Endocrine therapy mediated hot flushes in breast cancer: can exercise help?

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Abstract

Introduction: Hot flushes are a sudden, intense sensation of heat causing skin reddening, flushing and profuse sweating. Hot flushes are a side effect of endocrine therapy for breast cancer treatment; and are possibly more frequent and severe than post-menopausal hot flushes. Many breast cancer patients do not adhere to the endocrine therapy guidelines due to the frequency and severity of hot flushes, which significantly increases the risk of cancer recurrence. There are currently few treatments able to reduce the frequency and severity of hot flushes in breast cancer. Exercise training has shown promise in alleviating menopausal hot flushes. Therefore, the overall aim of this thesis was to examine subjective and physiological frequency and severity of endocrine mediated hot flushes in breast cancer patients; and determine the effectiveness of an exercise training intervention in ameliorating endocrine mediated hot flushes.

Methods: Eight breast cancer patients undergoing endocrine therapy (age 53 ± 8, BMI 29 ± 6) were recruited and 16 postmenopausal women experiencing hot flushes (age 53 ± 4, BMI 29 ± 5) from a previously reported study were included. A 7-day subjective hot flush frequency and severity questionnaire was used to examine subjective frequency and severity. To measure physiological frequency and severity sweat rate, skin and cerebral blood flow were measured in the laboratory. To do this women wore a tube-lined suit, which was perfused with 34°C water followed by a passive heat stress where water temperature increased to 48°C. A hot flush was objectively defined as a transient and pronounced increase in sternal sweat rate (>0.002 mg cm^{-2} min^{-1}). Sweating and cutaneous vasodilatory temperature thresholds and sensitivities were identified during the passive heating. Cardiorespiratory fitness and vascular health were also measured. Breast cancer patients (n=5) then completed a progressive 16-week moderate-intensity exercise training intervention (30-60 minutes, 3-5x per week). After which, measurements were repeated. Data were analysed using t-tests and/or general linear modelling, and presented as mean (95% CI).

Results: Despite similar subjective hot flush frequency (49, 58; 95%CI = -45, 26; P = 0.56) and severity (81, 125; 95%CI = -102, 13 AU; P = 0.12) in breast cancer patients and postmenopausal women, respectively breast cancer patients demonstrated attenuated sweating, skin and cerebral blood flow responses during a hot flush (P > 0.05). 16 weeks of

exercise training did mediate reductions in subjective hot flush frequency by 37 hot flushes per week (52, 12; 95%CI = -21, 102, *P* = 0.14) and severity by 68 AU (78, 27; 95%CI = -39, 166 AU; *P* = 0.16) but this did not reach statistical significance. Exercise training did not attenuate physiological responses to a hot flush as no differences in sweating, skin and cerebral blood flow responses occurred (*P* > 0.05).

Conclusion: These data indicate that endocrine mediated hot flushes in breast cancer are not more severe than those experienced by post-menopausal women. Exercise training has shown promise as a potential non-pharmacological treatment for endocrine mediated hot flushes in breast cancer but requires further study.

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Declaration

I declare that the work contained in this thesis is entirely my own. Previous paper data from our laboratory has been utilised with permission for study 1.

Publications completed by the candidate during MPhil tenure

Jones, H., **France, M**., Low, D. Chapter 19 – Menopause and the Exercising Female – Forsyth, J. J., Roberts, C. The Exercising Female: Science and its Application. 2018. Publisher Taylor Francis Routledge. (*In Press*). **Aspects from this review article are included in the literature review section of this thesis.**

Jones, H., Barr, D. A., Bailey, T. G., Lucas, R., Crandall, C., **France, M**., Low, D. A. (2018). The trigger of a menopausal hot flush: core temperature revisited. (BJOG *under review*).

Oral communications

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Poster communications

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Aromatase inhibitors	(AIs)
Blood pressure	(BP)
Body mass index	(BMI)
Bone mineral density	(BMD)
Botulinum toxin A	(BTX)
Cardiac output	(CO)
Cardiovascular disease	(CVD)
Cognitive behavioural therapy	(CBT)
Cutaneous vascular conductance	(CVC)
Diastolic blood pressure	(DBP)
Final menstrual period	(FMP)
Flow mediated dilation	(FMD)
Functional magnetic resonance imaging	(fMRI)
Heart rate	(HR)
High density lipoprotein	(HDL)
Hormone replacement therapy	(HRT)
Intima-media thickness	(IMT)
Low density lipoprotein	(LDL)

Mean arterial pressure	(MAP)
Middle cerebral artery velocity	(MCAv)
Neurokinin B	(NKB)
Nitric oxide	(NO)
Oestrogen-related receptor alpha	(ERRα)
Oestrogen receptor positive	(ER positive)
Peak end tidal volume of carbon dioxide	($P_{ET}CO_2$)
Peak oxygen uptake	(VO_{2peak})
Physical Activity Readiness Questionnaire	(PAR-Q)
Quality of Life	(QOL)
Resistance training	(RT)
Selective oestrogen receptor modulators	(SERMs)
Stroke volume	(SV)
Sweat rate	(SR)
Systolic blood pressure	(SBP)
Transcranial Doppler	(TCD)
Type 2 Diabetes Mellitus	(T2DM)

1 General Introduction

1.1 Background

A hot flush is defined as a sudden, intense sensation of heat causing skin reddening, flushing and profuse sweating (Low et al, 2008). Post-menopausal women without breast cancer have displayed elevations in skin blood flow (80%), sweating (5-fold) and reductions in cerebral blood flow during a hot flush (Sloan et al, 2001; Low et al, 2008; Lucas et al, 2013).

A type of endocrine therapy that suppresses oestrogen is prescribed after active breast cancer treatment. This prescription, however, causes hot flushes in ~70% of breast cancer patients (Antoine et al, 2008). Hot flushes are extremely debilitating and cause anxiety, depression, sleep disturbances and chronic fatigue (Gupta et al, 2006). Importantly, hot flushes may be more frequent and severe in breast cancer patients relative to naturally menopausal women (Carpenter et al, 2002) and are the main reason up to 90% of patients stop endocrine treatment early or reduce their dosage (Kligman and Younus, 2010). This has a significant impact on breast cancer prognosis (Murphy et al, 2012; Makubate et al, 2013).

The physiological changes that occur during a hot flush have not been thoroughly investigated in breast cancer patients. In one study, Carpenter *et al* (2004) reported that breast cancer patients subjectively under report hot flush occurrence in comparison to an objective measure of skin conductance. This could possibly be due to breast cancer patients being more or less 'attuned' to physical sensations. A subsequent study reported that subjective hot flush frequency and severity was greater in breast cancer patients by 4 hot flushes and 4 arbitrary units (AU) per 48 hours compared to postmenopausal females

(Carpenter et al, 2002). Substantiation of the severity between endocrine mediated and post-menopausal hot flushes and an objective physiological investigation is warranted.

Pharmacological treatment of hot flushes in breast cancer is limited as hormone replacement therapy (HRT) is contraindicated. Given the prevalence of hot flushes and their impact on adherence to endocrine therapy, alternative treatments are essential. One study has investigated the combined and individual effects of cognitive behavioural therapy (CBT) and exercise training on subjective frequency and severity of endocrine mediated hot flushes in breast cancer and found that the interventions combined were more beneficial in comparison to CBT or exercise training alone (Duijts et al, 2012). Along similar lines, the effect of exercise training on post-menopausal hot flushes has shown promising results as a non-pharmacological alternative treatment (Karacan, 2010; Luoto et al, 2012; Reed et al, 2014; Bailey et al, 2016a; Bailey et al, 2016b). In one study, Bailey *et al* (2016b) employed a 16-week moderate intensity aerobic exercise training intervention in postmenopausal women experiencing hot flushes. Subjective frequency and severity of hot flushes (Sloan et al, 2001) and physiological measures of hot flushes in a laboratory setting using sweating, skin and cerebral blood flow, were reduced following exercise training. The exercise training intervention was also successful in increasing cardiorespiratory fitness and improving thermoregulatory and vascular control, which may provide some explanation into how exercise can improve severity of hot flushes. Taken together, exercise training may be a useful intervention that requires further investigation into the objective and physiological changes that might occur in breast cancer patients.

1.2 Summary

The overall aim of this thesis was to examine subjective and objective endocrine mediated hot flushes in breast cancer patients and to determine the effect of exercise training. This aim will be realised via two experimental studies:

(i) To compare the subjective as well as objective physiological frequency and severity of hot flushes between post-menopausal women and breast cancer patients undergoing endocrine therapy

(ii) To investigate the effect of 16 weeks of moderate intensity aerobic exercise training on the frequency and severity of hot flushes as well as cardiovascular health in breast cancer patients taking endocrine therapy

2 Literature Review

2.1 Introduction

This literature review will provide an overview of available research studies that have investigated hot flushes in breast cancer patients undergoing endocrine therapy and postmenopausal women. Secondly, this review will highlight the potential of exercise as a non-pharmacological alternative to alleviate hot flushes.

2.1.1 Breast cancer

Breast cancer is the most common form of cancer in the UK and incidence in the UK has increased by ~4% over the last decade (Cancer Research UK, 2018). More than 80% of breast cancer occurs in women over the age of 50 (Breast Cancer Care, 2018). Due to increasing life expectancy, incidence rates of breast cancer are expected to further rise by 2% by 2035 (Cancer Research UK, 2018). Breast cancer survival rates in the UK have improved significantly over the past 50 years (Cancer Research UK, 2018), therefore, a large number of women are living with the after effects of the disease and its treatment.

