β deficient or normal mice (143). In muscle, GLUT4 expression is induced by ER α and inhibited by ER β (144). Again suggesting the possibility of ER β supporting diabetes formation, GLUT4 expression in male mice is reduced by ER β agonists; plus, ER α deficient mice treated with Tamoxifen (ER antagonist in some tissues) show increased GLUT4 expression in skeletal muscle, although Tamoxifen had no effect on insulin sensitivity or glucose tolerance in normal mice (144). After these and other experiments, the authors concluded that ER β mediates repression of GLUT4 in skeletal muscle but ER α mediates induction of GLUT4 in white adipose tissue, a relationship reflected by the predominance of ER β in skeletal muscle and ER α in white adipose tissue.

Estrogen and Exercise

The effect of physical activity on estrogen levels in postmenopausal women has recently been explored (145). Physical activity was assessed longitudinally using structured recall over 2 years in 194 postmenopausal women, 90 who were randomized to take 1 mg 17 β -estradiol daily, and 104 taking a placebo. Levels of serum sex hormone and serum sex-hormone binding globulin (SHBG) were correlated with physical activity. In women taking the placebo, total energy expenditure was positively associated with SHBG ($p < 0.001$) and inversely associated with testosterone and androstenedione ($p < 0.001$) and with estradiol ($p = 0.02$). In estradiol treated women, estradiol levels were inversely associated with total energy expenditure ($p = 0.002$) and weekly hours spent in moderate or more vigorous physical activity ($p = 0.001$). It was concluded that physical activity was associated with lower serum estradiol levels in all women, and that in placebo treated women, physical activity was associated with reduced androgen levels and elevated SHBG levels.

Aromatase Inhibitors for Cancer Therapy, Insulin and Exercise

Aromatase inhibitors (AIs) are often prescribed to reduce the risk of recurrence in postmenopausal women who have had hormone sensitive breast cancer. AIs act by preventing the conversion of androstenedione to estradiol by inhibiting the aromatase enzyme. Yet, as with many drug therapies, the use of AIs generally causes side effects. Most side effects are related to musculoskeletal events such as myalgias and arthralgias, bone fractures and decreased bone mineral density. Other side effects relate to gynecological and possibly cognitive health (146). The three main AIs used for breast cancer therapy are exemestane (Aromasin), anastrozole (Arimidex) and letrozole (Femara). Exemestane is an irreversible steroidal inhibitor of aromatase, while anastrozole and letrozole are reversible non-steroidal inhibitors.

Up to half of AI users experience AI-induced musculoskeletal syndrome, which involves arthralgia, carpal tunnel syndrome, pain associated with initiating movement, and stiffness. The pathogenesis of this AI

side effect is thought to involve the growth factor and insulin-like growth factor (GH/IGF-1) pathway, which is controlled by sex steroids (147). Many postmenopausal women who have not had cancer and are not taking aromatase inhibitors also have arthralgia, likely from reduced estrogen levels (148). Recently, in 18 AI users with rheumatic concerns (none previous to AI use), 10 had spondyloarthropathy, 2 had oligoarthritis and 6 had simple arthralgia, showing that arthritis is an emerging concern of AI use (149). Decreased bone mineral density and increased fracture risk are also side effects of aromatase inhibitor use for breast cancer treatment due to reduced estrogen levels(150). Although exercise is suggested to prevent AI induced osteoporosis, details of the exercise prescription have not been determined (150). An upcoming study will explore the effect of exercise therapy, and supplementations of vitamin D and calcium to prevent AI induced osteoporosis. Sixty postmenopausal women taking AIs will be randomized into exercise or control groups, with the exercise group participating 3 times per week in a gym based program involving resistance and impact exercises for 12 months, and both groups will receive vitamin D and calcium. The primary outcome will be total hip bone mineral density (151).

Exemestane treatment in a breast cancer patient has been shown to exacerbate diabetes, yet when the patient switched to letrozole, her diabetes symptoms lessened. It was hypothesized that the steroidal exemestane might mimic androgen structure, and impact insulin sensitivity similarly to androgens, whereas the non-steroidal letrozole would not have this effect (152). Other studies support that exemestane acts as a weak androgen (153,154). Women taking exemestane treatment after 2-3 years of Tamoxifen show reduced fat mass, increased ratio between free fatty acids and fat mass, decreased triglycerides and HDL and increased LDL compared to those remaining on Tamoxifen after one year (155). Androgen excess in women poses a risk of insulin resistance, intravascular thrombosis and cardiovascular disease, which can be reduced by exercising and maintaining normal weight (156). Letrozole has been found to suppress the adrenals and blunt the effect of the adrenocorticotropin hormone (ACTH) stimulation on cortisol and aldosterone secretion (157). Anastrozole (Arimidex) has not

been shown to affect the ACTH system. Importantly, none of the 3 main third generation aromatase inhibitors affect basal androgen levels (158).

Interestingly, sympathoadrenal activity increases proportionately with exercise intensity and is closely related to oxygen availability. The acute exercise responses of both norepinephrine and epinephrine are cycle and menstrual status dependent. In premenopausal women, increased exercise training and loss of cycle irregularity cause menstrual cycle dependent differences in resting plasma catecholamines to disappear, while baseline levels of norepinephrine significantly increase. Exhaustive training and loss of cycle regularity increase plasma norepinephrine at VO₂ max but do not affect epinephrine responses. An estrogen poor environment reduces catechol –O-methyltransferase activity by 20%. This enzyme inactivates catecholamines (159). This might impact the exercise induced changing levels of insulin in postmenopausal breast cancer survivors since catecholamines suppress insulin.

Summary

Insulin plays a vital role in glucose homeostasis but if this balance is interrupted by cells not adequately responding to insulin binding, hyperinsulinemia and insulin resistance occur and are linked to chronic diseases including breast cancer. Research supports the association between higher levels of insulin and glucose with increased risk and progression of breast cancer, and poorer prognosis. High levels of glucose and insulin provide a perfect microenvironment for cancer progression; hyperglycemia helps fuel glycolysis in cancer cells and creates carcinogenic oxidative stress, and hyperinsulinemia invokes a pathway leading to angiogenesis and increased glucose transport into cells. In addition, the insulin receptor initiates pathways that regulate cellular growth, proliferation and apoptosis, and hyperinsulinemia increases the synthesis and/or bioavailability of IGF-1 and sex hormones which are also strongly linked to carcinogenesis. So, reducing exposure of glucose and insulin may limit cancer progression. Pharmaceuticals treating insulin resistance, such as Metformin, are now being explored for breast cancer prevention and treatment. Physical activity also decreases insulin levels over time, and

insulin decreases can be to a similar degree as with Metformin. The ability of exercise to reduce insulin resistance depends on the type, intensity, duration and frequency of exercise, and although the ideal exercise prescription for improving insulin sensitivity is still under debate, the current guideline for diabetes prevention through glycemic control is at least 150 minutes of moderate exercise or at least 90 minutes of vigorous exercise per week distributed over at least 3 days with no more than 2 consecutive days without exercise. Exercise induced improvements in insulin sensitivity are noticeable after a single exercise session.

Acute exercise can affect transient glucose and insulin levels, and it is unknown how these short-term changes impact breast cancer risk or prognosis despite the likelihood that chronically high glucose and insulin levels support cancer. At intensities over 80% $\dot{V}O_2$ peak catecholamine levels rise and suppress insulin secretion, and glucose levels rise, but during recovery catecholamine levels drop and insulin levels peak allowing for glycogen stores to be replenished from circulating glucose. This effect is emphasized when exercise is to exhaustion. At lower intensities glucose production and utilization are matched so glucose levels are constant, and insulin secretion is suppressed.

Since higher levels of insulin are linked to breast cancer recurrence and mortality, breast cancer survivors may wish to partake in regular exercise because it improves insulin sensitivity and reduces insulin levels over time. Sport medicine and cancer associations have made recommendations for exercise prescriptions to prevent cancer and for cancer survivors. Exercise programs have been shown to reduce insulin levels in breast cancer survivors, and have also decreased levels of estrogen and body fat. The best exercise prescription for reducing recurrence in breast cancer survivors is unknown, although vigorous intensity and larger doses of exercise have been linked to greater reductions in breast cancer risk. Cancer patients are treated with exercise with the main aims of increasing fitness, physical well-being and quality of life, and to reduce recurrence and mortality.

There are several possible mechanisms linking physical activity with cancer inhibition, and rodent studies have allowed for advancements in these understandings. Preliminary evidence from cancer survivor studies suggest that diet and exercise may positively influence biomarkers associated with progressive disease and overall survival such as insulin levels, oxidative DNA damage and tumour proliferation rates. No known studies have assessed the impact of exercise intensity on insulin levels in breast cancer survivors, although survivors have been shown to react differently to exercise than controls. Survivors show lower lactate responses and tend to have elevated glucose responses to exercise than controls, and also have lower peak exercise stroke volume, cardiac output and $\text{VO}_{2\text{peak}}$.

Interestingly, aromatase inhibitor users have been shown to have less favorable cardiovascular risk profile than survivors taking Tamoxifen. Aromatase inhibitors greatly reduce the production of estrogen in postmenopausal women, and premenopausal estrogen levels are considered beneficial for insulin sensitivity whereas estrogen scarcity or excess androgen levels in women are linked to insulin resistance. Aromatase deficiencies have been linked to metabolic abnormalities including insulin resistance, and have been reduced by estrogen administration. Physical activity reduces estrogen and androgen levels in postmenopausal women. Exercise has been suggested to alleviate the side effects of aromatase inhibitor use, especially muscle and joint pains, although the prescription specifications are undecided.

Hence, exploring how exercise of different intensities affects glucose and insulin levels in postmenopausal breast cancer survivors is well warranted. Higher levels of glucose and insulin are associated with increased risk, progression and mortality of breast cancer, and although long-term exercise regimes improve insulin sensitivity, the impact of acute exercise of different intensities on glucose and insulin levels in postmenopausal breast cancer survivors is unknown. Exercise prescriptions are suggested to improve fitness, well-being and perhaps prevent cancer recurrence in survivors, so it is

crucial that we understand both the short-term and long-term effects of exercise on physiology and cancer etiology in this cohort.

Chapter 2: Body of Thesis

Introduction

For women with postmenopausal hormone receptor positive (HR+) breast cancer aromatase inhibitors (AIs) are recommended to reduce the risk of recurrence (160). The suggested duration of adjuvant endocrine therapy is 5 years, although this has been extended for an additional 5 years in some patients (161). Aromatase inhibitors induce a variety of well-known side effects related to estrogen deprivation (161). These include myalgia, arthralgia and bone loss.

Research is warranted to investigate the effects of estrogen deprivation therapy in breast cancer survivors on glucose homeostasis and insulin resistance. Insulin is a mitogen in both normal and malignant breast cells (162), and high fasting insulin levels are linked to distant recurrence and death in breast cancer survivors (52). Physiological levels of estrogen support normal insulin sensitivity and benefit pancreatic β -cell function however levels above or below this range may promote insulin resistance and type 2 diabetes (163). The effect of aromatase inhibitors on insulin resistance in postmenopausal breast cancer survivors has not been explored, although studies of aromatase deficient men and animals have been described. Maffei et al. (140) reported a case of a 29 year old male with an aromatase gene mutation who had increased serum levels of glucose, insulin and low density lipoprotein; all of these values were greatly reduced with estrogen administration. Mice without the ability to produce aromatase show three times the serum insulin levels of mice able to produce aromatase, and liver fat accumulation was reversed by estrogen treatment in mice lacking aromatase (164). Interestingly, there are also animal studies linking impaired estrogen receptor alpha (ER- α) to insulin resistance (165,166). Considering this literature, it is possible that sub-physiological levels of estrogen in aromatase inhibitor users may increase the risk of insulin resistance which may influence the risk of recurrence and insulin resistance linked comorbidities, such as type 2 diabetes and cardiovascular disease.

