Impairments in glucose and lipid metabolism in breast cancer patients

by

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Author's Declaration

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

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Abstract

BACKGROUND: Breast cancer patients typically present with unhealthy body composition (high fat mass and low muscularity) near diagnosis. These body composition characteristics often worsen during treatment and ultimately contribute to the development of secondary diseases like diabetes and cardiovascular disease in survivorship. Inflammation in overweight or obese individuals is associated with impaired glucose metabolism; the presence of the tumour may lead to greater impairments in glucose metabolism in breast cancer patients. OBJECTIVES AND HYPOTHESES: The objectives of this study were to: 1) evaluate breast cancer patients near the onset of treatment for metabolic measures including an oral glucose tolerance test (OGTT), cytokine profiles, as well as body composition, nutritional status and fitness and, 2) make comparisons between breast cancer patients, age- and BMI-matched females (HM females), and a group of young, non-malignant females with healthy BMIs (HY females) on these measures. We hypothesized that breast cancer patients would demonstrate impaired glucose metabolism relative to HM females, and that this would be attributed to systemic inflammation. We also hypothesized that both breast cancer patients and HM females would present with unhealthy body composition, impaired glucose and lipid metabolism, systemic inflammation, poor fitness and greater caloric intake compared to HY females. METHODS: We evaluated body composition using % body fat (skinfold callipers) and waist circumference. Following collection of fasting blood samples, an OGTT was conducted to assess glucose, insulin, c-peptide and glucagon dynamics. Fasting blood samples were analysed for lipids and pro- and anti-inflammatory cytokines. Incremental exercise tests were conducted to assess VO_{2peak}, and estimated 1-RM tests assessed strength of the biceps, triceps and quadriceps muscles. Baecke and CHAMPS questionnaires provided an indication of

habitual physical activity. A 3-day food record was used to analyze daily caloric intake and macronutrient distribution. Breast cancer patients and HM females were compared using paired t-tests. Patients and HM females were compared to HY females using t-tests. Statistical significance was accepted at $p < 0.05$.

RESULTS: Overall, breast cancer patients were overweight (BMI: 28.8 ± 6.0 kg/m^2) and presented with abdominal obesity (waist circumference: 94.6 ± 14.0 cm) and dyslipidemia (TAG: 1.84 ± 1.17 mM and HDL-c: 1.08 ± 0.23 mM), indicating risk for metabolic syndrome. Although fasting glucose concentrations did not differ between the 3 groups, breast cancer patients demonstrated higher glucose concentrations at 30 min during an OGTT. Similar to glucose, fasting insulin concentrations did not differ between the 3 groups, but patients demonstrated higher insulin at 150 min during an OGTT. Breast cancer patients had elevated fasting serum c-peptide (2.6 ± 1.2 ng/mL vs. 1.9 ± 0.8 ng/mL, $p = 0.005$). C-peptide remained elevated in patients compared to non-malignant females during the last hour of the OGTT, indicating that insulin secretion was sustained in breast cancer patients. We observed no difference in serum cytokines between patients and HM females or between patients and HY females. VO_{2peak}, although lower compared to HY females, was similar in patients and HM females. There were no differences in habitual physical activity or nutrition measures between any groups.

DISCUSSION AND CONCLUSIONS: Breast cancer patients presented with poorer glucose features during an OGTT compared to HM and HY females. However, systemic inflammation, body composition, energy expenditure and energy intake were similar in breast cancer patients and HM females. Thus, these impairments may be tumour-related. Future studies need to specifically elucidate the effects of the tumour in host glucose metabolism.

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1.0 Overview

Although breast cancer is the most commonly diagnosed form of cancer in females, patients have an 89% chance of achieving 5-year survival (Canadian Cancer Society 2011). These statistics are encouraging, however once patients enter into survivorship (here defined as the time post-treatment (Feuerstein 2007)), they are prone to developing secondary conditions such as diabetes, cardiovascular disease and even cancer recurrence. High fat mass, and perhaps low lean tissue, is associated with the development of breast cancer (Freedman 2004; Demark-Wahnefried et al. 2001; Rock and Demark-Wahnefried 2002). As breast cancer patients progress through treatment, and later as they enter into survivorship, they tend to experience further fat gains and lean tissue losses.

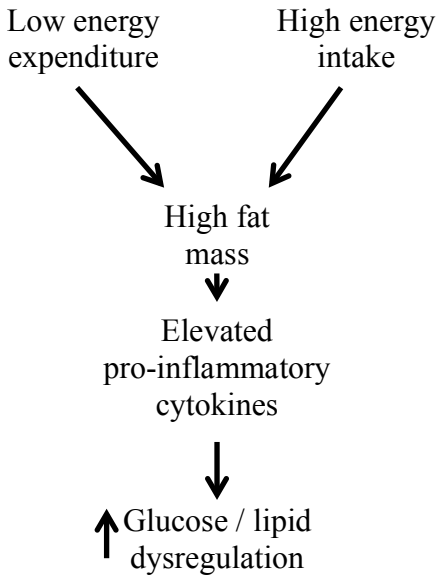
In non-malignant populations, obesity and sarcopenia (the term used to describe individuals with lower than normal muscularity) have been shown to contribute to the development of diabetes, cardiovascular disease and other metabolic complications (Choi 2010; Lee 2011). In breast cancer patients, obesity and sarcopenia at diagnosis, followed by fat gains and lean tissue losses during treatment and in survivorship, may have the same or exacerbated negative metabolic implications compared to non-malignant populations. Thus, it is important to understand the consequences of unhealthy body composition in breast cancer patients near the onset of treatment to develop future interventions that might attenuate the deleterious changes that occur during the disease trajectory and in survivorship.

In non-malignant populations, high fat mass and low lean tissue are associated with various metabolic complications including inflammation, glucose intolerance and dyslipidemia (Medalie et al. 1975). Increased serum concentrations of pro-inflammatory cytokines (i.e.

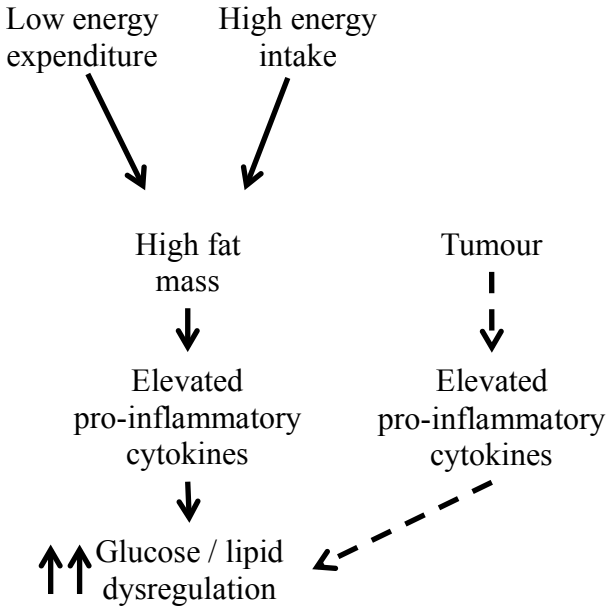
interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF-α)) (Park 2005) and decreased serum concentrations of anti-inflammatory cytokines (Matsubara 2002) accompany increases in fat mass and decreases in lean tissue mass. The increased concentrations of IL-6 and TNF-α that accompany high fat mass activate signal transduction pathways that may reduce insulin sensitivity and increase lipolysis (Plomgaard et al. 2005; Uysal et al. 1997; Kim et al. 2004). The resulting impairment in glucose metabolism and dyslipidemia have been shown in some cases to lead to an increased risk of developing serious metabolic diseases such as diabetes and cardiovascular disease in obese, non-malignant populations (Medalie et al. 1975). In obese, non-malignant populations, unhealthy body composition profiles (i.e. high fat mass and low levels of lean tissue) can be explained by energy imbalance. When energy expenditure is reduced (i.e. sedentarism (Weiss et al. 2006)) and energy intake increased (i.e. increased caloric intake (Racette et al. 2006)), the excess energy is stored as fat in adipose tissue, skeletal muscle and liver. Fat deposition may result in dysregulation of glucose and lipid metabolism in these tissues (Kelley et al. 2003; Kern et al. 2001; Corcoran et al. 2007).

Despite that high fat mass and low lean tissue have been characterized in breast cancer patients at different stages of the disease trajectory (Amaral et al. 2010; Demark-Wahnefried et al. 1997; Irwin et al. 2005), this unhealthy body composition profile may not necessarily lead to the same metabolic consequences as observed in non-malignant populations. Due to their underlying disease state, breast cancer patients may experience a greater degree of inflammation (Dehqanzada et al. 2007; Muraro et al. 2011). The combined effects of inflammation due to the tumour and high fat mass may result in a greater predisposition for the development of insulin resistance and dyslipidemia (Figure 1).

This thesis examines the body composition, metabolic characteristics, exercise and nutrition of a group of breast cancer patients at the onset of treatment. It is important to evaluate the patients before or at the early stages of chemotherapy, which can alter fat mass and inflammation (Demark-Wahnefried et al. 2001; Mills et al. 2005). We expected that patients would present with greater inflammation than can be explained due to their high fat mass. Inflammation would be associated with worse glucose and lipid metabolism compared to age and BMI-matched, non-malignant controls. Based on these concepts and results, this thesis provides a framework to design future exercise and nutrition interventions that will aim to counter the development of secondary diseases in survivorship.



a)



b)

Figure 1 Obesity and the tumour may increase systemic inflammation in breast cancer patients. A model indicating the potential role of inflammation to the development of metabolic dysregulation in **a)** non-malignant populations and **b)** breast cancer patients

2.0 Unhealthy body composition develops during treatment and in survivorship for breast cancer patients

Unlike other forms of cancer that are associated with pronounced weight loss (Di Sebastiano et al. 2012; Murphy et al. 2010), breast cancer is associated with weight gain over the disease trajectory and in survivorship (Goodwin et al. 1988; Goodwin et al. 1999). At diagnosis, patients tend to be overweight or obese with a large waist circumference and a high percentage body fat. Yaw et al. (2010) observed that at the time of diagnosis 42.4% of breast cancer patients were overweight or obese. In a large proportion of studies, newly diagnosed breast cancer patients are, on average, overweight or obese (Healy et al. 2010; Goodwin et al. 2009; Yaw et al. 2010) with BMI's reaching as high as 54.8 kg/m^2 (Goodwin et al. 2009). Interestingly, based on waist circumference, patients are also centrally obese (Healy et al. 2010; Goodwin et al. 2009) with values as high as 123.5 cm. Amaral et al. (2010) further investigated the tendency towards abdominal adiposity in breast cancer by comparing patients' waist circumferences to the International Diabetes Federation (IDF) cut-point for metabolic syndrome (a waist circumference > 88 cm (Alberti et al. 2006)). Metabolic syndrome is a set of risk factors that increase an individual's risk of developing cardiovascular disease and type II diabetes (Alberti et al. 2006). Amaral et al. (2010) observed that 62% of patients in their study had a waist circumference > 88 cm, and thus were at a higher risk of developing metabolic syndrome (Amaral et al. 2010). Furthermore, 89% of patients had a percentage body fat > 30%.

Unhealthy body composition profiles persist over the course of treatment for breast cancer (Prado et al. 2009; Van Londen et al. 2011), indicating the importance of potential interventions during treatment to attenuate these negative changes in body composition. In

addition to being characterized as overweight or obese during treatment, patients actually experience significant weight gain during this time. Demark-Wahnefried et al. (1993 and 1997) estimated that 50-96% of patients gained 2.5-6.2 kg over the course of treatment and Lankester et al. (2002) reported that 64% of patients experienced gains of at least 2 kg during 6 cycles of chemotherapy. It is noteworthy that the pattern of weight gain over the course of treatment for breast cancer is not the same as the pattern of weight gain in non-malignant females. Fat gains in non-malignant females are generally accompanied by gains in lean tissue, provided the individual is mobile (Forbes et al. 1986; Forbes 1987). Weight gain in breast cancer, on the other hand, is generally restricted to fat mass (Harvie et al. 2004; Demark-Wahnefried et al. 2001). In fact, patients may even lose lean tissue mass over the course of treatment. Dual energy x-ray absorptiometry (DXA) demonstrated that patients gained fat and lost muscle during a treatment regimen of 12 weeks of chemotherapy and radiation treatments in one study (Kutynec et al. 1999) and after a 6 month regimen of chemotherapy in another study (Cheney and Mahloch 1997). Prado et al. (2009) used computed tomography (CT) images to quantify muscle mass in patients undergoing treatment. They determined that 25.5% of patients were sarcopenic, with an average lean tissue mass of 34 ± 3.3 kg (average age of the patients was 54.8 ± 10.4 years) (Prado et al. 2009). As a comparison, a healthy lean tissue mass for females aged 50-59 years is 40.3 ± 4.0 kg (Tankó et al. 2002). Interestingly, loss of lean tissue is most pronounced in the lower limbs (Cheney and Mahloch 1997). This finding might suggest that breast cancer patients are performing less weight-bearing exercise and possibly becoming more inactive over treatment.

In survivorship, patients continue to gain weight. Irwin et al. (2005) observed that 68% of breast cancer survivors gained an average of 3.8 kg in the 3 years after completing

treatment, while Demark-Wahnefried et al. (2001) noted that breast cancer survivors gained an average of 2.1 kg in the 12 months after completing treatment. Weight gain in survivorship follows a pattern similar to weight gain during treatment: fat gain is accompanied by lean tissue loss. Demark-Wahnefried et al. (2001) demonstrated, using DXA, that patients gained fat mass (from 24.0 ± 1.6 kg to 26.3 ± 1.8 kg, p=0.04) and lost lean mass (from 45.4 ± 0.8 kg to 45.0 ± 0.8 kg, p=0.02) from the time they finished treatment to 12 months post-treatment. Not only is weight gain in survivorship significant, it is also progressive. When Makari-Judson et al. (2007) examined breast cancer survivors 1, 2 and 3 years post-treatment, they discovered that survivors gained 1.5 kg after 1 year, 2.7 kg after 2 years and 2.8 kg after 3 years.

It is clear that high fat mass and low lean tissue are associated with breast cancer at multiple points over the disease trajectory. This pattern of unhealthy body composition has negative metabolic implications in non-malignant populations, notably decreased insulin sensitivity and increased chronic inflammation (Goodpaster et al. 2005; Shoelson et al. 2007). Adipose tissue is not an inert storage depot, as it was previously thought to be. Rather, it is an active endocrine organ. When fat mass is high, there is an increase in secretion of pro-inflammatory cytokines into circulation (Mohamed-Ali et al. 1997; Fried et al. 1998), which may lead to impaired glucose tolerance and dyslipidemia (Wisse 2004).

It is possible that high fat mass has similar, if not more detrimental, metabolic consequences in breast cancer patients. Breast cancer patients have increased concentrations of circulating pro-inflammatory cytokines (Dehqanzada et al. 2007; Lyon et al. 2008), which have been shown to negatively alter aspects of glucose and lipid metabolism (Figure 1). A better understanding of unhealthy body composition and the associated metabolic perturbations at the

onset of treatment will advance our understanding of the underlying higher risk for secondary metabolic diseases in breast cancer survivorship.

3.0 High fat mass can develop from an imbalance of energy intake and energy expenditure in non-malignant populations

3.1 Reduced energy expenditure

Sedentarism, or low physical activity, reduces energy expenditure and is associated with increased risk of obesity (Hill and Peters 1998; Prentice and Jebbs 1995). The Centers for Disease Control and Prevention (1996) determined, from a sample of 25 164 overweight females, that 41% of overweight American females reported engaging in no physical activity during their leisure time. Further, the percentage of females not partaking in physical activity increased along with their degree of overweight. Body mass index (BMI), an indicator of obesity, was significantly and inversely correlated with physical activity, as measured in metabolic equivalent (MET) hours (MET-hr and BMI: $r = -0.11$, $p < 0.05$; MET-hr) (Fung et al. 2000). In a study of American indigenous peoples it was observed that Pima Indians who expend significantly more energy per day (3156 ± 415 kcal/day versus 2805 ± 415 kcal/day, $p<0.04$) have a lower percentage body fat compared to Pima Indians whose energy expenditure is reduced (29 ± 10% versus 41 ± 10%, $p<0.0001$) (Esparza et al. 2000).

These correlations between obesity and energy expenditure are supported by detraining studies. One such study, conducted by Liu et al. (2008), examined the effect of 1 month of detraining on elite level kayakers. Prior to initiation of the study, participants trained 18 hours per week. Their average maximal oxygen uptake (VO_{2max}) and BMI were 58.5 ± 1.77 mL/kg/min and 23.74 ± 0.54 kg/m^2, respectively. For 1 month, half of the kayakers (n=8) ceased all training; the remaining half of the kayakers (n=8) reduced their training volume by 50%. At the end of 1 month, waist circumference had significantly increased in both groups

(Liu et al. 2008). Liu et al. (2008) did not assess or control for caloric intake, which may also have contributed to the observed increases in waist circumference.

3.2 Increased energy intake

Overnutrition, or excess caloric intake, contributes to the development of obesity. Energy intake has traditionally been difficult to assess: food records, food frequency questionnaires and 24-hour diet recalls are commonly used but often provide underestimates of daily caloric intake (Trabulsi and Schoeller 2001). The doubly labelled water technique provides perhaps the most accurate assessment of energy expenditure: by measuring the clearance rates of water labeled with stable isotopes ^{2}H and ^{18}O over several days, total energy expenditure is calculated. In a weight stable individual, energy expenditure is equal to habitual caloric intake. Using this principle, doubly labelled water can provide an estimate of caloric intake. Park et al. (2011) used doubly labelled water to assess caloric intake in Japanese females across a spectrum of BMI's. They determined that females with higher BMI's had significantly higher caloric intakes compared to females with lower BMI's. Participants with BMI's between 24.7 – 40.0 kg/m^2 consumed on average 2373 ± 363 kcal/day. Their energy intake was significantly higher than participants with BMI's between 20.5 – 22.1 kg/m^2 (2038 ± 210 kcal/day, $p < 0.01$) and participants with BMI's between 22.3 – 24.7 kg/m^2 (2229 ± 297 kcal/day, $p < 0.05$) (Park et al. 2011). No difference in physical activity levels, measured using hip-mounted accelerometers, was observed between groups of females. Other researchers have used doubly labelled water to estimate energy intake in females with BMI's greater than 30 kg/m^2: females with BMI's of 32.9 ± 4.6 kg/m^2 consumed an average of 2445 ± 108 kcal/day

(Prentice et al. 1986), and females with BMI's of 37.4 ± 8.1 kg/m^2 consumed an average of 3708 ± 367 kcal/day (Platte et al. 1995). Participants in the latter study were selected only if they reported engaging in < 5 hours of physical activity per week.

High amounts of dietary fat may also contribute to the development of obesity. Using 3 and 7 day food records, both Miller et al. (1990) and Tucker and Kano (1992) found that obese females consume a significantly higher proportion of dietary fat versus lean females (Miller: 36.3 ± 1.5% for obese and 28.6 ± 1.5% for lean, $p<0.05$; Tucker and Kano: 42.58 ± 10.7% for obese and 37.64 ± 8.1% for lean, $p<0.05$). Although these findings support high fat diets being associated with obesity, given the challenges of recording dietary intake, they should be interpreted with caution.

4.0 Metabolic perturbations like glucose and lipid dysregulation develop as a result of unhealthy body composition in non-malignant populations

Glucose metabolism is a tightly controlled process in the healthy individual. In a fasted state or between meals, the liver releases glucose for use by other tissues (Stumvoll et al. 1998) and insulin secretion from the beta cells of the pancreas slows. As well, lipolysis occurs in the adipose tissue to provide non-esterified fatty acids (NEFAs) as an alternative fuel source to glucose (Jensen et al. 1987). When a meal is ingested, exogenous glucose enters the bloodstream, triggering increased secretion of insulin. Insulin suppresses glucose release from the liver and lipolysis in the adipose tissue (Korenblat et al. 2008). Insulin also facilitates glucose uptake in skeletal muscle (Korenblat et al. 2008): insulin binds to receptors on the sarcolemma and initiates an intracellular signalling cascade that results in the translocation of additional glucose transporter type 4 (GLUT4) transporters to the cell surface (Czech 1995). This process allows more glucose molecules to enter the muscle cell and become metabolized to fuel cellular functions. In an obese individual, high fat mass can disrupt this process and contribute to glucose intolerance and dyslipidemia in the following ways: 1) fat storage in tissues other than adipose tissue (like liver and skeletal muscle) (Goodpaster et al. 2005); 2) increasing lipolysis (Björntorp et al. 1969); and 3) decreasing glucose uptake by skeletal muscle (Bonen 2004).

4.1 Fat Storage in liver and skeletal muscle

Fat mass can contribute to metabolic dysregulation, particularly when it accumulates in visceral or ectopic depots. Body fat can be stored subcutaneously (beneath the skin), viscerally

(around the tissues of the abdomen, deep to the abdominal and paraspinal muscles) or ectopically (within and around organs not designed to harbour fat, like the liver, skeletal muscle, heart and blood vessels) (Goodpaster et al. 2005). Subcutaneous fat storage, particularly in the gluteal region is preferred to visceral or ectopic storage (Osama et al. 2006). Visceral adiposity is more strongly correlated with an increased risk of diabetes (Kapoor et al. 2007; Lebovitz and Banerji 2005), although the physiological mechanisms behind this correlation are unclear. It is possible that since visceral adipose tissue has direct access to portal circulation, the NEFAs and pro-inflammatory cytokines produced by visceral adipose tissue may be removed by and accumulate in the liver (Shoelson 2007). Accumulation of NEFAs and pro-inflammatory cytokines in hepatocytes increases liver inflammation and stimulates hepatic insulin resistance (Shoelson 2007).

Ectopic fat storage is positively associated with the incidence of cardiovascular disease: when excess fat accumulates around the heart and blood vessels, it impairs organ function, leading to vascular stiffness and hypertension (Montani et al. 2004). As well, intrahepatic fat and intramuscular fat are both associated with insulin resistance, a characteristic that predisposes one to diabetes: high intrahepatic fat content impairs insulin's ability to suppress glucose release from the liver (Samuel et al. 2004) and intramuscular fat impairs insulin's ability to stimulate glucose uptake in skeletal muscle (Perseghin et al. 1999).

4.2 Increased lipolysis

High fat mass can also influence metabolic dysregulation by increasing lipolysis (Jensen et al. 1989; Björntorp et al. 1969). Fat mass has a strong positive correlation with

lipolytic rates, meaning that circulating NEFA concentrations are elevated in obese compared to lean individuals (Horowitz et al. 1999; Gordon 1960). Obese individuals also have increased amounts of plasma membrane fatty acid transporters, which work to escort NEFAs into cells (Bonen 2004).

Once inside the cell, NEFAs compete with glucose as an energy substrate in muscle and adipose tissue (Fanelli et al. 1993), and inhibit insulin-stimulated glucose uptake. NEFAs may inhibit glucose uptake by disrupting GLUT4 synthesis, vesicle trafficking, budding and fusion (Roden et al. 1996). Griffin et al. (1999) reported that increased circulating NEFA concentrations may also inhibit insulin signalling within the muscle cell. When these circulating lipids accumulate within the cell they stimulate protein kinase C theta (PKCθ), a molecule with an inhibitory effect on phosphoinositide 3-kinase (PI3 kinase) (Griffin et al. 1999). As seen in Figure 2, PI3 kinase is directly involved in the cascade that leads to GLUT4 translocation to the cell membrane (Zierath et al. 2000). Stimulation of PKCθ therefore inhibits GLUT4 translocation and decreases insulin-stimulated glucose uptake in muscle.

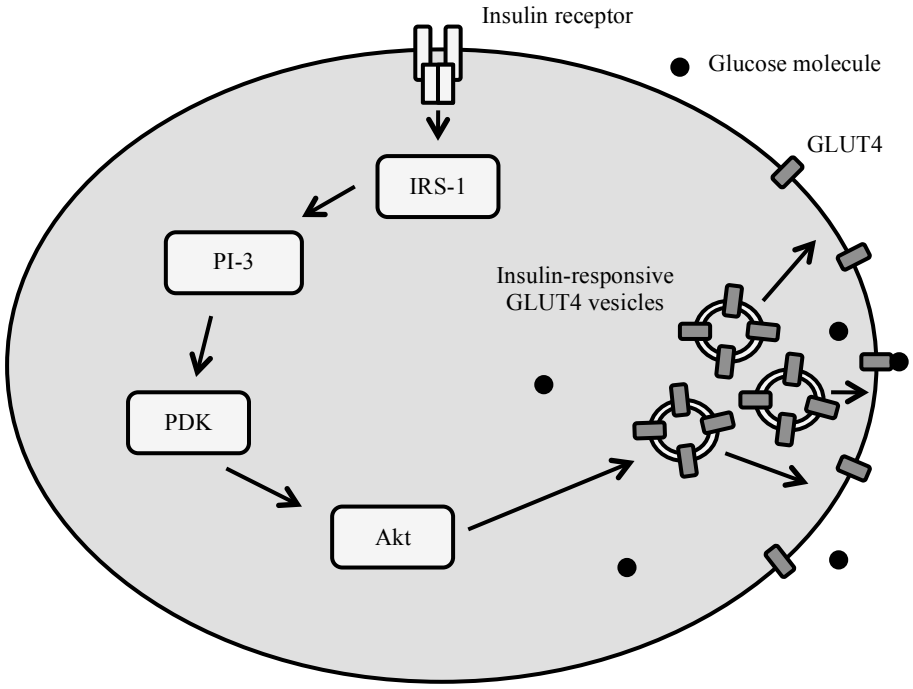


Figure 2 Insulin-stimulated glucose uptake in skeletal muscle. Insulin binds to its receptor, initiating a signal transduction pathway that increases GLUT4 translocation to the plasma membrane. Increased GLUT4 at the plasma membrane facilitates glucose uptake from the blood stream.

Increased lipolysis impacts cardiovascular health in addition to whole body glucose metabolism. High circulating levels of NEFAs can induce insulin resistance in cardiac muscle (Ferrannini and Iozzo 2006; Yanai et al. 2008). Reduced glucose uptake in cardiovascular tissue can impair cardiac function (Ferrannini and Iozzo 2006) and contribute to cardiovascular disease. Additionally, increased NEFA concentrations also stimulate endothelial nitric oxide production, which impairs endothelium-dependent vasodilation and leads to hypertension (Yanai et al. 2008).

4.3 Decreased glucose uptake by skeletal muscle

High fat mass is associated with decreased glucose uptake in skeletal muscle and this negatively impacts metabolic health. Skeletal muscle sarcolemma of both obese and diabetic individuals contains high amounts of the fatty acid transporter/cluster of differentiation 36 (FAT/CD36) compared to lean controls (Bonen 2004). Bonen et al. (2004) noted that this increased amount of fatty acid transporter not only served to increase intramyocellular triglyceride content but was also associated with a reduced amount of GLUT4 at the plasma membrane, limiting the amount of glucose that can be removed from circulation. In this study, localization of FAT/CD36 at the plasma membrane was increased 40% in obese individuals and 76% in diabetic individuals compared to a lean group.

It is generally accepted that high fat mass contributes to impaired glucose tolerance and dyslipidemia in various ways, for instance accumulating in the liver and muscle (Kelley et al. 2003), stimulating lipolysis (Björntorp et al. 1969; Jensen et al. 1989) and reducing glucose uptake in skeletal muscle (Bonen 2004). However, the emergence of adipose tissue as a potential new endocrine organ has stimulated further investigation into the mechanisms relating obesity and metabolic dysregulation. Researchers are beginning to uncover the role of inflammatory mediators in the development of glucose intolerance and cardiovascular disease. High amounts of adipose tissue are associated with elevated pro-inflammatory cytokine concentrations (Wisse 2004). Pro-inflammatory cytokines like IL-6 and TNF- α may interfere with insulin signalling pathways and facilitate the development of insulin resistance (Plomgaard et al. 2005; Uysal et al. 1997; Kim et al. 2004).

5.0 Inflammation may develop with unhealthy body composition, and may contribute to glucose and lipid dysregulation in non-malignant populations

Inflammation is a proposed link between obesity and metabolic diseases like cardiovascular disease and diabetes in non-malignant populations. In obese individuals, adipose tissue is a source of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8 (Xu et al. 2003). When adipocytes hypertrophy, monocyte-derived macrophages infiltrate the adipose tissue. Once inside the adipose tissue, macrophages release TNF-α, IL-6 and IL-8 (Weisberg et al. 2003). High circulating concentrations of these pro-inflammatory cytokines have been observed in obese compared to lean individuals (Ziccardi 2002; Xu et al. 2003; Visser et al. 1999).

TNF-α and IL-6 are associated with metabolic complications. Van Hall et al. (2003) noted an increase in lipolysis along with increasing serum concentrations of IL-6. Moreover, a positive correlation has been observed between serum concentrations of IL-6 and insulin resistance (Kern et al. 2001; Klover et al. 2003). TNF-α is also positively correlated with increased adipose tissue lipolysis and insulin resistance (Kern et al. 2001; Hotamisligil and Spiegelman 1994). TNF-α and IL-6 are products of the nuclear factor- κB (NF-κB) pathway (Shoelson et al. 2003), however they can also act as stimuli for the same pathway and in doing so, induce insulin resistance (Cifuentes et al. 2010). IKK-β, a serine kinase that is another product of the NF-κB pathway, promotes tyrosine phosphorylation and prevents serine phosphorylation of insulin receptor substrate 1 (IRS-1) when TNF- α or IL-6 activate the NF-κB pathway (Shoelson et al. 2003). Serine phosphorylation of IRS-1 is required for GLUT4 translocation; when tyrosine phosphorylation results from increased IKK-β, insulin resistance occurs (Yuan et al. 2001) (Figure 2). TNF-α and IL-6 also stimulate the c-Jun N-terminal

kinase (JNK) pathway; increased circulating JNK inhibits the activation of IRS-1 in the insulin signalling cascade, preventing the translocation of GLUT4 vesicles to the cell surface (Lee et al. 2003). This can also result in insulin resistance. IL-1β and c-reactive protein (CRP) are increased in obesity as well (Visser et al. 1999; De Lorenzo et al. 2007), and it has been shown that IL-10, an anti-inflammatory cytokine, is depressed in obesity (Gotoh et al. 2012). Fewer studies have examined the effects of obesity on other anti-inflammatory cytokines, although IL-4 is reportedly depressed in breast cancer patients compared to controls (Dehqanzada et al. 2007). It is possible that reduced circulating concentrations of anti-inflammatory cytokines, like IL-4 and IL-10, are associated with the low-grade chronic inflammation characteristic of obesity as well as the increased inflammation observed in breast cancer. Pro-inflammatory cytokine levels are reportedly elevated in breast cancer patients compared to non-malignant females (Dehqanzada et al. 2007).