Treatment for breast cancer is dependent on various factors including: (1) the type of cancer, (2) stage of the disease, and (3) whether the cancer has metastasized. Treatment can consist of chemotherapy, radiotherapy and surgery or any combination of these. Breast cancers can be hormone-receptor positive, for example oestrogen receptor positive (ER+) or progesterone receptor positive (PR+). In ER+ and PR+ breast cancers, cells contain oestrogen or progesterone receptors respectively that enable tumours to grow. There are also hormone-receptor negative breast cancers, for example human epidermal growth factor receptor 2 positive (HER2+). These proteins are receptors on breast cells that grow and divide exponentially. There are also triple-negative breast cancers, which possess no oestrogen or progesterone receptors and do not overexpress HER2. HER2+ only breast

cancer and triple-negative breast cancer are uncommon consisting of only 10-20% of breast cancers.

Approximately 80% of breast cancers are ER+ (Kohler et al, 2015) and the focus of this thesis is on ER+ breast cancers. Individuals with ER+ breast cancers are much more likely to respond to endocrine therapy that supress oestrogen. Endocrine therapy is usually prescribed to women recovering from ER+ breast cancer after completion of 'active' cancer treatment (e.g. chemotherapy, radiotherapy or surgery) as it can reduce the risk of breast cancer recurrence but in some instances it can be used to reduce the size of the cancerous tumour before surgery to remove it or can treat breast cancer that has returned or metastasized. Different types of endocrine therapy possess various strategies of action and are prescribed to women dependent on menopausal status. Strategies include providing ovarian ablation (blocking ovarian function), blocking oestrogen production or blocking oestrogens effects. Aromatase inhibitors (AIs) prevent the conversion of androgens into oestrogens in muscle and peripheral adipose tissue (Mom et al, 2006). AIs such as anastrozole, letrozole and exemestane, are prescribed solely to postmenopausal women as the ovaries in premenopausal women produce too large a quantity of aromatase for the inhibitors to block effectively (National Cancer Institute, 2017). Selective oestrogen receptor modulators (SERMs), such as tamoxifen, can be prescribed to pre and postmenopausal women. SERMs block the effects of oestrogen through binding to oestrogen receptors. AIs and SERMS are the most commonly prescribed endocrine therapy.

In recovery from breast cancer (i.e. following active treatment), endocrine therapy is recommended for a minimum of 5 years in ER+ breast cancer patients. Meta-analyses have shown that in ER+ breast cancers, full compliance with 5 years of tamoxifen can reduce

breast cancer mortality rate during the first 15-years after the start of treatment by at least a third (Early Breast Cancer Trialists' Collaborative, 2011). Similarly, AIs have been shown to reduce breast cancer mortality rate by around 40% throughout the first decade of treatment (Early Breast Cancer Trialists' Collaborative, 2015).

Importantly, 31-73% of breast cancer patients prescribed tamoxifen or AIs following active treatment discontinued treatment before the end of the recommended five-years and 41-72% did not adhere to the prescribed frequency of use (e.g. daily) (Murphy et al, 2012). The poor compliance to AIs or tamoxifen is predominately due to the side effect, hot flushes (Kligman and Younus, 2010). Hot flushes occur as a result of the loss of oestrogen action as a consequence of the endocrine treatment. In one study breast cancer patients subjectively reported significantly more frequent, severe and “bothersome” hot flushes compared to naturally postmenopausal women (Carpenter et al, 2002). In that study, Carpenter and colleagues recruited 69 breast cancer patients, 50% of which were taking tamoxifen, and 63 age-matched healthy pre-, peri- and postmenopausal females (Carpenter et al, 2002). A subjective hot flush questionnaire (Kronenberg, 1994) and a 48-hour hot flush diary were utilised. They found that hot flush frequency and severity were 4 hot flushes and 4 arbitrary units (AU) greater, respectively, per 48 hours in breast cancer patients compared to postmenopausal females. Moreover, hot flushes were 3 minutes greater in duration measured using subjective diaries in breast cancer patients undergoing endocrine therapy.

Tamoxifen increases the prevalence of hot flushes in comparison to placebo (Fisher et al, 1998; Fallowfield et al, 2001). Aromatase inhibitors are also associated with a similar prevalence of hot flushes as tamoxifen (Fallowfield and Cella, 2002). Intriguingly, despite potential greater severity, breast cancer patients have been shown to subjectively under

report hot flushes, which have met the objective criteria required to be considered a hot flush (Carpenter, Monahan and Azzouz, 2004). An increase in skin conductance of at least 2 µmho within a 30 second period was required to define an objective hot flush (Carpenter, Monahan and Azzouz, 2004). Carpenter *et al* (1999) recruited 19 postmenopausal breast cancer patients, three of which were taking tamoxifen, and five healthy premenopausal women. 24-hour ambulatory sternal skin conductance monitoring was employed in this study as well as a subjective hot flush diary; skin conductance monitoring during waking hours was associated with a 30% false-negative rate. This could imply that hot flush frequency data reported in studies utilising skin conductance monitoring may be underestimated (Carpenter et al, 2002). Nevertheless, sternal skin conductance data should also be interpreted with caution given that sternal skin conductance monitoring could not differentiate between sweating from a hot flush and sweating due to performing exercise/physical activity (Carpenter, Monahan and Azzouz, 2004). Research studies with more reliable measurement of objective frequency and severity of hot flushes, e.g. physiological changes, other than skin conductance, are required.

2.1.2 Postmenopausal hot flushes

The most common side effect of the menopause are hot flushes (Thurston, 2018). A menopausal hot flush is a sudden, intense sensation of increasing heat causing skin reddening, flushing and profuse sweating (Low et al, 2008). Research studies have shown during a menopausal hot flush, elevations in skin blood flow (80%), sweating (500%) a reduction (5%) in cerebral blood flow occur (Sloan et al, 2001; Low et al, 2008; Lucas et al, 2013).

2.2 The physiology of a hot flush

Previous studies measuring physiological hot flushes using measures such as Laser Doppler flowmetry and capacitance hygrometry have found that increases in skin temperature and skin blood flow occur prior to an increase in sweating (Figure 2.1) (Low et al, 2008; Hubing et al, 2010; Low et al, 2011; Lucas et al, 2013; Bailey et al, 2016a). Blood from the central regions is redistributed to the peripheral regions, which can lead to large increases in systemic vascular conductance and reductions in blood pressure (Crandall et al, 2008; Low et al, 2008). Low et al. (2011) found that increases in skin blood flow and cutaneous vascular conductance (CVC) during a hot flush are inhibited by botulinum toxin A (BTX), which blocks the release of neurotransmitters from sympathetic cholinergic nerves. In the absence of an increase in core body temperature, increases in efferent sympathetic nerve activity to the skin were observed prior to and during a hot flush (Low et al, 2011), likely influencing changes in sweating and skin blood flow. Additionally, increases in activity in the brainstem using function magnetic resonance imaging (fMRI) were observed prior to the onset of a hot flush, followed by significant increases in insula and prefrontal cortex activity (Diwadkar, Murphy and Freedman, 2013). This suggests that increases in skin blood flow and sweating may be neurally mediated rather than 'locally' or non-neurally mediated (Freedman, Woodward and Mayes, 1994).

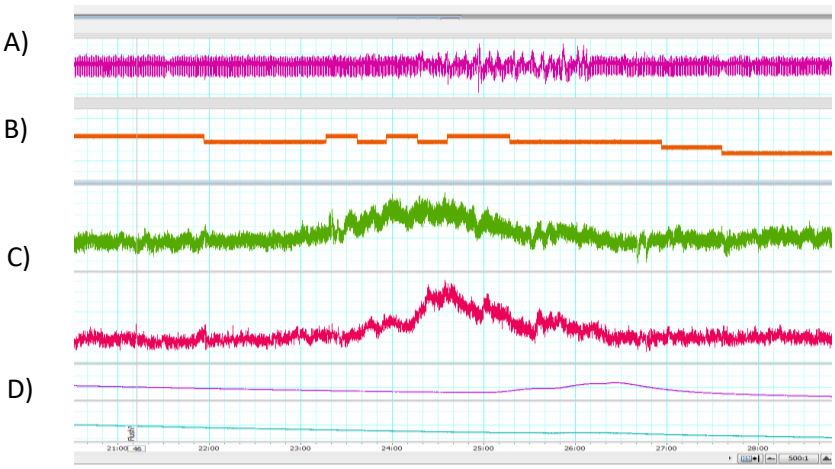


Figure 2.1 Example of thermoregulatory response during a hot flush A) pink trace is ECG B) orange trace is core temperature C) green trace and red trace are chest and forearm skin blood flow, respectively D) purple and blue trace are chest and forearm sweat rate, respectively.

A reduction in central blood volume during a hot flush may be accompanied by a reduction in end-tidal carbon dioxide, which could contribute to a drop in cerebral blood flow as seen during passive heat stress (Brothers et al, 2009; Nelson et al, 2011). According to Lucas *et al.* (2013), 66% of hot flushes in 11 healthy postmenopausal women were accompanied by a 5% reduction in cerebral blood flow. The transient decrease in middle cerebral artery velocity (MCAv) could be responsible for the possible feelings of dizziness or nausea associated with a hot flush. Collectively, there is a large suite of studies describing the physiological changes during a menopausal hot flush. It is assumed that such changes also occur in a hot flush mediated by endocrine treatment in recovery from breast cancer. This, however, warrants further investigation as endocrine therapy may mechanistically mediate hot flushes differently in breast cancer patients compared to naturally occurring postmenopausal hot flushes.