Physical exercise has been prescribed to breast cancer survivors to help lessen fatigue and improve physical fitness and functioning during and after treatment for breast cancer (167). Exercise is recommended for aromatase inhibitor users specifically to alleviate the adverse effects of bone loss and joint pain (161). It is also recommended to prevent and treat insulin resistance (168). However, a specific exercise prescription for women receiving AIs has not been developed.

Exercise training is thought to reduce serum levels of insulin (168). During exercise intervention studies, blood is generally taken while subjects are in a resting state, and does not represent the short-term fluctuations in serum proteins related to exercise. If a single bout of exercise causes a transient increase in insulin, the overall exposure to insulin may be increased during an exercise intervention, even if long-term resting values decrease.

When considering the effect of exercise on serum insulin, the intensity of the exercise prescription is a critical factor. At intensities above 80% of peak oxygen consumption (VO_{2peak}) catecholamine levels rise and suppress insulin secretion. During recovery, however, catecholamine levels decrease and insulin levels rise substantially. At intensities $<60\% VO_{2peak}$, insulin levels remain constant, or may even decrease slightly (74). It appears that high intensity exercise may cause insulin spikes during recovery and low intensity exercise may keep insulin levels stable.

Relationships involving estrogen, insulin, insulin resistance, breast cancer and exercise have been noted in the literature. Estrogen deprivation may promote insulin resistance resulting in increased serum insulin levels, which may pose health risks in breast cancer survivors. Exercise is suggested for breast cancer survivors yet high intensity exercise may cause substantial increases of serum insulin in recovery. Whether or not these effects might influence the well-being of breast cancer survivors is unknown.

Also, proinsulin is cleaved into insulin and C-peptide and an equal proportion of these cleaved substances are secreted simultaneously. C-peptide is often used as a marker of insulin secretion since it has a longer half-life than insulin of 35 compared to 5 minutes (169).

Are breast cancer survivors taking aromatase inhibitors at greater risk of insulin resistance than breast cancer survivors not taking this medication? Does the intensity of an exercise session affect serum glucose, C-peptide and/or insulin levels in breast cancer survivors? Do breast cancer survivors taking aromatase inhibitors show different levels of serum glucose, C-peptide and/or insulin in response to exercise bouts, compared to survivors not taking this medication? Questions such as these are pertinent considering the role of exercise prescription in breast cancer survivorship.

In this study we assessed glucose, C-peptide, and insulin levels before, immediately after and 45 minutes after moderate and intense exercise sessions in 3 groups: controls, breast cancer survivors taking aromatase inhibitors (AI) and breast cancer survivors not taking aromatase inhibitors (BCS).

Methods

Breast cancer survivors were recruited by email and personal contact with breast cancer survivor dragon boat teams in the Vancouver Regional District and Fraser Valley of British Columbia. Control subjects were recruited from a paddling club (Ridge Canoe and Kayak Club) or referred to this study from other researchers who had informed the public of their studies using ethically approved posters in recreation centres and word of mouth. Women who expressed interest in participating were sent consent forms to be reviewed and they were assessed for eligibility. After viewing the forms, interested and eligible subjects scheduled dates for their participation in the study.

Eligibility

Subjects were eligible to participate if they met the following criteria:

- Breast cancer survivor taking an aromatase inhibitor, or breast cancer survivor not taking an aromatase inhibitor, or women who have never been diagnosed with breast cancer
- Did not smoke
- Were postmenopausal women (no menses for at least 12 months)
- Were able to provide written consent in English or French

Exclusion Criteria

- uncontrolled hypertension, cardiac disease, a psychiatric condition or any other medical problem that prohibits exercise
- metastatic disease

Protocol

Each subject followed the same procedure, and participated in 3 research sessions. Subjects were not taking any medications that would influence the results. Caffeine was prohibited for 12 hours prior to each session. Before the initial visit each subject fasted for a minimum of 12 hours. Upon arrival, the Participation Readiness Questionnaire (PAR-Q) and duplicate consent forms were completed, and breast cancer survivors gave brief descriptions of their cancer histories. Height and weight were measured. Body mass index (BMI) was calculated and blood was drawn for insulin and glucose analysis. After a snack and at least 30 minutes of rest, a graded exercise test on a treadmill was performed to measure maximal oxygen consumption.

During a 5-10 minute warm up, the treadmill speed that would remain constant for the test was determined based on the cadence, perceived fitness, and treadmill comfort of each subject, with the aim of finding a speed that would enable a 10-15 minute incremental test to be performed. The subject was assisted with putting on a mask, which was attached to a calibrated metabolic cart (SensorMedics Vmax Series 29, Yorba Linda, California) recording expired gases. The test began with the treadmill at 0

grade, and the grade was increased by 2% every 2 minutes. Heart rate was recorded every minute using a Polar Electro heart rate monitor (Kempele, Finland). Subjects were allowed to place their fingertips on the treadmill if balance support was desired. The test ceased when a respiratory ratio of 1.1 was reached, or at the request of the subject.

Oxygen consumption values were displayed as 20 second averages for the test. Peak oxygen consumption was calculated as the average of the highest three consecutive values. To determine target heart rates for the high and moderate intensity exercise bouts, heart rate and VO_2 values for each minute of the exercise test were graphed. The desired intensity of percent VO_2 peak for each was divided by 100 then multiplied by peak VO_2 . For example, 85% of a VO_2 peak of 25.63 ml/kg/min is $0.85 \times 25.63 = 21.79$. A trend line was used to find the heart rate corresponding with each percent VO_2 of interest (60% and 85%). Exercise intensities for the next 2 sessions were monitored by heart rate.

Standard breakfasts were distributed at the first sessions and were to be eaten 3 hours prior to the commencement of each of the following sessions. The quantities of food varied depending on the mass of the subject (table 1) but all breakfasts included at least one Sunrype fruit bar (32 g carbohydrate, 130 Calories), Mott's Fruitsations unsweetened fruit sauce (13 g carbohydrate, 50 Calories) and Western Family Multigrain Rice Cake (8 g carbohydrate, 40 calories). Similar to the recommendation of ingesting 1 g carbohydrate per kg of human mass 1 hour prior to exercise, subjects were asked to consume this amount 3 hours prior to exercise with the aim of subjects arriving in postabsorptive states. The high proportion of carbohydrate in the meal was chosen because it could be absorbed more quickly than a mixed meal, helping to ensure all subjects were in postabsorptive states at the beginning of each session.

Table 1. Types and Amounts of Breakfast Foods Depending on Subject Mass

Weight of subject	Breakfast
under 59.5kg/130.9lbs	1 Bar, sauce and 1 cake (53 g, 220 Cal)
60kg/131lbs to 70kg /154lbs	1 Bar, 2 sauces and 1 cake (66g, 270 Cal)
70kg /154lbs to 79.5kg/174.9 lbs	1 bar, 2 sauces, 2 cakes (74 g, 310 Cal)
over 80 kg or 175 lbs	2 bars, 1 sauce and 1 cake (85g, 350 Cal)

The next 2 visits to the lab had similar formats but the intensities of exercise were applied randomly by rolling a die and assigning moderate and intense exercise sessions to odd and even numbers, respectively. Subjects ate their standard breakfasts between 6 am and 9 am, depending on scheduling, and travelled to the laboratory by motor vehicle. Three hours after eating, blood was drawn into serum separating tubes by venepuncture. Subjects warmed-up for 5-10 minutes, and then performed the prescribed intensity treadmill session, followed by a cool down. Blood was taken immediately after the session, and again 45 minutes into recovery. The exercise testing and laboratory sessions were supervised by a physician, and emergency equipment such as a defibrillator and oxygen were available.

The high intensity exercise session was 10 minutes at 85% VO_2peak and the low intensity session was 45 minutes at 60% VO_2peak , as determined by the initial graded treadmill test. At exercise intensities above 80% VO_2max , catecholamine hormones reach their highest plasma concentrations, with epinephrine and norepinephrine levels 17 to 20 times greater than at rest (13). This compares to a two to four fold increase of these catecholamines at moderate exercise intensities (170,171). Despite insulin suppression during even moderate intensity exercise, glucose production is matched by glucose utilization, and insulin levels are not dramatically affected after the bout due to post-exercise euglycemia. Conversely, intense exercise induces a marked rise in glucose production that is not

matched by glucose utilization; however, the high catecholamine levels provoked by intense exercise are thought to prevent glucose stimulation of insulin secretion. After the exercise session ceases, catecholamine levels quickly drop and insulin secretion is no longer inhibited, thus insulin levels rise in response to post-exercise hyperglycemia. Insulin levels are thought to remain heightened for up to one hour after exercise cessation (74). Hence, for this pilot study, we chose the high intensity bout to be 85% VO_2max , aiming to induce a post-exercise rise in insulin levels. With the aim of exercising long enough to provoke a substantial rise in catecholamines, yet not so long to be unmanageable by subjects, duration of 10 minutes was chosen for the high intensity exercise bout. For the low/moderate intensity bout, 45 minutes at 60% of VO_2peak was chosen, as exercise at this intensity and duration is not thought to have a great impact on catecholamine levels and it is consistent with a traditional 'aerobic workout' that an individual may undertake to meet the proposed physical activity guidelines for cancer survivors.

Blood Analyses

Serum glucose was measured immediately after each blood sample was taken using blood from the serum separating venepuncture tubes using a handheld glucose monitor (Bayer, Contour). Blood was kept on ice if it was to remain in the venepuncture tubes for more than 1 hour. The tubes were spun in a refrigerated centrifuge within 4 hours @ 7000 rpm for 10 minutes. Serum was separated from formed elements, and distributed into labeled epindorf tubes then frozen at -20 before being transferred to a -80 freezer. Serum C-peptide and insulin levels were assessed using an electrochemiluminescence immunoassay (ECLIA) (Cobas e 411, Roche Diagnostics GmbH, Mannheim) with sensitivities of 0.003 nmol/L and 1.30 pmol/L, respectively. The fasting levels of insulin and glucose were used to estimate insulin resistance using the HOMA-IR calculation (172). The Diabetes Trials Unit of Oxford University has created a convenient software package for HOMA-IR calculations (HOMA-IR Calculator, Version 2.2.2).

Statistical Analyses

Using SPSS, descriptive data was obtained for age, BMI, VO_2peak (relative and absolute), maximum heart rate, fasting glucose, C-peptide and insulin, and HOMA-IR for controls, breast cancer survivors taking aromatase inhibitors, and breast cancer survivors not taking aromatase inhibitors. For these factors, Analysis of Variance (ANOVA) compared means to assess homogeneity between the 3 groups, and Fisher's Least Significant Difference (LSD) post-hoc explored values at or near significance. For both exercise intensities, the glucose, C-peptide and insulin levels changed from pre-exercise to post-exercise, post-exercise to 45 minute recovery and pre-exercise to 45 minute recovery in each subject. These changes were calculated and then compared. To see if groups affected the changing glucose, C-peptide, and insulin levels depending on exercise intensity the General Linear Model (GLM) was used with groups as covariates. In this test, the difference between intensities for each post- to pre-, 45-post- to post- and 45-post- to pre-exercise change was used. Paired t-tests compared the pre-exercise to post-exercise, post-exercise to 45 minute recovery and pre-exercise to 45 minute recovery differences in glucose, C-peptide and insulin between exercise intensities in separate cohorts using the false discovery rate (FDR) correction for multiple tests. There were nine tests performed since each difference (post- to pre-, 45-post- to post- and 45-post- to pre-exercise) in each substance (glucose, insulin and C-peptide) was compared between intense and moderate exercise.