6.0 Investigation into the metabolic consequences of unhealthy body composition in breast cancer is limited

Unhealthy body composition (high fat mass in particular, as well as low muscularity) has been characterized and documented in breast cancer patients (Demark-Wahnefried et al. 1993; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 2001). While metabolic consequences attributed to high fat mass and low lean tissue (i.e. inflammation, glucose intolerance and dyslipidemia) in non-malignant individuals have been well-described (Kern et al. 2001; Modan et al. 1985; Howard et al. 2003; Klover et al. 2003), these effects have been minimally explored in breast cancer. Studies have reported that breast cancer patients have increased chronic inflammation due to their underlying disease state (Kozlowski et al. 2003; Zhang and Adachi 1999; Tessitore et al. 2000), which may exacerbate the metabolic consequences anticipated due to high fat mass. Kozlowski et al. (2003) noted that breast cancer patients had increased serum concentrations of IL-6 compared to non-malignant controls. Kozlowski and colleagues (2003) also observed that the serum concentrations of this cytokine correlated positively with clinical disease stage. Zhang et al. (1999) confirmed a strong positive correlation between tumour progression and cytokine concentrations, and also observed that CRP is elevated in the serum of breast cancer patients. Serum TNF- α has also been documented to be significantly higher in breast cancer patients compared to non-malignant controls (Tessitore et al. 2003). It is possible that the combined effects of the tumour and high fat mass may result in higher concentrations of circulating pro-inflammatory cytokines, and thus a worse degree of glucose intolerance and dyslipidemia, than would be expected of a non-malignant female of a similar percentage body fat (Figure 1). To date,

however, no studies have examined the association between inflammation, glucose tolerance and serum lipids in breast cancer patients.

Despite that IL-6 and TNF- α are known to hinder insulin sensitivity in non-malignant individuals (Kern et al. 2001; Klover et al. 2003; Hotamisligil and Spiegelman 1994), investigation of the effects of these cytokines on impairing glucose and lipid metabolism in breast cancer patients remains extremely limited. The majority of studies on metabolism in breast cancer have focused on evaluating risk of cancer development in association with various metabolic disorders such as diabetes (Bjørge et al. 2010; Lipscombe et al. 2006; Mink 2002). However, understanding the links between body composition and metabolism as a mechanism to potentially explain these increased risks in breast cancer patients is unclear or lacking.

The vast majority of studies that have examined the metabolic disorders associated with breast cancer are large-scale population-based risk assessment studies. Bjørge et al. (2010) collected blood samples and anthropomorphic measurements from 290 000 European females between 1974 and 2005. Of these females, 4862 developed breast cancer. By comparing the cases and controls, Bjørge showed that post-menopausal females who demonstrated symptoms of metabolic syndrome such as impaired fasting and 2-hr glucose, abdominal obesity, high TAG concentrations and low high density lipoprotein (HDL) cholesterol concentrations were more likely to develop breast cancer. With similar study designs high fasting glucose, high fasting insulin and insulin resistance were confirmed as other risk factors for breast cancer (Muti et al. 2002; Mink et al. 2002; Goodwin et al. 1988). Diabetes is another risk factor for breast cancer. Using Ontario health databases, Lipscombe et al. (2006) determined that the chance of a diabetic female developing breast cancer is 2.97/1000, compared the chance of a

non-diabetic female developing breast cancer, which is 2.75/1000 (p=0.021). These findings were further supported by Mink et al. (2002) who demonstrated that breast cancer incidence is 60% higher in diabetic females compared to females with a healthy fasting glucose (< 5.6 mM). Dyslipidemia is also prevalent in breast cancer patients (Kökoğlu et al. 1994). Goodwin et al. (1997) examined fasting lipids in females who underwent breast biopsies. They discovered that plasma TAG concentrations were significantly higher in breast cancer patients compared to patients whose biopsies were negative for proliferative disease (0.94 ± 1.04 mg/ml vs 0.83 ± 1.04 mg/ml, p=0.03) (Goodwin et al. 1997).

These risk assessment studies have established a relationship between breast cancer incidence and metabolic disorders. However, no studies to date have quantified fasting glucose or 2-hr glucose during an oral glucose tolerance test (OGTT), fasting lipids or markers of inflammation like CRP, IL-6 or TNF-α in recently diagnosed breast cancer patients. During an OGTT a standard 75g load of glucose is administered orally to a fasted individual and blood is drawn at regular intervals for 2-3 hours. OGTTs crudely assess whether an individual is glucose tolerant, insulin resistant or diabetic based on their fasting and 2 hour serum glucose concentrations. A handful of studies have performed OGTTs in breast cancer patients to evaluate the effects of treatment (Chala et al. 2006; Polushina et al. 2002; Elefsiniotis et al. 2004). Chala et al. (2006) and Elefsiniotis et al. (2004) did not include a non-malignant female group for comparison. Polushina et al. (2002) evaluated breast cancer patients relative to a non-malignant female group, however they did not indicate whether measures were taken prior to or during chemotherapy.

The aforementioned studies on breast cancer have not examined metabolism, exercise capacity and nutrition in association with breast cancer. The majority of these studies

examined metabolism, exercise capacity or nutrition independently. Due to the interrelationships between body composition, metabolism, nutrition and exercise, it is methodologically important to take an integrative approach and study these topics together in a single group of breast cancer patients. It is also important to examine these patients near diagnosis before further gains in fat and losses in muscle occur over treatment and in survivorship. With the data from this thesis, we can further understand the metabolic disorders that may develop in treatment and survivorship, and design effective exercise and nutrition interventions to prevent or counter co-morbidities.

7.0 Rationale

Eighty-nine percent of breast cancer patients achieve 5-year survival and this number increases each year (Canadian Cancer Society 2011). As such, it is increasingly important to address the secondary diseases like diabetes and cardiovascular disease that develop once patients enter into survivorship. It is known that breast cancer patients have high fat mass and low muscle mass upon diagnosis (Amaral et al. 2010; Healy et al. 2010; Yaw et al. 2010) and that these profiles worsen as patients progress through treatment and enter into survivorship (Demark-Wahnefried et al. 2001; Demark-Wahnefried et al. 1993; Demark-Wahnefried et al. 1997). In non-malignant populations, similar unhealthy body composition profiles are associated with a variety of metabolic complications, like chronic inflammation, glucose intolerance and dyslipidemia, all of which increase the risk of diabetes and cardiovascular disease. It is well supported that obesity in non-malignant populations can be attributed to reduced energy expenditure and increased energy intake.

The metabolic consequences of high fat mass and low lean tissue in breast cancer patients are not well known, as they are in non-malignant populations. It is possible that inflammation resulting from lingering effects of the recently excised tumour combined with inflammation due to high fat mass may cause patients to present with a greater degree of impaired glucose tolerance and dyslipidemia at diagnosis compared to non-malignant controls of a similar age and BMI. The increased concentrations of circulating pro-inflammatory cytokines may explain patients' predisposition to the development of secondary diseases in survivorship.

Before we can begin countering the development of diabetes and cardiovascular disease in survivorship, we need to examine the extent of metabolic complications such as

inflammation, glucose intolerance and dyslipidemia at baseline. The onset of chemotherapy is an important baseline to avoid the confounding effects of body composition changes as a result of treatment and treatment-associated fatigue. Once we understand how patients differ from non-malignant controls, we can begin designing effective and targeted nutrition and exercise interventions over treatment that will counter the development of diabetes, cardiovascular disease and cancer recurrence in survivorship. For instance, we may determine that breast cancer patients have a significantly higher fat mass compared to age/BMI-matched controls, and that this excess fat mass is associated with reduced glucose tolerance. With exercise data from patients we can start to identify potential population specific exercise prescriptions for duration and intensity to reduce fat mass and improve other health and metabolic outcomes.

8.0 Objectives

1. To independently evaluate the body composition, glucose/lipid metabolism, inflammation, exercise capacity and nutrition of breast cancer patients near diagnosis and compare these parameters to IDF cutpoints for metabolic syndrome. A healthy group of non-malignant females who were matched to breast cancer patients by age and BMI (HM) as well as young healthy non-malignant females (HY) will also be compared to IDF cutpoints for metabolic syndrome.

2. To compare metabolic parameters of breast cancer patients to HM females to determine whether breast cancer patients are associated with a greater degree of metabolic dysregulation and inflammation.

3. To compare metabolic parameters of breast cancer patients and HM females with a non-malignant group of young females who have healthy BMIs (HY females).

9.0 Hypotheses

1. Breast cancer patients will present with:
    a. BMI's in the overweight or obese categories, which will be supported by a high percentage body fat and a large waist circumference relative to IDF cutpoints for metabolic syndrome.

    b. TAG, HDL cholesterol and fasting glucose concentrations that are outside the IDF cut-points and indicate risk for metabolic syndrome. The breast cancer patients will also demonstrate unfavourable partitioning of other serum lipids: high fasting total cholesterol, low-density lipoprotein (LDL) cholesterol and TAG concentrations.

    c. High fasting and 2-hr blood glucose concentrations during an OGTT, and elevated insulin and c-peptide responses during an OGTT compared with HM females.

    d. High circulating concentrations of TNF-α, IL-6, IL-8 and CRP; low circulating concentrations of IL-4 and IL-10, compared to HM females.

    e. Poor VO_{2peak} and strength tests relative to their age and relative to the HM females.

f. Low energy expenditure and high caloric intake compared to HM females. This will be associated with their high percentage body fat.

2. Both breast cancer patients and HM females will have similar circulating concentrations of pro-inflammatory cytokines, lower circulating concentrations of anti-inflammatory cytokines and impairments in glucose metabolism and dyslipidemia; however, these metabolic parameters will be exaggerated in breast cancer patients compared to HM females. Although there will be impairments to glucose and lipid metabolism, overall HM females will not present with metabolic syndrome.

3. Compared to the HY females, both the breast cancer patients and the HM females will have a higher BMI, a higher percentage fat mass, a greater waist circumference, elevated circulating concentrations of pro-inflammatory cytokines, depressed circulating concentrations of anti-inflammatory cytokines, impaired glucose metabolism, dyslipidemia, poor relative VO_{2peak} and strength measures, low energy expenditure and high energy intake. HY females will not present with any factors that indicate risk of metabolic syndrome.

10.0 Methods

10.1 General Study Design

This study involved 26 participants: 8 breast cancer patients, 8 HM females and 10 HY females. All participants received 4 evaluations within a 1 week period: 1) body composition using skinfolds and circumference measurements; 2) blood sampling after an overnight fast and during an OGTT to measure aspects of glucose and lipid metabolism, as well as inflammation; 3) exercise capacity using a cardiovascular test, a strength test, and physical activity questionnaires (PAQs); and 4) nutrition using a 3 day food diary. This study was reviewed and received ethics clearance by the University of Waterloo Office of Research Ethics (for all participants) and by the Tri-Hospital Research Ethics Board (for breast cancer patients). Copies of the forms indicating ethics clearance was received have been included in Appendix I.

10.2 Participants

Eligibility criteria for the breast cancer patients, HM and HY females are presented in Table 1. All participants completed a Health Questionnaire (Appendix II) to assess whether they were free from metabolic disease (i.e. cardiovascular disease and diabetes) and free of any respiratory conditions or injuries that would have prevented them from safely participating in exercise testing.

Table 1. Eligibility criteria for breast cancer patients, HM females and HY females

Breast Cancer Patients	HM	HY
Inclusion Criteria		
• Females ≥ 18 years	• Age within ± 3 year of matched patient	• Females 18-25 years
• Recent diagnosis of breast cancer and up to 4 weeks following first cycle of chemotherapy	• BMI within ± 2 kg/m^2 of matched patient	• BMI between 18.5-24.9 kg/m^2
• Clinical stages I-II		
	• Use/no use of hormonal contraception matched to patient	• Not currently using hormonal contraceptives and have not used hormonal contraceptives within the past 6 months
	• Menopausal status matched to patient	
		• Recreationally active 3-5 days per week
• Fasting glucose <7.0 mM	• Fasting glucose <7.0 mM	• Fasting glucose <6.0 mM
Exclusion Criteria		
• Previous diagnosis of cancer in the last 5 years (other than carcinoma *in situ*)		
• Cardiovascular disease or thyroid disease that is not currently managed with medication		
• Diabetes or HIV		
• Injuries or health conditions that prevent participants' safe participation in exercise		

10.3 Recruitment

Breast cancer patients were recruited from the Grand River Regional Cancer Center (GRRCC) in Kitchener. Oncologists and the Clinical Trials Department from GRRCC completed a Hospital Screening Form detailing the eligibility requirements listed in Table 1 for each newly diagnosed breast cancer patient. Completed screening forms were forwarded to the

Nutrition and Metabolism Laboratory and our laboratory contacted eligible patients with details about the study.

HM females were recruited from the city of Kitchener-Waterloo through recruitment posters displayed in community centers, grocery stores, fitness centers and online bulletins. HY females were recruited from the University of Waterloo through announcements made in undergraduate classes and recruitment posters that were placed around campus. Additional information on breast cancer patient and HM female recruitment can be found in Appendix III.

10.4 Screening

Potential participants were screened for eligibility before beginning their assessments, using the eligibility criteria outlined in Table 1. Interested individuals who consented arrived at the laboratory after an overnight fast (no food or drink except water after midnight the night before) and received a finger-prick test (Aviva Accu-Check; Roche Diagnostics) to confirm that blood glucose was < 7.0 mM for patients and HM females, and < 6.0 mM for HY females. Potential participants were also weighed and measured to determine BMI, and completed a Health Status Screening Form to determine if they had any prior cancer or metabolic disease that would have excluded them from participating in the study. All screening sessions took place between 8:30am and 9:00am so that all potential participants were fasted for 8-12 hours.

10.5 Data Collection

Eligible participants received the following 4 assessments within a 1-week period: 1) body composition; 2) blood sampling; 3) cardiovascular and strength assessments, as well as physical activity questionnaires; and 4) nutrition.

10.5.1 Body Composition

Skinfold caliper (Harpenden Skinfold Caliper; Burgess Hill, West Sussex) and circumference measurements were taken by an exercise physiologist at the UW-WELLFIT center to minimize variation between measurements. Skinfold measurements were taken on the triceps, suprailiac region and thighs (2-3 trials per site) using the method described by Jackson and Pollock (1985). Sites were located using boney landmarks on the right side of the body while the participant was standing with their feet hip width apart. The triceps skinfold site was marked midway between the acromion process and the tip of the olecranon process while the forearm was supinated and flexed at 90°. The triceps skinfold measurement was taken with the arm returned to a neutral position at the side of the body. The suprailiac skinfold site was marked along the anterior superior iliac spine and taken while the participant's right arm was abducted to 90° with the fingertips of their right hand resting on their right shoulder. The thigh skinfold site was marked midway between the base of the patella and the inguinal crease. All skinfold measurements were taken once in the order in which they have been described, and then repeated in the same order once the subcutaneous adipose tissue has had approximately 30 seconds to relax. If the difference between measurements at any site was greater than 0.4 mm, the measurement was taken a third time. We used the average of the closest 2 trials as the final measurement for each site. Body fat percentage was estimated from the skinfold measurements taken at the triceps, suprailiac region and thigh. The skinfold measurements was entered into the Jackson 3-Site Equation for body density:

$$\textit{Body density} = 1.0994921 - (0.0009929 \times [\text{sum of 3 skinfolds in mm}]) + (0.0000023 \times [\text{sum of 3 skinfolds in mm}]) - (0.0001392 \times \text{age})$$

The body density value was then converted to percentage body fat using the Siri Equation:

$$\%\ body\ fat = (495/\text{body density}) - 450$$

Circumferences were measured using a tape measure. Landmarking and measuring for the mid-humerus circumferences were done using the same positioning as the triceps skinfolds. The waist circumference was taken at the top of the iliac crests, in accordance with the American College of Sports Medicine (ACSM 2009). The mid-thigh circumference was landmarked with the participant's right leg bent at 90°. This positioning causes the midpoint between the base of the patella and the inguinal crease to shift distally, so the midpoint was re-marked for the circumference measurement. Circumference measurements were taken once at each site, on the participant's right side.

One breast cancer patient, as well as all HM and HY females received a full-body DXA scan (Hologic QDR Series, Discovery W S/N 84474; Bedford, MA) for additional quantification of % body fat and lean tissue mass. Scans were completed by the same certified medical radiation technologist on the same day as the anthropomorphic body composition measurements. All participants wore a cotton hospital gown during the scan, removed all jewellery and lay in a supine position with their legs extended but separated by at least 2 cm, and feet turned in (toes touching). Their upper limbs lay at their sides, leaving 2 cm gap between torso and limb, with the forearms in a supinated position, and their neck and spine in a neutral position. This positioning allowed for easier separation of individual body compartments during analysis of the scan (i.e. upper limbs, lower limbs and pelvic region).

The remaining 7 breast cancer patients did not receive DXA scans since they were recruited and assessed before this lab acquired the DXA equipment. Percent body fat was determined using the skinfold calipers and was compared between all participants.

We estimated lean tissue mass in the breast cancer patients without DXAs indirectly from their skinfold caliper measurements:

$$\textit{Estimated lean tissue} = (100 - \%\ \text{body fat})\% * \text{weight}$$

To determine whether indirect calculations of lean tissue using skinfold measurements were comparable to measurements from DXA, we compared estimated and measured lean tissue in all participants who received DXA scans (n=19) using a paired t-test (Appendix IV). Lean tissue was calculated from skinfold measurements by multiplying % body fat by body weight to attain fat mass and, subsequently, subtracting fat mass from body weight. On average, our calculations using skinfolds provided an overestimation of lean tissue compared to DXA for the majority (14/19) of participants (mean ± SD for lean tissue measured by skinfold and DXA: 42.8 ± 5.5 kg vs. 40.4 ± 5.0 kg, respectively, $p = 0.018$); the average difference between estimate and measurement was 2.5 ± 4.1 kg. Considering the average difference between estimated and measured lean tissue was consistent and relatively small (2.5 kg represents < 6.5% of the lean tissue in a 70 kg adult) we used estimated lean tissue mass for breast cancer patients lacking DXA scans with caution.

10.5.2 Blood Sampling

Participants arrived at the University of Waterloo after an overnight fast (8-12 hours with no food or drink except for water) for an OGTT (see Figure 3). A sterile catheter was inserted into the antecubital vein of the participant's preferred arm and 25 mL of blood was drawn. Thirty minutes later a second fasting sample of 5 mL was drawn. Immediately after the second fasting sample, participants were instructed to consume a 75 g glucose drink (Trutol Glucose Tolerance Beverage, Thermo Fisher Scientific; East Providence, RI). All participants consumed the glucose drink within 10 min. Fifteen minutes after participants consumed the glucose drink, 5 mL of blood was drawn. Blood was also drawn at 30, 45 and 60 minutes post-glucose drink. After 60 minutes post-glucose drink, blood was drawn every 30 minutes for a total time of 180 minutes post-glucose drink (total time of OGTT = 210 minutes).

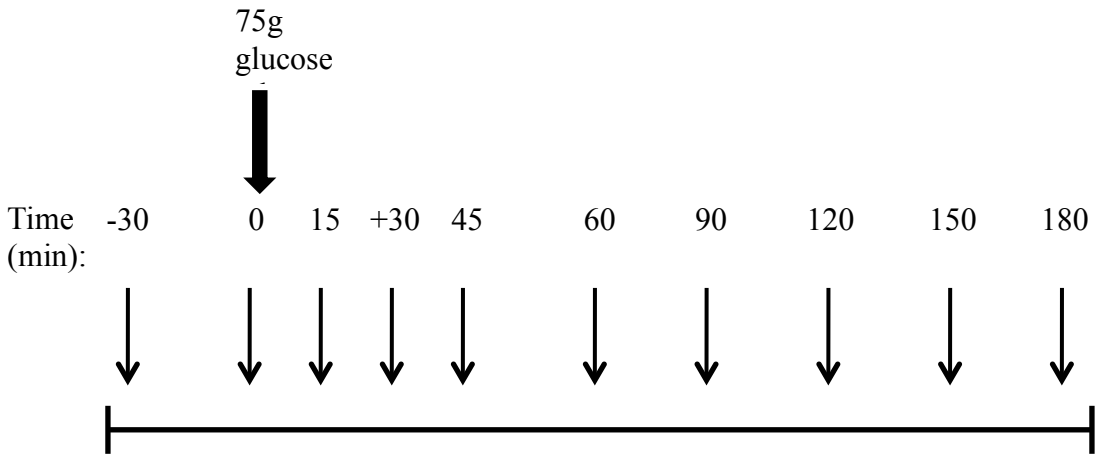


Figure 3 Timing of events during the 3 hour OGTT protocol. The thin black arrows indicate when blood was sampled. Participants were fasted and ingested a 75g bolus of glucose after the 0 min blood draw.

Blood collected during the first blood draw (i.e. at -30 minutes) was analyzed for the parameters listed in Table 2. Blood samples collected at all timepoints during the OGTT were

assessed for glucose, insulin, c-peptide and glucagon. We assessed insulin sensitivity using the homeostatic model assessment of insulin resistance (HOMA-IR), which considers fasting values only, and the Matsuda Index (Matsuda and Defronzo 1999), which takes into account glucose and insulin values during a 75g OGTT. The Matsuda index has been validated against the euglycemic insulin clamp.

$$HOMA\text{-}IR = \frac{(fasting\ glucose * fasting\ insulin)}{22.5}$$

$$Matsuda\ Index = \frac{10\,000}{\sqrt{(fasting\ glucose * fasting\ insulin * mean\ OGTT\ glucose * mean\ OGTT\ insulin)}}$$

The molar ratio of glucagon to insulin provides an indication of whether gluconeogenesis and insulin action are balanced. To calculate this ratio we divided plasma glucagon by serum insulin (both in pM). We multiplied insulin concentrations by 6.945 to convert them from μIU/mL to pM; we multiplied glucagon concentrations by 3.485 to convert them from pg/mL to pM.

Table 2. Metabolic parameters assessed using blood collected at the -30 min timepoint

Glucose metabolism	Fasting lipids	Inflammatory mediators
Glucose	Total cholesterol	IL-6
Insulin	LDL-cholesterol	TNF-α
C-peptide	HDL-cholesterol	IL-8
Glucagon	TAG	IL-4
	NEFA	IL-10
	Glycerol	CRP

10.5.3 Cardiovascular and Strength Assessments, and Physical Activity Questionnaires

All exercise testing took place in the UW WELL-FIT Center. Predictive VO_{2peak} tests were conducted on a cycle ergometer (Ergometrics er800s; Ergoline, Bitz, Germany) using the Vmax breath-by-breath system (Vmax; SensorMedics, Yorba Linda, CA) as an indicator of cardiovascular fitness. Predictive VO_{2peak} tests were used instead of VO_{2max} tests since most exercise tests on clinical populations are symptom limited and VO_{2max} is generally not achieved. All participants wore a Hans Rudolph facemask (7400 Vmask Series Oro-Nasal MASK) during the test. The mouthpiece was not used since many patients experience dry mouth and mouth sores as a result of chemotherapy; the facemask is a valid, and more comfortable, alternative (Evans and Potteiger 1995; Bell et al. 2012). Heart rate was monitored throughout the test using an electrocardiogram (EK10 Spacelabs Burdick Inc.; Deerfield, WI).

Participants rested in a seated position for 5-10 minutes on the cycle ergometer while the set-up was completed. We then collected 2 minutes of resting values with the participant

seated on the cycle ergometer, after which time instructed the participant to begin pedaling at a resistance of 25 W. Participants maintained a cadence of 50 – 70 rpm throughout the test. Blood pressure, using a manual sphygmomanometer, and rating of perceived exertion were recorded every 2 minutes. Resistance was increased between 10 – 50 W depending on the participant's rating of perceived exertion in the previous increment. Tests were terminated when participants reach 85% of their age-predicted maximal heart rate (HR), when they were no longer able to maintain a cadence of 50 – 70 rpm or requested to stop. VO_{2peak} was extrapolated using HR and VO_2 data for each stage. VO_2 and HR are linearly correlated (Darby and Yaekle 2000). It is therefore possible to predict VO_{2peak} using a participant's maximal HR. VO_2 is plotted against HR at the end of each exercise increment, and a linear trend line is created. VO_{2peak} is then extrapolated using the participant's age-predicted maximal HR and the equation of the trend line.

Strength was assessed using predictive repetition maximum (1RM) tests for biceps curl, triceps extension and leg extension. Bench press is conventionally used to assess upper body strength, however many breast cancer patients may endure surgery during the treatment time-course, which may lead to lymphedema or tightening of the tendons and ligaments ('cording') on one side of their body. This often results in strength differences. Biceps curls and triceps extensions can be assessed unilaterally and allows for the accurate measurement of muscle strength for each side of the body. A squat is used by convention to measure lower body strength. However, this compound exercise is difficult to instruct to participants who have limited exercise experience. We chose to assess leg extension since it can be measured using a weight machine, which restricts extraneous movement. Biceps and triceps were assessed unilaterally using a cable machine and stacked weights; quadriceps were assessed bilaterally.

For each exercise, following a demonstration on proper technique, participants performed 10 repetitions at 40-60% of their 1RM as a warm-up (estimated using data from the Institute for Aerobics Research based on the weight of the participant). After 1-3 minutes of rest, the weight was increased to the participant's estimated 60-80% and participants completed up to 10 repetitions with proper technique. If participants reached 10 repetitions during this first stage, after 3-5 minutes of rest the weight was increased to the participant's estimated 80-95% 1RM. They completed as many repetitions as possible using proper technique and the first stage was then discounted. 1RM was predicted using the O'Connor Equation (O'Connor et al. 1989):

$$1\text{RM} = \frac{weight\ lifted}{repetitions} \div \left(\frac{1}{repetitions} - 0.025\right)$$

Habitual physical activity was assessed using the Baecke PAQ (Baecke, Burema, and Frijters 1982) and the CHAMPS Activities Questionnaire for Older Adults (Stewart et al. 2001). These questionnaires provide information regarding the participant's habitual patterns of physical activity that may not necessarily be represented by their cardiovascular and strength test results. The Baecke PAQ provides 3 quantitative indices of physical activity that can be compared across participants: the Work Index, which evaluates occupational physical activity; the Sport Index, which evaluates voluntary exercise during leisure time; and the Leisure Index which accounts for other types of physical activity during leisure time. The CHAMPS Activities Questionnaire for Older Adults provides an estimate of exercise-related caloric expenditure per week. The Baecke questionnaire has been validated for use in adults aged 20-70 (Pols et al. 1995); the CHAMPS questionnaire has been validated for use in older adults, clinical populations as well as in individuals of different ethnic backgrounds (Feldman et al.

2009; Resnicow et al. 2003). The use of both of these questionnaires captured a wide range of ages for this study.

Caloric expenditure per week was estimated using the CHAMPS Activities Questionnaire for Older Adults using the equation described by the ACSM. The participant provided an indication of the duration of each activity described in the questionnaire. Each activity is assigned a metabolic equivalent of task (MET) value; a MET is a physiological measure that expresses the energy required for various physical activities. One MET is generally considered to reflect resting energy expenditure (Ainsworth et al. 1993). Caloric expenditure per activity per week was determined using the following:

kcal/activity/week = duration(hours/week)*METs(/minute)*3.5*60*(weight in kg/200)

Caloric expenditure per week was estimated by summing the expenditure of each activity.

Resting energy expenditure was calculated using the equation described by Ainsworth et al. (1993):

1 MET = weight in kg * 1 kcal * 24 hours

Total energy expenditure (TEE) per day was estimated using the following equation (Brooks et al. 2004):

TEE = 354 – (6.91 * age) + PA [(9.36 * weight in kg) + (726 * height in m)]

Physical activity level (PAL) scores between 1.2 – 2.0 were assigned to participants depending on their CHAMPS and Baecke scores, and PAL was used to calculate the physical activity coefficient (PA).

10.5.4 Nutrition

Participants completed a 3-day food record, as described by Thompson and Byers (1994), over the course of 2 weekdays and 1 weekend day during the same week of the body composition, metabolic and exercise capacity evaluations (Appendix V). We chose the 3-day food record over other methods of dietary assessment such as the 24-hr diet recall and food frequency questionnaire because it has shown a stronger correlation when compared to the gold standard of measurement for energy intake, doubly labeled water (Trabulsi and Schoeller 2001). The 3-day food record is also advantageous because of its prospective nature and ability to provide quantitative information about dietary intake (Thompson and Byers 1994). Previous studies that have evaluated energy intake in cancer patients who are undergoing chemotherapy have employed 3-day food records (Kutynec et al. 1999; Ovesen et al. 1993).

We determined daily caloric intake and macronutrient breakdown (% fat, % carbohydrate and % protein) from these records using ESHA Food Processor software. The Canadian Nutrient File was primarily used during data analysis with ESHA, however data from the USDA National Nutrient Database for Standard Reference was used when Canadian information was not available. At the end of each day, participants were instructed to indicate whether they consumed more, the same or less than they usually did, as well as record any supplements or vitamins taken. Participants also rated their appetite and level of tiredness on a scale of 0 – 10 at the end of each day, where 0 indicated their best appetite and no feelings of tiredness, and 10 indicated their worst appetite and worst feelings of tiredness. In addition, breast cancer patients rated their level of nausea on a scale of 0 – 10 at the end of each day, where 0 indicated no feelings of nausea and 10 indicated their worst feelings of nausea. At the end of the 3-day period, patients completed a short questionnaire where they indicated any self-

reported weight change or symptoms of chemotherapy that might have affected their eating habits (i.e. mouth sores, changes in taste of certain foods) in the past 2 weeks, and whether their food intake and activities levels had changed in the past month.

10.6 Analyses

10.6.1 Blood Analyses

Serum was collected for glucose, insulin, c-peptide, lactate, glycerol, NEFAs, TNF-α, IL-6, IL-4, IL-8, IL-10 and CRP. Whole blood was given 30 minutes to clot at room temperature in borosilicate glass tubes. The blood was then be centrifuged at 2500 rpm for 10 minutes at room temperature. For the analysis of total cholesterol, LDL cholesterol, HDL cholesterol and TAGs, 2 mL of whole blood from the -30 minute time-point was added to a gold-capped serum-separting tube (SST) provided by Lifelabs Medical Laboratory Services. These tubes contained clot activators and a serum separating gel, and yielded serum. The blood in the SSTs were also centrifuged at 2500 rpm for 10 minutes at room temperature.

Plasma samples for glucagon were prepared by adding 2.0 mL of whole blood to test tubes containing 50 μL of a solution of combined EDTA and aprotinin ([EDTA] = 40 mg/mL saline; [aprotinin] = 0.4 mg/mL saline). Heparin and EDTA are anticoagulants. Aprotinin is an antiproteolytic protein, which prevents the breakdown of rapidly degraded proteins like glucagon. These tubes were kept on ice for the duration of the OGTT and then centrifuged at 2800 rpm at 4° C for 15 minutes.