2.2.1 Mechanisms contributing to menopausal hot flushes

The physiological mechanisms associated with menopausal hot flushes are unclear. One explanation for the onset of a hot flush is an increase in core body temperature (Freedman and Woodward, 1996; Freedman, 2014). An increase in core body temperature of 0.03-0.05°C in the 30 minutes prior to a hot flush was apparent in 65% of hot flushes in 8 symptomatic postmenopausal women (Freedman and Krell, 1999). Later findings suggested that core body temperature fluctuates by 0.035°C during a 24-hr period in both symptomatic and non-symptomatic post-menopausal women and importantly, these fluctuations in core body temperature do not trigger a hot flush (Freedman, 2002). As a consequence, other mechanisms have been proposed. Changes in neurotransmitters in the brain have been suggested, for example hypothalamic neurokinin B (NKB) that is increased during the menopause. Intravenous infusion of hypothalamic NKB resulted in 8 out of 10 women experiencing a hot flush, measured using sternal skin conductance (Jayasena et al, 2015). Furthermore, alterations in the sympathetic nervous system may be associated with hot flushes. Low *et al.* (2011) observed increases in sympathetic nerve activity to the skin during a hot flush and proposed that these increases are potentially due to an elevation in a singular or multiple neural component such as cutaneous vasoconstrictor, sudomotor and/or vasodilator neural activities.

Vascular (dys)function may be implicated in the severity of hot flushes (Mendelsohn and Karas, 1999; Bechlioulis et al, 2010; Bechlioulis et al, 2012; Sassarini et al, 2012; Sassarini et al, 2014). Postmenopausal women exhibit reduced flow mediated dilation (FMD) (a marker of large artery nitric oxide function) compared to males and premenopausal females (Thurston et al, 2008; Moreau et al, 2012) and possess higher levels of vascular inflammatory markers indicative of vascular dysfunction compared to premenopausal

females (Nyberg et al, 2014). Moreover, Bechlioulis *et al.* (2010) found women with more severe hot flushes had significantly lower FMD compared with menopausal women with no/mild hot flushes (Bechlioulis et al, 2010). It has been suggested that postmenopausal women with severe hot flushes may possess negatively altered vascular regulatory mechanisms (Najjar, Scuteri and Lakatta, 2005) or elevated sympathetic tone (Ganong, 2001).

There is emerging research evidence that post-menopausal women who demonstrate vasomotor symptoms (e.g. hot flushes) have a higher prevalence of cardiovascular disease (CVD) risk factors and novel markers of vascular function (FMD) using Doppler ultrasound (Thurston et al, 2011; Gray et al, 2018). Vasomotor symptoms (increased severity and duration of hot flushes) were associated with an 18% increased type 2 diabetes mellitus (T2DM) risk (Gray et al, 2018). Taken together the presence of hot flushes may be an important and independent marker of health.

2.2.2 Treatment for the endocrine meditated hot flushes

The clinical need for therapies to alleviate hot flush symptoms in breast cancer patients has been highlighted by the National Cancer Research Institute (NCRI) Breast Cancer Symptom Management group (Morgan and Fenlon, 2013) and the National Institute for Health and Care Excellence (NICE) guidelines for menopause (NICE, 2015). Yet, little is known about by endocrine mediated hot flushes.

Cognitive behavioural therapy is one non-pharmacological treatment that has been examined. Cognitive behavioural therapy is a talking therapy, which encourages individuals to change their behaviour and mind-set to help relieve problems. Various methodologies for program implementation has been employed, including internet-based (Atema et al,

2017) and group sessions (Duijts et al, 2012; Mann et al, 2012). Each program strategy of CBT found significant reductions of 1-3 AU in hot flush problem ratings. Duijts et al. (2012) investigated the effect of CBT and physical exercise on overall menopausal symptoms. Duijts and colleagues found that both physical exercise and CBT interventions combined significantly decreased short- and long-term overall endocrine symptoms, specifically hot flushes, compared with the control group. The study demonstrated a significant decrease of 1.3 AU in hot flushes and night sweats problem ratings. These findings suggest that physical exercise may be beneficial for reducing menopausal symptoms in breast cancer patients. Physical exercise alone, however, demonstrated an insignificant decrease of 0.45 AU. Nonetheless, this intervention did not include participant exercise supervision and may not have been of a sufficient exercise intensity. Given the knowledge of the importance of exercise intensity and supervision for optimal training intervention effects (see below) and the positive impact of exercise training in post-menopausal women, further research into the effect of exercise training on endocrine therapy hot flushes is warranted.

2.2.3 The effect of exercise training on hot flushes in postmenopausal women

Various studies exist that have directly measured the impact of exercise training on subjective hot flushes (Lindh-Astrand et al, 2004; Luoto et al, 2012; Moilanen et al, 2012; Reed et al, 2014; Daley et al, 2015; Bailey et al, 2016a; Bailey et al, 2016b). Exercise training has elicited a decrease in hot flush frequency in the range of 4 to 64% and severity in the range of 5 to 70% (Table 2.1). As an example, Bailey *et al.* (2016) compared the mean number of self-reported hot flushes per week between an exercise intervention and no-intervention control. After adjusting for baseline hot flush frequency/severity, the mean frequency of hot flushes per week was 48 events (70%) lower following the exercise intervention (mean: 18 events/week) vs. following a same period of no-intervention

control (95%CI for mean difference: 39 to 56 events/week, $P < 0.001$). It is important to highlight that not all studies have reported a statistically significant reduction in hot flush frequency following a period of exercise training compared with control (Newton et al, 2014; Sternfeld et al, 2014; Daley et al, 2015). Nevertheless, a number of these studies have employed a lower intensity exercise stimulus and duration as discussed previously or with appropriate guidance to cause an increase in cardiorespiratory fitness; or, in some studies, they did not even assess cardiorespiratory fitness. Overall, higher exercise intensity and longer duration of intervention as well as monitoring adherence generally mediated the largest improvements in hot flush frequency and severity (Table 2.1). For example, aerobic exercise training interventions performed at moderate-high intensity for a duration of ≥ 12 weeks demonstrated significant improvements in hot flush frequency and severity (Lindh-Astrand et al, 2004; Karacan, 2010; Luoto et al, 2012; Bailey et al, 2016a; Bailey et al, 2016b).

Taken together, all of the exercise training studies performed prior to 2015 (Table 2.1), were included in the recommendations for the National Institute for Health and Care Excellence and North American Menopause Society guidelines on menopausal treatment (Carpenter et al, 2015; Sarri, Davies and Lumsden, 2015). This included systematic reviews on exercise and menopausal symptoms which reported that the data on exercise training is inconclusive due to a lack of controlled studies (n = 5 included) (Daley, Stokes-Lampard and Macarthur, 2009; Daley, Stokes-Lampard and Macarthur, 2011). Nevertheless, one focus of these systematic reviews was a comparison of exercise training interventions to HRT. Whilst it is well established that hot flushes are alleviated by hormone replacement therapy (HRT), any lifestyle intervention will be different from HRT mechanistically as oestrogen will still be low. The systematic review data led to exercise not being

recommended for menopause management or included in these guideline documents. Yet, the research studies that have been published since, including the suite of studies from Copenhagen Women's study (Agil et al, 2010; Moilanen et al, 2012; Nyberg et al, 2014; Bailey et al, 2016a; Bailey et al, 2016b; Egelund et al, 2017; Mandrup et al, 2017a; Mandrup et al, 2017b; Nyberg et al, 2017; Seidelin et al, 2017), suggest positive benefits of exercising in the menopausal transition. One study examined the impact of exercise training on objective physiological responses to menopausal hot flushes for the first time (Bailey et al, 2016a). In the aforementioned study, participants completed a 16-week moderate intensity exercise training intervention, and physiological measurements of core body temperature, sweat rate, skin and cerebral blood flow were recorded. They found that exercise training reduced the sweating and skin blood flow response typically observed during a hot flush episode as well as an attenuated decrease in cerebral blood flow (Bailey et al, 2016a). The physiological changes were also related to reductions in the subjective description of sweating, skin reddening, and feeling faint/light headedness. This provided direct evidence that exercise training reduced the physiological and subjective severity of menopausal hot flushes through the improvement of thermoregulatory and systemic vascular responses during a hot flush.

Table 2.1 The exercise training-mediated changes in hot flush frequency and severity measured using subjective rating scales.

	Author	Exercise modality	Exercise frequency	Exercise duration (weeks)	Time since menopause (years)	Scale	Change in frequency	Change in severity
High intensity	Sternfeld et al., 2014	Aerobic exercise	60 min 3x/week	12	N/A	Daily diaries	33%	17%
	Karacan., 2010	Aerobic exercise	55 mins 3x/week	24	N/A	Menopause Rating Scale	N/A	5%*
Moderate intensity	Bailey et al., 2016a,b	Aerobic exercise	60 min 5x/week	16	1-4	Sloan	64%*	72%*
	Lindh-Åstrand et al., 2004	Aerobic exercise	60 min 3x/week	36	N/A	Kupperman's Index	50%	39%
	Daley et al., 2015	Aerobic exercise	30 min 5x/week	24	N/A	Hot flush rating scale	22%	N/A
	Luoto et al., 2012	Aerobic exercise	50 min 3x/week	12	0.5-2	Women's Health Questionnaire	4%*	17%
	Moilanen et al., 2012	Aerobic exercise	50 mins 4x/week	24	0.5-3	Women's Health Questionnaire	†	†
Low intensity	Newton et al., 2014	Yoga	4x/week	12	N/A	Daily diaries	35%	15%
	Reed et al., 2014	Aerobic exercise	60 mins 3x/week	12	< 5	Menopausal Quality of Life	N/A	22%*
	Reed et al., 2014	Yoga	1x/week + 20mins practice daily	12	< 5	Menopausal Quality of Life	N/A	23%*

N/A: not recorded; †unable to determine values; *statistically significant change compared to baseline

2.3 How does exercise mediate improvements in hot flushes?

Based on the physiology of a hot flush and the suggested mechanisms mediating the frequency and severity of menopausal hot flushes (described above), the cardiovascular and thermoregulatory control systems are logical physiological targets in the causal pathway for an exercise exposure for alleviating hot flush outcomes. Both of these physiological systems become dysfunctional during the menopause and are implicated in the causal pathway for menopausal hot flushes (Deecher and Dorries, 2007). Only one study to date, described above, has attempted to investigate the pathways of how exercise training could alleviate menopausal hot flushes (Bailey *et al.* 2016a, 2016b). After 16 weeks of exercise training that would have induced regular, episodic increases in shear stress, increases in FMD, and reductions in body temperature thresholds for sweating and cutaneous vasodilation were observed indicating improved endothelial function and thermoregulatory efficiency respectively. These improvements in endothelial function and thermoregulation coincided with reductions in physiological and subjective hot flush frequency and severity. This provides support that these physiological systems could be involved in the alleviation of hot flushes. Whilst, there may be other systems involved (e.g. sympathetic nervous system), exercise-mediated changes require further study in post-menopausal and endocrine mediated hot flushes.