Results

Thirty-two subjects were recruited. Two subjects dropped out of the study after their first sessions due to scheduling challenges. One subject revealed that she was in a clinical trial and that she might have been taking Letrozole or a placebo, so her group could not be determined. Upon exploration of raw data, some outlier values were discovered and the data from one subject was removed from the AI group due to several extremely high values (glucose: 7.9 mmol/L, C-peptide: 1461 pmol/L, insulin: 148.7 pmol/L). Thus, upon statistical analyses there were 10 controls, 8 aromatase inhibitor users and 10

survivors not using aromatase inhibitors; when survivor groups were combined there were 19 survivors because the subject with unknown aromatase inhibitor status was included.

Descriptive Analyses

Cancer history for the AI and BCS groups is displayed in table 2. Women in the AI group were diagnosed much more recently than those in the BCS group, and this difference was statistically significant ($p = 0.01$). A greater proportion of women in the AI group had had chemotherapy, radiation and surgery than women in the BCS group. An equal proportion of women in both groups had used Tamoxifen, including one woman in the BCS group who was currently taking Tamoxifen. One woman in the BCS group had taken an aromatase inhibitor in the past but was not currently taking it.

Table 2. Cancer History of the AI and BCS Groups

	AI	BCS
Years since diagnosis	4.25 \pm 2.6	12.10 \pm 7.5
Chemotherapy (%)	87.5 (7 of 8)	60 (6 of 10)
Radiation (%)	75 (6 of 8)	40 (4 of 10)
Surgery (%)	100 (8 of 8)	90 (9 of 10)
Tamoxifen	50 (4 of 8)	50 (5 of 10)

Means and standard deviations describing pertinent subject characteristics are located in table 3.

ANOVA found the 3 groups to be homogeneous in terms of age, BMI, relative VO_2peak , absolute VO_2peak , maximum heart rate (HRmax), fasting glucose, C-peptide and insulin, and HOMA-IR values.

Still, age differences between groups approached significance ($p = 0.08$) and using LSD Post Hoc, the AI and BCS groups were determined to be significantly different in age ($p = 0.03$). Other variables with p values approaching significance ($0.05 < p < 0.1$) were BMI between control and BCS groups, and AI and BCS groups, and absolute VO_2max between AI and BCS groups.

Table 3. Means and Standard Deviations of Baseline Data for Each Group

	Control (n=10)	AI (n=8)	BCS (n=10)	Survivors (n=19)
Age	60.2 ± 6.3	56.6 ± 5.4	63.2 ± 5.9	60.9 ± 6.8
BMI	26.0 ± 2.3	26.0 ± 2.7	23.7 ± 3.0	24.7 ± 2.9
Relative VO ₂ peak	29.6 ± 5.6	31.1 ± 7.0	28.9 ± 4.1	29.6 ± 5.5
Absolute VO ₂ peak	1.93 ± 0.4	2.19 ± 0.4	1.84 ± 0.3	1.97 ± 0.4
HRmax	175.7 ± 10.8	174.0 ± 7.5	171.5 ± 7.5	171.4 ± 8.5
Fasting glucose (mmol/L)	5.28 ± 0.5	5.25 ± 0.8	5.55 ± 0.8	5.35 ± 0.8
Fasting C-peptide (pmol/L)	625.71 ± 212.1	542.73 ± 124.2	619.96 ± 255.7	577.54 ± 203.5
Fasting insulin (pmol/L)	47.51 ± 12.6	40.18 ± 17.4	43.63 ± 21.3	42.35 ± 18.6
HOMA-IR	0.91 ± 0.2	0.75 ± 0.3	0.87 ± 0.4	0.82 ± 0.3

General Linear Model (GLM)

The differences in the levels of glucose, C-peptide and insulin between post and pre-exercise, 45 minutes post and immediately post-exercise, and 45 minutes post- and pre-exercise for each intensity were calculated for each subject. Then, the difference between each of these differences was calculated for each subject between the intense and moderate exercise sessions, creating 9 value sets. These values were used for the GLM. In this model, all data was analyzed with the two survivor groups separately used as covariates. This model determined if there was statistical evidence for the results from simple t-tests performed without differentiating the groups to be different from the results when each survivor group was a covariate. In this way, the impact of group on the differences between exercise intensities between glucose, C-peptide and insulin levels and between pre-, immediately post-, and 45 minutes post-exercise were assessed.

Importantly, the GLM indicated that the results of the tests did not change when the covariates were used. Each survivor group, regardless of aromatase inhibitor use, was not significantly different from the control group when assessing the differences between intense and moderate exercise for the differences between post- and pre-exercise, 45 minutes post- and immediately post-exercise, and 45 minutes post- and pre-exercise levels of glucose, C-peptide and insulin. The p-values, ranging from 0.16 to 0.91 (table 4), demonstrate that there was no statistical evidence obtained for the results of the GLM

being different when either survivor group was used as a covariate compared to when the groups were not differentiated. There is no statistical evidence that the 3 groups differ in their changing values of glucose, C-peptide and insulin between moderate and intense exercise.

Table 4. Adjusted P-values for GLM Assessing Changes Between Post- and Pre-, 45 Recovery- and Post- and 45 Recovery- and Pre-Exercise Between Exercise Intensities Using AIs and BCSs as Separate Covariates

		p-value	p-value
Blood substance	Difference between intensities	AI	BCS
Glucose	Post – pre-exercise	0.91	0.91
Glucose	45 recovery – post-exercise	0.52	0.87
Glucose	45 recovery – pre-exercise	0.57	0.87
C-peptide	Post – pre-exercise	0.58	0.87
C-peptide	45 recovery – post-exercise	0.25	0.87
C-peptide	45 recovery – pre-exercise	0.81	0.91
Insulin	Post – pre-exercise	0.29	0.87
Insulin	45 recovery – post-exercise	0.16	0.87
Insulin	45 recovery – pre-exercise	0.68	0.88

Regardless, graphs depicting the glucose, C-peptide and insulin levels for the 3 groups separately are in Appendix 2.

Survivor Paired T-Tests

The two survivor groups were combined (n = 19) since the groups were not proven to be statistically different in their changing levels of glucose, C-peptide and insulin between exercise intensities, the combined group has more statistical power than either group alone, and it is a clinically relevant cohort. Paired t-tests analyzed the differences in post- and pre-exercise, 45 minutes post- and immediately post-exercise, and 45 minutes post- and pre-exercise levels of glucose, C-peptide and insulin between intense and moderate exercise. Nine t-tests were performed, and the False Discovery Rate (FDR) method of correction for multiple tests was used.

The p-values in table 5 show how glucose, C-peptide and insulin level changes impacted by exercise and its recovery differ between moderate and vigorous exercise intensities in survivors (Figures 1-3).

Table 5. Adjusted P-values for Post- Minus Pre-, 45 Recovery- Minus Post-, and 45 Recovery Minus Pre-Exercise Differences Between Intensities in All Survivors (n=19)

Substance	Test	p-value (FDR)
Glucose	Post – pre-exercise	0.01
Glucose	45 recovery – post-exercise	0.01
Glucose	45 recovery – pre-exercise	0.52
C-peptide	Post – pre-exercise	0.04
C-peptide	45 recovery – post-exercise	0.04
C-peptide	45 recovery – pre-exercise	0.24
Insulin	Post – pre-exercise	0.09
Insulin	45 recovery – post-exercise	0.24
Insulin	45 recovery – pre-exercise	0.24

Glucose

The glucose levels before, immediately after, and 45 minutes after moderate and intense exercise in survivors are displayed in Figure 1. The difference between pre- and post-exercise glucose levels between moderate and intense exercise in survivors was significant ($p = 0.01$); glucose increased significantly more during intense exercise than during moderate exercise in survivors. The difference between post-exercise and post-45 minute recovery glucose levels between moderate and intense exercise in survivors was also significant ($p = 0.01$); glucose levels decreased significantly more during recovery from intense exercise than during recovery from moderate exercise. Yet, the difference in pre- and 45 minute post-exercise glucose was not significantly different between moderate and intense exercise in survivors ($p = 0.52$); the change between pre-exercise and 45-minute-post –exercise glucose levels was similar for both intensities, still slightly increased after recovery compared to before exercise.

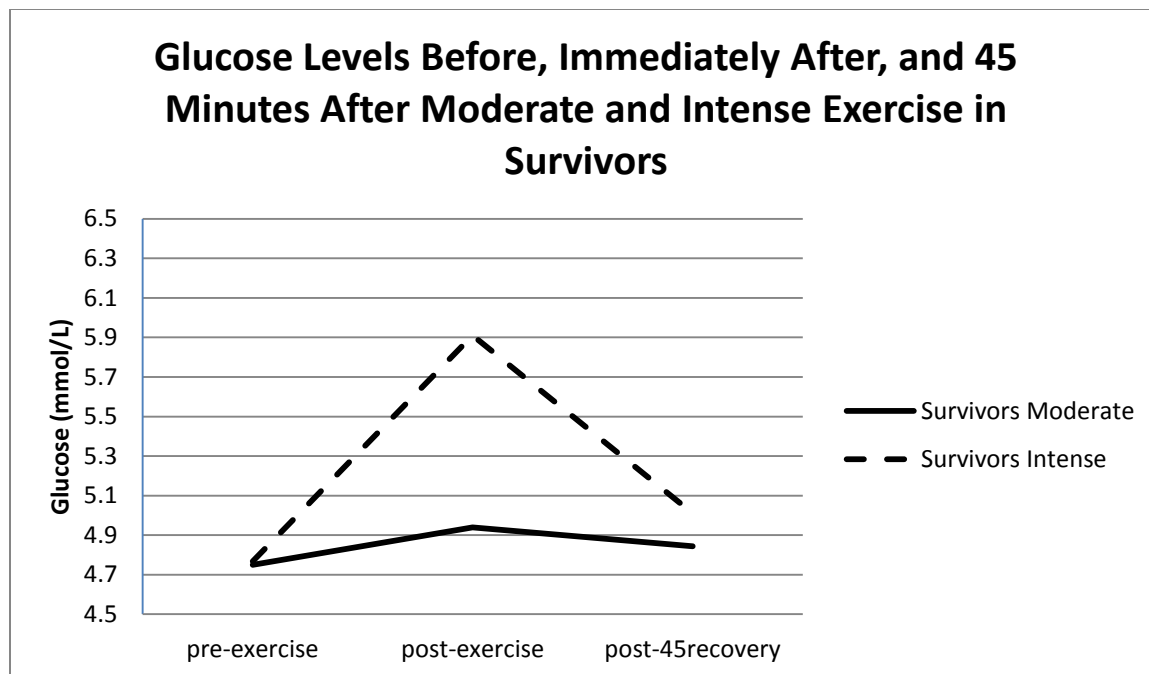


Figure 1. Glucose Levels Before, Immediately After and 45 Minutes After Moderate and Intense Exercise in Survivors

C-Peptide

The C-peptide levels before, immediately after and 45 minutes after moderate and intense exercise in survivors are displayed in Figure 2. The difference between pre- and post-exercise C-peptide levels between moderate and intense exercise in survivors was significant ($p = 0.04$); C-peptide increased during intense exercise and decreased during moderate exercise creating a significant difference in its changing levels between exercise intensities. The difference between post-exercise and post-45 minute recovery C-peptide levels between moderate and intense exercise in survivors was also significant ($p = 0.04$); C-peptide decreased more during recovery from intense exercise than for moderate exercise. Yet, the difference in pre- and 45 minute post-exercise C-peptide was not significantly different between moderate and intense exercise in survivors ($p = 0.24$); the change between pre-exercise 45-minute-post-exercise C-peptide levels was similar for both intensities, with lower C-peptide levels after recovery than before exercise.