After centrifugation, the serum and plasma were then extracted from the borosilicate glass tubes and SSTs, aliquoted into separate eppendorf tubes to avoid multiple thaw/re-freeze

cycles, and stored at -80° C for subsequent analysis. Plasma samples for glucagon analysis were stored in borosilicate glass test tubes because of glucagon's tendency to adhere to plastic.

10.6.2 Serum Glucose

Serum glucose was measured using a spectrophotometer (Shimadzu UV160U UV-Visible Recording Spectrophotometer; Columbia, MD) and peroxidase/glucose oxidase enzymatic reaction. Test tubes were filled in triplicate with 10 μL distilled water (blank), glucose standard or serum sample. We added 2.5 mL of a reagent solution containing peroxidase, glucose oxidase and o-dianisidine dihydrochloride to each tube. Tubes were then vortexed and incubated at 37° C for 30 minutes. During the incubation period, glucose in the blanks, standards and samples reacted with glucose oxidase, releasing hydrogen peroxide. Peroxidase then catalyzed a reaction between the liberated hydrogen peroxide and o-dianisidine dihydrochloride, forming oxidized o-dianisidine. Oxidized o-dianisidine produces a colour that was read at 450 nm by the spectrophotometer. The intensity of the colour in the samples in comparison to the intensity of the colour in the standards provides an indication of glucose concentration.

10.6.3 Serum Insulin

Serum insulin was measured in duplicate using a Coat-A-Count Insulin Radioimmunoassay kit (Siemans Healthcare Diagnostics; Deerfield, IL). We added 200 μL of blank, standard or sample to polypropylene tubes pre-coated with insulin antibody. To all tubes we added 1.0 mL of ^{125}I-labeled insulin. Tubes were then vortexed and incubated at room temperature for 24 hours. During the incubation period, ^{125}I-labeled insulin competes

with insulin in the sample or standard for binding sites on the insulin antibody molecules, which are fixed to the polypropylene tube walls. After 24 hours the supernatant was aspirated and tubes were counted for 1 minute using a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences; Woodbridge, ON). Samples or standards with high concentrations of insulin would bind less ^{125}I-labeled insulin and be less radioactive.

10.6.4 Serum C-Peptide

Serum c-peptide was assessed in duplicate using a C-Peptide Double Antibody Radioimmunoassay kit (Siemans Healthcare Diagnostics; Deerfield, IL). During the assay, 25 μL of standard or serum was combined with 100 μL ^{125}I-labeled c-peptide and 100 μL c-peptide antibody in polypropylene tubes. The tubes were vortexed and incubated for 4 hours at room temperature. During the incubation, c-peptide in the standard and sample competes with ^{125}I-labeled c-peptide for binding sites on the c-peptide antibody. After the 4 hour incubation, 1.0 mL of cold (4° C) precipitating solution was added to each tube. The tubes were vortexed and then centrifuged at 3000g for 15 minutes. After centrifugation, the supernatant was aspirated and the tubes were counted for 1 minute in a gamma counter. Radioactivity is inversely correlated with c-peptide concentration. Concentration of c-peptide was determined by interpolating samples from a graph of known c-peptide concentrations.

10.6.5 Plasma Glucagon

Plasma glucagon was assayed in duplicate using a Glucagon Double Antibody Radioimmunoassay kit (Siemans Healthcare Diagnostics; Deerfield, IL). 200 μL of plasma

sample or standard was added to a borosilicate glass test tube, along with 100 μL of glucagon antibody. The tubes were vortexed and incubated at 4° C for 24 hours. After the first incubation period, 100 μL of ^{125}I-labeled glucagon was added to all tubes. The tubes were vortexed and incubated at 4° C for 24 hours again. After the second incubation period 1.0 mL of cold (4° C) precipitating solution was added to each tube. The tubes were vortexed once more and centrifuged at 1500g for 15 minutes. When centrifugation was complete, the supernatant was aspirated and the tubes were counted for 1 minute in a gamma counter. Over the course of the 2 incubation periods, glucagon in the standards and samples competes with ^{125}I-labeled glucagon for binding sites on the glucagon antibodies. Thus, radioactivity of the samples is inversely related to glucagon concentration. Plotting the radioactivity (counts per minute) of the standards (known concentrations of glucagon) generates a standard curve from which concentrations of the samples were interpolated.

10.6.6 Perchloric Acid (PCA) Extraction for Glycerol Samples

Serum contains extraneous proteins that interfere with the true reading of certain metabolites like glycerol. To remove this extra protein, a solution of 0.6 M perchloric acid was prepared by combining perchloric acid stock solution and water. 500 μL of this solution was combined with 100 μL serum in an eppendorf tube. All tubes and solutions were kept on ice throughout the procedure. Tubes were vortexed and centrifuged at 4° C at 15 000g for 2 minutes. After centrifugation, 250 μL of 1.25 M potassium bicarbonate was added to each tube. After tubes were allowed to sit for 10 minutes, centrifugation was repeated and the supernatant was extracted, transferred to new eppendorf tubes and stored at -80 °C. A dilution

factor of 8.5 is introduced with the PCA extraction; the dilution factor was taken into account when calculating final concentrations of glycerol.

10.6.7 Serum Glycerol

Serum glycerol was measured in triplicate using a glycerol kinase-based enzymatic reaction and a spectrofluorophotometer. A dilute reagent of hydrazine, glycine, NAD+, ATP, $MgCl_2$, Cysteine and 3-GPDH was prepared. The first 6 reagents were combined, the solution was adjusted to the desired volume using distilled water and then the pH was adjusted to 9.2 (for enzyme stability). 3-GPDH was added after the pH was adjusted. 50 μL of standard or sample was added to each test tube and combined with 1.0 mL dilute reagent and vortexed. The test tubes were then read on the spectrofluorophotometer at 365 – 455 nm (baseline). A solution of 20 μL glycerol kinase and 1.0 mL dilute reagent was then prepared. 10 μL of the glycerol kinase solution was added to each test tube. Tubes were incubated at room temperature in the dark for 60 min and then re-read. During the incubation, glycerol kinase catalyzes the reaction of glycerol in the samples and standards with ATP. This reaction forms glycerol-3-P which then reacts with NAD^+ in a reaction catalyzed by 3-GPDH. This second reaction forms NADH, which fluoresces in direct proportion to glycerol in the sample or standard.

10.6.8 Serum Non-Esterified Fatty Acids

NEFAs were measured in quadruplicate using the Wako NEFA-HR(2) Microtiter Procedure (Wako Diagnostics; Richmond, VA). 5 μL of blank, standard or sample were added

to separate wells in a 96 well microplate. 200 μL of Colour Reagent A (acyl-coenzyme A synthase, coenzyme A, ATP and 4-aminoantipyrine) were added to each well and the microplate was then incubated at 37° C for 5 minutes inside the spectrophotometer (Spectramax Plus 384; Molecular Devices; Sunnyvale, PA). The absorbance was read at 550 nm (baseline) and then 100 μL of Color Reagent B (acyl-coenzyme A oxidase and peroxidase) was added to each well. The microplate was incubated again and read a second time. During the first incubation, serum NEFAs react with Color Reagent A to produce acyl-COA and several byproducts. In the second incubation, acyl-COA is oxidized to produced hydrogen peroxide. Hydrogen peroxide then catalyzes the reaction of 4-aminoantipyrine, whose produce fluoresces in direct proportion to the concentration of NEFAs in each sample or standard. Concentrations were determined from the equation provided in the Wako Diagnostics kit. NEFA concentrations were determined using the following equation provided by Wako Diagnostics:

$$\textit{Sample concentration (mM)} = \text{standard concentration} * \frac{sample\ absorbance}{standard\ absorbance}$$

10.6.9 Serum Triacylglycerol, Total Cholesterol, LDL Cholesterol and HDL Cholesterol

Total cholesterol, LDL cholesterol, HDL cholesterol and TAGs were analyzed by Lifelabs Medical Laboratory Services.

10.6.10 Serum TNF-α, IL-6, IL-4, IL-8, IL-10

Serum cytokines were analyzed using the BD Cytometric Bead Array Human Soluble Protein Master Buffer Kit and BD FACSCalibur flow cytometer (BD Biosciences; Mississauga, ON). The kit provides 6 types of capture beads coated with an antibody specific to TNF-α, IL-6, IL-4, IL-8 or IL-10. Each bead has a matching detection reagent that fluoresces at a specific activity. Prior to beginning the assay, all beads were combined into a single tube labelled 'Mixed Capture Beads' and vortexed. All detection reagents were combined into single tube labelled 'Mixed Detection Reagents'.

During the assay, 50 μL of each standard or sample was added to appropriately labelled tubes. 50 μL of the Mixed Capture Beads was then added to each tube: tubes were then vortexed and incubated at room temperature for 1 hour. During the incubation, the beads form complexes with the cytokines matching their antibodies. After 1 hour, 50 μL of Mixed Detection Reagents was added to each tube, tubes were vortexed and then incubated for 2 hours at room temperature. During the 2 hour incubation, the detection reagent specific to each bead associates with the bead/cytokine complex, forming a sandwich structure. After 2 hours, 1 mL of wash buffer was added to all tubes. Tubes were vortexed and centrifuged at 200g for 5 minutes, after which time the supernatant was aspirated. Each pellet was then resuspended with 300 μL wash buffer and acquired on the flow cytometer. Each of the 6 bead/cytokine/detection reagent complexes is reflected as a different population. Concentrations were determined by comparing the mean fluorescence of the population to the standard curve for each cytokine. Due to difficulty detecting TNF-α in several samples, we verified TNF- α concentrations in all samples using a Quantikine HS ELISA Human TNF-α Immunoassay (R&D Systems Inc.; Minneapolis, MN; Appendix VI).

10.6.11 Serum CRP

Serum CRP was measured using an enzyme-linked immunosorbant assay (ELISA) (ALPCO Immunoassays; Salem, NH). Wells of the ELISA plate were pre-coated with rabbit polyclonal antibody specific for human CRP. After washing each well thoroughly with dilute wash buffer, 100 μL of standard or sample (diluted 100-fold) was added and the plate was incubated at room temperature for 1 hour. During the incubation period, CRP in the standards or samples became bound to the antibody coated microwells. Following the incubation, the plate was washed to remove all unbound substances and 100 μL of immunoconjugate (peroxidase-labeled rabbit anti-CRP) was added to each well and the plate was incubated for another hour. During the second incubation period, the immunoconjugate became bound to the CRP-antibody complex affixed to the wells. Following the second incubation, the plate was washed again and 100 μL of tetramethylbenzidine (TMB, a peroxidase substrate) was added to each well. The plate was incubated in the dark at room temperature for 20 minutes, during which time a blue colour developed as the TMB reacted with peroxidase. The reaction was stopped, and the colour turned yellow, when 50 μL of 0.45 M sulphuric acid was added to each well. The absorbance of each well was read at 450 nm. The amount of colour is directly proportional to the concentration of CRP in the sample.

10.7 Statistical analysis and calculations

Values are presented as mean ± SD. Area under curve (AUC) calculations were completed using Sigma Plot ® version 11.2 (Systat Software Inc.; San Jose, CA); t-tests and paired t-tests were completed using Microsoft Excel. Areas under the curves (AUC) for

glucose, insulin, c-peptide and glucagon were calculated using the incremental area method (Allison et al. 1995). For all analyses, statistical significance was accepted at $p < 0.05$. Trends were identified as $p < 0.10$. Values > ± 2 SD were excluded as outliers.

As described in the following sections, 2-tailed student t-tests and paired t-tests were used in place of 2-way ANOVA (time *vs.* participant group) for the OGTT data and in place of 1-way ANOVA (participant group) for the fasting serum data (i.e. lipids, cytokines etc.). Our consulting biostatistician (Dr. Joel Dubin) had suggested that time should be considered a continuous variable and therefore should not be used as a treatment in 2-way ANOVA. T-tests were therefore used for these comparisons instead. 1-way ANOVA was inappropriate for the fasting data since paired t-tests, and not student t-tests, were conducted between breast cancer patients and HM females.

10.7.1 Comparison of breast cancer patients and HM females

We used paired t-tests and 95% confidence intervals (CI) to compare body composition, fasting metabolites, OGTT measurements and AUC, exercise and nutrition measurements between breast cancer patients and HM females. In all comparisons between breast cancer patients and HM females, paired t-tests and 95% CI analyses agreed. The p-values reported in *11.0 Results* refer to the paired t-tests.

10.7.2 Comparison of breast cancer patients and HM females to HY females

Two-tailed two-sample t-tests were used to compare breast cancer patients to HY females, and HM females to HY females.

10.7.3 Comparison between fasting and OGTT values for glucose, insulin, c-peptide and glucagon

Two-tailed two-sample t-tests were used to compare average fasting glucose, insulin, c-peptide and glucagon to values obtained 15, 30, 45, 60, 90, 120, 150 and 180 min post-glucose ingestion for all groups.

11.0 Results

11.1 Clinical description of breast cancer patients

The breast cancer patient group was fairly homogeneous based on clinical criteria, despite our small sample size. All patients were Stage I or II, indicating that the disease was confined to the breast tissue or the surrounding lymph nodes. The primary tumour mass was either < 5 cm with metastases to surrounding lymph nodes or > 5 cm with no metastases to surrounding lymph nodes (National Cancer Institute 2012). Patients had no metastases to other areas of the body. All patients had undergone either tumour resection or mastectomy prior to participating in the present study. The length of time between surgery and the initiation of chemotherapy was not reported in most cases. However, no more than 4 months elapsed between diagnosis and the first cycle of chemotherapy for all patients. Chemotherapy regimens prescribed to patients were AC (doxorubicin + cyclophosphamide), ACT (doxorubicin + cyclophosphamide + paclitaxel) or TC (docetaxel + cyclophosphamide). All patients had received 1-2 cycles of chemotherapy prior to data collection.

11.2 Breast cancer patients were overweight and exhibited similar body composition compared to HM females

On average at the time of data collection, breast cancer patients were 46 ± 10 years of age and overweight (BMI: 28.8 ± 6.0 kg/m^2; Table 3); 2 patients had BMIs in the normal weight category (18.5 – 24.9 kg/m^2), 4 patients were overweight (25 – 30 kg/m^2) and 2 were obese (> 30 kg/m^2). Breast cancer patients were individually matched to HM females for age and BMI, so it follows that we did not observe any significant differences in these parameters

between patients and HM females (age: 46 ± 10 yrs vs. 47 ± 10 yrs respectively, p = 0.160; BMI: 28.8 ± 6.0 kg/m^2 vs. 28.9 ± 6.4 kg/m^2 respectively, p = 0.892). Percent body fat and waist circumference were not controlled for during HM recruitment however we found that these parameters were not different between patients and HM females (body fat: 41.3 ± 10.9% vs. 43.9 ± 12.7% respectively, p = 0.524; waist circumference: 94.6 ± 14.0 cm vs. 97.4 ± 18.5 cm respectively, p = 0.507). On average HY females were younger (21 ± 2 yrs), had a normal BMI (22.0 ± 2.4 kg/m^2), lower percentage body fat (26.4 ± 4.7%) and a smaller waist circumference (75.1 ± 5.5 cm) compared to patients and HM females.

Using skin-fold measurements, calculated lean tissue mass in patients was on average 43.7 ± 5.4 kg. We observed no difference between this estimate of lean tissue in breast cancer patients and the estimates of lean tissue HM and HY groups (HM: 42.7 ± 6.2 kg, p = 0.877, HY: 42.7 ± 5.4 kg, p = 0.690). Further, the estimate of lean tissue in breast cancer patients was not different from the more precise measurements of lean tissue using DXA in HM females and HY females (HM: 42.6 ± 4.3 kg, p = 0.633, HY: 38.6 ± 5.3, p = 0.062).

Table 3. Physical characteristics and body composition of all participant groups

	Breast cancer patients	HM females	HY females
Age (years)	46 ± 10#	47 ± 10#	21 ± 2
BMI (kg/m^2)	28.8 ± 6.0#	28.9 ± 6.4#	22.0 ± 2.4
Weight (kg)	76.8 ± 17.8#	75.9 ± 17.8#	59.0 ± 9.3
Height (m)	1.63 ± 0.05	1.64 ± 0.06	1.63 ± 0.07
Waist circumference (cm)	94.6 ± 14.0#	97.4 ± 18.5#	75.1 ± 5.5
Body fat (%)	41.3 ± 10.9#	43.9 ± 12.7#	26.4 ± 4.7
Estimate of lean tissue mass (kg)	43.7 ± 5.4	42.6 ± 6.1	42.7 ± 5.4

Data are presented as mean ± SD. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

11.3 Overall, breast cancer patients presented with symptoms of metabolic syndrome attributed to dyslipidemia and abdominal obesity

Individuals with abdominal obesity (a waist circumference > 88 cm) along with any 2 of the following criteria were characterized as having metabolic syndrome according to IDF guidelines: fasting serum glucose ≥ 5.6 mM, fasting TAG ≥ 1.7 mM, fasting HDL-cholesterol ≤ 1.3 mM or high blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg). When considered individually, 2 patients presented with metabolic syndrome. Overall, patients had a waist circumference of 94.6 ± 14.0 cm (6 of 8 patients were centrally obese; Table 4), fasting TAG of 1.84 ± 1.17 mM, and HDL-cholesterol of 1.08 ± 0.23 mM. On average, these parameters were in line with the ranges of metabolic syndrome in breast cancer patients. HM females demonstrated central obesity (average waist circumference: 97.4 ± 18.5 cm; 6 of 8 participants were centrally obese) similar to breast cancer patients, which was expected considering patients and HM females were matched for BMI. Unlike the patient group, however, average fasting TAG (0.93 ± 0.27 mM), HDL-cholesterol (1.58 ± 0.42 mM) for the HM females were within the IDF cutpoints, indicating that HM females were not at risk for metabolic syndrome. Average fasting serum glucose and blood pressure were also within IDF cutpoints for the HM group (Table 4). When considered individually, 1 participant in the HM group was identified as having metabolic syndrome. As expected, HY females did not demonstrate central obesity (average waist circumference: 75.1 ± 5.5 cm) and all other criteria for metabolic syndrome were within IDF cutpoints (Table 4). None of the HY females was identified as having metabolic syndrome.

Table 4. Description of breast cancer patients, HM females and HY females relative to IDF cutpoints for metabolic syndrome.

	IDF cutpoint	Breast cancer patients	HM females	HY females
Waist circumference (cm)	≥ 88	94.6 ± 14.0 (6 of 8)	97.4 ± 18.5 (6 of 8)	75.1 ± 5.5 (0 of 10)
Fasting serum glucose (mM)	≥ 5.6	5.11 ± 1.17 (2 of 8)	4.58 ± 1.01 (1 of 8)	4.29 ± 1.09 (1 of 10)
TAG (mM)	≥ 1.7	1.84 ± 1.17 (2 of 8)	0.93 ± 0.27 (0 of 8)	0.74 ± 0.33 (0 of 10)
HDL-cholesterol (mM)	< 1.3	1.08 ± 0.23 (5 of 8)	1.58 ± 0.42 (2 of 8)	1.31 ± 0.20 (5 of 10)
SBP (mmHg)	≥ 130	126 ± 16 (3 of 8)	120 ± 7 (1 of 8)	114 ± 5 (0 of 10)
DBP (mmHg)	≥ 85	77 ± 11 (3 of 8)	77 ± 6 (0 of 8)	73 ± 8 (0 of 10)

Data are presented as mean ± SD. Number of participants in each group outside the IDF cutpoints supporting the presence of metabolic syndrome is indicated in brackets. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

We did not observe any significant differences between breast cancer patients and HM females in fasting total cholesterol, LDL-cholesterol, NEFA, glycerol or TAG, however there was a trend towards lower HDL-cholesterol in patients compared to HM females (1.08 ± 0.23 mM vs. 1.58 ± 0.42 mM, p = 0.083; Table 5). Breast cancer patients demonstrated lower fasting HDL-cholesterol, higher fasting glycerol and higher fasting TAG, relative to HY females whereas HM females demonstrated higher fasting total cholesterol and glycerol compared to HY females. Overall, these data suggest that breast cancer patients may be at increased risk for developing type 2 diabetes (Canadian Diabetes Association 2012) and cardiovascular disease (Heart and Stroke Foundation 2012) due to their central obesity and the presence of dyslipidemia, which are independent risk factors for both of these conditions.

11.4 Breast cancer patients demonstrated elevated serum glucose, insulin and c-peptide during an OGTT compared to HM and HY females

Fasting serum glucose was not different between the 3 groups (Table 4). Serum glucose was significantly elevated at 15 min post-ingestion of the carbohydrate drink compared to fasting concentrations in all 3 groups (Figure 4). Interestingly, serum glucose concentrations returned to <6.0mM at 150 min, 120 min and 60 min post-ingestion in breast cancer patients, HM females and HY females, respectively. As we hypothesized, this suggests that breast cancer patients are less adept at clearing glucose following an oral carbohydrate challenge compared to HM females, and that both patients and HM females are less adept at clearing glucose compared to HY females, despite that average fasting serum glucose was not different between the 3 groups.

Table 5. Fasting lipids in breast cancer patients, HM females and HY females

	Breast cancer patients	HM females	HY females
Total cholesterol (mM)	4.12 ± 0.75	4.47 ± 0.79#	3.74 ± 0.37
HDL-C (mM)	1.08 ± 0.23#	1.58 ± 0.42	1.31 ± 0.20
LDL-C (mM)	2.20 ± 0.36	2.47 ± 0.57	2.09 ± 0.45
NEFA (mM)	0.574 ± 0.220	0.708 ± 0.288	0.462 ± 0.214
Glycerol (μM)	115.7 ± 59.2#	167.1 ± 95.3#	55.7 ± 23.0
TAG (mM)	1.84 ± 1.17#	0.93 ± 0.27	0.74 ± 0.33

Data are presented as mean ± SD. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

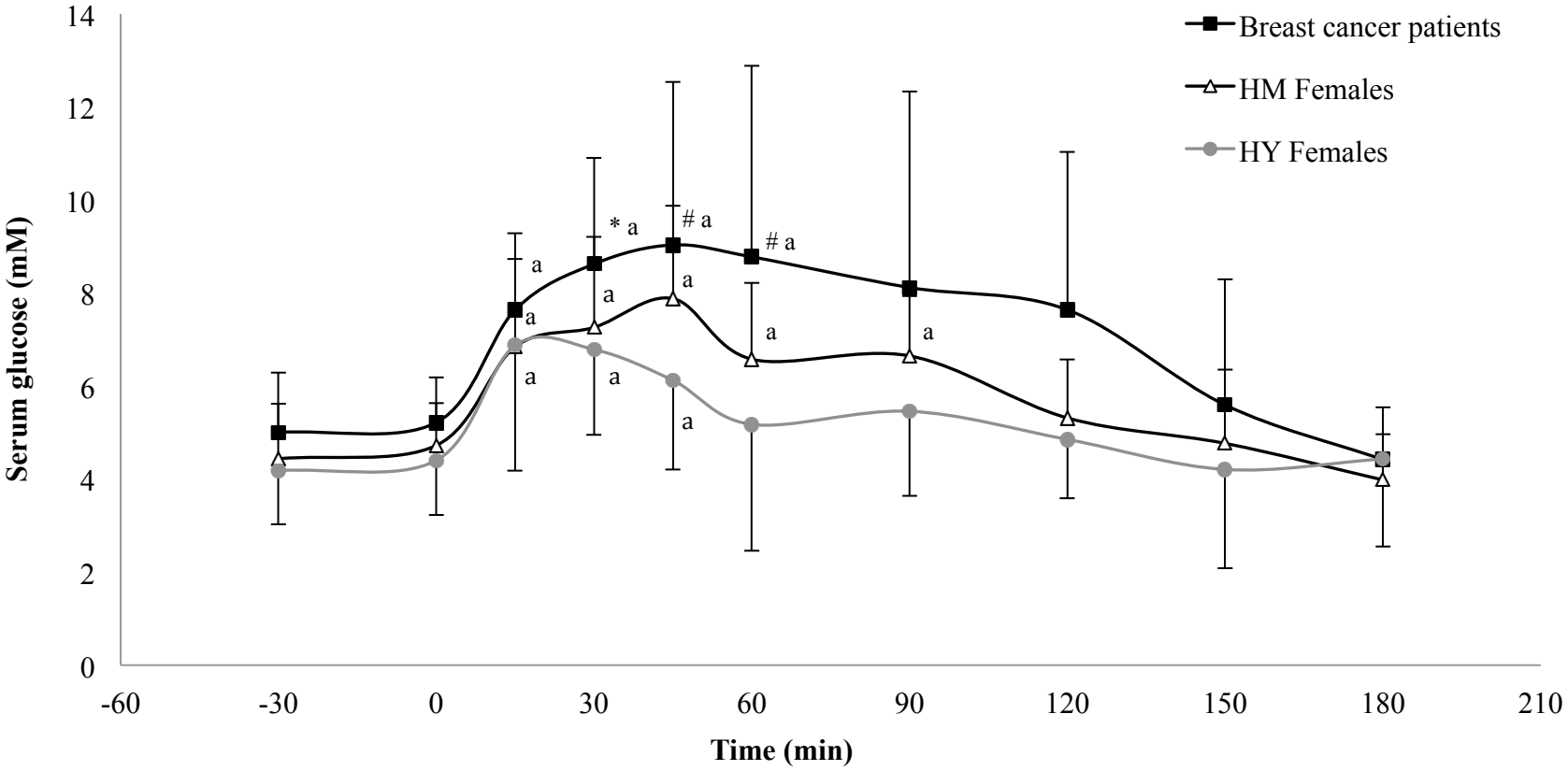


Figure 4 Serum glucose during an OGTT for all participant groups. Error bars represent standard deviation. * denotes significant difference from HM females; # denotes significant difference from HY females; [a] denotes significant difference from fasting values within group. Statistical significance was accepted at $p < 0.05$.

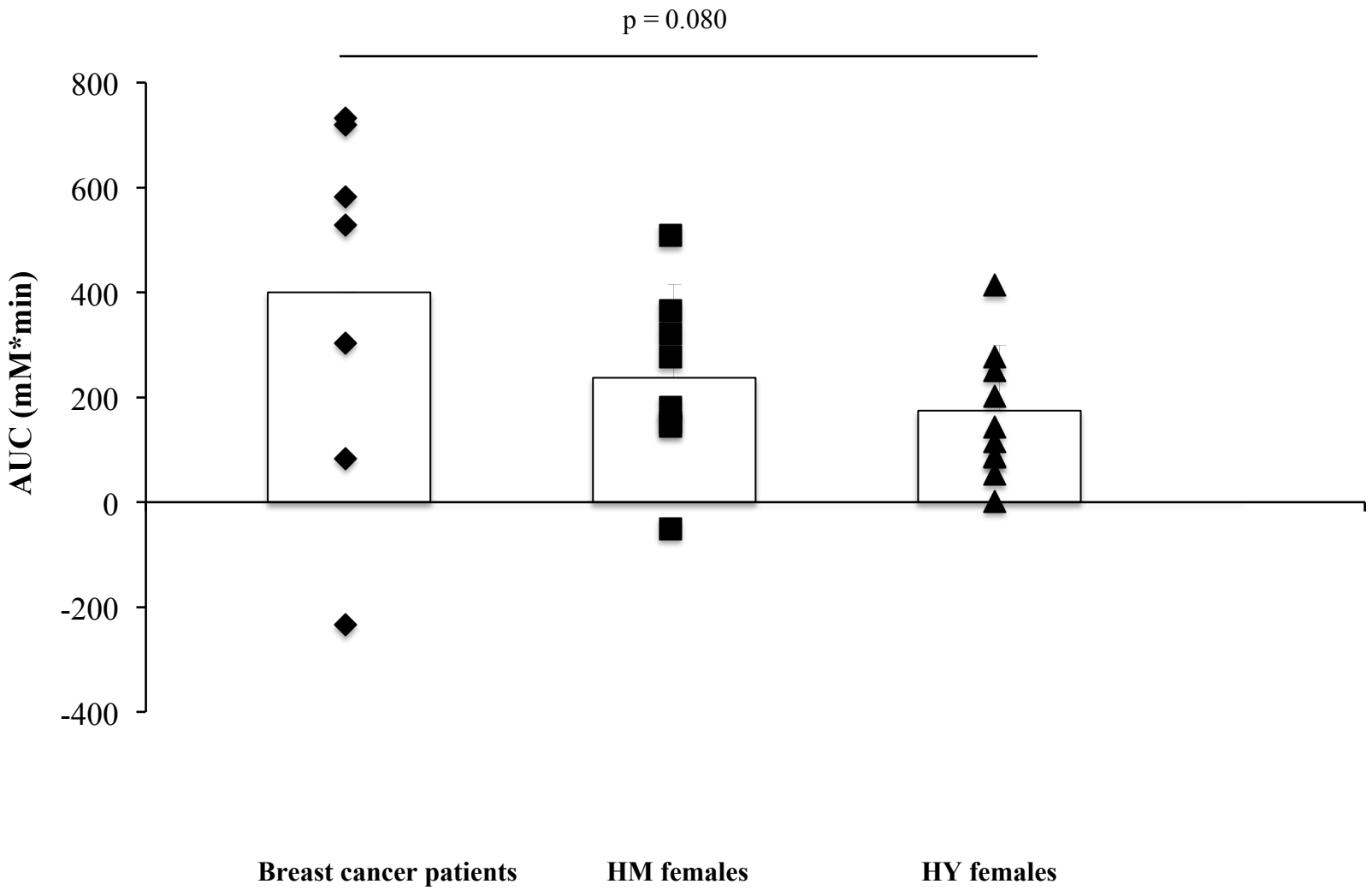


Figure 5 Serum glucose AUC during an OGTT across all participant groups. Black diamonds, squares and triangles represent individual data points for breast cancer patients, HM females and HY females, respectively. No significant differences were observed between breast cancer patients, HM females or HY females.