2.4 Associated benefits of exercise on menopausal symptoms in postmenopausal women

Recently, a small number of research studies have examined the impact of exercise training on menopausal symptoms (Lindh-Astrand et al, 2004; Agil et al, 2010; Karacan, 2010; Luoto et al, 2012; Mansikkamaki et al, 2012; Moilanen et al, 2012; Newton et al, 2014; Reed et al, 2014; Sternfeld et al, 2014; Daley et al, 2015; Bailey et al, 2016a; Bailey et al, 2016b).

Predominantly, the studies have utilised menopause specific measurement tools including the Menopause Quality of Life (MQOL) questionnaire (Hilditch et al, 1996), Menopause Rating Scale (MRS) (Heinemann et al, 2004) or Greene Climacteric Scale (Greene, 1998), which provide subjective data as the primary outcome. Generally, all of the exercise training studies show benefit in improving quality of life and subjective menopausal symptoms in post-menopausal women (Lindh-Astrand et al, 2004; Agil et al, 2010; Karacan, 2010; Luoto et al, 2012; Mansikkamaki et al, 2012; Moilanen et al, 2012; Newton et al, 2014; Reed et al, 2014; Sternfeld et al, 2014; Daley et al, 2015; Bailey et al, 2016a; Bailey et al, 2016b). For example, some studies have shown that exercise training improves psychological symptoms such as depression, anxiety and insomnia (Agil et al, 2010; Mansikkamaki et al, 2012; Moilanen et al, 2012; Newton et al, 2014; Sternfeld et al, 2014; Daley et al, 2015) whilst others reported benefits in physical symptoms such as urogenital symptoms (Karacan, 2010). Importantly, and in line with the exercise training benefits on fitness, the magnitude of the intensity and duration of exercise training likely mediates the extent of the benefits. Low intensity exercise, including yoga, have observed improvements in menopause related quality of life (Elavsky and McAuley, 2007; Reed et al, 2014), whereas other studies that employed higher intensity aerobic exercise training found improved somatic, psychological and urogenital symptoms (Karacan, 2010).

2.5 Breast cancer and exercise

2.5.1 Can exercise increase cardiorespiratory fitness in breast cancer patients?

Whilst, cardiorespiratory fitness in breast cancer patients is impaired (Jones et al, 2007; Peel et al, 2014), the majority of exercise training studies show fitness can be improved (Nieman et al, 1995; Burnham and Wilcox, 2002; Kolden et al, 2002; Courneya et al, 2003;

Hutnick et al, 2005; Lane, Jespersen and McKenzie, 2005; Pinto et al, 2005; Cheema and Gaul, 2006; Herrero et al, 2006), with only one study showing non-significant increases (Turner, Hayes and Reul-Hirche, 2004). Generally, exercise training protocols utilising aerobic exercise with longer intervention durations are able to show the largest improvements in cardiorespiratory fitness in breast cancer patients. Taken together, these studies suggest that exercise training can mediate positive health effects even with evidence of fatigue and lymphedema, common side effects of cancer treatment (Courneya et al, 2003; Pinto et al, 2005).

2.5.2 Associated benefits of exercise on menopausal symptoms in breast cancer patients undergoing endocrine therapy

Few studies have investigated the effect of exercise on menopausal symptoms in breast cancer patients. It is plausible to assume that increases in cardiorespiratory fitness in breast cancer patients, results in benefits in QOL and menopausal symptoms, likewise to postmenopausal women. Studies investigating the impact of exercise training on QOL outcomes have utilised subjective questionnaires such as World Health Organisation QOL Assessment Scale (World Health Organization, 1995) and Functional Assessment of Cancer Therapy-Breast (FACT-B) scales (Brady et al, 1997). Overall QOL has been shown to increase after exercise training consisting of aerobic exercise and/or resistance training (Courneya et al, 2003; Cheema and Gaul, 2006). Low intensity exercise, such as yoga, has improved menopausal symptoms such as joint pain, fatigue and negative mood (Carson et al, 2009). Yet, the generalisability of these findings is restricted by the small sample size and use of only subjective diaries (Sloan et al, 2001). Although only a small amount of evidence exists to date, data suggests that exercise training is an effective intervention to improve

menopausal symptoms, in breast cancer survivors, yet only one study to date has investigated hot flushes as an outcome (Duijts et al, 2012).

Table 2.2 Summary of cardiorespiratory fitness changes from exercise training studies performed in breast cancer patients.

	Author	Exercise modality	Exercise frequency	Exercise duration (weeks)	Time since treatment (months)	Change in fitness (ml. $kg^{-1}.min^{-1)}$
High intensity	Courneya et al., 2003	Aerobic exercise	15-35 mins 3x per week	15	12	2.7*
	Herrero et al., 2005	Mixed. Aerobic exercise and RT	Aerobic exercise: 20-30 mins 3x per week. RT: 3x per week	24	24-60	2.2*
	Turner et al., 2004	Mixed. Aerobic exercise and RT	Aerobic exercise and RT 1x per week.	8	Median of 17	2.0
	Nieman et al., 1994	Mixed. Aerobic exercise and RT	60 mins 3x per week	8	<48	Increase in walk test distance*
Moderate intensity	Thorsen et al., 2005	Aerobic exercise	30 minutes home-based exercise 2x per week	14	1	6.4*
	Burnham & Wilcox., 2002	Aerobic exercise	14-32 mins 3x per week	10	2	6.1*
	Kolden et al., 2002	Aerobic exercise	One hour group classes 3x per week	16	N/A	4.6*
	Hutnick et al., 2005	Mixed. Aerobic exercise and RT	Aerobic exercise: 40-90 mins 3x per week. RT: 3x per week.	24	2 weeks	3.3*
	Cheema & Gaul., 2006	Mixed. Aerobic exercise and RT	Aerobic exercise: 15-30 mins 3x per week. RT: 2x per week	8	>6	1.5*
	Pinto et al., 2005	Aerobic exercise	2-5x per week	12	<60	Decrease in walk test time*

Abbreviations: RT, Resistance training; N/A, not recorded, *statistically significant change from baseline, $P < 0.05$

Summary

This literature review aimed to highlight the similarities and differences between hot flushes experienced during the menopause compared to those mediated by endocrine therapy in breast cancer. The role of exercise training as a non-pharmacological tool to alleviate hot flushes associated with breast cancer is highlighted.

3 General Methods

3.1 General methods

The measurements and protocols undertaken in this thesis are utilised in both studies. This general methods chapter describes participant information, techniques of subjective and physiological measurements, protocols and data reduction methods. Specific research designs, protocols and statistics for each study are outlined within the respective chapters.

3.1.1 Participants

Participants were informed of the experimental procedure and any associated risks verbally and in writing before providing written informed consent. All participants reported experiencing at least four hot flushes over a 24-hour period. Participants were recruited from one of two studies, both having conformed to the *Declaration of Helsinki* and gained ethical approval by the National Research ethics committee.

3.2 Measurements

3.2.1 Subjective hot flush frequency and severity:

Participants completed a 7-day hot flush frequency and severity diary (Sloan et al, 2001). The use of subjective hot flush diaries is a validated method to acquire subjective data for hot flush symptoms and perceptions (Sloan et al, 2001) and has been utilised in a number of hot flush research studies (Luoto et al, 2012; Moilanen et al, 2012; Sternfeld et al, 2014; Bailey et al, 2016a; Bailey et al, 2016b). Participants recorded the number of hot flushes per day along with the severity of each hot flush on a scale of 1 to 4 (1 being mild, 2 moderate, 3 severe, and 4 very severe). This data provided a weekly hot flush score. Daily hot flush severity was calculated using the sum of hot flushes recorded into each severity rating, that is [(3 x 1 (mild)) + (4 x 2 (moderate)) + (1 x 3 (severe)) + (0 x 4 (very severe)) =

daily severity score of 14]. A hot flush severity index was then calculated by the total sum of daily severity scores over the 7-day period.