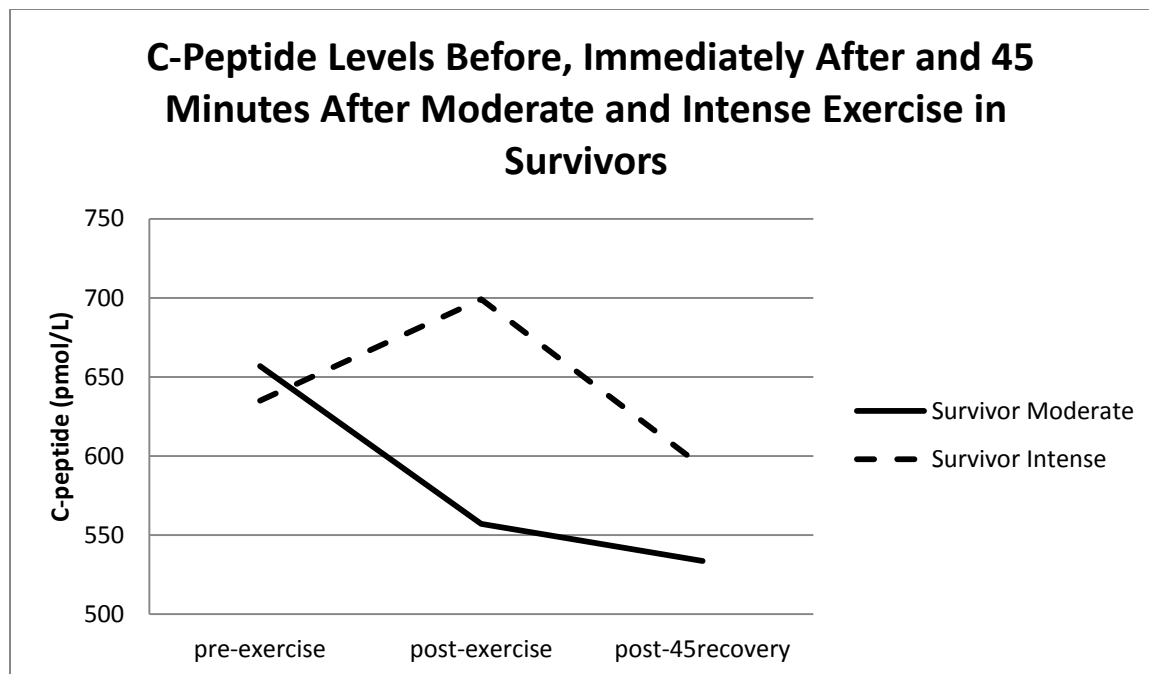


Figure 2. C-Peptide Levels Before, Immediately After and 45 Minutes After Moderate and Intense Exercise in Survivors

Insulin

Insulin levels before, immediately after and 45 minutes after moderate and intense exercise in survivors are displayed in Figure 3. The difference between pre- and post-exercise insulin levels between moderate and intense exercise in survivors was noteworthy but not statistically significant ($p = 0.09$); insulin increased more during intense exercise than for moderate exercise in survivors. The difference between post-exercise and post-45 minute recovery insulin levels between moderate and intense exercise in survivors was not statistically significant ($p = 0.24$); the decrease in insulin during recovery was similar for both intensities. The difference in pre- and 45 minute post-exercise insulin was not significantly different between moderate and intense exercise in survivors ($p = 0.24$); the change pre-exercise and 45 minute post-exercise insulin was similar for both intensities with insulin levels slightly lower after recovery than before exercise.

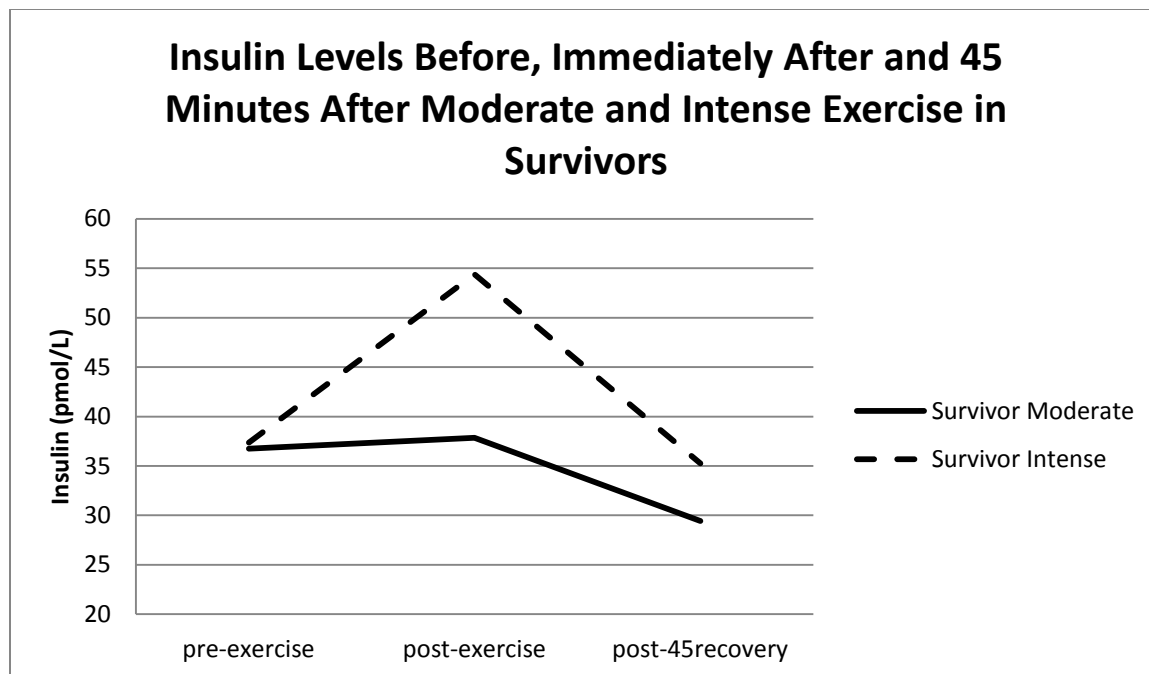


Figure 3. Insulin Levels Before, Immediately After and 45 Minutes After Moderate and Intense Exercise in Survivors

Control Paired T-Tests

For the sake of curiosity, the nine FDR corrected paired t-tests were also performed for the control group (n = 10). None of the tests had statistically significant results; there was no evidence that the changing glucose, C-peptide and insulin levels before, immediately after and 45 minutes after exercise were affected by exercise intensity in the control group. Still, an ANOVA test comparing the changing levels of blood substances between survivors and controls showed no statistical differences between groups (Figures 4-6).

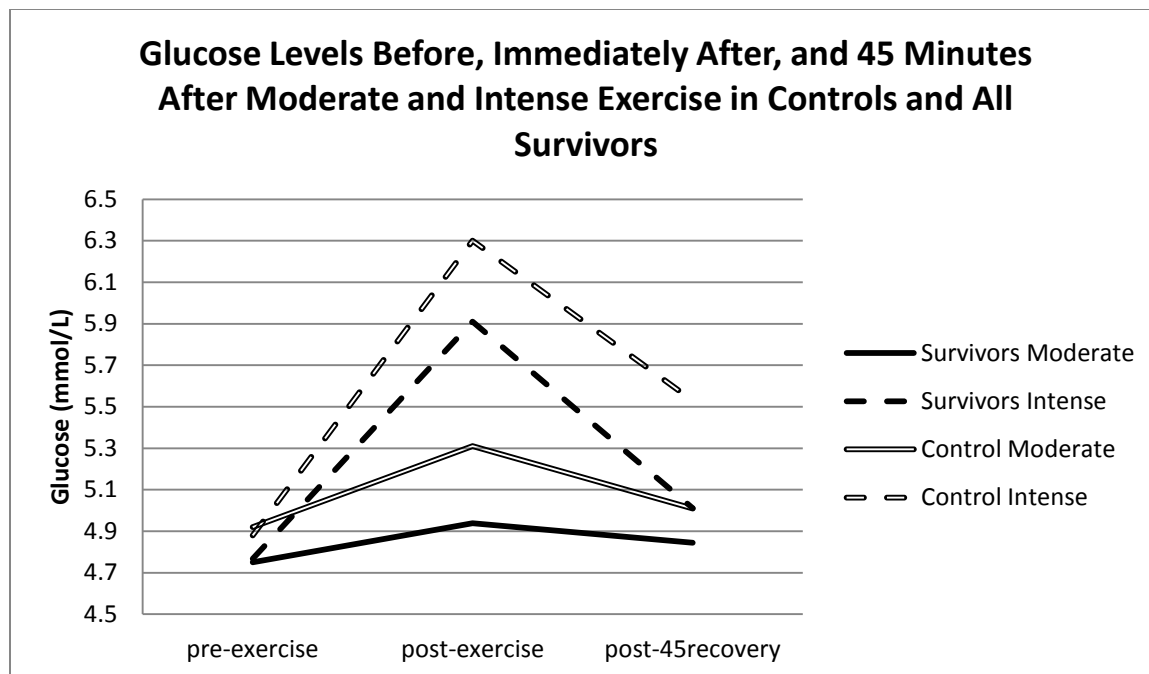


Figure 4. Glucose Levels in Controls and All Survivors Before, Immediately After and 45 Minutes After Moderate and Intense Exercise.

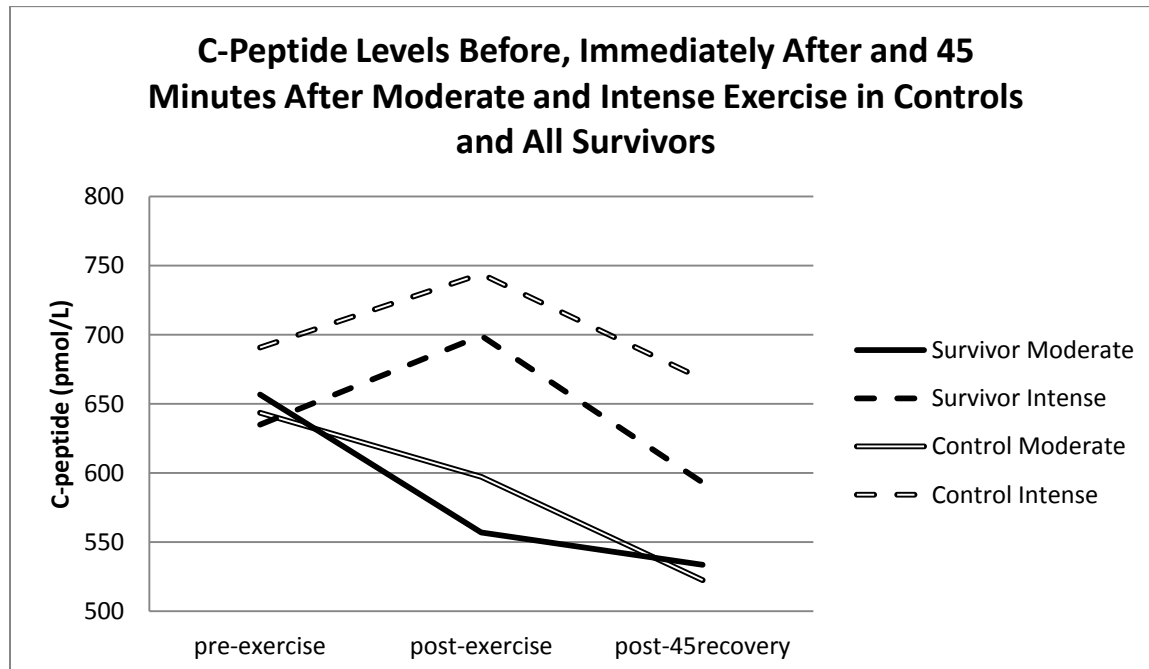


Figure 5. C-Peptide Levels in Controls and All Survivors Before, Immediately After and 45 Minutes After Moderate and Intense Exercise