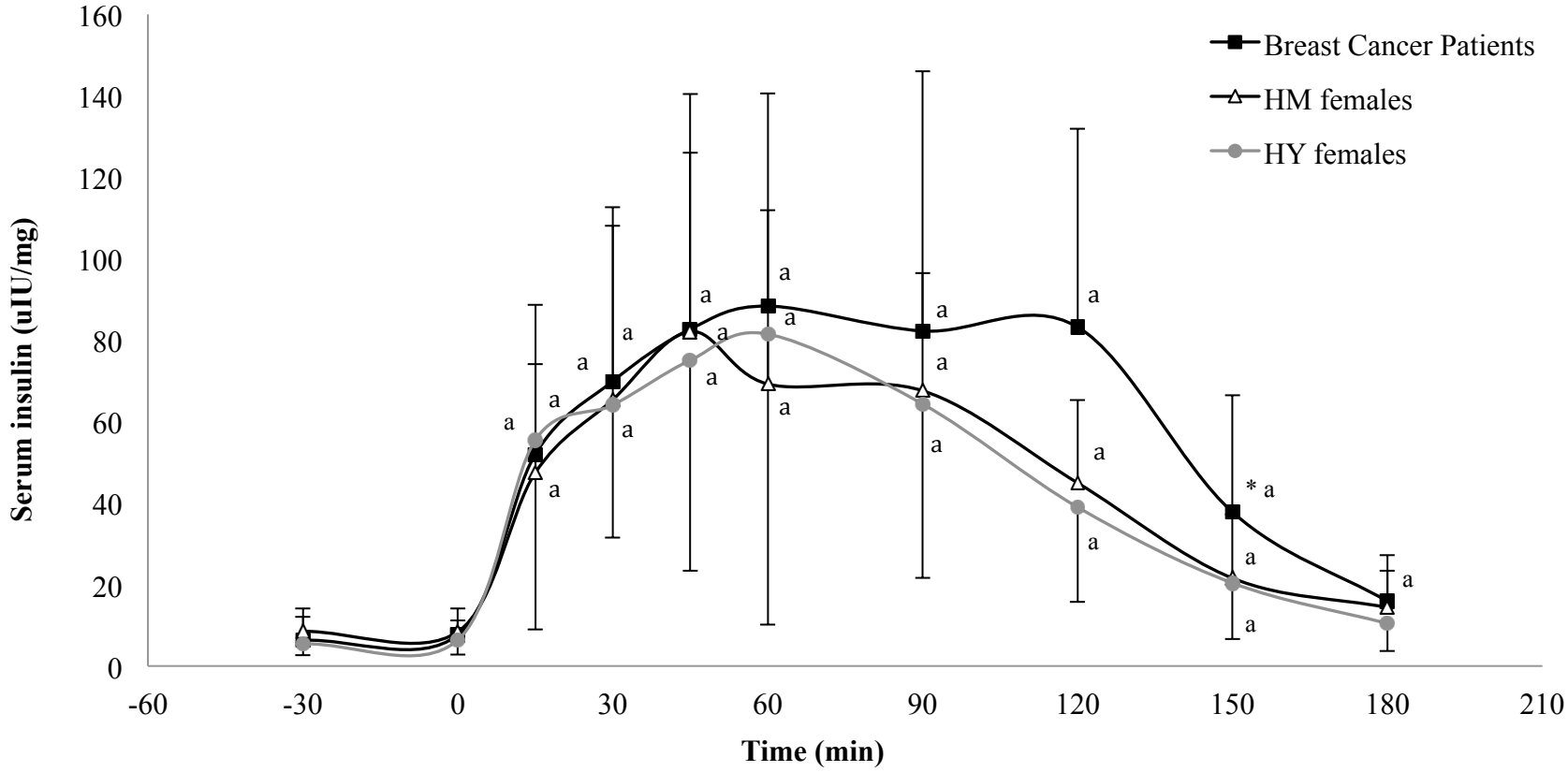


Figure 6 Serum insulin during an OGTT for all participant groups. Error bars represent standard deviation. * denotes significant difference from HM females; [a] denotes significant difference from fasting values within group. Statistical significance was accepted at $p < 0.05$.

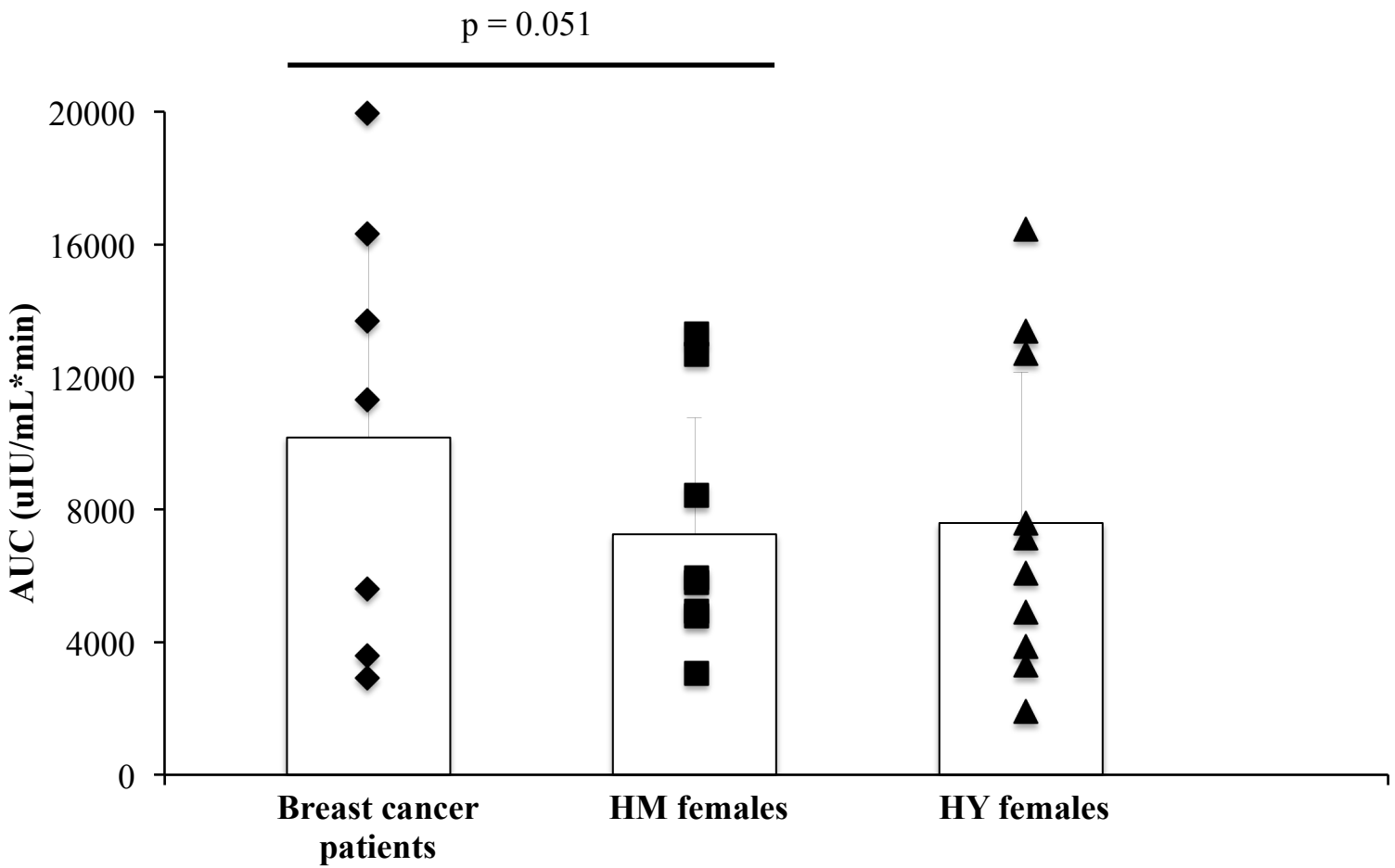


Figure 7 Serum insulin AUC during an OGTT across all participant groups. Black diamonds, squares and triangles represent individual data points for breast cancer patients, HM females and HY females, respectively. No significant differences were observed between breast cancer patients, HM females or HY females.

When we examined differences in glucose concentrations between groups at individual time-points during the OGTT, breast cancer patients demonstrated higher serum glucose at 30 min compared to HM females (8.62 ± 2.28 mM vs. 7.26 ± 1.94 mM, respectively, $p = 0.010$; Figure 4A), and higher serum glucose at 45 and 60 min compared to HY females. There was no significant difference in serum glucose between HM females and HY females at any time-point during the OGTT. Glucose AUC between patients, HM and HY groups did not differ (400 ± 359 mM*min vs. 237 ± 179 mM*min vs. 175 ± 124 mM*min; Figure 5).

We observed no difference in fasting insulin concentrations between any of the groups. However, similar to glucose, serum insulin was significantly elevated at 15 min post-ingestion of the carbohydrate drink compared to fasting concentrations for all groups (Figure 6). Interestingly, insulin remained elevated for the duration of the OGTT for the breast cancer patients and until 150 min for the HM and HY groups. When we compared serum insulin concentrations between groups at individual time-points during the OGTT, we observed that patients demonstrated almost double the concentrations of serum insulin compared to HM females at 150 min (37.8 ± 26.5 μIU/mL vs. 21.6 ± 15.7 μIU/mL, $p = 0.025$). Although serum insulin concentrations tended to be higher in breast cancer patients compared to HY females at 120 and 150 min, these differences were not significant. We also observed a trend towards higher insulin AUC in the breast cancer patients compared to HM females (10170 ± 6193 μIU/mL*min vs. 7257 ± 3530 μIU/mL*min, $p = 0.051$; Figure 7).

Fasting serum c-peptide was significantly elevated in breast cancer patients compared to HM and HY females (2.6 ± 1.2 ng/mL, 1.9 ± 0.8 ng/mL and 1.5 ± 0.6 ng/mL, respectively; $p = 0.005$ in patients vs. HM; $p = 0.015$ in patients vs. HY; Figure 8). Serum c-peptide followed a similar pattern to serum glucose and insulin and was elevated at 15 min post-ingestion of the

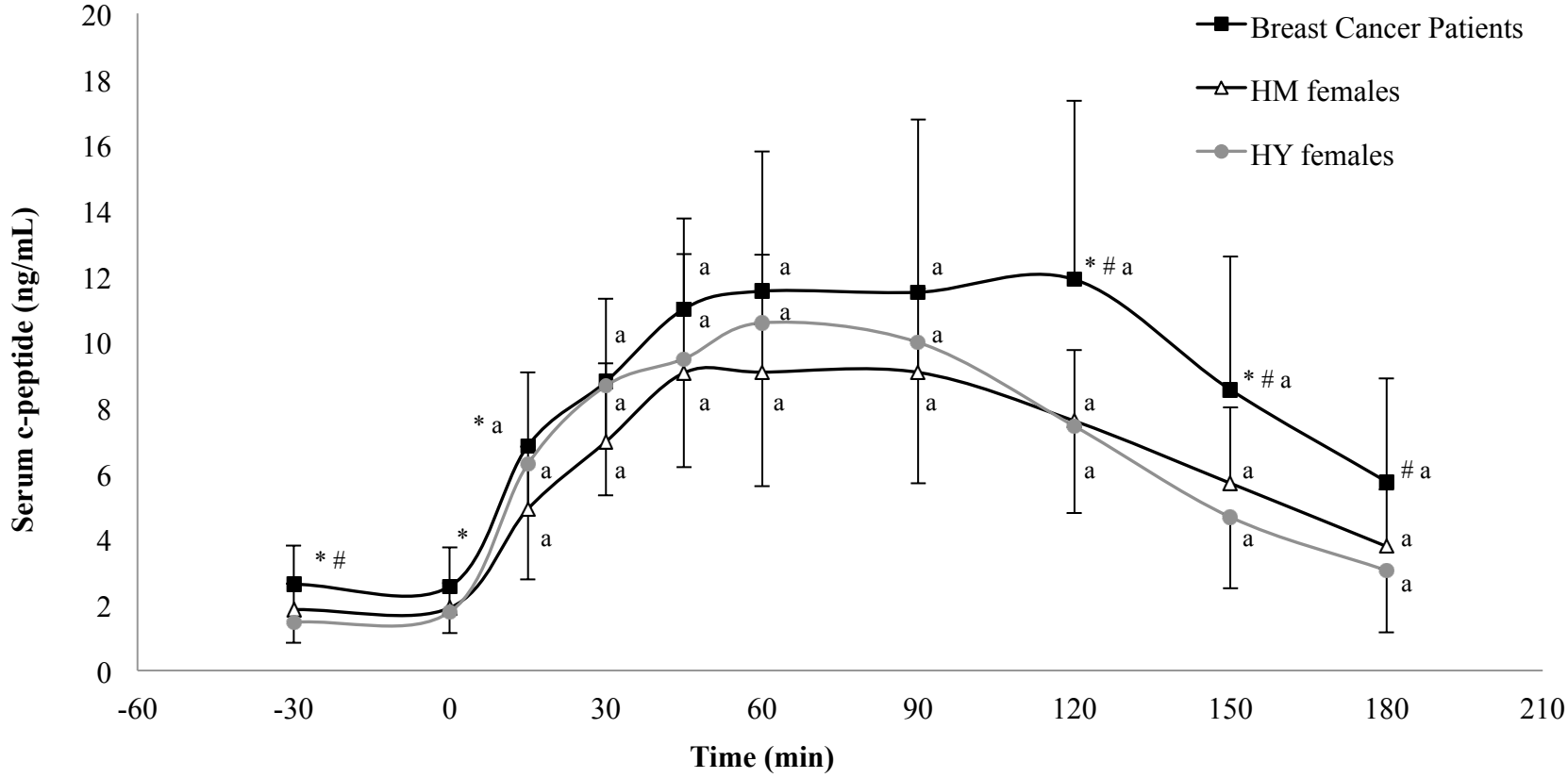


Figure 8 Serum c-peptide during an OGTT for all participant groups. Error bars represent standard deviation. * denotes significant difference from HM females; # denotes significant difference from HY females; [a] denotes significant difference from fasting values within group. Statistical significance was accepted at $p < 0.05$.

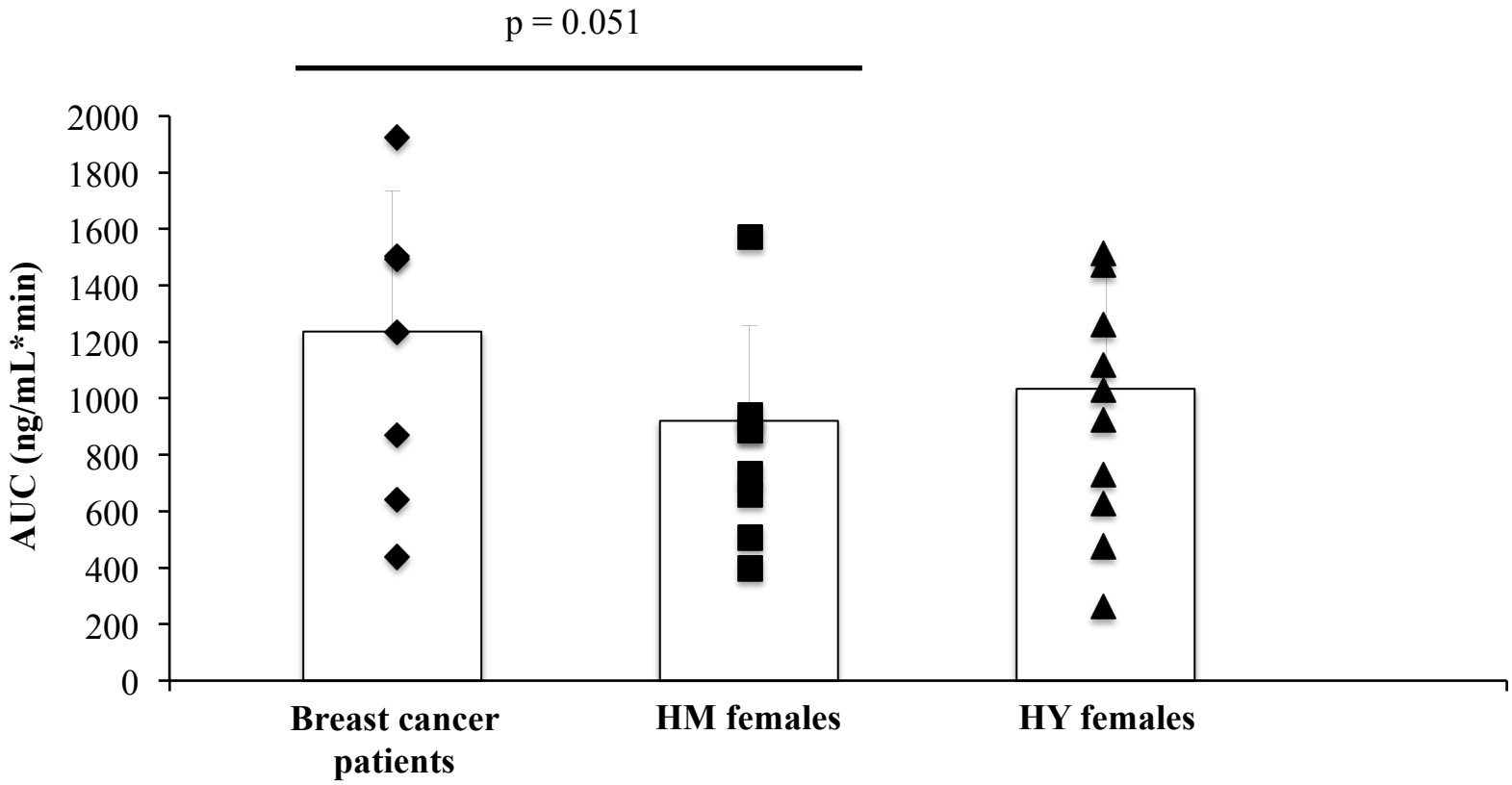


Figure 9 Serum c-peptide AUC during an OGTT across all participant groups. E Black diamonds, squares and triangles represent individual data points for breast cancer patients, HM females and HY females, respectively. No significant differences were observed between breast cancer patients, HM females or HY females.

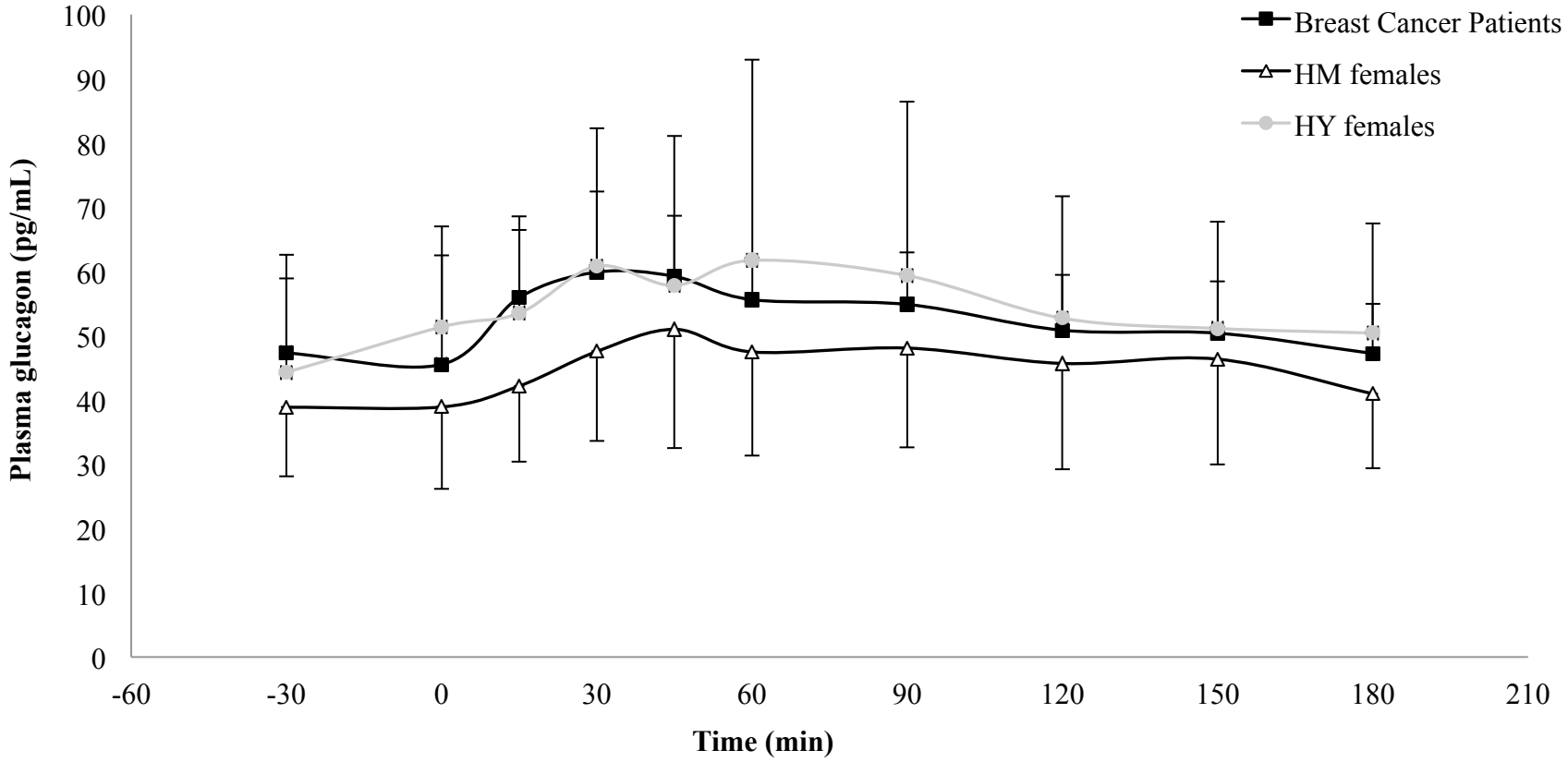


Figure 10 Plasma glucagon during an OGTT for all groups. Error bars represent standard deviation. No significant differences from fasting concentrations were observed within groups. When individual time-points were compared across groups, no significant differences were observed between breast cancer patients, HM females or HY females.

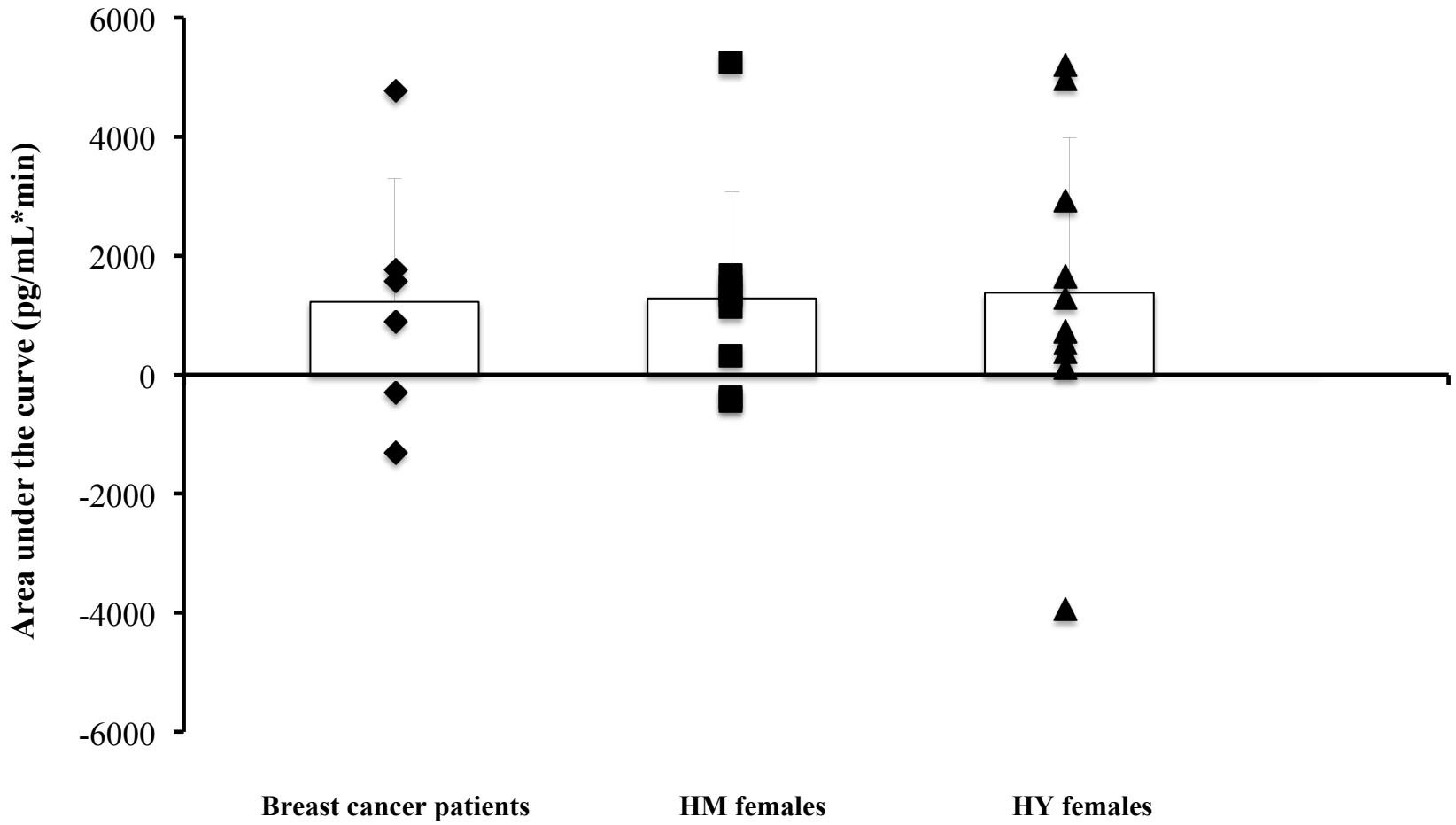


Figure 11 Plasma glucagon AUC during an OGTT across all participant groups. Black diamonds, squares and triangles represent individual data points for breast cancer patients, HM females and HY females, respectively. No significant differences were observed between breast cancer patients, HM females or HY females.

carbohydrate drink compared to fasting values for breast cancer patients, HM females and HY females, and remained elevated in all 3 groups until 180 min. When we compared the 3 groups at individual time-points throughout the OGTT we observed that c-peptide concentrations in breast cancer patients remained elevated relative to both non-malignant groups until 120 (breast cancer patients), 150 (HM group) and 180 (HY group) min, indicating that there was continued insulin secretion later in the OGTT protocol for breast cancer patients compared to the non-malignant groups. This is in line with the elevated insulin concentrations observed in patients compared to the non-malignant females. We observed no significant differences in serum c-peptide between HM and HY females. However, similar to the insulin results, we observed a trend towards greater c-peptide AUC in the breast cancer patients compared to HM females (1236 ± 499 ng/mL*min vs. 921 ± 336 ng/mL*min, $p = 0.051$; Figure 9).

Plasma glucagon was not elevated post-ingestion of carbohydrate compared to fasting values for any of the groups (Figure 10), and we did not observe any significant differences in plasma glucagon between any of the 3 groups at any time point during the OGTT. Further, there were no differences in plasma glucagon AUC between breast cancer patients (Figure 11; 1224 ± 2076 pg/mL), HM females (1286 ± 1786 pg/mL) and HY females (1374 ± 2612 pg/mL).

Glucagon-to-insulin ratios decreased 15 min post-ingestion of the carbohydrate drink relative to fasting values for the breast cancer patients (Table 6; 0.05 ± 0.03 vs. 0.38 ± 0.21, $p = 0.004$), HM females (0.05 ± 0.03 vs. 0.29 ± 0.27, $p = 0.026$) and HY females (0.07 ± 0.05 vs. 0.43 ± 0.24, $p < 0.001$), and this depression was maintained for all groups until 120 min. We observed no difference in the glucagon-to-insulin ratio between any groups at any time point during the OGTT, suggesting that the balance between gluconeogenesis and insulin action is

Table 6. Glucagon-to-insulin ratio during the OGTT and insulin sensitivity indices for all participant groups

	Breast cancer patients	HM females	HY females
Glucagon:Insulin			
Average Fasting	0.38 ± 0.21	0.29 ± 0.27	0.43 ± 0.24
15 min	0.05 ± 0.03[a]	0.05 ± 0.03[a]	0.07 ± 0.05[a]
30 min	0.04 ± 0.01[a]	0.04 ± 0.03[a]	0.05 ± 0.02[a]
45 min	0.03 ± 0.01[a]	0.03 ± 0.02[a]	0.04 ± 0.02[a]
60 min	0.04 ± 0.03[a]	0.04 ± 0.02[a]	0.05 ± 0.05[a]
90 min	0.05 ± 0.04[a]	0.03 ± 0.02[a]	0.06 ± 0.05[a]
120 min	0.06 ± 0.04[a]	0.05 ± 0.03[a]	0.08 ± 0.06[a]
150 min	0.16 ± 0.19	0.17 ± 0.19	0.21 ± 0.23
180 min	0.29 ± 0.31	0.22 ± 0.22	0.31 ± 0.23
Insulin sensitivity			
HOMA-IR	1.63 ± 1.53	1.91 ± 1.69	1.14 ± 0.60
Matsuda Index	7.98 ± 7.37	7.66 ± 4.83	7.68 ± 4.06

Data are presented as mean ± SD. * denotes significant difference from HM females; # denotes significant difference from HY females; [a] denotes difference from average fasting value within group. Statistical significance was accepted at $p < 0.05$.

similar between patients, HM females and HY females. As shown in Table 6, there appeared to be no difference in insulin sensitivity between the 3 groups, as assessed by HOMA-IR (breast cancer patients: 1.63 ± 1.53; HM females: 1.91 ± 1.69; HY females: 1.14 ± 0.60) and Matsuda Index (breast cancer patients: 7.98 ± 7.37; HM females: 7.66 ± 4.83; HY females: 7.68 ± 4.06).

11.5 Inflammation was not elevated in breast cancer patients compared to HM and HY females.

Although we did not observe a difference in serum CRP between breast cancer patients and HM females (Table 7; 7.84 ± 10.97 pg/mL in patients vs. 1.44 ± 1.50 pg/mL in HM females, $p = 0.193$), CRP in the HM females was elevated relative to HY females (0.13 ± 0.09 pg/mL, $p = 0.019$). Additionally, we observed a trend towards elevated CRP in the breast cancer patients relative to the HY females ($p = 0.051$). Despite that serum CRP concentrations tended to be greater in patients and HM females relative to HY females, HY females demonstrated similar values to breast cancer patients and HM females for TNF-α, IL-8, IL-4 and IL-10. There was no difference between breast cancer patients and HM females for serum TNF-α, IL-6, IL-8, IL-4 or IL-10. Serum IL-6 was elevated in HM females relative to HY females (10.84 ± 2.72 pg/mL vs. 7.98 ± 1.69 pg/mL respectively, $p = 0.017$; Table 7), and there was a trend towards elevated IL-6 in patients relative to HY females (10.04 ± 2.41 pg/mL vs. 7.98 ± 1.69 pg/mL respectively, $p = 0.055$).