3.2.2 Cardiorespiratory fitness:

A fitness test for peak oxygen uptake (VO_{2peak}) was performed on a treadmill (H/P Cosmos, Germany) using a modified Bruce protocol. A facemask was worn which was connected to an online gas analysis system (Oxycon Pro, Jaegar, Germany). After a 2-minute warm-up at 2.2 km/h on a flat gradient, the initial workload was set at 2.7 km/h at a 5° gradient. Thereafter, continuous increments in speed and gradient were performed each minute until volitional exhaustion. Heart rate was measured continuously using short-range telemetry (RS800, Polar, Finland) and ratings of perceived exertion (Borg, 1998) were collected throughout. VO_{2peak} was calculated from expired gas fraction (Oxycon Pro, Jaegar, Hochberg, Germany) as the highest 15-second period of data in the final minute before volitional exhaustion.

3.2.3 Vascular function:

Brachial artery endothelium-dependent function was measured using the FMD technique. Measurements were performed in a supine position after 20 minutes of rest. A Duplex ultrasound image was recorded for 1-minute (T300; Terason, Burlington, MA) to obtain baseline diameter and velocity. After baseline, recording was paused and a rapid inflation pneumatic cuff (D.E. Hokanson, Bellevue, UK) positioned on the forearm, distal to the brachial artery and was inflated to ~220 mmHg for a period of 5 minutes to induce ischemia. Diameter and velocity recordings were resumed 30 seconds before cuff deflation and were recorded for an additional 3 minutes, in accordance with recent technical specifications (Thijssen et al, 2011).

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. A detailed description of the analysis methodology can be found in recent papers (Black et al, 2008; Thijssen et al, 2011). Blood flow was calculated at 30 Hz from synchronized diameter and velocity data. Shear rate was calculated as four times mean blood velocity per vessel diameter. Allometric scaling was subsequently performed to consider baseline vessel diameter.

3.2.4 Temperature and blood flow responses to thermal heating:

Participants were fitted in a tube-lined jacket and trousers (Meg-Eng, Ottawa Canada), which covered the entire body except for the head, feet, and the right forearm. Participants rested for 15 minutes in a semi recumbent position whilst 34°C water was perfused through the suit. Ten minutes of thermoneutral rest ensued before a passive heat stress was induced with 48°C water perfused through the suit for 60 minutes or until a rise of approximately 1°C in core body temperature occurred. The following measurements were recorded throughout.

Heart rate was obtained using a 3-lead ECG (PowerLab; ADInstruments, Oxford, UK), alongside continuous beat-by-beat arterial blood pressure (BP) via finger photoplethysmography (Finapress, Amsterdam, the Netherlands). Stroke volume (SV) and cardiac output (CO) were calculated using the BP waveform using the Modelflow method, incorporating age, height, sex, and weight as moderator variables (Beatscope 1.0 software; TNO, Biomedical Instruments, Amsterdam, the Netherlands). Intermittent arterial BP was measured to verify continuous BP measurements by brachial auscultation using an autosphygmomanometer (Dinamap, Germany).

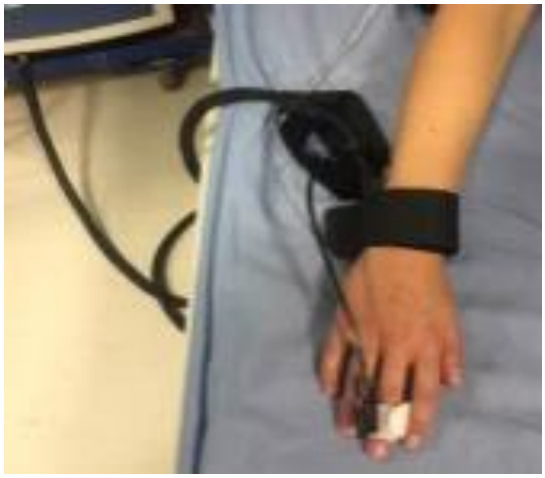


Figure 3.1 Beat-by-beat arterial blood pressure measurement via finger photoplethysmography (Finapress).

Mean skin temperature was measured using the weighted average of four regional temperatures measured from thermocouples (iButtons data logger; Maxim Integrated, San Jose, CA) fitted to a lateral calf, a lateral thigh, a tricep and the chest (Ramanathan, 1964). Core body temperature was measured via an ingestible telemetry pill (CorTemp, HQ Inc, Palmetto, FL) that was ingested approximately 6 hours prior to measurements. Mean body temperature was calculated using the weighted product of core and mean skin temperature (Stolwijk and Hardy, 1966).

a)

b)

c) 

Figure 3.2 a) Thermocouple skin temperature sensor b) CorTemp™ sensor c) CorTemp™ data recorder.

Cutaneous blood flow was measured using multi-fibre laser-Doppler flowmetry probes (Periflux System 5001; Perimed AB, Stockholm, Sweden) that were fixed with an adhesive heating ring. The laser-Doppler flow probes were positioned in an area that did not appear by visual inspection to be overly vascular. Calibration of the laser Doppler probes was performed using 2 generic points in accordance with manufacturers calibration guidelines using a zeroing disk and motility standard (Periflux System, Perimed AB). Sweat rate (SR) was continuously recorded via capacitance hygrometry with the ventilated capsule technique. Dry 100% nitrogen gas was supplied through acrylic capsules attached to the skin surface at a flow rate of 300 mL/min, with the humidity of the gas flowing out of the capsules measured by the capacitance hygrometer (Viasala HMP 155, Helsinki, Finland). Laser Doppler flowmetry probes and ventilated sweat capsules were secured to the chest and exposed forearm using tape.

Cutaneous vascular conductance was calculated as the ratio of LDF to mean arterial pressure (MAP) and expressed as both CVC and a percentage of maximum CVC (%CVC_{max}). The sensitivity of the sweating responses was estimated using the slope of the linear relationship between sweat-rate-per-unit change in core temperature beyond the core

temperature threshold for sweating; any plateau was omitted from the slope calculation (Wingo et al, 2010). The same method was employed to estimate skin blood flow sensitivity but instead using the rate of CVC-per-unit change in core temperature (Wingo et al, 2010).

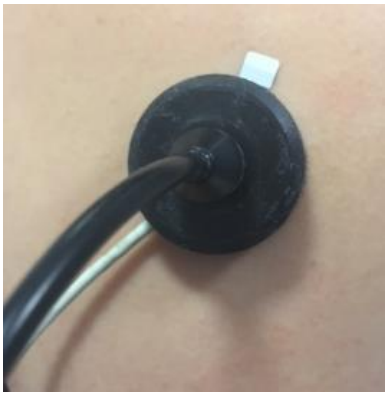
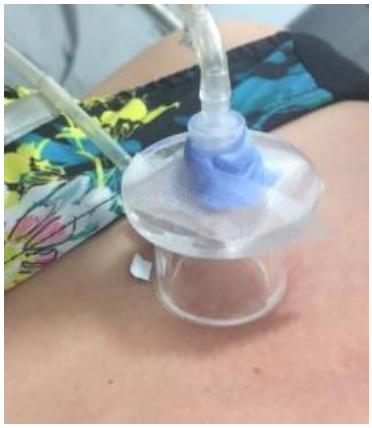
(a)  (b) 

Figure 3.3 (a) Laser Doppler flowmetry probes fixed with an adhesive heating ring (b) capacitance hygrometry using ventilated capsule technique.

Middle cerebral artery velocity (MCAv) was assessed bilaterally using transcranial Doppler (TCD) ultrasonography through the temporal window to insonate the middle cerebral artery (MCA), 1 cm distal to the MCA-anterior cerebral artery bifurcation. Vessel identification was achieved by probe direction, velocity, depth and waveforms. A Marc 600 head frame (Spencer Technologies, Seattle, USA) secured 2-MHz Doppler probes (Spencer Technologies, Seattle WA, USA) and were adjusted until an optimal signal was found. Water-based gel was placed between the participant's skin and the probe to reduce noise and artefact. Probe direction and TCD settings (depth, gain and power) were recorded once an optimal signal was identified to ensure comparable measurement post-intervention. Participants were asked to use a two-way valve-breathing (MLA1028, ADInstruments,

Colorado Springs, Colorado, USA) mouthpiece (MLA1026, ADInstruments) from which peak end tidal CO_2 ($P_{ET}CO_2$) was measured every 10 minutes for approximately 60 seconds using a calibrated gas analyser (ML206, ADInstruments). All data were sampled at 50 Hz with data acquisition hardware (PowerLab, ADInstruments, Oxford, UK) and displayed using LabChart data analysis software (ADInstruments, Colorado Springs, Colorado, USA).

3.2.5 Cutaneous blood flow responses to local heating:

Following whole body passive heating, local cutaneous heating was performed simultaneously at the chest and forearm laser-Doppler flowmetry sites to assess maximal cutaneous blood flow. The temperature of the adhesive heating ring was increased by 1°C every 5 seconds starting from 34°C and rising to 42°C.

3.3 Data reduction

Data from the passive heat stress test were extracted from LabChart. An average from ten minutes of thermoneutral rest was extracted as baseline. After which, 30 seconds averages throughout thermal heating were extracted. The onset of sweating and cutaneous vasodilation body/core temperature thresholds were identified via transient increases in SR and CVC. These thresholds were determined as the body/core temperature at the onset of chest and forearm SR and CVC in a blinded fashion by the same analyst. Sweating sensitivity was estimated using the slope of the linear relationship between SR per-unit change in mean body temperature beyond the mean body temperature threshold. Hot flushes during passive heating were omitted from the slope calculation to ensure accuracy of CVC and SR sensitivity calculations. This method was also employed for the estimation of skin blood flow sensitivity, using the rate of CVC per-unit-change in core temperature.

Data from the localised thermal heating test were extracted from LabChart using 30-second averages.