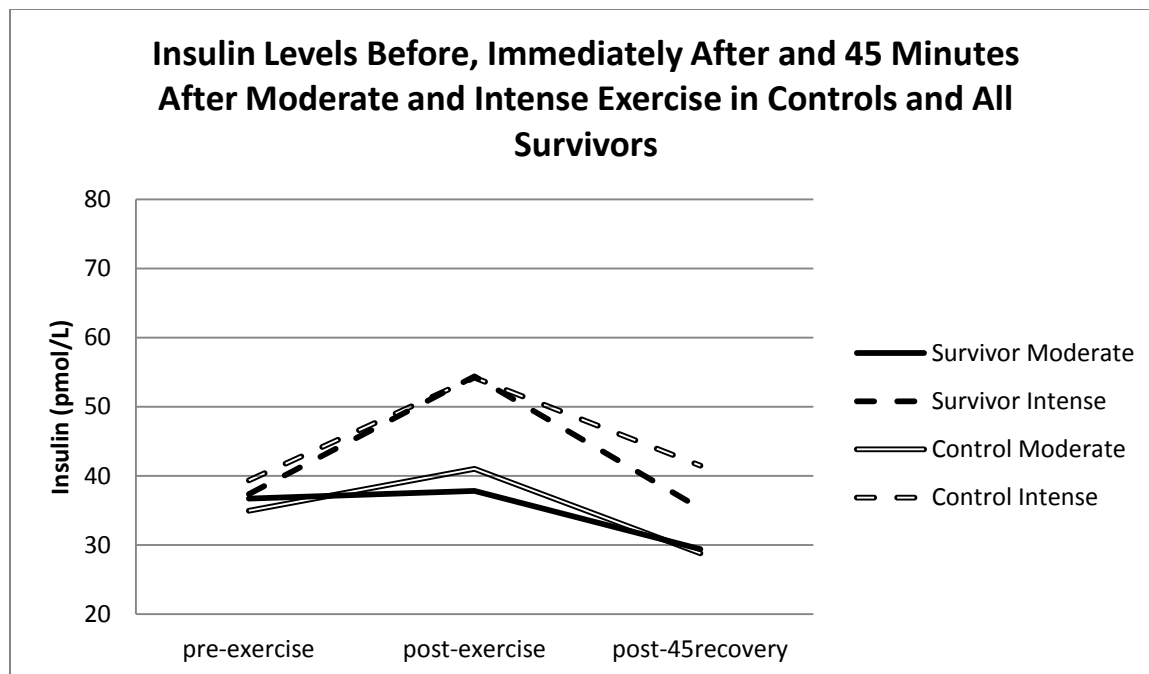


Figure 6. Insulin Levels in Controls and All Survivors Before, Immediately After and 45 Minutes After Moderate and Intense Exercise.

Discussion

One of the main findings in this study is that there are no significant differences in the fasting levels of glucose, C-peptide, and insulin between the control, AI and BCS groups, leading to non-significant differences in insulin resistance (HOMA-IR). Another major finding is that the levels of glucose, C-peptide and insulin change similarly after exercise and its recovery in all groups. Importantly, in all breast cancer survivors combined ($n = 19$) there were significant differences between intensities in the changing levels of glucose and C-peptide during exercise and its recovery (glucose, $p = 0.01$ and 0.01 , C-peptide, $p = 0.04$ and 0.04 , respectively) and a difference approaching significance for the increase in insulin during exercise ($p = 0.09$) – all resulting in higher transient levels of glucose, C-peptide and insulin during intense exercise compared to moderate exercise.

Although the risk of developing post-menopausal breast cancer has been significantly associated with elevated fasting insulin (16), this study did not find significantly increased levels of fasting insulin in breast cancer survivors compared to controls. Women in the AI group had been diagnosed 4.25 ± 2.6

years ago. Women in the BCS group had been diagnosed 12.10 ± 7.5 years ago. If higher insulin levels had predisposed these breast cancer survivors to their diagnoses, there is no evidence of it several years after their cancer treatments. All of the subjects in this study were physically active; regular exercise can maintain and increase insulin sensitivity and corresponding insulin levels. Perhaps differences in insulin sensitivity would have been noticed between sedentary controls and breast cancer survivors opposed to this active cohort.

The results of this study do not support that estrogen deficiency secondary to use of aromatase inhibitors promotes insulin resistance in breast cancer survivors. There were no significant differences in HOMA-IR values between any of the 3 groups. It was hypothesized that AI users may have had insulin resistance due to their theoretically lower estrogen levels compared to postmenopausal women not taking AIs. Although estrogen was not measured in this study, disturbances with estrogen levels have been linked with insulin resistance, and the mechanism of aromatase inhibitor treatment is to reduce the conversion of androgens to estrogens thus reducing circulating estrogen levels. The uniformity in HOMA-IR values between the control, AI and BCS groups does not support the notion that aromatase inhibitor use may be linked to insulin resistance. Interestingly, the aromatase inhibitor exemestane (Aromasin) has been linked to the exacerbation of diabetes in a case study (152). When the patient receiving exemestane showed increased fasting glycemia, she was removed from treatment and her glycemia reduced. She was again given exemestane and glycemia returned; this time exemestane was replaced with letrozole, and glycemia decreased to the same level as without aromatase inhibitor treatment. The authors concluded that exemestane's androgen-like steroid structure may have enabled its negative impact on insulin sensitivity rather than its effect on estrogen levels. The authors suggested that although exemestane may reduce insulin sensitivity, letrozole may have no effect or increase insulin sensitivity. None of the women in the AI group were taking exemestane; four women were taking letrozole, and five were taking anastrozole, neither of which have steroid structures. Interestingly, a

man with aromatase deficiency has shown increased fasting glucose and insulin levels that are reduced with estrogen administration (140). Perhaps the ratio between androgen and estrogen levels has a greater impact on insulin resistance than the levels of these hormones alone; men without aromatase and women taking the androgen-like exemestane may have higher androgen/estrogen ratios than the normal population or than women taking aromatase inhibitors not mimicking androgens. This notion also makes sense when considering the high prevalence of insulin resistance in women with polycystic ovarian syndrome, in which patients have abnormally high androgen levels. If the androgen/estrogen ratio affects insulin resistance rather than simply low levels of estrogen, women taking non-steroidal aromatase inhibitors may not be at risk of insulin resistance.

Importantly, intense exercise resulted in transiently higher levels of glucose, C-peptide, and insulin compared to moderate exercise. What clinical relevance can be obtained from these results for breast cancer survivors? High levels of glucose, C-peptide and insulin have all been associated with breast cancer progression.

In postmenopausal women, high serum levels of glucose have been linked to breast cancer risk (8) and worse prognosis (41,42). Elevated serum glucose helps increase DNA synthesis of tumour cells and provokes free radical production disrupting DNA and its repair mechanisms and initiating damaging pathways of carcinogenesis. Hyperglycemia also leads to glycated protein structures that enhance production of free radicals, cytokines and growth factors (9,15). Glucose measurements from studies linking hyperglycemia and cancer have been taken from resting women, and are thought to represent chronic states of glucose levels. Transient hyperglycemia experienced by postmenopausal survivors performing intense exercise, as occurred in this study, has not been explored for its effect on cancer risk or prognosis. Since glucose levels increased significantly greater during intense exercise than during moderate exercise in survivors participating in this pilot study, the effect of exercise induced glucose

increases on cancer etiology in breast cancer survivors should be examined in future studies, especially considering that chronic hyperglycemia is already indicated in promoting carcinogenesis and development of other chronic diseases such as type 2 diabetes and cardiovascular disease.

In postmenopausal women, high serum levels of C-peptide have been linked to increased mortality. A prospective, observational study of 604 women assessed the association between serum C-peptide, a marker of insulin secretion, and death from all causes and breast cancer (43). Fasting C-peptide levels, measured 3 years after breast cancer diagnosis, were associated with an increased risk of death from all causes and breast cancer in women without type 2 diabetes. A 1-ng/mL (333 pmol/L) increase in C-peptide was associated with a 31% increased risk of any death (HR 1.31; 95% CI, 1.06 to 1.63; $P = .013$) and a 35% increased risk of death as a result of breast cancer (HR 1.35; 95% CI, 1.02 to 1.87, $P = .048$). In this study focusing on exercise intensity, the average increase in C-peptide during intense exercise was 58 pmol/L. Proinsulin is cleaved to produce insulin and C-peptide, and although C-peptide is often measured to assess insulin secretion it has been historically considered an inert substance.

Interestingly, C-peptide has been shown to serve its own functions, likely via a G-protein coupled receptor and Ca^{2+} -dependent intracellular signalling pathways that lead to MAPK, Na^+/K^+ -ATPase and eNOS stimulation, and insulin mimicry(173). Although C-peptide has mainly been researched due to its deficiency in type 1 diabetics, its downstream effects can also be considered in the light of cancer.

Disregulation of the MAPK pathway leading to excessive activation is fundamental to many cancers, including breast cancer (174). The enzyme Na^+/K^+ -ATPase has been implicated in the development and progression of different cancers and is a recent target for anti-cancer therapy (175). Plus, eNOS enables angiogenesis, a necessary component of cancer progression (176). The substances stimulated by C-peptide seem to favour the presence of cancer. In this study, C-peptide increased during intense exercise whereas it decreased during moderate exercise, resulting in statistically significant changes in C-peptide levels between exercise intensities in survivors. Intense exercise showed a transient increase

in C-peptide whereas moderate exercise resulted in decreasing C-peptide levels during and up to 45 minutes after exercise. Since lower C-peptide levels have been found to reduce all cause mortality and breast cancer mortality in women diagnosed with breast cancer, and C-peptide signalling pathways might be linked to cancer, reducing C-peptide levels via moderate exercise might be beneficial for breast cancer survivors. More research is required to discover the duration of decreased C-peptide levels after recovery from exercise of different intensities, and whether exercise induced decreases in C-peptide affect carcinogenesis and mortality in breast cancer survivors. Additionally, further research could explore the possibility of cancer related negative impacts of the transient increase of C-peptide from intense exercise.

In postmenopausal women, high serum levels of insulin have been linked to breast cancer risk (16) and worse prognosis (44,45). Aside from insulin's metabolic role, it is a growth factor that enhances the production and mitogenic properties of insulin-like growth factors leading to cell proliferation (9). Insulin and insulin-like growth factors bind to the insulin receptor, which activates pathways that centrally control cellular growth, proliferation and apoptosis – abilities needed for tumour development. Hyperinsulinemia can increase the synthesis and/or availability of sex-steroid hormones that can enhance tumour development (19). Increased serum insulin reduces hepatic synthesis and serum levels of sex hormone binding globulin (SHBG), increasing bioavailable estrogen which would support estrogen dependent cancer (12,17). The link between increased insulin levels and breast cancer is so strong that some oncologists now treat their patients with the insulin lowering drug metformin. The prognostic effect of insulin is important because insulin levels are modifiable using metformin or lifestyle interventions focusing on weight loss or physical activity (47). In this study, the difference in the pre- to post-exercise insulin levels approached significance between exercise intensities in survivors. Interestingly, insulin levels decreased after 45 minutes of recovery after both intensities, most obviously after moderate exercise. There was a notable increase in insulin levels during intense exercise. These

observations may have clinical implications. It is uncertain if this temporary insulin increase with intense exercise can impact cancer prognosis through insulin's mitogenic and signaling effects. More research is required to assess the effect of exercise induced transient increases of insulin on breast cancer considering the known adverse connection between chronic hyperinsulinemia and breast cancer prognosis. Plus, the tendency for moderate exercise to lower insulin levels after recovery may have clinically beneficial effects for survivors.