It is important to note that TNF-α, IL-6, IL-4 and IL-10 were not detectable in all samples (Table 7). In particular, TNF-α was undetectable in 4/8 breast cancer patient samples, 6/8 HM females samples and 6/10 HY female samples. TNF-α results should therefore be

Table 7. Pro- and anti-inflammatory cytokine concentrations for all participant groups

	Breast cancer patients	HM females	HY females
Pro-inflammatory			
TNF-α (pg/mL)	8.94 ± 3.06 (n=4) range: 6.39 – 12.49	10.04 ± 3.97 (n=2) range: 7.23 – 12.85	7.63 ± 1.15 (n=4) range: 6.39 – 9.17
IL-6 (pg/mL)	10.04 ± 2.41 (n=7) range: 7.33 – 13.76	10.84 ± 2.72$^{\#}$ (n=7) range: 7.02 – 15.23	7.98 ± 1.69 (n=10) range: 5.22 – 9.93
IL-8 (pg/mL)	18.26 ± 4.40 (n=8) range: 13.48 – 26.14	16.49 ± 2.85 (n=8) range: 11.67 – 19.46	19.54 ± 9.79 (n=10) range: 11.67 – 23.51
CRP (mg/L)	7.84 ± 10.97 (n=8) range: 0.17 – 26.82	1.44 ± 1.50$^{\#}$ (n=7) range: 0.08 – 3.92	0.13 ± 0.09 (n=9) range: 0.05 – 0.31
Anti-inflammatory			
IL-4 (pg/mL)	8.81 ± 1.04 (n=7) range: 7.37 – 10.80	8.65 ± 1.16 (n=8) range: 6.56 – 10.29	8.19 ± 1.49 (n=8) range: 6.56 – 11.05
IL-10 (pg/mL)	8.83 ± 1.38 (n=8) range: 7.14 – 10.96	9.14 ± 2.36 (n=8) range: 6.73 – 12.95	7.98 ± 1.14 (n=9) range: 5.64 – 9.23

All cytokines were not detectable in every participant. Data are presented as mean ± SD for detected values, along with number of participants who had detectable values in brackets. The range of values that were detected is also provided. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

interpreted with caution, however the values we observed in the present study are similar to what has been observed in other studies on breast cancer patients (Appendix VII) and healthy overweight and lean females (Appendix VIII). Additionally, other studies that assessed serum cytokines using bead array analysis have reported undetectable TNF-α levels (Appendix IX).

11.6 Cardiovascular fitness and habitual physical activity was poor in breast cancer patients and HM females

Breast cancer patients and HM females achieved similar VO_{2peak} measurements during an incremental exercise test (27.33 ± 10.90 mL/kg/min vs. 30.61 ± 10.86 mL/kg/min respectively, $p = 0.207$; Table 8), however both patients and HM females' VO_{2peak} was significantly lower than HY females (43.41 ± 9.95 mL/kg/min). According to the American College of Sports Medicine (ACSM 2009), a 'good' VO_{2max} for females aged 40 – 49 yrs is 35 – 38 mL/kg/min (Appendix X). Breast cancer patients and HM females fall into the 'poor' category of aerobic fitness ($VO_{2max} \leq 31$ mL/kg/min), indicating that both groups are unfit. By comparison, HY females fall into the 'good' category of aerobic fitness for females aged 20 – 29 yrs, which is 40 – 43 mL/kg/min. This is not surprising since the eligibility criteria for females in this group included a normal BMI and engaging in recreational physical activity 3-5 times per week. Interestingly, 1RM for leg extension was greater in patients (59 ± 19 lbs; Table 8) and HM females (45 ± 8 lbs) compared to HY females (32 ± 9 lbs), suggesting that quadriceps strength was greater in heavier participants. We observed no differences in 1RM for forearm flexion or extension.

Table 8. Exercise assessment results (cardiovascular and strength) for all participant groups

	Breast cancer patients	HM females	HY females
Cardiovascular fitness			
VO_{2peak} (mL/kg/min)	27.33 ± 10.90#	30.61 ± 10.86#	43.41 ± 9.95
Strength (1RM)			
L Forearm flexion (lbs)	31 ± 5	32 ± 6	31 ± 8
R Forearm flexion (lbs)	31 ± 6	33 ± 6	32 ± 8
L Forearm extension (lbs)	33 ± 7	33 ± 8	28 ± 7
R Forearm extension (lbs)	33 ± 7	34 ± 7	29 ± 7
Leg extension (lbs)	59 ± 19#	45 ± 8#	32 ± 9

Data are presented as mean ± SD. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

Table 9. Comparison of subjective measures of habitual physical activity between participant groups

	Breast cancer patients	HM females	HY females
Baecke Questionnaire			
Work Index	2.03 ± 0.47	2.76 ± 1.00	2.29 ± 0.48
Sport Index	2.81 ± 1.16	3.06 ± 0.79	3.23 ± 0.79
Leisure Index	3.00 ± 0.63	2.92 ± 0.72	3.45 ± 0.55
CHAMPS Questionnaire			
All exercise-related activity			
kcal/d	489 ± 290	569 ± 272	504 ± 313
frequency/d	4 ± 1	3 ± 2	3 ± 1
Moderate-intensity activity			
kcal/d	299 ± 245	344 ± 210	420 ± 272
frequency/d	1 ± 1	1 ± 1	2 ± 1
Total Energy Expenditure (kcal/d)	2351 ± 490	2564 ± 429	2171 ± 570

Data are presented as mean ± SD. Each activity on the CHAMPS questionnaire was assigned a MET value. Kcal/d for all exercise related activity was calculated by summing energy expended on all activities with a MET value ≥ 2.0, for each participant; kcal/d for moderate-intensity activity was calculated by summing energy expended on activities with a MET value ≥ 3.0, for each participant. Frequency/d was calculated by dividing the number of times per week participants reported engaging in an activity by 7. * denotes significant difference from HM females; # denotes significant difference from HY females. Statistical significance was accepted at $p < 0.05$.

Using weight and physical activity level (PAL), we estimated that the average TEE for the breast cancer patients was 2351 ± 490 kcal/d. Although we observed a trend towards lower TEE in the breast cancer patients compared to HM females (2351 ± 490 kcal/d vs. 2564 ± 429 kcal/d, $p = 0.053$; Table 9), no significant differences in TEE existed between groups. We used 2 physical activity questionnaires to assess habitual physical activity due to the wide range in age of all participants in the study. Using the Baecke questionnaire, breast cancer patients achieved a work index of 2.03 ± 0.47 (Table 9), a sport index of 2.81 ± 1.16 and a leisure index of 3.00 ± 0.63. These indices represent physical activity level during work, sport and leisure time, with higher numbers (> 2.9, > 2.4 and > 3.1, respectively) indicating relatively high levels of physical activity and lower numbers (< 2.9, < 2.4 and < 3.1, respectively) indicating relatively low levels of physical activity (Baecke et al. 1982). We did not observe any differences in any of the Baecke indices between breast cancer patients, HM or HY females. Furthermore, the CHAMPS questionnaire revealed no differences between groups for estimates of all exercise-related energy expenditure and moderate-intensity energy expenditure. Although it appears that habitual physical activity levels are similar across all groups, our methods of assessment were crude and subjective; the questionnaires may not have been sensitive or objective enough tools to discern differences in activity levels, particularly when compared to the VO_{2peak} results.

11.7 Energy intake did not differ between breast cancer patients, HM females and HY females

The 3-day food record revealed that daily caloric intake was similar between breast cancer patients (1857 ± 422 kcal/d; Figure 8), HM females (1829 ± 431 kcal/d) and HY females (1830

± 409 kcal/d). The average macronutrient distribution was similar between groups and ranged from 47 – 54% for carbohydrate, 30 – 33% for fat and 16 – 19% for protein. Alcohol intake did not differ across groups. When we examined the Scored Patient-Generated Subjective Global Assessments, 3 patients indicated a self-reported decrease in weight in the 2 weeks prior to completing the food diary, and 5 patients reported experiencing symptoms of chemotherapy relating to loss of appetite (i.e. 'foods taste funny', 'mouth sores', 'feeling full quickly'). Despite these reports, the average nausea rating for patients during the 3 days they recorded their food intake was minimal (0.6 ± 1.0 out of 10), and appetite and tiredness rating did not differ between patients and control groups. We examined the 3-day variation in caloric intake, macronutrient distribution and appetite and tiredness ratings between breast cancer patients, HM females and HY females, to explore whether breast cancer patients demonstrated greater inconsistency in their diets. However variation across days was similar for all parameters for all groups.

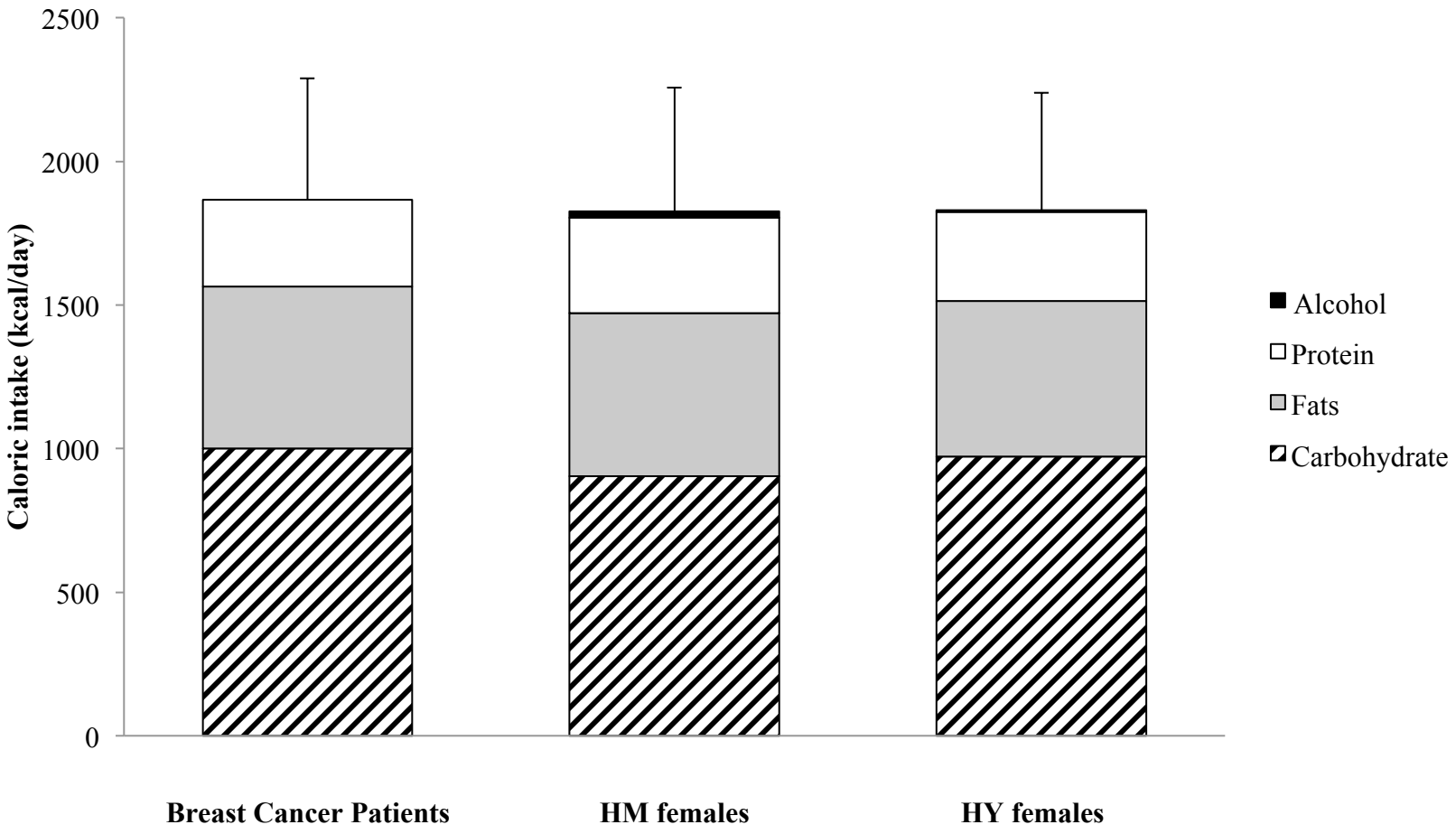


Figure 12 Daily caloric intake and macronutrient distribution for all participant groups. Error bars represent standard deviation. No significant differences were observed between breast cancer patients, HM females and HY females.

12.0 Discussion

In the present study, we successfully matched the breast cancer patients to HM females for age and BMI. Patients and HM females were overweight according to BMI and demonstrated similarities in other aspects of body composition, including a high percent body fat (>30%) and abdominal adiposity. Furthermore, both of these groups differed significantly from the HY females, who were younger, with a normal BMI. Thus, we were successful in establishing our proposed model (Figure 1): a comparison of metabolic parameters, exercise and nutrition a) between breast cancer patients and non-malignant females of similar age and body composition, and b) between non-malignant, young females with a normal BMI and the two aforementioned groups.

Both breast cancer patients and HM females exhibited abdominal obesity, which is an independent risk factor for secondary diseases like diabetes and cardiovascular disease. Overall, patients also presented with metabolic syndrome and dyslipidemia. During an OGTT, serum glucose concentrations were greater at early time points and took longer to reach <6.0mM in breast cancer patients compared to HM and HY females. Further, breast cancer patients demonstrated insulin and c-peptide concentrations that remained elevated throughout the entire OGTT compared with the HM and HY females, indicating continued insulin secretion compared to the non-malignant females. These metabolic characteristics are of particular concern given that breast cancer patients are expected to gain weight in the form of adipose tissue over the course of treatment. Further deleterious changes in body composition may worsen glucose handling, further increase insulin secretion and, ultimately, increase risk of secondary disease in survivorship. We observed no differences in markers of inflammation

between breast cancer patients and HM females, suggesting that the differences in glucose metabolism between these two groups may not be attributed to systemic inflammation.

Based on our results, neither reduced energy expenditure nor increased caloric intake explained the differences in glucose metabolism between breast cancer patients and HM females. Both groups demonstrated similar VO_{2peak} and strength measurements, and we observed no differences in habitual physical activity, as assessed by the Baecke and CHAMPS questionnaires. Caloric intake, macronutrient distribution, appetite, tiredness and variation between days did not differ between groups. It is possible that other factors, independent of physical activity level, nutrition or inflammation, are responsible for the difference in glucose metabolism between breast cancer patients and HM females.

12.1 Breast cancer patients on average presented with poor indicators of metabolic health including abdominal obesity, dyslipidemia and metabolic syndrome

Few studies have evaluated the metabolic health of patients at diagnosis, and to our knowledge, this is the first study to examine patients near the onset of treatment for symptoms of metabolic syndrome. Despite that not all patients could be described as having metabolic syndrome – only 2 patients were considered to have metabolic syndrome – it is apparent that this group was not healthy. On average, patients were dyslipidemic in 2 elements of the lipid profile and 6 out of 8 patients demonstrated abdominal obesity. Dyslipidemia and abdominal obesity are both strong, independent risk factors for cardiovascular disease and diabetes (Sattar 2003). HM females were also centrally obese, and as such, may have an increased risk of diabetes or cardiovascular disease. Since, on average, HM females did not present with

metabolic syndrome and presented with fewer indicators of metabolic disease compared to breast cancer patients, the risk of diabetes or cardiovascular disease is likely lower in HM females compared to patients.

Based on the similarities in body composition between our patient population and the patients examined in other studies, our patient characteristics reflect those of a typical population of recently diagnosed breast cancer patients. Patients generally presented with BMIs in the overweight category, as did patients in studies by Healy et al. (2010), Goodwin et al. (2009) and Yaw et al. (2010). The proportion of patients in our study with a waist circumference > 88cm (6/8 patients or 75%) and > 30% body fat (7/8 patients or 88%) was similar to the proportion of patients in a study by Amaral et al. (2010) who demonstrated that 62% of patients (or 44/71 patients) had a waist circumference of > 88cm, and 89% of patients (or 63/71 patients) had >30% body fat.

The present study has revealed that breast cancer patients have unhealthy metabolic characteristics that accompany their breast cancer diagnosis, and that they are at risk for secondary disease. Patients examined in the present study were Stage I and II; it is unknown whether later stage patients have worse body composition or display a higher prevalence of metabolic syndrome. Advanced stage breast cancer patients tend to exhibit muscle and fat wasting (Prado et al. 2009; Hortobagyi et al. 1983). Further, patients in the present study are likely representative of the general population of breast cancer patients based on the comparisons of the existing literature; however, larger scale studies are still needed. It is important to bear in mind that individuals with metabolic syndrome are 5-times more likely to develop diabetes compared to individuals without metabolic syndrome (Stern et al. 2004). Furthermore, the risk of experiencing or dying from a heart attack or stroke is 3-fold and 2-fold

greater respectively in individuals with metabolic syndrome (Alberti et al. 2006). Breast cancer patients tend to gain weight over the course of treatment, which contributes to the development of secondary disease in survivorship (Demark-Wahnefried et al. 2001; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 1993). The findings of the present study underscore the importance of exercise and nutrition interventions to prevent patients from developing further deleterious metabolic perturbations during treatment trajectory.

12.2 Breast cancer patients demonstrated impaired glucose metabolism compared to HM and HY females

We are the first group to conduct OGTTs on breast cancer patients near the onset of treatment and compare the patients to an age- and BMI-matched non-malignant control group. By conducting an OGTT we were able to reveal differences in glucose metabolism between breast cancer patients and non-malignant females that would not have been apparent by examination of fasting values only. Fasting serum glucose and fasting serum insulin were the same across all three groups, however during the OGTT, serum glucose concentrations took longer to return to <6.0 mM in patients compared to both groups of non-malignant females, and in HM females compared to HY females. Interestingly, the OGTT also revealed that fasting insulin secretion, measured by c-peptide, was elevated in breast cancer patients relative to both non-malignant groups. Moreover, breast cancer patients continued to secrete insulin during an OGTT for a longer period of time than HM and HY females, contributing to the relatively elevated insulin concentrations during the OGTT in breast cancer patients. Despite that there were no differences in the insulin sensitivity indices that were calculated amongst the

different groups, the results of the OGTT demonstrated that glucose metabolism in patients may be dysregulated.

Although patients in the present study were measured at the onset of chemotherapy, all patients had recently undergone surgical resection to remove the primary tumour mass. Despite tumour resection, it is possible that the impairments in glucose metabolism relative to non-malignant females that we observed resulted from lingering effects of the tumour. Previous studies have reported that impairments in glucose metabolism can persist in cancer patients post-tumour resection for 6 months (Litwin et al. 2008) to 1 year in some cases (Rex and Duckworth 1984), and as long as 3 years in other cases (Fogar et al. 1994). If this is the case, we expect that the breast cancer patients in the present study would have exhibited a greater degree of glucose dysregulation had we measured them prior to tumour resection. However, since no studies to date have compared glucose metabolism pre- and post-tumour resection in breast cancer patients, we cannot be certain.

12.3 Inflammation may not explain differences in glucose metabolism between breast cancer patients and HM females

No differences in serum cytokines were observed between breast cancer patients and HM females. IL-6 and CRP concentrations were significantly elevated in HM females compared to HY females. Breast cancer patients and HM females demonstrated similar unhealthy body composition, whereas HY females were normal weight according to BMI and demonstrated a low percentage body fat. Previous studies have shown that serum concentrations of IL-6 and CRP increase along with BMI and percentage body fat (Visser et al.

1999; Bastard et al. 2000). Therefore, high BMI and high percentage body fat in the breast cancer patients and HM females compared to the HY females is a possible explanation for the differences in IL-6 and CRP. No differences in other pro- or anti-inflammatory cytokines were apparent between the matched participants and the HY females. This is not altogether surprising, given that cytokines are distinct molecules with integrated, yet unique, roles in human physiology (Coppack 2007).

Previous studies have reported significantly increased systemic concentrations of pro-inflammatory cytokines, and significantly decreased systemic concentrations of anti-inflammatory cytokines, in breast cancer patients compared to non-malignant females (Dehqanzada et al. 2007; Kozlowski et al. 2003; Tessitore et al. 2000). There is a vast range in the magnitude of these differences: for example, Kozlowski et al. (2003) reported a 10-fold increase in IL-6, whereas Dehqanzada et al. (2007) reported a mere 1.5-fold decrease in IL-4, in patients versus non-malignant females.

Obesity is also associated with increased systemic inflammation (Weisberg et al. 2003), likely because adipose tissue has been observed to be a source of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8 (Xu et al. 2003). Further, TNF-α and IL-6 have been shown to interact with the insulin signaling pathway (Uysal et al. 1997; Plomgaard et al. 2005; Kim et al. 2004), preventing GLUT4 translocation to the sarcolemma and thereby contributing to reduced overall glucose uptake (Lee et al. 2003; Shoelson et al. 2003; Yuan et al. 2001). In light of the evidence supporting increased systemic inflammation in breast cancer patients, as well as the high prevalence of obesity in this population, we were led to hypothesize: firstly, that patients would demonstrate impairments in glucose metabolism compared to non-malignant females of

the same age and BMI; and secondly, that increased concentrations of pro-inflammatory cytokines may be responsible for this difference.

Elevated systemic pro-inflammatory cytokines have not been consistently reported in breast cancer patients: Sheen-Chen et al. (1997) observed average TNF-α concentrations of 1.47 ± 0.58 pg/mL in patients with invasive breast cancer, which is lower than the average TNF-α concentrations observed in non-malignant females in the present study (HM females: 10.04 ± 3.97 pg/mL; HY females: 7.63 ± 1.15 pg/mL). Other studies have reported similarly low concentrations for IL-6 and IL-8 in breast cancer (Pusztai et al. 2004; Dehqanzada et al. 2007). Additionally, high concentrations of pro-inflammatory cytokines have been observed in normal-weight non-malignant females (De Lorenzo et al. 2007; Ferguson et al. 2004). Kim et al. (2006) observed IL-8 concentrations as high as 120 pg/mL in a relatively young group of non-malignant females (age: 37 ± 6 yrs). Discrepancies regarding concentrations of pro-inflammatory cytokines in patients and non-malignant individuals may have arisen due to different methods of measurement (i.e. ELISA vs. bead array), staging of patients or timing of testing patients. Not all studies stratified their patients into clinical or tumour stage, which may have had an effect on systemic inflammation. As well, it was unclear whether these studies controlled for cycles of chemotherapy; concentrations of pro-inflammatory cytokines might have varied depending on whether patients were tested immediately prior to or immediately post a dose of chemotherapy.

In light of the ambiguous evidence regarding systemic inflammation, it is possible that some other factor(s) is(are) responsible for the difference in glucose metabolism between breast cancer patients and HM females in the present study. This unknown factor may be tumour-related since the present study observed no differences in other factors that might play

a role in impairments in glucose metabolism, such as unhealthy body composition, excess energy intake and reduced energy expenditure. Patients in the present study had previously undergone tumour resection, however previous studies have shown that impairments in glucose metabolism can persist for months following tumour resection in cancer patients (Fogar et al. 1994; Litwin et al. 2008).

12.3.1 Body composition, activity levels and nutrition may not explain differences in glucose metabolism between breast cancer patients and HM females

Body composition (adiposity and lean tissue mass) (Kohrt and Holloszy 1995; Shoelson et al. 2007), activity levels (Karelis et al. 2008) and nutrition (Park et al. 2011) have been well established as factors that can influence glucose metabolism. However, we observed no differences in any of these parameters between breast cancer patients and HM females. Excess adiposity contributes to glucose dysregulation in several ways. Increased rates of lipolysis allow fatty acids to accumulate in the liver, where they inhibit the suppression of glucose release (Shoelson et al. 2007), and in skeletal muscle where they interfere with insulin signaling and glucose uptake (Griffin et al. 1999; Perseghin et al. 1999). When NEFAs accumulate in muscle cells they compete with glucose as an energy substrate (Fanelli 1993). Additionally, NEFAs have been shown to stimulate PKCθ, an inhibitor of PI3 kinase (Griffin et al. 1999). PI3 kinase is an integral component of the insulin signaling pathways that leads to translocation of GLUT4 to the sarcolemma for glucose uptake. Skeletal muscle of individuals with greater adiposity also contains more fatty acid transporters (FAT/CD36), at the expense of GLUT4 transporters (Bonen 2004). This reduces the amount of glucose that can be taken up

by skeletal muscle. As such, individuals with greater adiposity often demonstrate glucose dysregulation.

Similarly, individuals with reduced lean tissue mass also tend to demonstrate glucose dysregulation. Lean mass, or more specifically skeletal muscle mass, is a major site for glucose uptake during an OGTT (Katz et al. 1983; Ferrannini et al. 1985). Kalyani et al. (2012) performed lower-limb CT scans and 2-hr OGTTs on 587 non-diabetic adults aged 26-95 years. Kalyani et al. (2012) found that at 2 hours during the OGTT, serum glucose was greater in participants with muscle cross-sectional area (CSA) in the lowest quartile compared to participants with muscle CSA in the highest quartile (Q1: 6.69 ± 1.72 mM vs. Q4: 5.81 ± 1.64 mM, $p < 0.001$). Muscle CSA was also inversely correlated with fasting glucose, fasting insulin, glucose and insulin AUC, and insulin resistance. Similarly, Srikanthan et al. (2010) observed a positive association between sarcopenia and insulin resistance in 9892 middle-aged non-obese and obese adults. We observed no difference in percent body fat or waist circumference in breast cancer patients compared to HM females in the present study. Although we did not measure lean tissue, our lean tissue estimates (based on skin-fold measures) in breast cancer patients were similar to the lean tissue estimates and measurements in HM females. Without DXA measurements for the breast cancer patient group we cannot be certain that no differences in lean tissue mass exist between patients and HM females. However, based on our results adiposity and lean tissue likely do not account for differences in glucose metabolism.

Active, normal weight females typically exhibit healthier glucose metabolism compared to sedentary females of similar body composition (Karelis et al. 2008), and this distinction has also been demonstrated in overweight and obesity. In an observational study,

Ekelund (2007) assessed physical activity energy expenditure (PAEE), % body fat, fasting glucose and 2-hr glucose in 217 overweight females at baseline and after 5.6 years. After 5.6 years, females who had increased their PAEE of their own volition had also exhibited improvements in fasting and 2-hr glucose despite no change in body fat or VO_{2max}. In the present study, both breast cancer patients and HM females achieved an average VO_{2peak} significantly lower than HY females; HY females achieved a 'good' cardiovascular fitness level for their age. The discrepancy in VO_{2peak} between breast cancer patients and HM females compared to the HY females alludes to an increased level of physical activity in the HY females. This increased physical activity is likely a contributing factor to the improvements in glucose metabolism in HY females compared to the 2 older groups. We observed no difference in objective measures of physical fitness (VO_{2peak}, strength) or subjective measures of habitual physical activity (Baecke and CHAMPS questionnaire scores) between patients and HM females. Activity levels therefore may not explain the observed differences in glucose metabolism between breast cancer patients and HM females.

Excess energy intake, or overnutrition, may contribute to glucose dysregulation (Mott et al. 1986). When an individual is in positive energy balance the body stores excess calories as adipose tissue. Excess adipose tissue is associated with increased lipolysis and glucose dysregulation. We did not observe any differences in daily caloric intake, macronutrient distribution, tiredness, appetite or variation in diet between groups when we examined the 3-day food records. Similar dietary intake across breast cancer patients, HM females and HY females does not support our hypotheses, and is not consistent with the literature regarding breast cancer and dietary habits. This suggests that overnutrition may not be a contributing factor to impaired glucose metabolism in the patient group.

It is important to bear in mind that daily caloric intakes reported by participants in the present study are likely underestimates. We estimated, using 3-day food records, that breast cancer patients and HM females consumed 1857 ± 422 kcal/d and 1829 ± 431 kcal/d respectively. Park et al. (2011) observed using doubly labeled water that healthy Japanese females of a similar age and BMI (mean age: 52.4 ± 9.4 years, BMI: 24.7 – 40.0 kg/m^2) consumed 2373 ± 363, which is roughly 30% greater than the estimates for breast cancer patients and HM females. Underestimates of daily caloric intake were observed in the HY control group as well: females of normal BMI in the study by Park et al. (2011) consumed roughly 22% more per day compared to HY females (2229 ± 297 kcal/d vs. 1830 ± 409 kcal/d, respectively). Studies validating 3, 7 and 14-day food records against doubly labeled water have observed under-reporting in food records by 32% (Prentice et al. 1986), 46% (Platte et al. 1995) and 58% (Trabulsi and Schoeller 2001). It is not surprising that participants in the present study under-reported because the act of completing a food diary can both consciously and unconsciously cause participants to alter their eating habits. Further, under-reporting is more prevalent among overweight and obese individuals. A more objective measurement of nutrition might have revealed differences in caloric intake or macronutrient distribution between groups.

Chemotherapy may have an effect on glucose metabolism, however investigation into this hypothesis is limited. The development of diabetes and glucose intolerance has been observed in patients with acute lymphocytic leukemia (Weiser et al. 2004) and colorectal cancer (Feng et al. 2012) who were treated with chemotherapy and who were non-diabetic prior to treatment. Only one study to date has examined the effects of chemotherapy on glucose metabolism in breast cancer patients: Hickish et al. (2001) observed that fasting blood

glucose was increased following the completion of all cycles of chemotherapy relative to diagnosis in non-diabetic patients. Patients in the studies by Weiser et al. (2004), Feng et al. (2012) and Hickish et al. (2001) had all received 5-fluorouracil-based chemotherapy, and it may be that this particular chemotherapy regimen is responsible for the observed impairments in glucose metabolism. In the present study, no patients received 5-fluorouracil-based chemotherapy. Furthermore, the study by Hickish et al. (2001) suggests that the effects of chemotherapy on glucose metabolism may only become apparent in later cycles, towards the completion of treatment, and patients in the present study had received only 1 or 2 cycles of chemotherapy at the time of assessment. Therefore chemotherapy likely is not responsible for the impairments in glucose metabolism observed in the present study. However, investigation into the effects of AC, ACT and TC chemotherapy on glucose metabolism is necessary before these regimens can be discounted as contributors to impairments in glucose metabolism.

The differences in glucose metabolism between breast cancer patients and HM females align with our hypotheses; however, in contrast to our hypotheses, pro-inflammatory cytokines may not have contributed to this glucose dysregulation. Systemic concentrations of pro-inflammatory cytokines were not elevated in patients compared to HM controls. It is possible that the tumour is still responsible for the impairment in glucose metabolism in breast cancer patients but not in the capacity we hypothesized. Glucose is the preferred energy substrate for tumour tissue (Reitzer et al. 1979) and it is well documented that tumours metabolize glucose at a high rate (Warburg 1956; Cori and Cori 1925; Gullino et al. 1967). Alterations to glucose metabolism in patients has been observed in various types of cancer (Norton et al. 1984; Carter et al. 1975; Yoshikawa et al. 2001; Muscogiuri et al. 2011). While the mechanism is unknown, it is possible that the tumour may reduce glucose uptake in the host to increase glucose

available for the tumour itself. Increased insulin secretion, as indicated by elevated c-peptide concentrations, might be a compensatory effect of reduced glucose uptake in peripheral tissues; if skeletal muscle glucose uptake is somehow inhibited, one might expect that more insulin would be secreted in an attempt to promote glucose uptake.