Nineteen participants experienced a hot flush during laboratory testing. These hot flushes were objectively recorded in both normothermia (spontaneous) and during passive heating. A hot flush was defined as a transient and pronounced increase in sternal SR (>0.002 mg cm^{-2} min^{-1}) (Freedman, 2001; Low et al, 2008; Bailey et al, 2016a). The end of a hot flush was recorded as the return of SR to baseline values prior to the hot flush. When hot flushes occurred with an absence of an increase in sweat rate, they were identified via a disruption in the ECG signal and a reduction in MCA*v* and BP. Following identification of a hot flush, each hot flush was divided into 8 equal segments, representing 12.5% of hot flush duration to account for variance in length of hot flushes. An average of every 30 seconds from 2 minutes pre and 2 minutes post hot flush were extracted from LabChart.

4 Comparison of endocrine therapy mediated hot flushes vs postmenopausal hot flushes

4.1 Introduction

Oestrogen receptor positive (ER+) breast cancer patients are prescribed endocrine therapy (e.g. SERMs and AI) after completion of active cancer treatment to reduce the recurrence of breast cancer. Nevertheless, 31-73% of patients prescribed endocrine therapy discontinued treatment before the end of the 5-year recommended use and 41-72% did not adhere to the prescribed frequency of use (Murphy et al, 2012). This is due to the side effect of hot flushes.

A small number of studies have investigated the frequency and severity of hot flushes associated with breast cancer. Carpenter and colleagues employed ambulatory sternal skin conductance monitoring for 24 hours as well as subjective hot flush diaries and event markers to measure hot flushes in 19 postmenopausal breast cancer patients, three of which were taking tamoxifen, and five healthy premenopausal women. The study indicated that breast cancer patients subjectively under reported hot flush occurrence in comparison to objective measures as only 59% of hot flushes identified by skin conductance were recorded subjectively using either a diary entry or event marker (Carpenter et al, 1999). In the same study, 15 'hot flushes' were observed in premenopausal participants using skin conductance monitoring, but these increases in skin conductance were associated with increases in physical activity and/or performing exercise, suggesting that sternal skin conductance may not be able to differentiate between sweating from hot flushes and onset of sweating from increases in core temperature (Carpenter et al, 1999). In a subsequent study, Carpenter *et al* (2002) recruited 69 breast cancer patients, 50% taking tamoxifen, and 63 age-matched healthy pre-, peri- and postmenopausal females. A subjective hot flush questionnaire (Kronenberg, 1994) and a 48-hour hot flush diary were utilised to record frequency and severity of hot flushes. Overall hot flush frequency and severity were greater

in breast cancer patients over 48 hours by 4 hot flushes and 4 AU respectively compared to postmenopausal females. Carpenter and colleagues concluded that sternal skin conductance should not be utilised as a proxy measure of subjective hot flush intensity given that 47% of 569 subjective hot flushes were insufficient to meet objective criteria whilst 26% showed no change in skin conductance (Carpenter et al, 2005). A more reliable objective measure of hot flush frequency and severity is required to determine if hot flushes associated with breast cancer are indeed more frequent and severe compared to menopausal hot flushes.

There are a greater number of studies, with more in-depth physiological measurement, that have investigated the frequency and severity of postmenopausal hot flushes (Low et al, 2008; Lucas et al, 2013). Indeed postmenopausal hot flushes are physiologically defined as a transient and pronounced increase in cutaneous vascular conductance (CVC) and skin blood flow (by ~80%), followed by a subsequent increase in sweat rate (500%) (Low et al, 2008) and a reduction in MCA*v* (by ~5%) (Lucas et al, 2013). These physiological changes reflect the subjective descriptions from the hot flush questionnaires (e.g. Sloan *et al,* 2001). For example, the transient decrease in cerebral blood flow is likely responsible for the possible feelings of dizziness or nausea associated with a hot flush. Nevertheless, these objective physiological changes have not been described in endocrine therapy mediated hot flushes. Moreover, postmenopausal hot flushes have been linked with thermoregulatory dysfunction following the menopause and the severity of hot flushes have been associated with changes in vascular function (Bechlioulis et al, 2010). Again, this has not been explored in breast cancer patients receiving endocrine therapy and may provide important information for alleviating hot flushes. Therefore, the aim of the current study was to compare the subjective and physiological severity of hot flushes between

postmenopausal women and breast cancer patients undergoing endocrine therapy. It was hypothesised that the subjective and physiological frequency and severity of hot flushes would be increased in breast cancer patients undergoing endocrine therapy. A secondary aim was to compare thermoregulatory and vascular function in these 2 groups and it was hypothesised that thermoregulatory and vascular function in breast cancer patients would be impaired.

4.2 Methods

4.2.1 Participants

Twenty-four participants (aged 53 ± 8 years; BMI 29.03 ± 5.27 kg/m^2) were included in the current study. Participants were breast cancer patients taking aromatase inhibitors or tamoxifen (n=8), or age-matched naturally postmenopausal women measured from a previous study (n=16, Bailey *et al*, 2016b). Participants were recruited from Liverpool Women's Hospital, Royal Liverpool and Broadgreen University Hospitals, local G.P. practices and via local advertisement. Postmenopausal females were 1-4 years since their last menstrual period and had not used hormone replacement therapy within the last 6 months whilst breast cancer patients were > 3 months post active cancer treatment. All participants were experiencing > 4 hot flushes over a 24-hour period and postmenopausal females experienced a hot flush during the laboratory monitoring period. Both studies conformed to the Declaration of Helsinki and were approved by NHS research ethics committees.

4.2.2 Research Design

Participants attended the laboratory on two separate occasions. Participants fasted over-night or at least 6 hours prior to the session, not having consumed alcohol for 24 hours and

caffeine for 12 hours or performed strenuous exercise for 24 hours before each visit. Visit one included anthropometric measurements, brachial artery endothelial function using FMD and a cardiorespiratory test (VO_{2peak}). At conclusion of visit 1, a hot flush frequency and severity questionnaire was given to the participant to complete and return at visit 2. Visits were completed after 7 days and all measurements took place in a temperature-controlled laboratory (24 ± 1 °C). Six hours prior to visit 2, participants consumed a thermometric pill to measure gut temperature as an index of core body temperature. At visit 2, temperature, local sweating, local skin and cerebral blood flow responses were measured during normothermia and throughout a thermal challenge and during any hot flushes that occurred during either period.

4.2.3 Measurements

Anthropometric measurements, brachial FMD, and VO_{2peak} were undertaken as described in chapter 3 (sections 3.2.2 and 3.2.3). Subjective hot flush questionnaires (Sloan et al, 2001) were completed over a 7-day period, between testing sessions (Chapter 3, sections 3.2.1). MCAv, BP, HR, SV, cardiac output, $P_{ET}CO_2$, LDF, CVC, SR, skin temperature and core temperature were measured throughout baseline and thermal test as described previously (Chapter 3, section 3.2.4). Following the thermal challenge, local cutaneous heating was performed at the chest and forearm using laser Doppler flowmetry, outlined in Chapter 3 (section 3.2.5).

4.2.4 Data Reduction

Data were extracted before, during and after a hot flush identified as a transient and pronounced increase in sternal SR (>0.002 mg cm^{-2} min^{-1}). During passive heating, data were extracted to establish thresholds for the onset of cutaneous vasodilation and

sweating and skin blood flow:core temperature, sweating:body temperature slopes. This is described in detail in Chapter 3 section 3.3.

4.2.5 Statistical analysis

All data was analysed using Statistical Package for Social Sciences (SPSS 23; Chicago, IL). Statistical significance was defined at $p < 0.05$ and exact p values are reported (p-values of "0.000" are reported as "< 0.001"). An independent t-test was employed for comparison of anthropometric measures, subjective hot flushes, resting MCAv, VO_{2peak}, FMD, chest and forearm CVC and SR thresholds and CVC/SR: core temperature slopes of breast cancer patients undergoing endocrine therapy and postmenopausal women. A general linear model was used to analyse the relative change in chest and forearm CVC, chest and forearm SR, MCAv, CBVC, MAP and HR during a hot flush and to compare differences in breast cancer patients undergoing endocrine therapy and postmenopausal women. Data in the text are expressed as means and 95% confidence intervals, unless stated otherwise.

4.3 Results

4.3.1 Participants

Age, height, weight, BMI, resting SBP, resting heart rate, resting MCA*v* and FMD were similar in breast cancer patients and postmenopausal women ($P > 0.05$, table 4.1). DBP was 9 (-16, -2) mmHg lower and VO_{2peak} was 3.48 (0.60, 6.37) ml.kg.min^{-1} higher in breast cancer patients undergoing endocrine therapy compared to postmenopausal women ($P = 0.02$, Table 4.1). Breast cancer patients were taking tamoxifen (n=5), letrozole (n=2) or anastrozole (n=1).

Table 4.1 Participant characteristics

Characteristic	Postmenopausal	Breast cancer	*P* value

Age (years)	53 ± 4	53 ± 8	0.87
Height (cm)	165.2 ± 5.7	162.9 ± 5.2	0.34
Weight (kg)	78.0 ± 16.7	77.0 ± 16.1	0.89
BMI (kg/m^2)	29.0 ± 5.7	29.0 ± 5.7	0.99
Resting SBP (mmHg)	129 ± 6	124 ± 17	0.45
Resting DBP (mmHg)	77 ± 9	68 ± 7	0.02*
Resting Heart Rate (beat.min^{-1})	69 ± 7	69 ± 13	0.49
Resting MCAv (cm/s)	51.4 ± 5.3	58.9 ± 6.8	0.07
VO_{2peak} (ml.kg.min)	22.4 ± 2.3	25.9 ± 3.0	0.02*
FMD (%)	5.1 ± 1.5	6.2 ± 2.7	0.29
Subjective frequency of hot flushes	58 ± 20	49 ± 38	0.56
Subjective severity of hot flushes (AU)	125 ± 49	81 ± 60	0.12

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MCAv, middle cerebral artery velocity; FMD, flow-mediated dilation; AU, arbitrary units. * Significant difference between groups.