There are a few points to consider regarding study outcomes and design. Importantly, low power limited the results of this pilot study. For example, the changes in insulin levels approached a significant difference during exercise between intensities. Insulin measurements had high standard deviations. The high standard deviations limiting the power of statistical testing could have been counteracted by having a higher number of study participants. On another note, continuous blood monitoring measuring glucose, C-peptide and insulin would have offered more data than 3 isolated samples for each exercise intensity session. For example, insulin levels were expected to remain stable during both moderate and intense exercise, and then spike immediately after intense exercise only. However, it was observed that insulin levels rose during intense exercise and decreased during recovery. Because there was no observed insulin suppression during intense exercise it is possible that the catecholamine response to intense exercise is blunted in postmenopausal women compared to the cohort used in previous catecholamine/exercise research, a finding worthy of future research. Still, continuous blood monitoring measurements would have identified the exact point that insulin peaked, and whether its incline and/or decline were gradual or sudden, helping to understand the physiology of blood substances related to metabolism, cancer and exercise. Aside, it is interesting to note that both C-peptide and insulin levels returned to levels lower than pre-exercise measurements after 45 minutes of recovery. Increased insulin levels were expected to remain for up to one hour after intense exercise; the possibility of a blunted

catecholamine preventing insulin suppression during exercise and spiking during recovery may again be worth investigating.

Chapter 3: Conclusion

This pilot study has shown that intense exercise exerts temporary increases in metabolic substances which have also been linked to breast cancer. Although this provides an incentive for further research on the ideal exercise prescriptions for breast cancer survivors, it must be noted that any potential negative implications of acute exercise may be counterbalanced by the benefits of long-term exercise involving high intensity. For example, exercise interventions of more intense exercise or longer duration moderate exercise affect insulin sensitivity for up to 1 or 2 days as blood glucose is being used to replenish glycogen stores used by the activity whereas lower intensity exercise has less impact on insulin sensitivity (61). Breast cancer survivors are at a greater risk of recurrence than women who have not had cancer. Understanding how lifestyle choices affect the internal balance of substances that may impact cancer will empower breast cancer survivors to make educated choices. Although the long-term benefits of exercise for breast cancer survivors cannot be disputed, the appropriate balance of exercise type, duration and intensity continue to be honed.

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Appendices

Appendix 1. Recruitment Flyer

EXERCISE AND BREAST CANCER SURVIVOR STUDY SEEKS POST-MENOPAUSAL WOMEN



This UBC study will explore exercise intensity and levels of insulin. We seek 3 groups of post-menopausal participants:

- Breast cancer survivors taking aromatase inhibitors
- Breast cancer survivors who are not taking aromatase inhibitors
- Women who have never had cancer

To learn more please contact Sherry Hunt:

*****@*****.ca

(604)-***-****



Appendix 2. Initial contact email

The University of British Columbia



*Division of Sports Medicine
3055 Wesbrook Mall
Vancouver, B.C. Canada V6T 1Z3
Phone: ***-**** Fax: ***-*****

Hello,

My name is Sherry Hunt, and I am a graduate student at UBC under the supervision of Dr. Don McKenzie. We are doing a study to determine if exercises of different intensities affect the levels of insulin in the blood of breast cancer survivors.

I am sending this email to the contact people of breast cancer survivor teams in the Lower Mainland. If you are the contact, I would sincerely appreciate it if you would forward this email to your teammates as well.

For women who have had breast cancer, exercise is often suggested to reduce the chances of recurrence, and to combat some of the negative effects of drugs that affect estrogen. Yet, exercises of different intensities may have different impacts on short-term insulin levels. Our research group wants to gain a better understanding of the intensities of exercise that may prevent breast cancer recurrences and decrease the risk of other illnesses such as diabetes and heart disease.

Our study involves visiting a laboratory at UBC on 3 occasions to exercise and have blood taken.

You will be able to take part in the study if you meet the following criteria:

- Have been diagnosed with breast cancer, finished treatment and are either taking aromatase inhibitors or not receiving hormonal therapy
- A woman that has not had breast cancer
- Do not smoke
- Are post-menopausal (no menses for at least 12 months)
- Are able to provide written consent in English or French
- Do not have uncontrolled hypertension, cardiac illness, a psychiatric condition or any other medical problem that would make exercise inadvisable.

I have attached a consent form to this email for you to view. Please email me at *****@****.ca, or call me at 604-***-**** if you are interested in participating.

Sincerely,

Sherry Hunt, BSc.

Appendix 3. Consent Form

The University of British Columbia



*Division of Sports Medicine
3055 Wesbrook Mall
Vancouver, B.C. Canada V6T 1Z3
Phone: ***-**** Fax: ***-*****

SUBJECT INFORMATION AND CONSENT FORM

Exercise Intensity and Insulin Levels in Post-Menopausal Breast Cancer Survivors Receiving Aromatase Inhibitors: A Pilot Study

Principal Investigator:

Donald C. McKenzie, MD, PhD

Sports Medicine

UBC ***-****

Co-Investigators:

Karen Gelmon, MD

BC Cancer Agency

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Sherry Hunt

UBC Graduate Student

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Emergency Telephone Number (24-hours, 7-days a week):

Phone your doctor, local hospital, or 911 in the case of emergency complications related to performing exercise or having blood taken from your vein

Non-Emergency contact numbers are noted in sections 19 and 20 of this document.

1. INTRODUCTION

You are being invited to take part in this research study because you are a postmenopausal breast cancer survivor either taking non-steroidal aromatase inhibitors, or no hormonal therapy at all. You are also invited as a control subject with no history of breast cancer.

2. YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you do not have to provide any reason for your decision not to participate.

Please take time to read the following information carefully and to discuss it with your family and friends before you decide.

3. WHO IS CONDUCTING THE STUDY?

The University of British Columbia is conducting the study. It is not funded by any external agencies.

4. BACKGROUND

Many breast cancer survivors who have had estrogen linked breast cancers take aromatase inhibitors to prevent recurrence. Several short-term side-effects of aromatase inhibitors are known, such as bone

loss and joint pain. There may be other long-term side effects that have not yet been discovered because aromatase inhibitors have only been prescribed by doctors for a short time. It is possible that aromatase inhibitors affect the insulin level in the blood.

Exercise is recommended to treat the musculoskeletal side effects of aromatase inhibitors and also to prevent insulin resistance. The ideal frequency, intensity type, or duration of exercise to combat aromatase inhibitor side effects and insulin resistance has not been determined. This study examines if different exercise intensities will influence insulin levels in women taking aromatase inhibitors.

5. WHAT IS THE PURPOSE OF THE STUDY?

The purpose of this pilot study is to examine the effect of aromatase inhibitors and low and high intensity exercise on insulin and glucose in post-menopausal breast cancer patients.

6. WHO CAN PARTICIPATE IN THE STUDY?

You are eligible for this trial if you meet the following criteria:

- Have been diagnosed with breast cancer, finished treatment and are either taking aromatase inhibitors or not receiving hormonal therapy
- A woman that has not had breast cancer
- Do not smoke
- Are post-menopausal (no menses for at least 12 months)
- Are able to provide written consent in English or French

7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

If you have uncontrolled hypertension, cardiac illness, a psychiatric condition, or any other condition that limits your ability to exercise, you should not participate in this study.

8. WHAT DOES THE STUDY INVOLVE?

The food that you eat will affect the levels of the substances we are measuring in your blood. For this reason, we are asking that you do not eat breakfast before the first visit, and that you eat a standard breakfast that will be supplied to you in advance, at 7 am on the mornings of the 2nd and 3rd visits. Please bring breakfast for you to eat during the first visit, after the blood test and 30 minutes before the exercise test. Also, please do not consume caffeine or alcohol for 12 hours, or perform physical activity for 12 hours before any visit.

This study will take place in Vancouver, at the Allan McGavin Sport Medicine Centre, UBC, and involves 30 participants. All sessions will be monitored by Dr. Don McKenzie, whom is a physician specializing in Sport Medicine.

If you agree to take part in the study, you can expect the following visits and activities:

First Visit

At this time, you will be given a Physical Activity Readiness Questionnaire (PAR-Q). You will complete the questionnaire in approximately 5 minutes, and you will not have to answer any questions that cause you to feel uncomfortable.

Height, weight and blood pressure will be measured and a physician or research nurse experienced with this procedure will take a blood sample from a vein in your arm.

At least 30 minutes after you have eaten breakfast, you will perform an exercise test that involves walking on a treadmill at an intensity that gradually increases until you are tired and can go no longer. The test will assess your aerobic fitness (the levels of oxygen and carbon dioxide gases that you breathe out will be examined, and your heart rate will be monitored).

You will be given the standard breakfasts for the next 2 visits during the first visit.

Second and Third Visits

The next 2 visits will have similar formats and will be assigned randomly. You will be asked to have eaten

your standard breakfast at 7 am, and then travel to the laboratory by car or transit. At 10 am, blood will be taken. You will then perform a warm-up followed by a high or low intensity treadmill session followed by a cool down. Blood will be taken from a vein in your arm immediately after the session, and again 45 minutes after it has ceased.

During one visit you will perform the high intensity exercise session, which will be 10 minutes in duration at an intensity of 85% of your maximum ability to use oxygen. During another visit you will perform the low intensity session, which will be 45 minutes at an intensity of 60% of your maximum ability to use oxygen. The intensity prescribed was determined by the initial graded treadmill test.

Time Required to Participate in the Study

The first laboratory session is expected to take approximately 1.5 hours the second and third sessions are expected to take ~2 hours, totaling about 6 hours of laboratory time.

9. WHAT ARE MY RESPONSIBILITIES?

You are responsible for not consuming alcohol or caffeine for the 12 hours prior to each visit, and to not exercise for the 12 hours prior to each visit. You are responsible for fasting for at least 12 hours before the first visit to the laboratory. You are responsible for eating the standard breakfast given to you at 7 am on the mornings of your 2nd and 3rd visits, and not eating again until the testing is completed no later than 12 pm.

You are also responsible for finding your own transportation to the laboratory and arriving at 9:30 am on the days scheduled for your appointments.

10. WHAT ARE THE POSSIBLE HARMS AND SIDE EFFECTS OF PARTICIPATING?

The treadmill test requires maximal effort. Maximal effort exercise is sometimes linked with health risk. During and immediately after the tests, it is possible to experience symptoms such as:

1. light-headedness (less common, 5-20%)

2. abnormal blood pressure (uncommon, 2-5%)
3. muscle cramps or strain (uncommon, 2-5%)
4. fainting (rare, less than 2%)
5. nausea (rare, less than 2%)
6. heart rhythm disturbances or heart attack (1.0 per 20,000 in testing facility)

These risks may be increased for those with a history of cancer and its treatments since these figures are drawn mainly from research on people without cancer history. Exercise testing will be supervised by a physician and occur in a medical facility. Your heart rate and expired gases will be assessed throughout the test. You are free to stop the test at anytime without impacting your participation in the study.

If you feel sharp pain or unusual discomfort while exercising during the study, stop the exercise and speak to the physician, at which point the situation will be assessed and appropriate action will occur. This may involve simply stopping the exercise session, or it may involve further medical assessment.

Mild muscle soreness is common after physical exercise, especially if the exercise is intense. You may feel stiffness in your legs 12 to 24 hours after using the treadmill. After each session, if you feel pain or discomfort aside from the usual muscle soreness associated with physical activity, notify the Dr. McKenzie by calling the phone number on the cover of this consent form (unless it is severe, in which case seek emergency medical assistance). He will assess the situation by phone, and may ask you to come back to the Allan McGavin Sport Medicine Centre for further assessment.

11. WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

You may not benefit directly from participation in this study however; information that you will gather from the graded exercise test will give you an estimate of your cardiovascular fitness level.