12.4 The tumour might be responsible for differences in glucose metabolism between breast cancer patients and HM females

Impaired glucose metabolism in cancer patients is not a new finding. In 1885, Freund first reported that patients with various types of cancer demonstrated alterations in glucose handling. These results were confirmed in 1888 by Tuffier and again in 1919 by Rohdenburg and Edwards, who used crude OGTTs to compare glucose handling between cancer patients and normal controls. In 1975, Carter et al. used a 100g 6-hr OGTT to compare breast cancer patients to normal controls, and reported results similar to results of the present study. Patients demonstrated normal fasting glucose and insulin concentrations that were not significantly different from normal controls, however serum glucose took longer to return to < 6.0 mM in patients compared to controls (roughly 240 min vs. 150 min, respectively). Patients and controls were of a similar age (mean[range]: 51[40 – 60] vs. 48[21-65]) however average BMI was in the normal range for the breast cancer patients and in the overweight range for the control group. Given that overweight individuals are more likely to experience impaired glucose metabolism compared to normal weight individuals (Shoelson et al. 2007), the difference in BMI between patients and controls in the Carter et al. (1975) study supports the tumour as a potential explanation for impaired glucose metabolism in these patients.

More recently, Yoshikawa (2001) used euglycemic hyperinsulinemic clamps to assess glucose dynamics in a group of stomach, colorectal and lung cancer patients and in a group of non-malignant controls. His results support previous studies that employed OGTTs: cancer patients demonstrated similar fasting and 120 min glucose and insulin concentrations compared to non-malignant controls, however overall glucose uptake in peripheral tissues was reduced in all cancer patients compared to non-malignant controls. Anabolic resistance and eventual wasting of skeletal muscle in stomach and lung cancer patients is a common characteristic of advanced disease, and may be a potential explanation for the impaired glucose metabolism observed in the study by Yoshikawa et al. (2001). Anabolic resistance is a state in which the rate of skeletal muscle protein synthesis is reduced, despite adequate supply of nutrients (Breen and Phillips 2011). However, since patients were weight stable and resting energy expenditure, measured using indirect calorimetry, was similar in patients and controls in the Yoshikawa et al. (2001) study, it is unlikely that anabolic resistance was responsible. Yoshikawa et al. (2001) postulated that reduced glucose uptake in cancer was tumour-driven. If this is the case, lingering impairments in glucose metabolism might persist in patients who have undergone tumour resection (Litwin et al. 2008).

Tumour size is positively correlated with degree of glucose impairment. Muscogiuri et al. (2011) used euglycemic hyperinsulinemic clamps to assess glucose dynamics in adrenal incidentaloma patients and non-malignant controls, and CT imaging to assess size of the tumour. Muscogiuri et al. (2011) observed that glucose uptake in peripheral tissues was lower in patients compared to non-malignant controls (25.4 ± 10.0 μmol/kg/min vs. 32.5 ± 13.3 μmol/kg/min, $p < 0.05$). There was no statistical difference in BMI, fat mass or lean tissue mass (measured using DXA) observed between patients and controls. Further, tumour mass

was inversely correlated with insulin sensitivity ($R = - 0.57$, $p = 0.04$). Muscogiuri et al. (2011) hypothesized that the relationship between tumour size and degree of glucose intolerance in cancer patients might be explained by the high glucose requirement of tumours. They suggested that the tumour might secrete some unknown factor to increase glucose availability or prevent glucose uptake by the host's peripheral tissues. If the hypothesis put forth by Muscogiuri et al. (2011) is correct, removal of the tumour should improve glucose metabolism in the patient, at least to a certain extent. It is unlikely that the patient's tissues will resume a metabolic state equivalent to that of a non-malignant individual immediately post-surgery.

Surgical removal of tumours is associated with improved glucose handling in cancer patients. Saruc et al. (2009) examined glucose tolerance of pancreatic cancer patients pre- and post-surgery: pre-tumour resection 12/18 patients exhibited impaired glucose tolerance and 6 weeks post-tumour resection only 5/18 patients exhibited impaired glucose tolerance. Similar improvements in glucose handling post-tumour resection were observed by Permert et al. (1993) and Ohtsuka et al. (2009). Other studies have suggested that while improvements in glucose handling following tumour-resection do occur, there is likely a lingering impairment in glucose handling that persists for an unidentified length of time. For example, Litwin et al. (2008) observed that the percentage of patients with normal glucose tolerance improved following tumour-resection for pancreatic cancer, and continued to improve for at least 6 months post-tumour resection (proportion of patients with normal glucose tolerance: 15% pre-tumour resection, 39% 2 months post-tumour resection and 45% 6 months post-tumour resection). Cancer patients in a study by Fogar et al. (1994) showed improvements in glucose tolerance following tumour resection, however these improvements were observed as early as 2

months in some patients and as late as 40 months (3.3 years) in other patients. Patients in the present study underwent tumour resection prior to the OGTT, however we still observed impairments in glucose metabolism at the time of evaluation that cannot be attributed to adiposity, physical activity levels or nutrition.

It is important to bear in mind that although Permert et al. (1993), Fogar et al. (1994), Litwin et al. (2008) and Midorikawa et al. (2001) observed improvements in glucose metabolism following tumour-resection, none of these studies compared glucose metabolism in patients to a non-malignant control group. Furthermore, none of these studies reported glucose or insulin values during the OGTTs conducted in their studies, nor did they present the glucose or insulin curves. They only reported whether the status of patients had changed from diabetic to impaired glucose tolerance or normal glucose tolerance, or from impaired glucose tolerance to normal glucose tolerance. Importantly, although glucose metabolism may improve following tumour resection in cancer patients, patients may still be impaired relative to non-malignant individuals. Post-tumour resection in the study by Midorikawa et al. (2001), steady-state plasma glucose (SSPG; a measure of glucose metabolism) improved to approximately 7.5 mM. However, Suzuki et al. (1999) has previously reported that SSPG in normal glucose tolerant, non-malignant females was 4.72 ± 0.28 mM. We have seen in the present study that although breast cancer patients are considered to have normal glucose tolerance (fasting glucose < 5.6 mM and 120 min glucose < 7.8 mM), glucose concentrations take longer to return to < 6.0 mM compared to HM females. Thus, although breast cancer patients in the present study were considered to have normal glucose tolerance, their glucose handling was impaired compared to HM females.

Impairments in glucose metabolism have been observed in numerous studies on various types of cancer (Norton et al. 1984; Rohdenburg et al. 1919; Freund 1885). Further, the impairments observed in these studies were similar to the impairments observed in the present study (i.e. normal fasting glucose but reduced glucose clearance compared to non-malignant controls during an OGTT) (Carter et al. 1975; Norton et al. 1984; Yoshikawa et al. 2001). Considering that the degree of glucose impairment is correlated with tumour size (Muscogiuri et al. 2011), and that surgical resection of the tumour may improve glucose metabolism to a certain degree (Saruc et al. 2009), it is possible that impairments in glucose metabolism observed in breast cancer patients in the present study may have been worse if we had collected the data pre-tumour resection. Therefore the pattern of glucose, insulin and c-peptide that we observed during the OGTT post-tumour resection may in fact be relative improvements compared to pre-tumour resection.

Cell culture work has been conducted to elucidate the mechanism responsible for impaired glucose metabolism in cancer. Isaksson et al. (2003) observed that skeletal muscle isolated from pancreatic patients was less responsive to physiological concentrations of insulin compared to non-malignant controls. Further, they observed that the impairments in insulin signaling were specific to glucose transport across the sarcolemma and PI3K activity. Decreased glycogen synthase activity was also observed in skeletal muscle of humans and rodents with pancreatic cancer compared to controls. Basso et al. (2002) attempted to identify the postulated factor secreted by tumours that may impair glucose metabolism in cancer patients by fractionating tumour-conditioned media into portions of separate molecular weights. They then observed that impairments in glucose metabolism in rat hepatocytes were induced by fractions of the media comprised of proteins with molecular weights as low as 10

kDa. Most cytokines have molecular weights greater than 20 – 30 kDa, suggesting that the factor responsible for impaired glucose metabolism in patient tissues may not be a pro-inflammatory cytokine. It is important to note that Isaksson et al. (2003) and Basso et al. (2002) both conducted their experiments using tissues from humans and animals with pancreatic cancer. Due to the location of the tumour, inherent alterations to glucose metabolism may be expected, and it difficult to know whether these alterations would be present in tissues from breast cancer patients. Nonetheless, further investigation into this unidentified protein is warranted, and should be the focus of future studies.

12.5 Methodological Limitations

This study was underpowered owing to its small sample size (power calculations are provided in Appendix XI). We observed many trends that might have reached significance had we had more participants (for example, differences in insulin and glucose AUC between groups). A larger sample size would have reduced the heterogeneity of all groups; 2 breast cancer patients had BMIs in the normal weight category and 1 patient demonstrated healthy glucose handling, which likely contributed to the large standard deviations in the present study. Other important limitations include lack of DXA scans in the breast cancer patient group, sub-optimal measurement tools for assessment of energy expenditure, and not controlling for lean tissue mass or physical activity levels when recruiting HM females. Based on our results, it appears as though there is no difference in lean tissue between patients and HM females, however since we have shown in *Methods (Section 10.5.1 Body Composition)* that lean tissue calculated from skinfold measurements tends to underestimate true lean tissue mass by roughly

2.5 kg, it is difficult to draw accurate conclusions. It is possible that this error may be more pronounced in obese individuals or in breast cancer patients. Additionally, skinfolds are less sensitive than DXA; differences in lean tissue or even fat mass between breast cancer patients and HM females might have been apparent if DXA was used. Therefore, differences in body composition between breast cancer patients and HM females potentially may have been masked by the relatively low precision of the skinfold measurements.

It is possible that the questionnaires are not sensitive enough to detect differences between patients and HM females. This is particularly supported by the lack of differences observed in habitual physical activity between HY females and the other two participant groups. Considering that the HY females had achieved significantly higher V_{O2peak} compared to breast cancer patients and HM females, one would expect that physical activity questionnaires would align with the differences in VO_{2peak}. Physical activity questionnaires rely on subjects' accuracy, perception and honesty in reporting type, time, duration and intensity of physical activity. Moreover, they often provide an overestimation of physical activity, particularly in subjects with high percent body fat (Timperio et al. 2003; Buchowski et al. 1999), like the patients and HM females in the present study. Hip-mounted accelerometers are objective tools for the measurement of habitual physical activity, and have been validated against the doubly labeled water technique for assessing energy expenditure (R = 0.73 (Bouten et al. 1996) and R = 0.80 (Westerterp and Bouten 1997)). HM females may have had greater habitual physical activity compared to patients, and this might contribute to healthier glucose metabolism. Accelerometers should be considered for use in future studies for assessing habitual physical activity.

TNF-α detection rate is low, particularly when bead array assays are employed (Appendix IX). Detection rates have been reported to be as low as 0% (Dehqanzada et al. 2007; Dabitao et al. 2011) and, what is particularly disconcerting, these low detection rates appear to be unpredictable. Undetectable TNF-α concentrations were reported in populations with widely varying characteristics, including the elderly (Van Munster et al. 2008), breast cancer patients (Dehqanzada et al. 2007; Pusztai et al. 2004), HIV-positive males (Dabitao et al. 2011), periodontitis patients (Andrukhov et al. 2011) as well as healthy females and males (Mussi et al. 1997). It has been suggested that certain therapeutic drugs and small molecules have the potential to induce the production of antibodies against TNF-α and produce undetectable serum concentrations (Tarrant 2010). Drugs with this purported ability have not been catalogued, and it remains possible that certain chemotherapy drugs possess this cytokine-neutralizing effect. Additionally, it has been suggested that certain unknown properties of anti-coagulants might enable the detection of serum cytokines. Acid citrate dextrose, citrate and lithium heparin are such anticoagulants that may enhance the detection of given cytokines (Tarrant 2010).

Cytokine concentrations were undetectable in several samples, and this may also be due to the short half-lives of serum cytokines, of TNF-α in particular. As outlined in our model (Figure 1), cytokines are secreted by adipose and tumour tissue and released into circulation. If cytokine decay rate is high, serum concentrations would constitute an underrepresentation of cytokine production in these tissues. The average half-life of a typical serum protein is 2-3 weeks (Schultze and Heremans 1966); serum albumin, for example, has a half-life of 20 days. Therefore, most serum proteins are quite stable and can be reliably measured, provided diurnal and menstrual cycles, or fed/fasted state is controlled for. Few studies have attempted to

characterize the kinetics of serum cytokines, and as such, no reference values for the half-lives of the cytokines measured in the present study exist. Further, the studies that have assessed cytokine half-lives have reported widely differing values. Oliver et al. (1993) observed that TNF-α had a half-life of 18.2 min, whereas Waage et al. (1989) reported a half-life of 70 ± 11 min. Both of these groups reported that other cytokines measured, including IL-6 and IL-8, peaked within 6 hours of an endotoxin challenge, after which time their serum concentrations declined rapidly. The authors alluded to a complex pattern of cytokine release and interaction in response to septic shock and it is possible that these effects can be extrapolated to chronic inflammation, however no studies have examined this. Nevertheless, it appears that the cytokine response to stress is transient and difficult to capture in serum.

12.6 Future Directions

The emphasis of future studies should be on evaluating glucose metabolism in breast cancer patients prior to any form of treatment, including surgery and neo-adjuvant chemotherapy. Cancer patients tend to present with impairments in glucose metabolism (Freund 1885). The degree of impairment is positively correlated with tumour size (Muscogiuri et al. 2011), and tumour-resection has been shown to restore glucose metabolism (Saruc et al. 2009). However, no studies to date have compared the glucose metabolism of breast cancer patients to non-malignant females using an OGTT both pre- and post-tumour resection. Resultantly, the degree to which removal of the primary tumour mass can restore glucose metabolism is unknown, as well as the length of time post-tumour resection necessary for glucose metabolism to return to the level of a non-malignant female.

Glucose metabolism should be evaluated: 1) prior to tumour resection; and 2) immediately post-tumour resection. Ideally, researchers should evaluate glucose metabolism at regular intervals prior to initiation of adjuvant chemotherapy or radiotherapy, to help establish whether lingering effects of the tumour on glucose clearance exist, as well as whether glucose handling returns to the pattern observed in non-malignant controls. However, it is becoming increasingly common to initiate chemotherapy as soon as possible after tumour resection, which may render this study design unfeasible.

Euglycemic hyperinsulinemic clamps would be the ideal technique to include in future studies because they would indicate whether abnormalities in glucose metabolism were due to reduced glucose uptake in peripheral tissues or impaired suppression of hepatic glucose release. Clamp procedures are long and it may be an excessive burden in a cancer patient population. For this reason, although OGTTs are inferior, they may be a more feasible choice in this type of study. In the present study we presented evidence against inflammation as an explanation for impaired glucose metabolism in breast cancer patients. These results should be verified, and a comparison of systemic cytokine levels pre- and post-tumour resection would be instrumental in determining whether the lack of differences in systemic cytokines between groups in the present study can be attributed to prior removal of the primary tumour mass.

Animal and cell culture studies should also be conducted to investigate some of the mechanisms for impaired glucose metabolism in cancer proposed in this thesis. One such study might evaluate cause and effect by inducing mammary tumours in lean mice or rats. If glucose handling worsens during an intraperitoneal glucose tolerance test after induction of the tumour, it would suggest the tumour is the cause of impairments in glucose metabolism (Ahrén and Andrén-Sandberg 1993). A secondary study might evaluate whether removal of tumours

in these animals would improve glucose metabolism (Norton et al. 1984). This series of studies should also be repeated in obese animals to observe whether, and to what degree, glucose metabolism is worse in obese animals with mammary tumours compared to lean animals. The study conducted by Isaksson et al. in 2003 on the response of skeletal muscle from a pancreatic cancer patient to insulin should be repeated in skeletal muscle from breast cancer patients. If the cells are less responsive than expected to physiological concentrations of insulin it will confirm that insulin signaling in skeletal muscle of a breast cancer patient is dysregulated. Another interesting experiment would be to co-culture human myocytes with breast tumour tissue and observe the growth of myocytes (Larsen and Crowe 2009). Impairments in myocyte growth, glucose uptake or responsiveness to insulin might indicate a direct effect of the tumour. It may also be possible to isolate the proteins secreted by the tumour that might elicit this response in the myocytes and determine their identity.

The present study identified breast cancer patients as abdominally obese, dyslipidemic and as having metabolic syndrome. Our study highlighted the importance of exercise and nutrition interventions during treatment to prevent anticipated gains in fat mass and counter the development of diabetes and cardiovascular disease in survivorship. Large-scale nutrition and exercise intervention studies will reveal whether impairments in glucose metabolism can be prevented during treatment trajectory and improve the quality of life of breast cancer survivors. Future studies might also examine local fat and muscle biopsies to evaluate local versus systemic inflammation.

13.0 Conclusions

The first major conclusion of the present study is that breast cancer patients are at increased risk for cardiovascular disease and diabetes at the onset of treatment. Patients presented on average with metabolic syndrome, and as such are at an increased risk of heart attack or stroke compared to individuals without metabolic syndrome (Alberti et al. 2006). Further, patients presented with abdominal obesity and dyslipidemia, which are independent risk factors for cardiovascular disease (Eckel and Krauss 1998) and diabetes (Chan et al. 1994). A large body of evidence suggests that breast cancer patients gain fat and lose muscle during treatment and in survivorship (Demark-Wahnefried et al. 2001; Demark-Wahnefried et al. 1997; Demark-Wahnefried et al. 1993). Fewer studies have examined patients at or near diagnosis, however these studies consistently suggest that patients are overweight or obese prior to beginning chemotherapy (Amaral et al. 2010; Yaw et al. 2010; Healy et al. 2010). The results of the present study lend additional support to this body of literature. Importantly, our study highlights the need for exercise and nutrition intervention studies to counter the further unhealthy changes in body composition that are anticipated during treatment.

The second major conclusion of the present study is that glucose metabolism is impaired in breast cancer patients compared to non-malignant females of the same age and BMI. During an OGTT, serum glucose in breast cancer patients took longer to return to < 6.0 mM compared to HM and HY females. Insulin secretion, as indicated by serum c-peptide concentrations, was both elevated at fasting and prolonged during the OGTT in patients compared to HM females. Although we hypothesized that this difference in glucose metabolism would be related to increased systemic inflammation as a result of the tumour, we observed no differences in circulating cytokines between patients and HM females. Body

composition, activity levels and caloric intake, which are known to influence glucose metabolism, did not differ between breast cancer patients and HM females. Alterations to glucose metabolism in cancer patients has been observed in other studies (Norton et al. 1984; Carter et al. 1975), and it is possible that an unknown factor secreted by the tumour is responsible. Although patients in the present study had previously undergone tumour resection, lingering effects of the tumour might persist (Litwin et al. 2008) and might reduce glucose uptake in peripheral tissues.

This is the first study to evaluate body composition, glucose metabolism, inflammation, exercise capacity and nutrition in a group of breast cancer patients near the onset of treatment. We are also the first to conduct OGTTs in this population and compare the patients to a non-malignant group of females of the same age and BMI. Our results therefore offer a unique perspective into the physiology and metabolic health of breast cancer patients. Patients tend to develop secondary diseases like cardiovascular disease and diabetes in survivorship (Patnaik et al. 2011; Lipscombe et al. 2006), potentially due to unhealthy body composition near diagnosis and weight gain during treatment. Based on our results, we have concluded that a separate tumour-related factor might be responsible for differences in glucose metabolism in breast cancer patients compared to HM females, and that lingering effects may be observed post-tumour resection. Further investigation into this factor may lead to a better understanding of its effects on glucose metabolism. This may translate into more effective exercise and nutrition interventions that are targeted to the specific physiology and metabolism of breast cancer patients.

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Appendix I Ethics clearance forms

UNIVERSITY OF WATERLOO
OFFICE OF RESEARCH ETHICS

Feedback on Ethics Review of Application to Conduct Research with Humans

All research involving human participants at the University of Waterloo must be carried out in compliance with the Office of Research Ethics Guidelines for Research with Human Participants and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.

ORE File #: 17079

Project Title: A nutrition and metabolic evaluation of healthy females

Faculty Supervisor: Marina Mourtzakis **Department/School:** Kinesiology
Student Investigator: Kirsten Bell **Department/School:** Kinesiology

---

The above research application has undergone ethics review through the Office of Research Ethics and received the following ethics review category:

☐ **Ethics Clearance**. The application is considered acceptable on ethical grounds and complies with ORE Guidelines for Research with Human Participants and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. No revisions are required.

CONDITIONS ASSOCIATED WITH ETHICS CLEARANCE:
1. Ethics clearance is valid for five years from the date ethics clearance is granted.
2. Projects must be conducted in accordance with the description in the application for which full ethics clearance is granted. All subsequent modifications to the protocol must receive prior ethics clearance through the Office of Research Ethics.
3. An annual progress report (ORE Form 105) must be submitted for ethics review for each year of an ongoing project.
4. Any events, procedures, or unanticipated problems that adversely affect participants must be reported to the ORE using ORE Form 106.

☐ The application is considered acceptable on ethical grounds and complies with ORE Guidelines for Research with Human Participants and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. * **Minor/editorial revisions are required** as outlined in a transmitted email. Revised materials must be provided for the ORE file.

☑ Acceptance of the application on ethical grounds is **conditional on revisions and/or additional information**. The following revisions and/or additional information must be provided for ethics review and are requested within **10 days**. A study may not begin until it receives ethics clearance.

- ☐ Information Letter was not provided and is required for ethics review.
- ☐ Information Letter provided is incomplete and requires revisions outlined in the email message.
- ☐ Information Letter and Consent Form were not provided and are required for ethics review.
- ☑ Information Letter and Consent Form provided are incomplete and require revisions outlined in the email message.
- ☐ Copy of interview/survey questions was not provided and is required for ethics review.
- ☑ Other revisions/information are required as outlined in the email message.

☐ Due to the level and/or number of questions and concerns raised during the ethics review process the application is considered not acceptable on ethical grounds at this time . Comments are summarized in the attached ethics review feedback. A new application is required.

[redacted]

Susan E. Sykes, Ph.D., C.Psych.
Director, Office of Research Ethics

26 May 11
Date

OR
Susanne Santi, M. Math
Senior Manager, Research Ethics

OR
Julie Joza, B.Sc.
Manager, Research Ethics

Marina Mourtzakis

From: ORE Ethics Application System <OHRAC@uwaterloo.ca>
Sent: Thursday, May 12, 2011 4:46 PM
To: Marina Mourtzakis
Cc: ke2bell@uwaterloo.ca
Subject: Ethics Clearance (ORE # 17079)

Dear Researcher:

The recommended revisions/additional information requested in the ethics review of your ORE application:

Title: A nutrition and metabolic evaluation of healthy females ORE #: 17079 Faculty Supervisor: Marina Mourtzakis (mmourtza@uwaterloo.ca) Student Investigator: Kirsten Bell (ke2bell@uwaterloo.ca)

have been reviewed and are considered acceptable. As a result, your application now has received full ethics clearance.

A signed copy of the Notification of Full Ethics Clearance will be sent to the Principal Investigator or Faculty Supervisor in the case of student research.

****

Note 1: This clearance is valid for five years from the date shown on the certificate and a new application must be submitted for on-going projects continuing beyond five years.

Note 2: This project must be conducted according to the application description and revised materials for which ethics clearance have been granted. All subsequent modifications to the protocol must receive prior ethics clearance through our office and must not begin until notification has been received.

Note 3: Researchers must submit a Progress Report on Continuing Human Research Projects (ORE Form 105) annually for all ongoing research projects. In addition, researchers must submit a Form 105 at the conclusion of the project if it continues for less than a year.

Note 4: Any events related to the procedures used that adversely affect participants must be reported immediately to the ORE using ORE Form 106.

Best wishes for success with this study.

Susan E. Sykes, Ph.D., C. Psych.
Director, Office of Research Ethics
519.888.4567 x 36005
ssykes@uwaterloo.ca

UNIVERSITY OF WATERLOO

OFFICE OF RESEARCH ETHICS

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

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Principal/Co-Investigator: Marina Mourtzakis	**Department:** Kinesiology
Principal/Co-Investigator: Rhona Hanning	**Department:** Health Studies & Gerontology
Principal/Co-Investigator: Mala Bahl	**Department:** Grand River Hospital
Student Investigator: Megan Bedbrook	**Department:** Kinesiology
Student Investigator: Vivienne Vance	**Department:** Health Studies & Gerontology
Collaborator: Caryl Russell	**Department:** Kinesiology

ORE File #: 14826

Project Title: Nutrition and metabolic evaluation of breast cancer patients

---

This certificate provides confirmation that the additional information/revised materials requested for the above project have been reviewed and are considered acceptable in accordance with the University of Waterloo's Guidelines for Research with Human Participants and the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. Thus, the provisional ethics clearance status has been removed and the project now has received full ethics clearance. This clearance is valid for a period of ***four years*** *from the date shown below and is subject to an* ***annual ethics review process*** *(see Note 2). A new application must be submitted for on-going projects continuing beyond four years.*

Note 1*: This project must be conducted in accordance with the description in the application and revised materials for which full ethics clearance has been granted. All subsequent modifications to the application must be submitted for prior ethics review using ORE Form 104 and must not be intiated until notification of ethics clearance has been received.*

Note 2*: All ongoing research projects must undergo annual ethics review. ORE Form 105 is used for this purpose and must be submitted by the Faculty Investigator/Supervisor (FI/FS) when requested by the ORE. Researchers must submit a Form 105 at the conclusion of the project if it continues for less than a year.*

Note 3*: FIs and FSs also are reminded that they must immediately report to the ORE (using ORE Form 106) any events related to the procedures used that adversely affected the participants and the steps taken to deal with these.*

ADDITIONAL COMMENTS:

[ ] No additional comments

[ ] Additional comments emailed on date shown below.

[redacted]

Susan E. Sykes, Ph.D., C.Psych.
Director, Office of Research Ethics
OR
Susanne Santi, M. Math
Manager, Research Ethics

Date July 8/08

CAMBRIDGE MEMORIAL HOSPITAL | GRAND RIVER HOSPITAL | St. Mary's GENERAL HOSPITAL

TRI-HOSPITAL RESEARCH ETHICS BOARD (THREB)

(A shared service for Cambridge Memorial Hospital, Grand River Hospital and St. Mary's General Hospital)
Grand River Hospital, 3570 King Street East, Kitchener, Ontario, N2G 1G3
Tel: (519) 749-4300 ext. 7460 Fax: (519) 894-8329

July 16th, 2008

CERTIFICATE OF APPROVAL
THREB # 08-187

Dr. Marina Mourtzakis
University of Waterloo
Department of Kinesiology
200 University Avenue West
Waterloo, ON
N2L 3G1

Dear Dr. Mourtzakis:

RE: THREB ID # 08-187: Nutrition and Metabolic Evaluation of Breast Cancer Patients

Study Identification Number: THREB # 08-187
1. Research Application dated May 13, 2008
2. Protocol (undated)
3. Information pamphlet
4. Consent Form (July 03, 2008)
5. Consent Form for future studies (July, 2008)
Study Approval Date: July 11, 2008
Anniversary Date for Renewal : June 4, 2009

The above study was reviewed at the Tri-Hospital Research Ethics Board (THREB) meeting of June 4, 2008 with a quorum present, and approved with conditions. The conditions have been met and you now have final THREB approval to begin the study. The study is to be reviewed in one year, before the next "Anniversary Date."

Approval is granted to conduct the research project in accordance with the above protocol. Requirements for ongoing approval include:

a. Annual progress reports for review and continued approval of the study by THREB;
b. Submission of any changes in the protocol, informed consent documents, information sheets, questionnaires, recruitment posters or other study materials;
c. Timely reporting of all local serious adverse events;
d. A final report, upon completion of the study, submitted within three months.

NOTE: The above Study Identification Number THREB # 08-187 has been assigned to your project. Please use this number on all future correspondence.

Sincerely

[redacted]

Michael D. Coughlin, Ph.D.
Chair, Tri-Hospital Research Ethics Board

Appendix II Health Status Questionnaire

HEALTH STATUS SCREENING FORM

STUDY: A nutritional and metabolic evaluation of healthy females

Participant ID: _____ **Date:_____**
Date of Birth:_____
Height:_____ **Weight:_____**
Has your weight been stable over the last 6 months (Y/N)?_____

SELF REPORT CHECKLIST:

Past Health Problems:

[ ] Epilepsy

[ ] Emphysema, Pneumonia, Asthma, Bronchitis

[ ] Heart Murmur

[ ] High Blood Pressure

[ ] Disease of the Arteries

[ ] Congenital Heart Disease

[ ] High Cholesterol

[ ] Heart Attack

[ ] Heart Operation

[ ] Cancer

[ ] Kidney and liver disease

[ ] Back Injuries

[ ] Diabetes (diet or insulin)

[ ] Varicose Veins

[ ] Heartburn

[ ] Enteritis/Colitis/Diverticulitis

[ ] Ulcers

[ ] Bleeding Disorders (including intestinal tract)

[ ] Diagnosis of any type of infectious disease

Please explain:_____
_____

Injuries:

Do you have any past or present injuries that would prevent you from participating in an exercise study (Y/N)?_____

If yes, what injuries have you sustained and when did they occur?_____
_____
_____

Present Health:

When was the date of your last menstrual cycle?_____

Are you in pre-menopause (Y/N)?_____

If **Yes**, are you pregnant (Y/N)? _____ Nursing (Y/N)? _____

Are you on any oral contraceptives (Y/N)?_____

If **No**, when was the last time you took oral contraceptives? _____

In the last 2 weeks, have you experienced:

[ ] Irregular Heart Beat

[ ] Fatigue

[ ] Chest Pain

[ ] Cough up blood

[ ] Shortness of Breath

[ ] Back Pain/Injury

[ ] Persistent Cough

[ ] Leg Pain/Injury

[ ] Wheezing (Asthma)

[ ] Dizziness

[ ] Pain; If yes, where?_____

Please list any additional current health problems and allergies:

_____
_____
_____

List medications you are currently taking:

_____
_____
_____

Current Physical Training Status:

I consider my physical training status to be: High [ ], Average [ ], Low [ ]

List the types of physical activities that you do on a regular basis:_____
_____

Smoking:

Never [ ] Ex-smoker [ ] Regular [ ] Average # cigarettes/day _____

Family Physician Contact Information:

Name: _____

Address: _____

_____

Phone: _____

_____

_____
Signature of Participant Date

Appendix III Screening and Recruitment of breast cancer patients and HM females

Breast cancer patients

Beginning February 2009, newly diagnosed breast cancer patients were screened for eligibility for the present study by oncologists and clinical trials staff at the GRRCC, and all screening sheets were forwarded to this lab. To date, 361 patients have been screened. Of this number, 8 were eligible and consented to participate in the study (recruitment rate = 2%). Six of the 8 consented patients continued on to complete the 16 week pilot study.

HM females

Recruitment for HM females began September 2011. I received 222 replies to the advertisement for the study. As shown in Figure 1, the majority of replies originated from the Graduate Studies Office (GSO) Mailing List. Online advertisements were the second most effective method of eliciting response from the community.