4.3.2 Hot flushes

4.3.2.1 Subjective hot flushes:

Breast cancer patients undergoing endocrine therapy experienced 9 (-45, 26) fewer hot flushes per week compared to postmenopausal women but this did not reach statistical significance (*P* = 0.56). Likewise, breast cancer patients rated their hot flushes as 44 (-102, 13) AU lower compared to postmenopausal women (*P* = 0.12).

Objective hot flushes:

In total 32 objective hot flushes were recorded (11 endocrine mediated and 21 menopausal).

Hot flush duration: On average hot flushes were 139 (-205, -72) seconds shorter in breast cancer patients undergoing endocrine therapy compared to postmenopausal women (*P* < 0.001).

Hot flush haemodynamics: There was no main effect of time on MAP (*P* = 0.41). There was a group x time interaction in MAP (*P* < 0.001). MAP decreased by 4 mmHg in

postmenopausal women whilst in breast cancer patients increased by 4 mmHg throughout a hot flush (Table 4.2). MAP was on average 16 mmHg higher throughout the hot flush in breast cancer patients compared to postmenopausal women (main effect of group, $P < 0.001$). On average, HR increased by 6 (4, 8) beat.min^{-1} during a hot flush (main effect of time, $P = 0.04$, Table 4.2). There was no group x time interaction in HR during hot flushes ($P = 0.84$). HR on average was 9 (1, 20) beat.min^{-1} lower in breast cancer patients undergoing endocrine therapy compared to postmenopausal women (main effect of group, $P = 0.03$).

Table 4.2 Haemodynamic responses during hot flushes in breast cancer patients and postmenopausal women undergoing passive heating.

	Breast cancer					**Postmenopausal**					**Group main effect**	**Time main effect**	**Group x time interaction**
Variable	Baseline (2 min)	0%	50%	100%	Post (+2 min)	Baseline (2 min)	0%	50%	100%	Post (+2 min)			
MAP (mmHg)	87 (9)	88 (8)	88 (8)	91 (10)	87 (11)	74 (8)	70 (9)	70 (9)	71 (10)	73 (9)	***P* < 0.001**	*P* = 0.41	***P* < 0.001**
HR (beat.min^{-1})	70 (10)	69 (11)	72 (11)	72 (13)	72 (9)	80 (13)	84 (13)	87 (13)	82 (13)	81 (18)	***P* = 0.03**	***P* = 0.04**	*P* = 0.84
CBVC (cm s^{-1} mmHg^{-1})	0.73 (0.04)	0.70 (0.07)	0.67 (0.10)	0.66 (0.14)	0.68 (0.18)	0.65 (0.07)	0.62 (0.09)	0.60 (0.08)	0.62 (0.09)	0.64 (0.07)	***P* = 0.02**	*P* = 0.09	*P* = 0.21

Data are presented as mean (SD). Hot flushes were statistically analysed over eight segments, but are represented over three time segments (0, 50, 100%) above. MAP, mean arterial pressure; HR, heart rate; CBVC, cerebrovascular conductance; SR, sweat rate.

Cutaneous vascular conductance: There was a group x time interaction in the change in chest CVC_{max} from 2 minutes prior to a hot flush ($P < 0.001$) as the peak increase in chest CVC_{max} occurred later in breast cancer patients (at ~62.5% time point) compared to postmenopausal women (at ~12.5% time point). Overall, the change in chest CVC_{max} was 5% (-1, 11) lower during a hot flush in breast cancer patients compared to postmenopausal women (main effect of group, $P = 0.07$, Figure 4.1). There was a group x time interaction for change in forearm CVC_{max} ($P < 0.001$). The peak increase in forearm CVC_{max} occurred later in breast cancer patients (at ~62.5%time point) compared to postmenopausal women (at ~12.5% time point). There was no main effect of group in change in forearm CVC_{max} ($P = 0.14$). The change in chest and forearm CVC_{max} increased by 12% (7, 16) and 16% (11, 20) during a hot flush, respectively (main effect of time, $P < 0.001$, Figure 4.1).

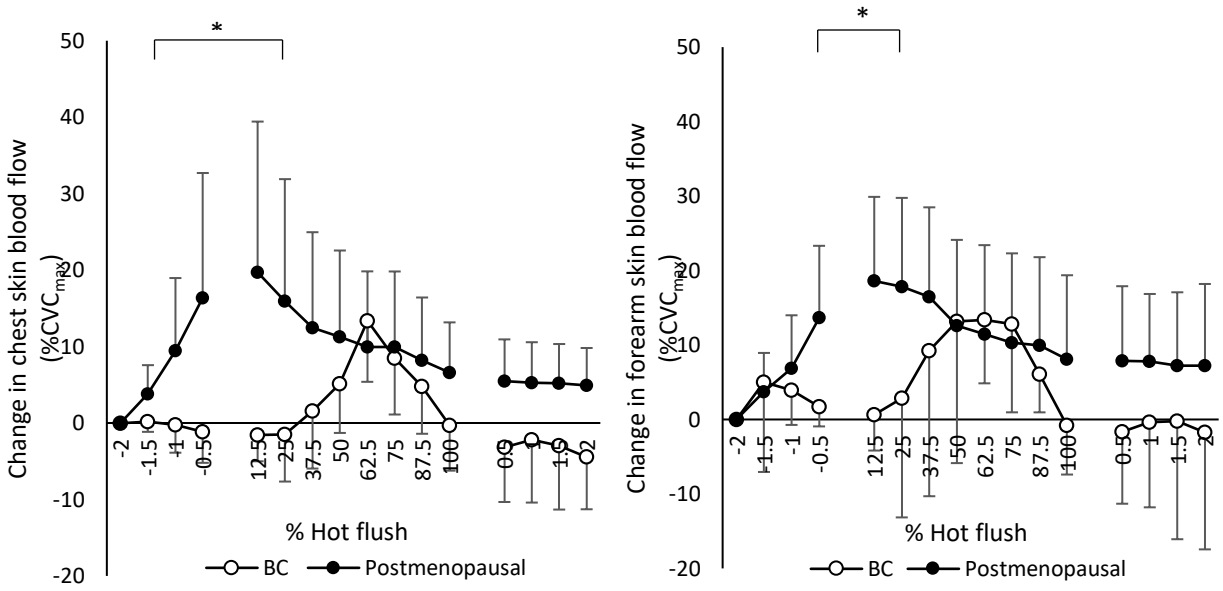


Figure 4.1 Changes in chest and forearm cutaneous vascular conductance during hot flushes in breast cancer patients undergoing endocrine therapy (n=5 hot flushes) and postmenopausal women (n=21 hot flushes). Error bars are SD. BC, breast cancer patients. *Significant difference between groups ($P < 0.05$).

Sweat rate: There was a significant main effect of group in chest and forearm sweat rate ($P < 0.05$; Figure 4.2), as well as a significant main effect of time ($P < 0.001$). There was a group x time interaction in the change in chest ($P = 0.004$) and forearm ($P < 0.001$) sweat rate compared to 2 minutes prior to a hot flush. Overall, the change in chest sweat rate was 0.05 (0.01, 0.09) $mg.cm^2.min^{-1}$ lower during a hot flush in breast cancer patients compared to postmenopausal women (Figure 4.2). Similarly, the change in forearm sweat rate was 0.04 (0.01, 0.08) $mg.cm^2.min^{-1}$ lower during a hot flush in breast cancer patients compared to postmenopausal women.

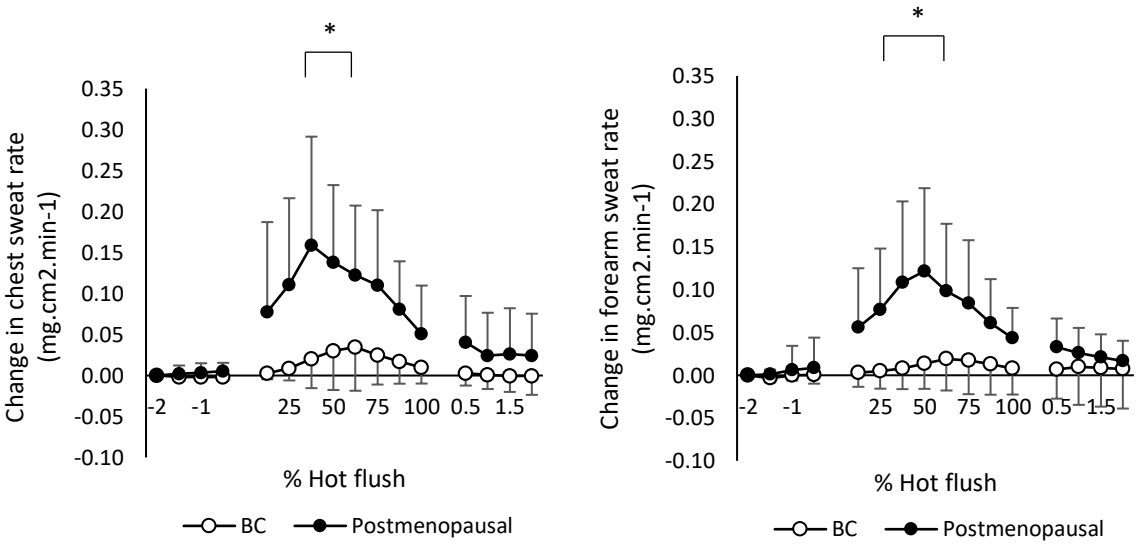


Figure 4.2 Changes in chest and forearm sweat rate during hot flushes in breast cancer patients undergoing endocrine therapy (n=5 hot flushes) and postmenopausal women (n=21 hot flushes). Error bars are SD. BC, breast cancer patients. *Significant difference between groups ($P < 0.05$).