We hope the information learned from this study can be used in the future to benefit breast cancer survivors. Exercise is suggested for breast cancer survivors to reduce chances of recurrence, new cancers, and other illnesses such as diabetes and heart disease. Exercise is also suggested for breast cancer survivors to lessen some of the

negative effects of adjuvant treatments, such as joint pain and bone loss. In this study we hope to gain a better understanding of how different intensities of exercise affect the body. With this knowledge, we will be able to help health care professionals prescribe the safest possible exercise programs to breast cancer survivors.

We will be able to give you the results of this study.

12. WHAT IF NEW INFORMATION BECOMES AVAILABLE THAT MAY AFFECT MY DECISION TO PARTICIPATE?

You will also be advised of any new information that becomes available that may affect your willingness to remain in this study.

13. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled. The study doctor(s)/investigators may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed.

14. WHAT HAPPENS IF SOMETHING GOES WRONG?

Signing this consent form in no way limits your legal rights against the investigators, or anyone else.

If you are injured as a consequence of participation in the study due to the administration of the study

procedures, your medical condition will be evaluated and medical care will be provided by one of the investigators or you will be referred for appropriate treatment. If you are injured as a result of participating in this study, the costs of your medical treatment will be paid for by your provincial medical plan to the extent that such coverage is available.

No funds have been set aside to compensate you in the event of injury or illness related to study treatment or procedures.

You do not waive any of your legal rights for compensation by signing this form.

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15. CAN I BE ASKED TO LEAVE THE STUDY?

If you are not complying with the requirements of the study or for any other reason, the study investigator/doctor may withdraw you from the study. On receiving new information about physical exercise and breast cancer survivorship, your research doctor might consider it to be in your best interests to withdraw you from the study without your consent if they judge that it would be better for your health.

16. AFTER THE STUDY IS FINISHED

We will send the study results to you by email. If you would prefer a hardcopy, please notify Sherry Hunt.

17. WHAT WILL THE STUDY COST ME?

You will not be paid for participating in this study. The fitness tests and standard breakfasts will be provided for you at no charge.

18. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. Your identity will not be used in any reports about the study. All information about this study will be kept behind locked doors or in secure computer files. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his representatives, Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

19. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or desire further information about this study before or during participation, you can contact Sherry Hunt at ***-***-**** or Dr. Don McKenzie at ***-***-****.

20. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services at 604-822-8598.

SUBJECT CONSENT TO PARTICIPATE

Check List:

- *I have read and understood the subject information and consent form.*
- *I have had sufficient time to consider the information provided and to ask for advice if necessary.*
- *I have had the opportunity to ask questions and have had satisfactory responses to my questions.*
- *I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.*
- *I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.*
- *I understand that I am not waiving any of my legal rights as a result of signing this consent form.*
- *I understand that there is no guarantee that this study will provide any benefits to me.*
- *I have read this form and I freely consent to participate in this study.*
- *I have been told that I will receive a dated and signed copy of this form.*

By checking this box you will consent for your serum to be used in future studies approved by the UBC ethics board relating to physical activity and breast cancer survivorship, excluding studies involving genetics

I consent to participate in this study.

Printed name of subject

Signature

Date

_____	_____	_____
Printed name of witness	Signature	Date

_____	_____	_____
Printed name of principal investigator/ designated representative	Signature	Date

_____	_____	_____
Printed name of translator (if applicable)	Signature	Date

Appendix 4. Raw Data

Table 6. Raw Data of Serum Substances for Each Group

		Control (n=10)	Control (n=10)	Control (n=10)	AI (n=8)	AI (n=8)	AI (n=8)	BCS (n =10)	BCS (n =10)	BCS (n =10)
		pre	Post	45- post	pre	post	45- post	pre	post	45- post
Moderate	Glucose (mmol/L)	4.92 ± 0.6	5.31 ± 0.8	5.01 ± 0.4	4.7 ± 0.4	4.85 ± 0.4	4.73 ± 0.4	4.79 ± 0.5	5.01 ± 0.6	4.94 ± 0.2
Moderate	C-pep (pmol/L)	643.6 ± 242.2	597.2 ± 226.2	522.5 ± 145.7	680.8 ± 141.5	542.4± 151.2	550.6 ± 150.0	635.4 ± 138.7	568.8± 154.3	520.1 ± 137.5
Moderate	Insulin (pmol/L)	35.0 ± 18.5	41.0 ± 20.0	28.8 ± 11.5	40.0 ± 13.6	38.3 ± 19.0	36.0 ± 15.6	33.8 ± 12.9	37.5 ± 23.6	24.1 ± 8.9
Intense	Glucose (mmol/L)	4.88 ±0.6	6.3 ± 2.0	5.53 ± 1.4	4.7 ± 0.4	5.675 ± 1.2	4.962 ± 0.7	4.82 ± 0.4	6.1 ± 0.9	5.05 ± 0.6
Intense	C-pep (pmol/L)	690.8 ± 282.0	743.8 ± 270.0	667.9 ± 212.1	693.7 ± 175.5	722.6 ± 197.8	615.9 ± 157.8	588.0 ± 80.0	680.4 ± 124.9	575.0 ± 157.2
Intense	Insulin (pmol/L)	39.3 ± 13.0	54.2 ± 26.9	41.5 ± 15.5	44.9 ± 16.5	70.4 ± 49.1	43.0 ± 17.1	31.3 ± 11.8	41.6 ± 18.6	29.1 ± 12.8

Table 7. Descriptive Data for Each Participant. Blood Was Drawn After a 12 Hour Fast.

ID	group	age	BMI	V02peak (ml/kg/min)	V02peak2 (L/min)	max HR	Glucose (mmol/L)	C-peptide (pmol/L)	Insulin (pmol/L)	HOMA- IR
12	Control	55	22.5	41.03	2.331	181	5.4	289.1	28.23	0.5
21	Control	57	28.6	30.3	2.503	182	5.8	623.6	44.44	0.9
20	Control	65	25.59	23.03	1.509	183	5.3	472.3	39.53	0.8
18	Control	58	25.63	27.5	1.137	188	5	569.9	41.04	0.8
19	Control	51	25.07	32.2	1.989	187	5.4	742.4	51.36	1
22	Control	62	23.79	30.8	1.948	172	6.1	706	66.56	1.3
27	Control	63	25.75	25.3	1.77	164	4.4	786	61.27	1.1
28	Control	54	25.56	35.63	2.384	177	5.1	522.7	39.49	0.7
30	Control	72	30.44	24.73	1.876	155	5.2	1060	63.36	1.2
32	Control	65	26.84	25.63	1.83	168	5.1	485.1	39.84	0.8
4	AI	59	23.4	27.4	1.584	167	6.4	558.9	37.42	0.7
9	AI	67	30.5	21.6	1.688	185	7.9	1461	148.7	3
10	AI	53	31.4	26	2.59	177	5.9	721.7	63.83	1.2
13	AI	53	23.63	47.2	2.859	180	4.2	331.4	21.05	0.4
14	AI	50	28.03	30.1	2.38	179	4.6	536.6	46.65	0.9
23	AI	57	26.04	33.3	2.364	177	5.9	462.3	22.64	0.4
25	AI	60	24.38	30.8	2.116	178	5.1	470.1	39.53	0.7
29	AI	54	26.61	25.93	1.699	140	5.4	674.6	64.98	1.2
31	AI	67	24.24	28.1	1.903	160	4.5	586.3	25.36	0.5
2	BCS	53	23.1	35.5	2.484	185	5.1	365.8	30.77	0.6
3	BCS	66	26.1	30.4	1.988	171	4.4	310.3	21.03	0.4
5	BCS	64	23.6	28.76	1.896	171	5.9	583	52.92	1
8	BCS	73	23.3	20.3	1.291	170	7.1	1161	59.17	1.2
11	BCS	54	21.3	32.2	1.904	182	4.9	674.2	56.5	1.1
15	BCS	66	21.84	27.7	1.545	160	5.4	492.8	20.88	0.4
16	BCS	65	26.56	26.8	2.132	165	5.9	889.2	83.53	1.6
17	BCS	66	22.14	30.4	1.617	167	6.3	629.9	49.62	1
24	BCS	62	29.8	26	1.934	175	5.8	671.9	45.59	0.9
26	BCS	63	19.63	30.87	1.627	169	4.7	421.5	16.27	
1	Survivor	72	24.6	23.7	1.544	153	4.1	431.8	46.88	0.8

Table 8. Moderate Exercise Data for Each Participant (Glucose: mmol/L; C-Peptide & Insulin: pmol/L)

ID	group	Pre glucose	Pre C-peptide	Pre insulin	Post glucose	Post C-peptide	Post insulin	45post glucose	45post C-peptide	45post insulin
12	Control	5	220.5	12.12	5.6	234.5	13.67	5.5	270.1	14.89
21	Control	4.8	599	29.52	5.1	573.1	45.28	5.1	549.9	40.04
20	Control	4.9	869.7	55.28	5.1	493.3	18.42	5	454.7	27.78
18	Control	5.7	523.7	29.26	5.4	531.6	44.01	4.7	395	13.42
19	Control	5.3	559.5	18.91	6.2	489.4	31.62	5.6	503.9	27.12
22	Control	5.7	510.1	16.08	6.9	1021	64.42	5.5	662	16.63
27	Control	3.8	906.7	72.54	4.4	804.1	60.86	4.6	719.5	45.64
28	Control	4.9	453.1	37.8	4.7	383.5	22.77	4.4	455	33.87
30	Control	4.4	1004	41.81	4.6	759.8	37.77	4.6	726.3	41.98
32	Control	4.7	789.7	36.42	5.1	681.6	71.43	5.1	488.6	26.47
4	AI	5.3	722.4	31.37	4.9	653.1	44.16	4.3	579.5	27.16
9	AI	6.3	1729	139.4	5.2	1207	47.97	5.2	1162	58.06
10	AI	4.8	673.3	39.64	4.7	628.4	38.92	4.4	757	58.49
13	AI	4.1	498.3	22.95	4.5	288.1	13.5	4.6	274.1	13.53
14	AI	4.3	668.3	53.31	4.4	446.7	27.88	4.4	600	47.59
23	AI	4.9	643	32.19	5.6	437.3	17.24	5.3	544.2	33.82
25	AI	5.1	521.5	31.75	4.5	623	71.94	4.4	421.2	21.66
29	AI	4.2	776.3	64.55	5	502.8	40.95	5	542.8	52.11
31	AI	4.9	943.3	44.12	5.2	760.1	51.71	5.4	686.1	33.53
2	BCS	4.7	531.1	31.22	4.7	447.7	44.51	4.8	369	16.74
3	BCS	4.6	467	32.71	4.2	291.6	11.83	5	341	16.81
5	BCS	5.6	666.8	34.95	5.2	698.7	44.89	5.1	488.8	28.68
8	BCS	5.3			6.1	804.7	36.21	5.1	778	17.98
11	BCS	5.2	630.7	49.68	5.2	545.9	49.5	5	491.8	27.84
15	BCS	4.4	623.6	14.37	4.7	464.2	9.62	5.3	479.9	17.19
16	BCS	4.3	916.7	57.53	5.7	711.1	78.63	5	581.5	30.76
17	BCS	4.2	778.7	30.77	4.3	619.7	21.3	4.8	565.2	29.67
24	BCS	4.9	548.6	30.34	5.1	640	65.22	4.6	689.7	41.77
26	BCS	4.7	555.7	23	4.9	464.3	13.13	4.7	416.1	14.26
1	Survivor	5.4	1324	94.95	5.6	685.7	56.95	5.3	490	25.84

Table 9. Intense Exercise Data for Each Participant (Glucose: mmol/L; C-Peptide & Insulin: pmol/L)