Seventy-five females were screened in the lab. The remaining 147 interested individuals were omitted from screening for a variety of reasons including, but not limited to:

1. time commitment or feasibility
2. disinterest upon learning details of the study
3. sex
4. no response

Twenty-eight females were a match for at least one patient. We identified as many as 8 matches for PT05, however we were only able to identify 1 match each for PT01 and PT08 (Figure 2).

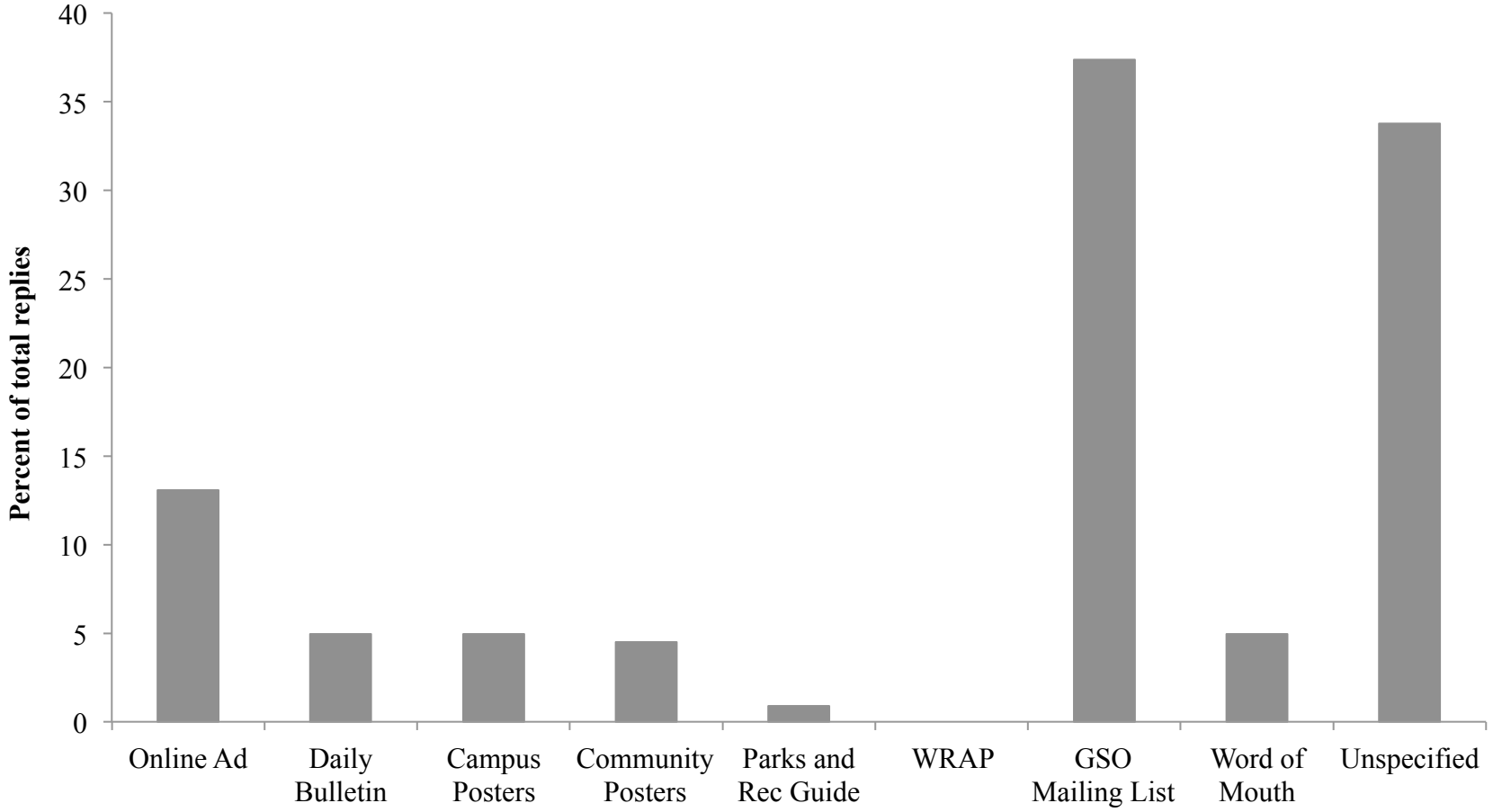

Figure 1 Percentage of replies originating from each recruitment strategy.

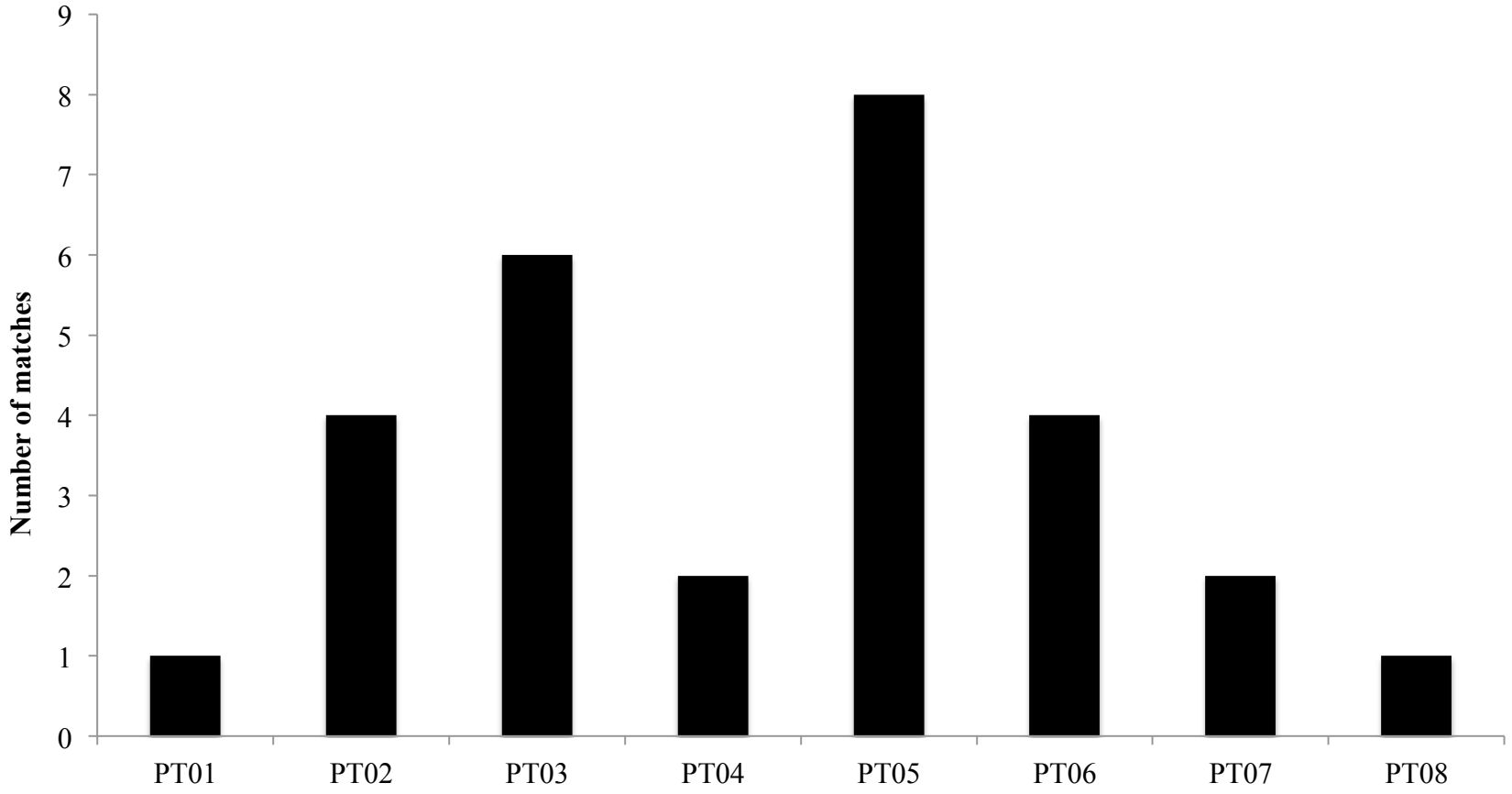

Figure 2 Total number of matches found for each breast cancer patient.

Appendix IV Comparison of lean tissue estimates (skinfolds) to lean tissue measurements (DXA)

Participant	Weight (kg)	% body fat (skinfolds)	Fat mass (kg)	Estimated lean tissue mass (kg)	DXA lean tissue mass (kg)	Difference
HM01	116.8	55.3	64.6	52.2	47.9	4.3
HM02	76.8	48.8	37.5	39.3	40.9	-1.6
HM03	66.0	26.9	17.8	48.6	43.6	5.0
HM04	84.8	52.8	44.8	39.9	43.6	-3.7
HM05	81.0	53.9	43.7	37.3	44.3	-7.0
HM06	72.7	48.6	35.3	37.3	42.4	-5.1
HM07	62.4	21.9	13.7	48.8	44.6	4.2
HM08	66.0	42.6	28.1	37.7	33.2	4.5
HY01	43.3	20.7	9.0	34.0	29.4	4.6
HY02	51.4	19.2	9.9	40.9	34.8	6.1
HY03	59.6	27.5	16.4	42.4	38.4	4.0
HY04	65.9	35.0	23.1	41.8	38.6	3.2
HY05	59.2	25.6	15.2	44.8	40.1	4.7
HY06	55.6	23.0	12.8	42.2	37.8	4.4
HY07	58.7	27.7	16.3	42.4	36.8	5.7
HY08	75.9	26.1	19.8	54.3	46.8	7.4
HY09	67.8	31.0	21.0	46.8	47.5	-0.7
HY10	52.1	27.8	14.5	37.4	36.1	1.2
PT01	57.4	19.9	11.4	46.0	40.6	5.4
			Average	42.8	40.4	2.5
			SD	5.5	5.0	4.1

Paired t-test for estimated lean tissue mass vs. DXA lean tissue mass: $p = 0.018$

Agreement between the skinfolds estimate of lean tissue mass and the DXA measurements was also assessed using a Bland-Altman plot (Figure 1) and the method described by Bland and Altman (1986). Bland-Altman plots are used to assess the validity of new equipment or techniques compared to a common practice method (Bland and Altman 1986). The plot is constructed by plotting the mean of the 2 measurements on the x-axis and the difference between these measurements on the y-axis. Lines are used to indicate the mean of the differences as well as ± 2 standard deviations (SD). If many data points that fall outside ± 2SD, it suggests that the agreement between the 2 measurement techniques is poor. Eighteen of 19 data points fell within ± 2SD of the mean when we compared the skinfolds estimate of lean tissue to the DXA measurements, suggesting that the skinfolds estimate may be similar to the DXA measurements. However, 13 of the 18 data points within ± 2SD were greater than the mean. This may indicate a tendency for the skinfolds estimate to overestimate lean tissue mass.

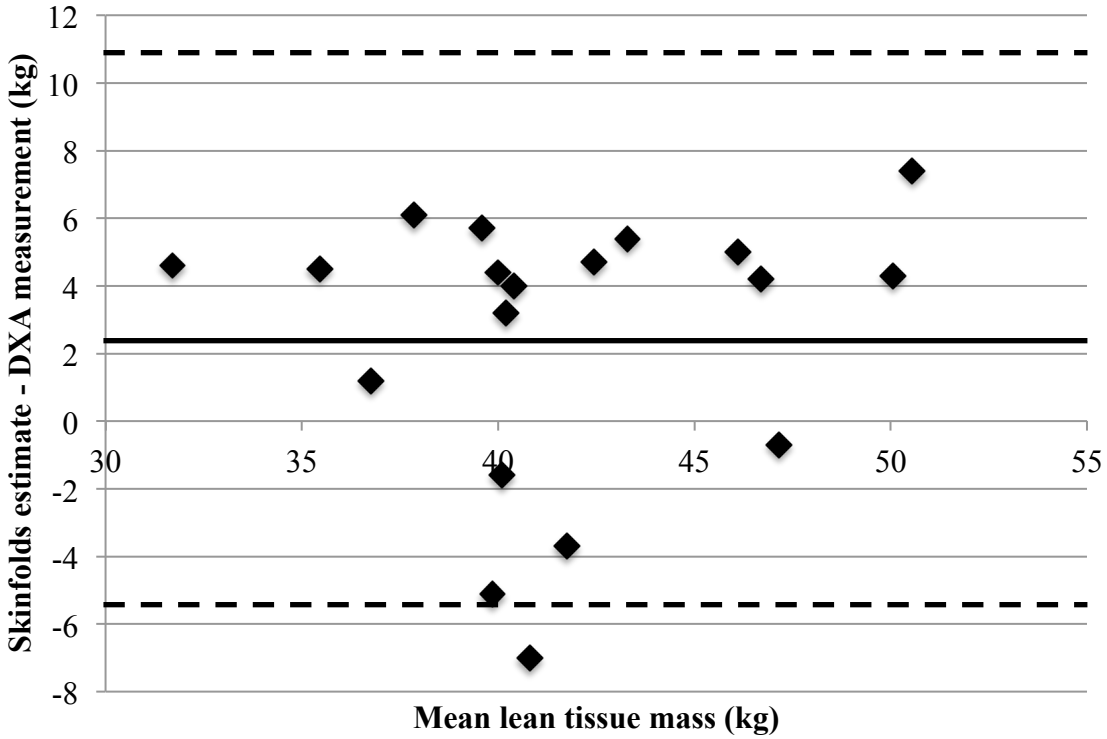

Figure 1 Bland-Altman plot to examine agreement between the estimate of lean tissue calculated from skinfolds and the measurements of lean tissue taken using DXA. Solid line represents the mean of the differences between the skinfolds and DXA; dashed lines represent ± 2 standard deviations from the mean.

Appendix V 3-day food record

3-DAY FOOD DIARY

Patient ID:_____

Phone Number: _____

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

Your Most Recent Treatment Date: _____ (DD/MM/YY)

University of Waterloo
Department of Kinesiology

Instructions for Recording Daily Food Intake

Your food diary will provide information for studying everything you eat and drink during a 3-day period. This information includes total calories, types of foods, amount of protein or carbohydrates or fats, as well as types of nutrients. It is important to **record ALL foods, beverages, and supplements** – whether it is a full course meal at home or a quick can of pop at work. Before you start recording your intake, please read the following instructions and the Sample Day.

The 3-Day Food Diary has a separate section for every day (see Day 1, Day 2, Day 3 on top each page). Each day is broken up into 6 eating times:

1. Morning meal
2. Mid-morning snack
3. Mid-day meal (lunch)
4. Afternoon snack
5. Evening meal
6. Evening snack

Please include the following information on your food record:

1. **FOOD AND BEVERAGE ITEMS**: Enter all foods and beverages consumed at the meal or snack time. Please record the specific type of food (for example: *WHOLE WHEAT* bread, *FROSTED FLAKES* cereal). In the same column, record all items added (examples: sugar, syrup, jam, butter, mayonnaise, gravy, milk, salt). For combination foods, please include detailed information on each item. For example: If you had a tuna sandwich, you would list the following detailed information: white bread, mayonnaise, carrot, solid white tuna, salt.

2. **DESCRIPTION OF ITEM**: For every food or beverage item listed, include the following (if applicable):
   - **Brand**: *MIRACLE WHIP* mayonnaise, *PIZZA HUT DEEP DISH* pizza, *OREO* cookie
   - **Type of flavour**: *BLUEBERRY* muffins, *STRAWBERRY* yogurt
   - **Method of cooking**: *FRIED, BAKED, BBQ'D*
   - **All relevant information on food label**: *LOW FAT* ranch salad dressing, *28% M.F.* cheddar cheese, *LEAN* Ground Beef

3. **UNIT OF MEASURE**: For every item consumed, enter the unit of measure you are using for this item. For example: enter the word "cup", "grams", "piece", "ounce", "teaspoon", or "tablespoon". Enter a unit of measure not only for the menu item, but for toppings or items added as well. Each entry must have its own unit of measure. Use measuring cups and spoons whenever possible.

4. **NUMBER OF UNITS**: In this area, record the number of units consumed. Include the amount of the food or beverage item and the amount of any topping or items added.

5. **Fill in the blanks on the bottom of each record.** Indicate the time of your meal or snack and where it was eaten (for example: at home, at a restaurant). If you did not eat a meal or snack, please place a check mark (✓) in the space provided on the bottom of the page, so that we do not think you forgot to record it.

6. **Daily check:** In the evening, go back over your entries to make sure you have included as much detail as possible for each item. At the end of each Day 1,2and 3, there are 2 questions that inquire about how the day you recorded compares to your normal diet. Don't forget to answer these questions.

All foods and beverages you consume are important (including water). Please be as accurate as possible. **Please do not change your normal eating habits** for the 3 days you are recording your food intake. Your **honesty is crucial to the success of this research study**.

Thank you for your participation in this study. Please look closely at the Sample Day before you start. **If you have any questions, please phone: Dr. Marina Mourtzakis (519-888-4567 x38459).**

Tips:

1. Carry your Food Diary with you & record your diet soon after you eat
2. Please record foods and beverages (including alcohol) consumed away from home (i.e. at the mall, at work, at a restaurant) these are just as important as those eaten at home
3. Don't forget to fill out the last 2 pages on supplements that you are currently taking and the nutritional questionnaire.

Sample Day

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units
Spaghetti with tomato/meat sauce:			
Pasta	Spaghetti, cooked	Cup	2
Tomato sauce	Hunt's canned sauce, roasted garlic flavor	Cup	1
Meat balls	Made with extra lean ground beef	Number (1 oz/ball)	5
Parmesan cheese, grated	Kraft, 30% Milk Fat (M.F.)	Tablespoon	1
Garlic Bread:			
Italian Bread	Toasted	Piece (large slice)	3
Garlic Butter		Teaspoon	3
Caesar salad:			
Lettuce	Romaine	Cup	1
Croutons	Safeway brand, garlic flavor	Tablespoon	2
Bacon bits	Simulated flavour, No Name Brand	Tablespoon	2
Caesar salad dressing	Kraft, Fat free	Tablespoon	2
Milk	1%	Cup	1
Tiramisu	Sarah Lee	Slice	1
Coffee	Black	Cup	1

Fill in blanks: Time of meal/snack: 6:00 pm **Location meal/snack was consumed:** at home

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

Day 1 – Morning Meal

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Mid-Morning Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Mid-day Meal (lunch)

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Mid-Afternoon Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Evening Meal

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 – Evening Snack

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 1 Meals

Compared to my normal diet, I ate:

☐ The same amount as I would usually eat

☐ More than I would usually eat

☐ Less than I would usually eat

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

0 1 2 3 4 5 6 7 8 9 10
Best Appetite Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10
Not nauseated Worst possible Nausea

0 1 2 3 4 5 6 7 8 9 10
Not Tired Worst possible Tiredness

Day 2 – Morning Meal

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 – Mid-Morning Snack

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 – Mid-day Meal (lunch)

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 – Mid-Afternoon Snack

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 – Evening Meal

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 – Evening Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 2 Meals

Compared to my normal diet, I ate:

☐ The same amount as I would usually eat

☐ More than I would usually eat

☐ Less than I would usually eat

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

0 1 2 3 4 5 6 7 8 9 10
Best Appetite Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10
Not nauseated Worst possible Nausea

0 1 2 3 4 5 6 7 8 9 10
Not Tired Worst possible Tiredness

Day 3 – Morning Meal

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 – Mid-Morning Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 – Mid-day Meal (lunch)

Food and Beverage Items	**DESCRIPTION OF ITEM**	**UNIT OF MEASURE**	**NO. OF UNITS**
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 – Mid-Afternoon Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 – Evening Meal

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 – Evening Snack

Food and Beverage Items	DESCRIPTION OF ITEM	UNIT OF MEASURE	NO. OF UNITS
Enter all foods and beverages consumed. For combination foods, please include detailed information on each item.	Include a detailed description of each food and drink item consumed including: - Brand name - Flavour - Method of cooking - All other relevant information on food/drink label	Enter unit of measure: for example: cup, grams, ounce, piece, teaspoon, tablespoon	Enter number of units

Fill in blanks: Time of meal/snack:_____ Location meal/snack was consumed:_____
Please CHECK (✓) if you did not eat or drink at this meal or snack time:_____

Day 3 Meals

Compared to my normal diet, I ate:

☐ The same amount as I would usually eat

☐ More than I would usually eat

☐ Less than I would usually eat

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

0 1 2 3 4 5 6 7 8 9 10
Best Appetite Worst Possible Appetite

0 1 2 3 4 5 6 7 8 9 10
Not nauseated Worst possible Nausea

0 1 2 3 4 5 6 7 8 9 10
Not Tired Worst possible Tiredness

VITAMINS, MINERALS & OTHER HERBAL / NUTRITIONAL SUPPLEMENTS

SUPPLEMENT BRAND (EXAMPLE: CENTRUM)	TYPE (EXAMPLE: 50+)	NUMBER OF PILLS PER DAY	DAYS PILLS WERE TAKEN (EXAMPLE: DAY 1,2 OR 3)

Patient Generated Subjective Global Assessment

Patient ID:

History (Boxes 1-4 are designed to be completed by the patient)

1. Weight

In **summary of my current and recent weight**:

My is height about _____ feet / inches tall
(or _____ cm)

My current weight is about _____ pounds
(or _____ kg)

One month ago I weighed about _____ pounds
(or _____ kg)

Six months ago I weighed about _____ pounds
(or _____ kg)

During the past two weeks my weight has:

- ☐ decreased
- ☐ not changed
- ☐ increased

2. Food Intake:

As compared to my normal intake, I would **rate the QUANTITY of my food intake during the past month** as:

- ☐ unchanged
- ☐ more than usual
- ☐ less than usual

I am now taking food of the following TYPE:

- ☐ *normal food*
- ☐ *normal food* but less than normal amount
- ☐ little solid food
- ☐ only liquids
- ☐ only nutritional supplements
- ☐ very little of anything
- ☐ only tube feedings or only nutrition by vein

3. Symptoms: I have had the following problems that **have kept me from eating enough** during the past two weeks (check all that apply):

- ☐ no problems eating
- ☐ no appetite, just did not feel like eating
- ☐ nausea ☐ vomiting
- ☐ constipation ☐ diarrhea
- ☐ mouth sores ☐ dry mouth
- ☐ things taste funny or have no taste
- ☐ smells bother me
- ☐ problems swallowing
- ☐ dental problems
- ☐ feel full quickly
- ☐ pain; where? _____
- ☐ other* _____

4. Activities and Function:

Over the **past month, I would generally rate my ACTIVITY** as:

- ☐ normal with no limitations
- ☐ not my normal self, but able to be up and about with fairly normal activities
- ☐ not feeling up to most things, but in bed or chair less than half the day
- ☐ able to do little activity and spend most of the day in bed or chair
- ☐ pretty much bedridden, rarely out of bed

Appendix VI TNF-α ELISA protocol

In addition to the cytometric bead array assay, serum TNF-α was measured using a Quantikine High Sensitivity ELISA kit (R&D Systems Inc.; Minneapolis, MN). Wells of the ELISA plate were pre-coated with mouse monoclonal antibody specific for human TNF-α. Following the addition of 50 μL of assay diluent to each well, 200 μL of standard, control or sample (undiluted) was added. The plate was then incubated at room temperature for 3 hour. During this incubation period, TNF-α in the standards, controls or samples became bound to the antibody coated microwells. Following the incubation, the plate was washed to remove all unbound substances and 200 μL of polyclonal antibody to TNF-α (conjugated to alkaline phosphatase) was added to each well and the plate was incubated for 2 hours. During the second incubation period, the immunoconjugate became bound to the TNF-α -antibody complex affixed to the wells. Following the second incubation, the plate was washed again and 50 μL of nicotinamide adenine dinucleotide phosphate (NADPH, an alkaline phosphatase substrate) was added to each well and the plate was incubated for 1 hour. During this incubation NADPH reacted with the alkaline phosphatase. 50 μL of an amplifier solution (INT-violet) was then added to each well so that a purple colour developed in response to the alkaline phosphatase activity. The plate was incubated for 30 minutes after which time 50 μL of 1M sulphuric acid was added to each well and the reaction was stopped. The absorbance of each well was read at 650 nm and 490 nm. Readings at 650 nm were subtracted from the readings at 490 nm to correct for optical imperfections in the microplate. The amount of colour following subtraction was directly proportional to the concentration of TNF-α in the sample.

Appendix VII Expected concentrations for serum cytokines in breast cancer patients

Table 1. Expected serum concentrations for TNF-α in breast cancer patients

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of assessment	Reference
1	1.47 ± 0.58	40	pre-surgery	ELISA	Sheen-Chen et al. 1997
2	1.49 ± 4.40 range: 1 – 27.9	55	age: 47 yrs range: 25 – 77 yrs stage I – III receiving chemotherapy	BD CBA	Pusztai et al. 2004
3	15.9 ± 0.9	20	stage IIIb post-surgery/pre-chemotherapy	ELISA	Berberoglu et al. 2004
4	21.6 ± 14.5	9	age: 50 yrs range: 38 – 62 yrs stage II	ELISA	Jablonska et al. 2001
5	37.1 ± 16.3	11	age: 50 yrs range: 38 – 62 yrs stage III/IV	ELISA	Jablonska et al. 2001

Table 2. Expected serum concentrations for IL-6 in breast cancer patients

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of assessment	Reference
1	7.55 ± 33.18 range: 0 – 244.2	55	age: 47 yrs range: 25 – 77 yrs stage I – III receiving chemotherapy	BD CBA	Pusztai et al. 2004
2	median: 18.7 range: 6.25 - 30	6	stage IIa	ELISA	Kozlowski et al. 2003
3	median: 19.3 rang: 7.8 – 36.4	23	stage IIb	ELISA	Kozlowski et al. 2003
4	median: 31.7 range: 6.25 - 100	45	age: 25 – 79 yrs	ELISA	Kozlowski et al. 2003
5	median: 40.9 range: 7.8 - 96	12	stage IIIa	ELISA	Kozlowski et al. 2003
6	median: 44.1 range: 8.4 - 100	4	stage IIIb	ELISA	Kozlowski et al. 2003

Table 3. Expected serum concentrations for IL-8 in breast cancer patients

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of assessment	Reference
1	median: 2.69 range: 1.36 – 26.34	22	age: 59.7 yrs 52% of patients on chemotherapy; terminated chemotherapy an average of 28.7 months before beginning study	Bead assay (Luminex plate)	Dehqanzada et al. (2007)
2	7.07 ± 5.21 range: 2.3 – 38.5	55	age: 47 yrs range: 25 – 77 yrs stage I – III receiving chemotherapy	BD CBA	Pusztai et al. 2004
3	median: 33.6 range: 7.8 – 60	6	stage IIa	ELISA	Kozlowski et al. 2003
4	median: 35.2 range: 7.8 – 76	23	stage IIb	ELISA	Kozlowski et al. 2003
5	median: 36.3 range: 7.8 – 60	12	stage IIIa	ELISA	Kozlowski et al. 2003
6	median: 40.1 range: 7.8 - 76	45	age: 25 – 79 yrs	ELISA	Kozlowski et al. 2003
7	median: 48.8 range: 8 – 75	4	stage IIIb	ELISA	Kozlowski et al. 2003

Table 4. Expected serum concentrations for IL-10 in breast cancer patients

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of assessment	Reference
1	median: 1.55 range: 0.67 – 6.30	22	age: 59.7 yrs 52% of patients on chemotherapy; terminated chemotherapy an average of 28.7 months before beginning study	Bead assay (Luminex plate)	Dehqanzada et al. (2007)
2	2.30 ± 5.23 range: 0 – 35.4	55	age: 47 yrs range: 25 – 77 yrs stage I – III receiving chemotherapy	BD CBA	Pusztai et al. 2004
3	median: 18.9 range: 5.6 – 29	6	stage IIa	ELISA	Kozlowski et al. 2003
4	median: 19.6 range: 6.4 – 32	23	stage IIb	ELISA	Kozlowski et al. 2003
5	median: 24.7 range: 5.6 – 37	45	age: 25 – 79 yrs	ELISA	Kozlowski et al. 2003
6	median: 26 range: 6.9 – 37	4	stage IIIb	ELISA	Kozlowski et al. 2003
7	median: 29.9 range: 6.2 – 35	12	stage IIIa	ELISA	Kozlowski et al. 2003

Table 5. Expected serum concentrations for IL-4 in breast cancer patients

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of assessment	Reference
1	median: 7.2 range: 5.64 – 157.74	22	age: 59.7 yrs 52% of patients on chemotherapy; terminated chemotherapy an average of 28.7 months before beginning study	Bead assay (Luminex plate)	Dehqanzada et al. (2007)

Table 6. Expected serum concentrations for CRP in breast cancer patients

Study no.	Mean ± SD (mg/L)	n	Population characteristics	Method of assessment	Reference
1	4.5 ± 5.8	14	age: 54.6 ± 8.3 years BMI: 30.1 ± 3.6 kg/m^2 Stage I-IIIa Had completed chemo at least 3 months prior to assessment	*Unspecified*	Campbell et al. 2012
2	10.1 ± 3.9	5	Stage IIa	RANDOX analyser	Ravishankaran and Karunanithi 2011
3	9.2 ± 4.6	8	Stage IIb	RANDOX analyser	Ravishankaran and Karunanithi 2011
4	13.8 ± 7.2	15	Stage IIIa	RANDOX analyser	Ravishankaran and Karunanithi 2011
5	12.8 ± 9.2	13	Stage IIIb	RANDOX analyser	Ravishankaran and Karunanithi 2011
6	21.5 ± 9.9	10	Stage IIIc	RANDOX analyser	Ravishankaran and Karunanithi 2011
7	37.5 ± 16.0	8	Stage IV	RANDOX analyser	Ravishankaran and Karunanithi 2011

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Appendix VIII Expected concentrations for serum cytokines in non-malignant females

Table 1. Expected serum concentrations for TNF-α in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	0.74 ± 0.09	8	age: 42 ± 5 yrs BMI: 20 kg/m^2 lean	ELISA	Bastard et al. 2000
2	1.48 ± 0.15	14	age: 45 ± 4 yrs BMI: 40 kg/m^2 obese non-diabetic	ELISA	Bastard et al. 2000
3	1.68 ± 0.12	7	age: 58 ± 2 yrs BMI: 37 kg/m^2 obese diabetic	ELISA	Bastard et al. 2000
4	1.93 ± 2.6 range: 0.2 – 14.9	58	age: 58 ± 6 yrs 46.7 ± 4.7 % body fat	ELISA	Ryan et al. 2004
5	2.25 ± 0.50	37	age: 57 ± 1 yrs 46.8 ± 0.8 % body fat	ELISA	Ryan et al. 2004
6	2.44 ± 0.55	37	age: 57 ± 1 yrs 43.5 ± 0.8 % body fat	ELISA	Ryan et al. 2004
7	median: 3.5 range: 3 – 25	10	median age: 28.5 yrs age range: 25 – 77 yrs 21 females, 9 males	ELISA	Mussi et al. 1997
8	3.5 ± 0.7	40	age: 35 ± 5 yrs BMI: 23.1 ± 1.5 kg/m^2	ELISA	Mafella et al. 2004
9	4.3 ± 2.1	48	age: 54 ± 8 yrs range: 48-63 yrs	ELISA	Cioffi et al. 2002
10	5.8 ± 1.5	67	age: 36 ± 5 yrs BMI: 37.6 ± 2.1 kg/m^2	ELISA	Mafella et al. 2004
11	20.10 ± 4.95	20	age: 20 – 35 yrs BMI: normal/lean	ELISA	De Lorenzo et al. 2007

Table 1 (Cont'd). Expected serum concentrations for TNF-α in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
12	42.77 ± 10.54	20	age: 20 – 35 yrs BMI: normal but 35% body fat	ELISA	De Lorenzo et al. 2007
13	56.37 ± 11.77	20	age: 20 – 35 yrs BMI: 25 – 35 kg/m^2	ELISA	De Lorenzo et al. 2007
14	34.3 ± 9.3	8	age: 27 yrs BMI: 26 kg/m^2	ELISA	Ferguson et al. 2004

Table 2. Expected serum concentrations for IL-6 in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	1.59 ± 0.13	37	age: 57 ± 1 yrs 43.5 ± 0.8 % body fat	ELISA	Ryan et al. 2004
2	1.7 ± 0.5	67	age: 36 ± 5 yrs BMI: 37.6 ± 2.1 kg/m^2	ELISA	Mafella et al. 2004
3	1.89 ± 0.12	37	age: 57 ± 1 yrs 46.8 ± 0.8 % body fat	ELISA	Ryan et al. 2004
4	2.29 ± 1.47 range: 0.45 – 10.00	58	age: 58 ± 6 yrs 46.7 ± 4.7 % body fat	ELISA	Ryan et al. 2004
5	3.9 ± 4.5	184	age: 59 ± 13 yrs BMI: normal	ELISA	Kip et al. 2004
6	4.2 ± 0.9	40	age: 35 ± 5 yrs BMI: 23.1 ± 1.5 kg/m^2	ELISA	Mafella et al. 2004
7	4.5 ± 4.9	269	age: 58 ± 11 yrs BMI: overweight	ELISA	Kip et al. 2004
8	4.7 ± 3.8	327	age: 57 ± 11 yrs BMI: obese	ELISA	Kip et al. 2004
9	median: 5.8 IQ range: 1.8 - 14	96	age: 38 ± 9 yrs BMI: 24 ± 4 kg/m^2	ELISA	Fernandez-Real et al. 2001
10	5.95 ± 2.28	20	age: 20 – 35 yrs BMI: normal/lean	ELISA	De Lorenzo et al. 2007
11	10.9 ± 4.1	48	age: 54 ± 8 yrs range: 48 – 63	ELISA	Cioffi et al. 2002
12	11.42 ± 1.77	20	age: 20 – 35 yrs BMI: normal but 35% body fat	ELISA	De Lorenzo et al. 2007
13	13.68 ± 2.29	20	age: 20 – 35 yrs BMI: 25 – 35 kg/m^2	ELISA	De Lorenzo et al. 2007

Table 3. Expected serum concentrations for IL-8 in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	0.9 ± 0.2	20	age: 20 – 35 yrs BMI: normal/lean	ELISA	De Lorenzo et al. 2007
2	2.0 ± 0.7	20	age: 20 – 35 yrs BMI: 25 – 35 kg/m^2	ELISA	De Lorenzo et al. 2007
3	2.3 ± 0.6	20	age: 20 – 35 yrs BMI: normal but 35% body fat	ELISA	De Lorenzo et al. 2007
4	median: 2.57 range: 0.8 – 6.63	13	age: 18 – 51 commercially available samples purchased (not collected by researchers)	Bead array (Luminex plate)	Dehqanzada et al. 2007
5	3.24 ± 1.07	24	age: 36 ± 8 yrs BMI: 23 ± 2 kg/m^2	ELISA	Straczkowski et al. 2002
6	3.6 ± 2.2*	50	age: 37 ± 6 yrs BMI: 21 kg/m^2	ELISA	Kim et al. 2006
7	4.31 ± 1.43	30	age: 40 ± 11 yrs BMI: 33 ± 3 kg/m^2	ELISA	Straczkowski et al. 2002
8	7.2 ± 0.3** range: < 5 - 9	15	age: 46 – 70 yrs 7 males, 8 females	Immulite Assay (Bead Assay)	Doganay et al. 2002
9	16.7 ± 22.4*	50	age: 37 ± 6 yrs BMI: 31 kg/m^2	ELISA	Kim et al. 2006
10	17.9 ± 0.4** range: 15 - 21	19	age: 57 – 72 yrs 9 males, 10 females diabetic	Immulite Assay (Bead Assay)	Doganay et al. 2002

* values as high as approximately 50 – 120 pg/mL were noted

** IL-1beta and IL-6 were below detection limit (5.0 pg/mL) in all samples; IL-8 was detectable in 87% of controls and 89% of diabetics; TNF-alpha detectable in 87% of diabetics

Table 4. Expected serum concentrations for IL-10 in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	median: 1.2* (25% - 0.7, 75% 2.9)	50	age: 36 ± 5 yrs BMI: 23.8 ± 1.2 kg/m^2	ELISA	Esposito et al. 2003
2	median: 1.66 range: 0.24 – 2.67	13	age: 18 – 51 commercially available samples purchased (not collected by researchers)	Bead array (Luminex plate)	Dehqanzada et al. 2007
3	median: 2.45* (25% - 1.1, 75% - 4.45)	50	age: 37 ± 5 BMI: 35.5 ± 2.9 kg/m^2	ELISA	Esposito et al. 2003
4	3.4 ± 0.8	20	age: 20 – 35 yrs BMI: normal/lean	ELISA	De Lorenzo et al. 2007
5	3.8 ± 1.3	20	age: 20 – 35 yrs BMI: normal but 35% body fat	ELISA	De Lorenzo et al. 2007
6	4.7 ± 1.9	20	age: 20 – 35 yrs BMI: 25 – 35 kg/m^2	ELISA	De Lorenzo et al. 2007
7	7.89 ± 2.4 median: 7.75 range: 3.85 – 12.5	27	age: 27 ± 5 yrs	ELISA	Sharma et al. 2007
8	13.5 ± 8.0	34	age: 32 ± 7 yrs range: 18-45	ELISA	Cioffi et al. 2002
9	16.0 ± 6.6	48	age: 54 ± 8 yrs range: 48 - 64	ELISA	Cioffi et al. 2002

* in this study, IL-10 was lower in individuals with non-obese and obese individuals who had metabolic syndrome

Table 5. Expected serum concentrations for IL-4 in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	3.4 ± 0.2	15	age: 55.1 yrs range: 31 – 62 yrs	ELISA	Famularo et al. 1990
2	8.26 95%CI: 5.48-12.45	17	age: 23 ± 2 yrs BMI: 21.06 ± 1.92 kg/m^2	ELISA	Corcos et al. 2004
3	median: 11.05 range: 6.68 – 15.38	13	age: 18 – 51 commercially available samples purchased (not collected by researchers)	Bead array (Luminex plate)	Dehqanzada et al. 2007
4	16.3 ± 4.5	34	age: 32 ± 7 yrs range: 18 – 45 yrs	ELISA	Cioffi et al. 2002
5	16.9 ± 5.6	48	age: 54 ± 8 yrs range: 48 – 63 yrs	ELISA	Cioffi et al. 2002
6	17.3 ± 7.2	36	age: 36 ± 9 yrs range: 17 – 55 yrs 4 males, 32 females	ELISA	Wong et al. 2000

Table 6. Expected serum concentrations for CRP in non-malignant females

Study no.	Mean ± SD (pg/mL)	n	Population characteristics	Method of Assessment	Reference
1	0.4 ± 0.1	20	age: 20 – 35 yrs BMI: normal/lean	ELISA	De Lorenzo et al. 2007
2	0.8 ± 0.3	20	age: 20 – 35 yrs BMI: normal but 35% body fat	ELISA	De Lorenzo et al. 2007
3	1.2 ± 0.3	40	age: 35 ± 5 yrs BMI: 24 ± 2 kg/m^2	ELISA	Mafella et al. 2004
4	2.2 ± 0.9	20	age: 21 – 35 yrs BMI: 25 – 35 kg/m^2	ELISA	De Lorenzo et al. 2007
5	3.4 ± 0.7	67	age: 37 ± 5 yrs BMI: 38 ± 2 kg/m^2	ELISA	Mafella et al. 2004
6	5.3 ± 1.0	37	age: 57 ± 1 yrs 43.5 ± 0.8 % body fat	Automated immunoanalyzer	Ryan et al. 2004
7	5.7 ± 0.6	37	age: 57 ± 1 yrs 46.8 ± 0.8 % body fat	Automated immunoanalyzer	Ryan et al. 2004
8	5.8 ± 11.0	184	age: 59 ± 13 yrs BMI: normal	High-sensitivity Hitachi analyzer	Kip et al. 2004
9	7.01 ± 5.74 range: 0.51 – 35.1	58	age: 58 ± 6 yrs 46.7 ± 4.7 % body fat	Automated immunoanalyzer	Ryan et al. 2004
10	8.9 ± 11.8	327	age: 57 ± 11 yrs BMI: obese	High-sensitivity Hitachi analyzer	Kip et al. 2004
11	9.7 ± 20.0	269	age: 58 ± 11 yrs BMI: overweight	High-sensitivity Hitachi analyzer	Kip et al. 2004

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Appendix IX TNF-α detectability in cytometric bead array analysis

Table 1. Studies that failed to detect TNF-α using cytometric bead array analysis

Study no.	Description	Reference
1	TNF-α detectable in 0% of breast cancer patients examined	Dehqanzada et al. 2007
2	TNF-α detectable in 46/53 (87%) of diabetic females	Doganay et al. 2002
3	TNF-α detectable in 10/55 (19%) of breast cancer patients	Pusztai et al. 2004
4	TNF-α detectable in 3/98 (4%) of elderly participants. Participants almost entirely females (average age: approx. 84yrs).	Van Munster et al. 2008
5	TNF-α not detected in any samples of males (n=12: 9 HIV-positive, 3 non-infected).	Dabitao et al. 2011
6	TNF-α detectable in 10/30 (33%) healthy controls	Mussi et al. 1997
7	TNF-α detectable in 8/25 (32%) of healthy controls and 32/37 (86.5%) periodontitis patients using BD CBA	Andrukhov et al. 2011

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Appendix X American College of Sports Medicine (ACSM) Cardiorespiratory fitness classification for women[1]

	VO_{2max} (mL/kg/min)				
Age (yrs)	Poor	Fair	Good	Excellent	Superior
20 – 29	≤ 35	36 – 39	40 – 43	44 – 49	50+
30 – 39	≤ 33	34 – 36	37 – 40	41 – 45	46+
40 – 49	≤ 31	32 – 34	35 – 38	39 – 44	45+
50 – 59	≤ 24	25 – 28	29 – 30	31 – 34	35+
60 – 69	≤ 25	26 – 28	29 – 31	32 – 35	36+
70 – 79	≤ 23	24 – 26	27 – 29	30 - 35	36+

[1] Adapted from Table 4.1 in Advanced Fitness Assessment and Exercise Prescription 5th ed. (Heyward, 2006)

Appendix XI Power Calculations

Table 1. Comparison of body composition between breast cancer patients (PT), HM females and HY females ($\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Variable	mean ± SD (n)			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
BMI (kg/m^2)	28.8±6.0 (8)	28.9±6.4 (8)	22.0±2.4 (10)	0.05	30 000	**0.843**	8	**0.843**	3
Weight (kg)	76.8±17.8 (8)	75.9±17.8 (8)	59.0±9.3 (10)	0.052	3100	0.751	10	0.751	10
Body fat (%)	41.3±10.9 (8)	43.9±12.7 (8)	26.4±4.7 (10)	0.09	145	**0.843**	8	**0.945**	6
Waist circumference (cm)	94.6±14.0 (8)	97.4±18.5 (8)	75.1±5.5 (10)	0.072	260	**0.977**	6	**0.977**	6

Table 2. Comparison of serum glucose during an OGTT between breast cancer patients (PT), HM females and HY females ($\alpha < 0.05$).

Time (min)	Serum glucose (mM, mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
-30	5.01 ± 1.28 (8)	4.44 ± 1.18 (8)	4.18 ± 1.16 (10)	0.209	38	0.278	34	0.073	320
0	5.22 ± 0.97 (8)	4.72 ± 0.91 (8)	4.40 ± 1.18 (10)	0.261	30	0.369	25	0.093	170
60	8.78 ± 4.10 (7)	6.58 ± 1.64 (8)	5.18 ± 2.72 (10)	0.372	17	0.625	12	0.284	33
120	7.64 ± 3.39 (7)	5.32 ± 1.26 (8)	4.85 ± 1.27 (10)	0.406	16	0.754	10	0.115	115
180	4.42 ± 1.13 (7)	3.98 ± 0.99 (8)	4.43 ± 1.88 (10)	0.156	47	0.050	350 000	0.110	125
AUC	340 ± 359 (7)	237 ± 179 (8)	175 ± 124 (10)	0.138	56	0.254	35	0.128	96

Table 3. Comparison of fasting serum glucose to serum glucose during an OGTT for breast cancer patients (t-test; $\alpha < 0.05$)

Time (min)	Serum glucose (mM, mean ±SD (n))		Power	n needed to achieve 80% power
	OGTT	Average fasting		
60	8.78 ± 4.10 (7)	5.84 ± 1.09 (8)	0.557	13
120	7.64 ± 3.39 (7)		0.252	32
180	4.42 ± 1.13 (7)		0.676	10

Table 4. Comparison of serum insulin during an OGTT between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Time (min)	Serum insulin (μIU/mL, mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
-30	6.32 ± 3.37 (8)	8.56 ± 5.58 (8)	5.44 ± 2.78 (10)	0.196	45	0.090	185	0.280	34
0	7.67 ± 5.32 (8)	8.42 ± 5.74 (8)	6.40 ± 3.61 (10)	0.064	410	0.097	160	0.140	85
60	88.38 ± 59.56 (7)	69.24 ± 42.72 (8)	81.49 ± 71.33 (10)	0.156	60	0.055	1400	0.073	325
120	83.24 ± 69.82 (7)	44.97 ± 20.27 (8)	39.00 ± 23.31 (10)	0.545	13	0.495	18	0.087	200
180	15.89 ± 11.04 (7)	14.38 ± 9.05 (8)	10.44 ± 6.77 (10)	0.066	350	0.272	35	0.164	68
AUC	10171±6193 (7)	7257±3530 (8)	7600±4537 (10)	0.268	24	0.149	70	0.053	2200

Table 5. Comparison of fasting serum insulin to serum insulin during an OGTT for breast cancer patients (t-test; $\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Time (min)	Serum insulin (μIU/mL, mean ±SD (n))		Power	n needed to achieve 80% power
	OGTT	Average fasting		
60	88.38 ± 59.56 (7)	6.99 ± 4.27 (8)	0.798	8
120	83.24 ± 69.82 (7)		**0.973**	5
180	15.89 ± 11.04 (7)		0.755	9

Table 6. Comparison of serum c-peptide during an OGTT between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Time (min)	Serum c-peptide (ng/mL, mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
-30	2.63 ± 1.16 (8)	1.86 ± 0.79 (8)	1.47 ± 0.63 (10)	0.549	13	0.723	11	0.197	52
0	2.56 ± 1.20 (8)	1.91 ± 0.76 (8)	1.78 ± 0.64 (10)	0.387	19	0.489	18	0.066	460
60	11.54 ± 4.26 (7)	9.08 ± 3.58 (8)	10.58 ± 4.97 (10)	0.279	23	0.068	370	0.101	145
120	11.90 ± 5.44 (7)	7.60 ± 2.16 (8)	7.44 ± 2.64 (10)	0.662	9	0.684	11	0.052	3600
180	5.75 ± 3.15 (7)	3.78 ± 1.72 (8)	3.04 ± 1.88 (10)	0.588	11	0.539	15	0.141	82
AUC	1236 ± 499 (7)	921 ± 336 (8)	1033 ± 393 (10)	0.389	16	0.139	77	0.093	170

Table 7. Comparison of fasting serum c-peptide to serum c-peptide during an OGTT for breast cancer patients (t-test; $\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Time (min)	Serum c-peptide (ng/mL, mean ±SD (n))		Power	n needed to achieve 80% power
	OGTT	Average fasting		
60	11.54 ± 4.26 (7)	2.60 ± 1.18 (8)	**1.000**	3
120	11.90 ± 5.44 (7)		**0.999**	4
180	5.75 ± 3.15 (7)		0.733	9

Table 8. Comparison of plasma glucagon during an OGTT between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Time (min)	Plasma glucagon (pg/mL, mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
-30	47.34 ± 15.23 (7)	38.91 ± 10.78 (8)	44.27 ± 14.63 (10)	0.325	20	0.069	350	0.144	80
0	45.53 ± 16.97 (7)	38.91 ± 12.77 (8)	51.38 ± 15.63 (10)	0.167	43	0.107	119	0.434	21
60	55.61 ± 6.56 (6)	47.48 ± 16.13 (8)	61.73 ± 31.21 (10)	0.291	18	0.094	138	0.233	42
120	50.86 ± 8.59 (6)	45.69 ± 16.47 (8)	52.73 ± 18.95 (10)	0.130	50	0.057	900	0.130	93
180	47.24 ± 7.68 (6)	40.97 ± 11.60 (8)	50.42 ± 17.08 (10)	0.252	21	0.074	252	0.268	36
AUC	1224 ± 2076 (6)	1286 ± 1786 (8)	1374 ± 2612 (10)	0.050	7700	0.052	3900	0.051	9800

Table 9. Comparison of fasting plasma glucagon to plasma glucagon during an OGTT for breast cancer patients (t-test; $\alpha < 0.05$)

Time (min)	Plasma glucagon (pg/mL, mean ±SD (n))		Power	n needed to achieve 80% power
	OGTT	Average fasting		
60	55.61 ± 6.56 (6)	46.44 ± 15.72 (7)	0.387	17
120	50.86 ± 8.59 (6)		0.101	99
180	47.24 ± 7.68 (6)		0.052	2500

Table 10. Comparison of fasting lipids between breast cancer patients (PT), HM controls and HY controls (t-test; $\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Variable	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
Total cholesterol (mM)	4.12 ± 0.75 (6)	4.47 ± 0.79 (8)	3.74 ± 0.37 (10)	0.149	40	0.279	28	**0.824**	9
HDL-C (mM)	1.08 ± 0.23 (6)	1.58 ± 0.42 (8)	1.31 ± 0.20 (10)	**0.899**	6	0.545	13	0.430	21
LDL-C (mM)	2.20 ± 0.36 (6)	2.47 ± 0.57 (8)	2.09 ± 0.45 (10)	0.270	20	0.079	210	0.325	28
NEFA (mM)	0.574 ± 0.220 (8)	0.708±0.288 (8)	0.462±0.214 (10)	0.277	27	0.185	56	0.528	16
Glycerol (μM)	115.7 ± 59.2 (8)	167.1 ± 95.3 (8)	55.7 ± 23.0 (10)	0.388	19	**0.843**	9	**0.979**	5
TAG (mM)	1.84 ± 1.17 (6)	0.93 ± 0.27 (8)	0.74 ± 0.33 (10)	0.664	8	0.786	8	0.241	40

Table 11. Comparison of glucagon:insulin ratio and insulin sensitivity between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Variable	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
Average Fasting	0.38 ± 0.21(7)	0.29 ± 0.27 (8)	0.43 ± 0.24 (10)	0.128	63	0.070	350	0.199	55
60 min	0.04 ± 0.03 (8)	0.04 ± 0.02 (8)	0.05±0.05 (10)	0.201	34	0.476	17	0.101	145
120 min	0.06 ± 0.04 (8)	0.05 ± 0.03 (8)	0.08±0.06 (10)	0.116	75	0.476	17	0.508	17
180 min	0.29 ± 0.31 (8)	0.22 ± 0.22 (8)	0.31±0.23 (10)	0.096	103	0.053	2500	0.128	96
HOMA-IR	1.63 ± 1.53 (7)	1.91 ± 1.69 (8)	1.14±0.60 (10)	0.072	260	0.154	67	0.333	28
Matsuda Index	7.98 ± 7.37 (7)	7.66 ± 4.83 (8)	9.59 ± 5.53 (10)	0.052	2900	0.076	260	0.115	115

Table 12. Comparison of cytokines between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Cytokine	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
TNF-α (pg/mL)	7.15±4.80 (5)	10.04±3.97 (2)	7.63±1.15 (4)	0.120	17	0.052	2000	0.191	12
IL-6 (pg/mL)	10.04±2.41 (7)	10.84±2.72 (7)	7.98±1.69 (10)	0.111	80	0.498	16	0.774	9
IL-8 (pg/mL)	18.26±4.40 (8)	16.49±2.85 (8)	19.54±9.79 (10)	0.409	18	0.065	475	0.139	85
CRP (mg/L)	7.84 ± 10.97(8)	2.37 ± 2.97(8)	0.21 ± 0.29(10)	0.516	14	0.765	10	0.746	10
IL-4 (pg/mL)	8.81±1.04 (7)	8.65±1.16 (8)	8.19±1.49 (8)	0.062	375	0.125	82	0.101	130
IL-10 (pg/mL)	8.83±1.38 (8)	9.14±2.36 (8)	7.98±1.14 (9)	0.067	330	0.276	33	0.201	50

Table 13. Comparison of exercise between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Variable	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
VO_{2peak} (mL/kg/min)	27.33±10.90 (6)	30.61±10.86 (8)	43.41±9.95 (10)	0.092	90	**0.825**	8	0.717	11
L Forearm flexion (lbs)	31±5 (7)	32±6 (8)	31±8 (10)	0.066	290	0.062	575	0.063	575
R Forearm flexion (lbs)	31±6 (7)	33±6 (8)	32±8 (10)	0.116	75	0.097	145	0.059	800
L Forearm extension (lbs)	33±7 (7)	33±8 (8)	28±7 (10)	0.059	500	0.274	32	0.294	32
R Forearm extension (lbs)	33±7 (7)	34±7 (8)	29±7 (10)	0.062	400	0.192	50	0.294	32
Leg extension (lbs)	59±19 (7)	45±8 (8)	32±9 (10)	0.493	13	**0.927**	6	**0.815**	9

Table 14. Comparison of habitual physical activity between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$). Bolded numbers indicate analyses where power was 80%.

Variable	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
Work Index	2.03±0.47 (8)	2.76±1.00 (8)	2.29±0.48 (10)	0.658	11	0.189	55	0.237	41
Sport Index	2.81±1.16 (8)	3.06±0.79 (8)	3.23±0.79 (10)	0.094	128	0.133	90	0.071	350
Leisure Index	3.00±0.63 (8)	2.92±0.72 (8)	3.45±0.55 (10)	**0.846**	8	0.318	29	0.417	22
All activity (kcal/wk)	3426±2029 (8)	4073±1799 (8)	3527±2193 (10)	0.133	70	0.051	6300	0.084	215
All activity (f/wk)	21±19 (8)	21±10 (8)	20±9 (10)	0.053	1800	0.052	3500	0.052	3500
Mod-int activity (kcal/wk)	1828±1753 (8)	2527±1421 (8)	2940±1903 (10)	0.188	45	0.232	45	0.081	235
Mod-int activity (f/wk)	8±11 (8)	8±5 (8)	11±6 (10)	0.064	390	0.115	115	0.168	65
TEE (kcal/d)	2351±490 (8)	2564±429 (8)	2171±570 (10)	0.213	37	0.104	135	0.344	27

Table 15. Comparison of nutrition between breast cancer patients (PT), HM females and HY females (t-test; $\alpha < 0.05$)

Variable (kcal/d)	(mean ± SD (n))			PT vs. HM (paired t-test)		PT vs. HY (t-test)		HM vs. HY (t-test)	
	PT	HM	HY	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power	Power	n needed to achieve 80% power
Caloric intake	1857±422 (7)	1829±431 (8)	1830±409 (9)	0.053	1850	0.052	3800	0.050	2 900 000
Carbohydrate intake	1000±289 (7)	903±172 (8)	973±283 (9)	0.179	39	0.054	1700	0.094	156
Protein intake	302±62 (7)	332±105 (8)	311±73 (9)	0.134	58	0.057	870	0.074	290
Fat intake	565±157 (7)	568±195 (8)	541±136 (9)	0.05	27 000	0.062	545	0.061	600
Alcohol intake	0±0 (7)	24±55 (8)	6±18 (9)	0.567	11	0.234	37	0.168	61

Appendix XII Individual patient data

Table 1. Individual patient data for age and body composition measurements

Patient	Age (years)	Weight (kg)	BMI (kg/m^2)	% body fat	Waist circumference (cm)
1	44	114.5	40.4	55.3	114.7
2	35	82.2	29.8	49.7	96.0
3	43	69.0	24.7	47.8	90.8
4	47	86.3	33.3	42.9	114.6
5	31	71.8	27.0	41.3	93.0
6	46	63.2	25.8	40.4	81.7
7	55	57.4	20.6	19.9	75.8
8	63	70.4	28.9	33.3	90.0
Mean	46	76.8	28.8	41.3	94.6
SD	10	17.8	6.0	10.9	14.0

Table 2. Individual patient data for selected OGTT parameters

Patient	Fasting serum glucose (mM)	2-hr glucose (mM)	Glucose AUC (mM*min)	Fasting serum insulin (μIU/mg)	Insulin AUC (μIU/mg*min)	Fasting serum c-peptide (ng/mL)	C-peptide AUC (ng/mL*min)
1	5.4	11.5	745	8.0	10951	3.3	1308
2	6.0	8.2	594	11.2	13174	2.6	1551
3	5.2	n/a	n/a	3.8	n/a	1.6	n/a
4	7.2	11.9	731	14.4	15612	5.0	1562
5	4.1	6.0	315	6.1	5608	2.5	966
6	3.8	4.0	95	2.4	3719	1.5	750
7	4.7	3.3	223	2.4	3103	1.5	560
8	4.6	8.7	540	7.5	19027	2.6	1956
Mean	5.1	7.6	400	8.7	10171	2.6	1236
SD	1.1	3.4	359	4.3	6193	1.2	499

Table 3. Individual patient data for select fasting lipids, cardiovascular fitness and energy intake

Patient	Fasting TAG (mM)	Fasting HDL-cholesterol (mM)	VO_{2peak}	Energy intake (kcal/d)
1	1.7	1.1	15.9	1937
2	n/a	n/a	23.8	n/a
3	n/a	n/a	21.1	1933
4	4.2	0.9	n/a	1837
5	1.3	1.2	23.7	1098
6	1.7	0.8	33.0	1936
7	1.2	1.1	46.5	2402
8	0.9	1.4	n/a	n/a
Mean	1.8	1.1	27.3	1857
SD	1.2	0.2	10.9	422

Table 4. Individual patient data for serum cytokines. * indicates that a value is an outlier, and was excluded from the mean calculation.

Patient	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	IL-4 (pg/mL)	CRP (mg/L)
1	n/a	9.4	18.1	7.7	n/a	3.5
2	6.4	7.3	13.5	7.5	8.4	0.6
3	6.4	13.8	16.6	9.9	7.4	26.8
4	12.5	10.1	26.1	11.0	8.7	4.3
5	10.5	12.9	15.1	10.2	10.8	1.5
6	n/a	8.2	15.1	7.1	8.7	1.8
7	0.0*	8.6	18.1	8.6	8.7	0.2
8	n/a	20.0*	23.5	8.7	9.2	24.0
Mean	8.9	10.0	18.3	8.8	8.3	7.8
SD	3.1	2.4	3.1	1.4	1.9	11.0

Appendix XIII Co-efficients of variation for biochemical assays

Table 1. Mean co-efficients of variation (CV) for each metabolic parameter

Metabolic parameter	Number of replicates per assay	% CV
Glucose	3	4.2
Insulin	2	10.0
C-peptide	2	9.0
Glucagon	2	8.9
Glycerol	3	
NEFA	4	
CRP	2	10.9

Appendix XIV Comparison of medication and supplements taken by patients and HM females

Table 1. Medication taken by patients and HM females (chemotherapeutic drugs excluded)

Pair	Patient	HM female
1	Apo-prochlorazine; Dexamethasone; Granisetron	Prozac (fluoxetine); none taken on testing days
2	Dexamethasone; Granisetron (both drugs taken on OGTT day, which was D3 of chemo)	None
3	Apo-prochlorazine; Dexamethasone; Granisetron (Kytril); Pegfilgrastim (Neulasta); Herceptin (took all drugs on OGTT day except Herceptin)	None
4	Novo-venlafaxine XR 150 mg 1x/d (depression); Apo-paroxetine 20 mg 2x/d (depression); Apo-prochlorazine; Granisetron; WBC booster day after chemo; Tylenol 3 as needed for bulging discs (L4-5) and arthritis (C3-4)	None
5	Apo-prochlorazine; Dexamethasone; Granisetron; Neulasta	Seasonale (levonorgestrel and ethinyl estradiol; extended cycle oral contraceptive)
6	Adicant Plus 16/12.5 OD; Bisaprolol 10 mg OD; Novo Spiroton 25 mg 2 tabs/evening; Pegfilgrastim (Neulasta); Dexamethasone; Stemetil PRN; Ondansetron (Zofran); (Adicand Plus and Novo-Bisoprolol taken on OGTT day)	None
7	Ondansetron; Dexamethasone; Filgrastim (Neupogen)	Synthyroid, florinef and hydrocortisone daily for hypothyroidism and adrenal insufficiency (condition managed in this way for 20 years)
8	Oxycodone in last 1.5 weeks for back pain aggrevated by chemo	None

Standard doses:

Apo-prochlorazine – 10 mg as needed
Dexamethasone – 4 mg 2x daily for D2-3 following chemotherapy
Granisetron – 1 mg D1-3 of chemotherapy
WBC booster (pegfilgrastim or filgrastim) – D2 following chemotherapy
Ondansetron – D1-3 of chemotherapy

Table 2. Supplements taken by patients and HM females

Pair	Patient	HM female
1	None	Melatonin (all days)
2	n/a	None
3	None	None
4	None	Mutivitamin (Preventive X); VitB; Ca; EPA (all days)
5	None	None
6	Omega 3, 6, 9 capsule; multivitamins; multiminerals; lutein; lycopene; Ca; VitD (days 2 and 3)	VitD; Ca; Mg; multivitamin; n-3 (all days)
7	Phosphytidyl serine; Ovol 180s (all days)	None
8	n/a	EFA oil; caproil; multivitamins; liquid chlorophyll; methylcobalamin; Vision Support II; Orthobone; Macasure; Strontium (all days)