ID	group	Pre glucose	Pre C-peptide	Pre insulin	Post glucose	Post C-peptide	Post insulin	45post glucose	45post C-peptide	45post insulin
12	Control	5	303.4	20.71	7.6	381.9	32.19	5.9	363.5	17.25
21	Control	4.8	637.5	48.1	7.7			4.9	555.7	36.27
20	Control	5.4	457.4	30.27	5.8	538.4	41.74	5.7	462.7	26.97
18	Control	4.7	594.9	34.61	6.1	802.8	76.16	4.9	518.3	28.53
19	Control	5.2	743.7	36.82	6.2	775	30.59	5.2	869.2	57.74
22	Control	5.6	691.4	26.9	10.8	562.1	24.51	9.4	1016	50.45
27	Control	4.3	980.8	66.96	5.2	1124	110.5	4.6	846.5	67.74
28	Control	5	538	39.52	4.3	604.4	60.5	4.6	575.7	50.57
30	Control	3.7	1313	47.66	3.6	1199	58.82	4.6	844.5	35.09
32	Control	5.1	647.6	41.86	5.7	706.9	52.81	5.5	626.7	44.44
4	AI	5.2	805.8	50.57	5.8	695	30	4.8	820.4	48.6
9	AI	5.5	1482	100.8	6.1	1553	89.79	5.9	1506	99.93
10	AI	4.6	626	39.11	5.2	629	46.06	5.4	596.5	48.42
13	AI	4.1	398.2	17.86	6.4	428.8	33.2	4.3	297.2	12.47
14	AI	4.6	830.4	64.85	4.6	794.3	58.71	5.1	724.8	65.51
23	AI	5.1	706.3	38.01	7.8	682.4	44.29	6.3	716.9	59.45
25	AI	4.5	561.7	29.88	6.4	1101	179.3	4.9	635.7	35.31
29	AI	4.5	656.7	60.59	4.3	606.7	93.56	5	526.8	45.84
31	AI	5	964.6	58.66	4.9	843.8	77.79	3.9	608.9	28.18
2	BCS	5.1	470.7	29.97	6.7	591.2	65.66	4.6	368.3	24.38
3	BCS	4.5	572.1	40.4	4.6	488.3	23.72	5.3	409.1	21.48
5	BCS	5.4	589.7	32.09	6.7	669.1	23.75	5.1	766	55.33
8	BCS	5.1	703.3	16.31	7	888.9	31.5	6.3	827.6	28.27
11	BCS	5.1	651	45.18	5.8	686	33.97	5.3	577.8	42.87
15	BCS	4.8	501.6	13.88	5.1	514.8	20.72	4.6	527.7	16.03
16	BCS	4.4	683	35.2	5.6	764.5	59.33	4.8	529.6	25.05
17	BCS	5.2	577.7	38.48	7.2	809.5	68.3	5.7	702.1	38.66
24	BCS	4	631.1	44.64	6.6	705.7	55.68	4.3	634	25.24
26	BCS	4.6	499.9	17.02	5.7	686.4	33.53	4.5	407.9	13.81
1	Survivor	4.7	652.4	45.63	4.8	696.8	62.13	4.1	456	30.91

Appendix 5. Graphs Depicting Serum Substances of 3 Groups at 2 Intensities

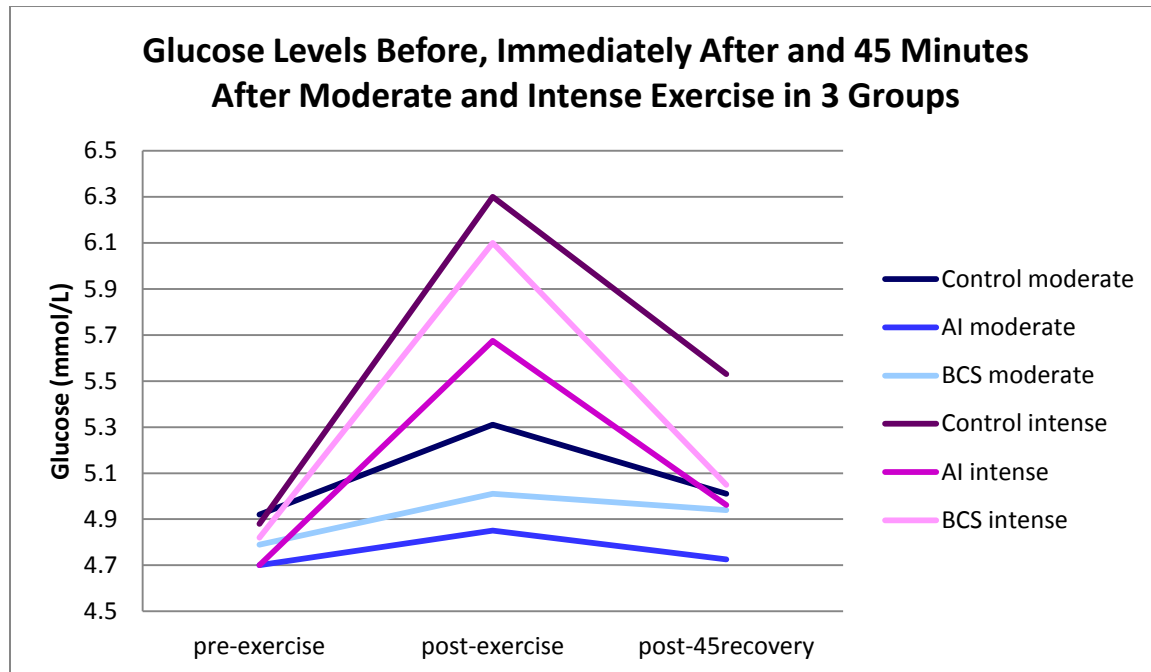


Figure 7. Glucose Levels Before, Immediately After and 45 Minutes After Moderate and Intense Exercise in Control, AI and BCS Groups

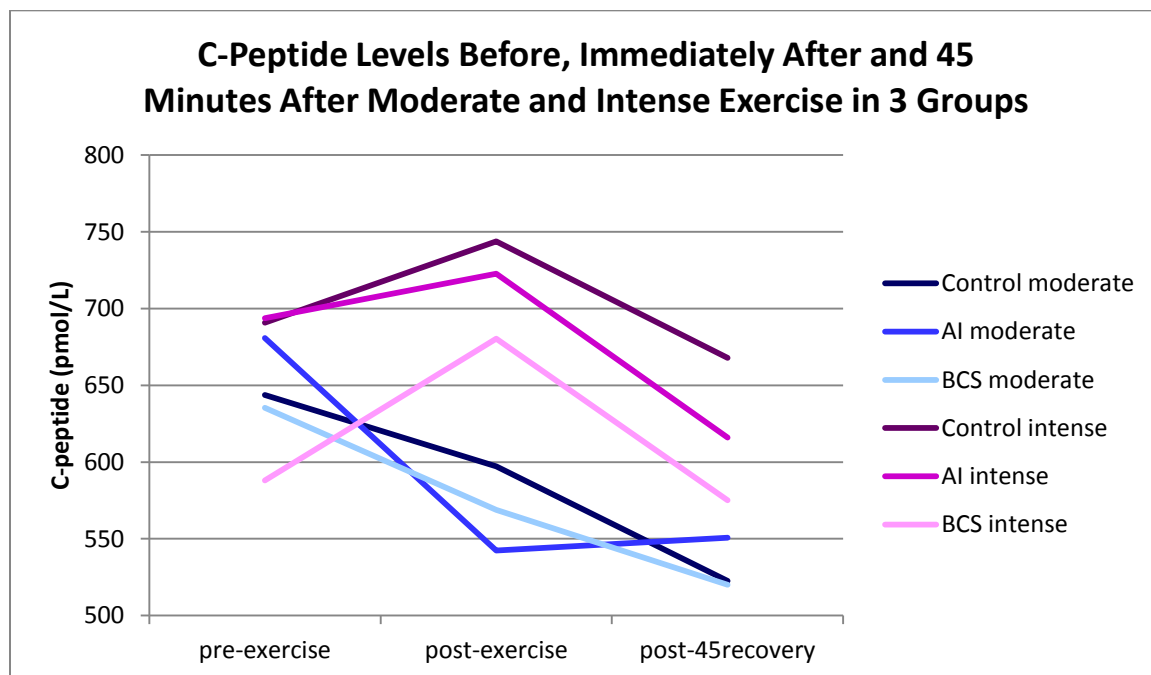


Figure 8. C-Peptide Levels Before, Immediately After, and 45 Minutes After Moderate and Intense Exercise in Control, AI and BCS Groups

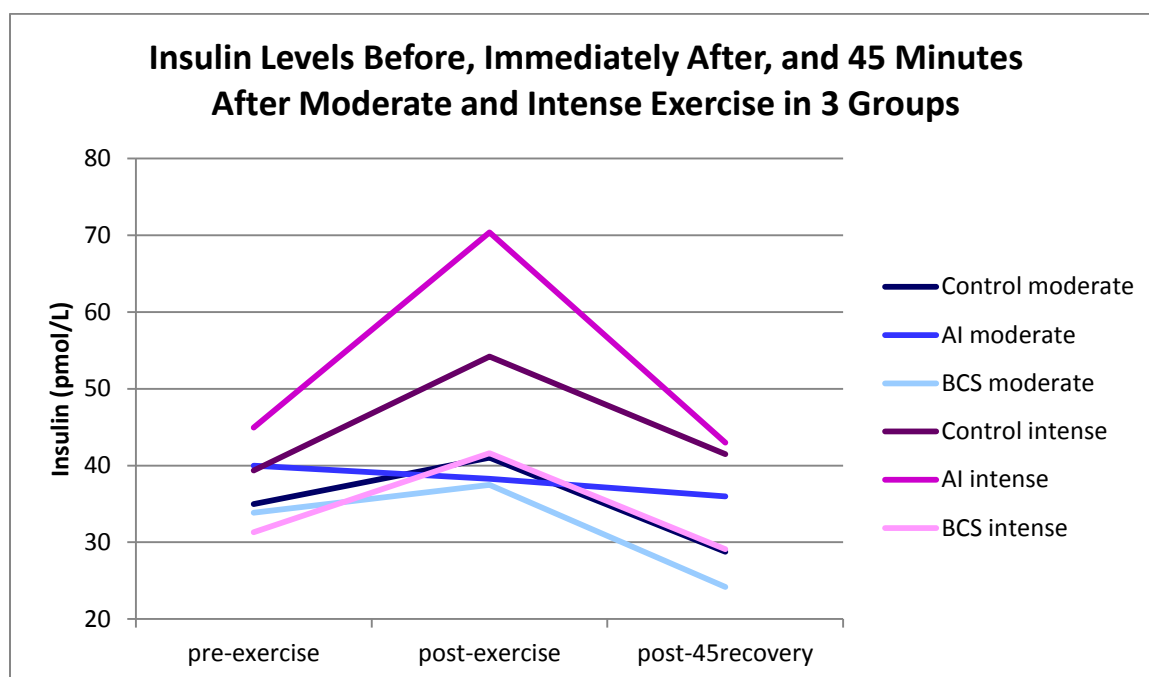


Figure 9. Insulin Levels Before, Immediately After, and 45 Minutes After Moderate and Intense Exercise in Control, AI and BCS Groups

Appendix 6. Adjusted P-Values for Nine T-Tests for Controls

Table 10. Adjusted P-Values for Post- Minus Pre-, 45 Recovery- Minus Post-, and 45 Recovery Minus Pre-Exercise Differences Between Intensities in Controls

Substance	Test	p-value (FDR)
Glucose	Post – pre-exercise	0.38
Glucose	45 recovery – post-exercise	0.38
Glucose	45 recovery – pre-exercise	0.38
C-peptide	Post – pre-exercise	0.83
C-peptide	45 recovery – post-exercise	0.80
C-peptide	45 recovery – pre-exercise	0.38
Insulin	Post – pre-exercise	0.83
Insulin	45 recovery – post-exercise	0.83
Insulin	45 recovery – pre-exercise	0.38