Cerebral blood flow: There was a significant main effect of time as MCA*v* decreased by 0.06 cm/s^2 during a hot flush (P = 0.002). Yet, there was no main effect of group (P = 0.80, Figure 4.3). There was a group x time interaction in MCA*v* (P = 0.01), the overall decrease in MCA*v* was 2.52 cm/s^2 in breast cancer patients (-5.18, 1.4 cm/s^2) whilst the decrease was 2.94 cm/s^2 in postmenopausal women (-5.11, -0.77 cm/s^2). When expressed as CBVC, there was no significant group x time interaction (P = 0.21) or main effect of time (P = 0.09). Overall, CBVC was 0.07 cm/s^2 (0.01, 0.14) mmHg higher during a hot flush by in breast cancer patients compared to postmenopausal women (main effect of group, P = 0.02, Table 4.2).

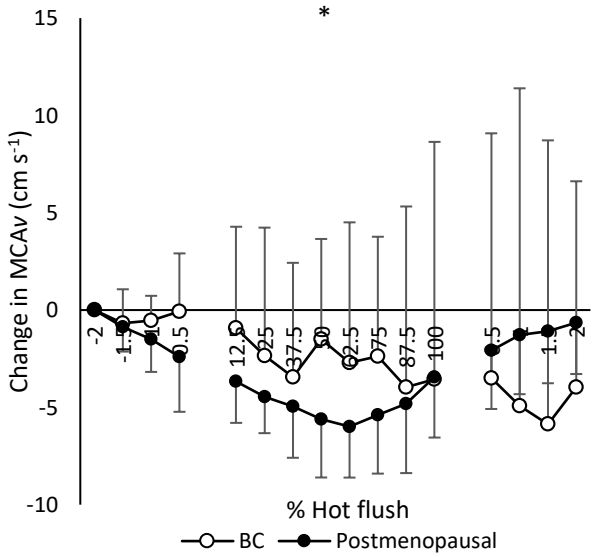


Figure 4.3 Changes in MCA*v* during hot flushes in breast cancer patients undergoing endocrine therapy (n=5 hot flushes) and postmenopausal women (n=21 hot flushes). Error bars are SD. BC, breast cancer patients; MCA*v*, middle cerebral artery velocity. *Significant difference between groups (P < 0.05).

4.3.3 Measurements during the heat stress challenge

Thermoregulation: Basal core body temperature (P = 0.69) resting skin blood flow at the chest (P = 0.81) and forearm (P = 0.83) were similar in breast cancer patients and postmenopausal women. Maximum cutaneous vascular conductance (CVC_{max}) at the chest

was 3.51 CVC_{max} higher in breast cancer patients undergoing endocrine therapy compared to postmenopausal women (P = 0.05), whilst at the forearm, CVC_{max} was 3.60 CVC_{max} lower in breast cancer patients compared to postmenopausal women (P = 0.04).

Cutaneous blood flow: The core temperature onset of chest (P = 0.42) and forearm (P = 0.39) cutaneous vasodilation as well as the rate of change in chest (P = 0.52) cutaneous vasodilation during passive heating were similar in breast cancer patients and postmenopausal women. The rate of change in forearm cutaneous vasodilation during passive heating was higher in breast cancer patients compared to postmenopausal women (4.15, 1.76 CVC/°C; 95%CI: 0.12, 4.67; P = 0.04).

Sweating: The core temperature onset of chest and forearm sweating were similar in breast cancer patients and postmenopausal women (P = 0.46; P = 0.56, respectively).

Table 4.3 Resting baseline data (mean ± SD) in breast cancer patients undergoing endocrine therapy (n = 7) and postmenopausal women (n = 16).

Variable	Breast cancer patients	Postmenopausal women	Mean difference	Main effect of group
Basal core body temperature (°C)	36.77 ± 0.64	36.87 ± 0.24	0.10	0.69
Basal chest CVC (AU/mmHg)	0.46 ± 0.34	0.49 ± 0.23	0.04	0.81
Basal forearm CVC (AU/mmHg)	0.27 ± 0.20	0.25 ± 0.10	0.02	0.83
Absolute chest vasodilatory threshold (°C)	36.83 ± 0.53	37.02 ± 0.27	0.18	0.42
Absolute forearm vasodilatory threshold (°C)	36.89 ± 0.50	37.07 ± 0.21	0.18	0.39
Rate of change in chest vasodilation (CVC.°C)	3.61 ± 2.37	2.90 ± 2.21	0.71	0.52
Rate of change in forearm vasodilation (CVC.°C)	4.15 ± 2.18	1.76 ± 1.01	2.39	**0.04**
Absolute chest sweating threshold (°C)	36.92 ± 0.60	37.11 ± 0.24	0.18	0.46
Absolute forearm sweating threshold (°C)	36.97 ± 0.58	37.11 ± 0.24	0.14	0.56

Data are presented as mean (SD). T_{core}, core temperature; CVC, cutaneous vascular conductance.

4.3.4 Cardiovascular Health

Cerebral blood flow: Basal MCA*v* was 7.5 (-0.82, 15.75) cm/s greater in breast cancer patients undergoing endocrine therapy compared to postmenopausal women (*P* = 0.07; Table 4.1). When accounting for BP, CBVC was 0.12 (-0.25, 0 cm/s^2) mmHg lower in breast cancer patients undergoing endocrine therapy versus postmenopausal women (*P* = 0.05).

Flow mediated dilation: Brachial artery FMD, time to peak diameter, peak response, shear rate area under curve and brachial artery diameter were similar in breast cancer patients undergoing endocrine therapy vs postmenopausal women ($P > 0.05$). Allometric scaling for baseline diameter did not alter results for the comparison of FMD between breast cancer patients undergoing endocrine therapy and postmenopausal women ($P = 0.48$).

4.4 Discussion

The primary aim of the current study was to compare the subjective and physiological severity of hot flushes between breast cancer patients undergoing endocrine therapy and postmenopausal women. Despite similar subjectively rated hot flush frequency and severity, breast cancer patients sweated less and had less pronounced skin and cerebral blood flow changes during a hot flush compared to postmenopausal women. Taken together, these data suggest that breast cancer patients undergoing endocrine therapy demonstrate less severe physiological hot flushes than postmenopausal women.

The current study suggests that breast cancer patients undergoing endocrine therapy experience less severe hot flushes when objective physiological severity is measured; contrary to previous studies employing sternal skin conductance monitoring in breast cancer (Carpenter et al, 2002; Carpenter, Monahan and Azzouz, 2004). Typically, subjective questionnaires are utilised to assess hot flush frequency and severity with severity reported by individuals dependent on their symptom-based interpretations of sweating, cutaneous vasodilation and dizziness (Sloan et al, 2001). Whilst, the subjective hot flush severity was not statistically different between breast cancer patients and post-menopausal women in the current study, subjective rating was lower in breast cancer patients, which broadly supports the physiological observations during a hot flush. It is acknowledged that the low

number of participants in this study is a limitation and possible explanation for the lack of significance in the subjective data collected. Together with the short duration of hot flushes in breast cancer, the physiological and subjective data indicate endocrine mediated hot flushes are less severe than post-menopausal hot flushes. Yet, the sensitivity of breast cancer patients to hot flushes could potentially be increased compared to postmenopausal women as it took a smaller physiological change in breast cancer patients to generate a similar subjective rating as postmenopausal women.

This is the first study to measure skin and cerebral blood flow responses during hot flushes alongside a measurement of sweating in breast cancer patients undergoing endocrine therapy. One noteworthy observation from the current study is that a small number of breast cancer patients did not demonstrate sweating during a hot flush. It is possible that breast cancer treatment e.g. chemotherapy, which can cause peripheral neuropathy, influences sweating responses during a hot flush or has a direct impact on the size and function of the sweat glands. Nevertheless, in the current study skin blood flow was also measured which is arguably a physiological response to the same signal (Johnson, Minson and Kellogg, 2014). Skin blood flow changes during a hot flush broadly show a similar temporal pattern to those observed in postmenopausal women (Low et al, 2008; Lucas et al, 2013; Bailey et al, 2016a; Bailey et al, 2016b), but the peak skin blood flow response during a hot flush occurred slightly later (i.e. in the middle of the flush rather than the beginning) in breast cancer patients undergoing endocrine therapy when compared to post-menopausal women. Typically, a hot flush is defined as an increase in skin blood flow prior to an increase in sweating (Low et al, 2008). It could be argued that the method utilised to identify the start of a hot flush contributed the temporal difference in the peak, as sweating above a certain threshold is the usual objective marker of a hot flush. In this

study, in the absence of sweating hot flushes were identified via other physiological variables including increases in skin blood flow and heart rate, decreases in MCAv and changes in MAP alongside any signal disruptions in ECG. Moreover, the overall magnitude of the change in skin blood flow was lower and the magnitude of the decline in cerebral blood flow was less in breast cancer patients undergoing endocrine therapy compared to postmenopausal women during a hot flush. Taken together, this data suggests that breast cancer patients undergoing endocrine therapy experience less skin reddening and dizziness during a hot flush and thus experience hot flushes of a reduced severity.

Thermoregulatory and vascular function were similar but VO_{2peak} and resting MCA*v* were higher in breast cancer patients compared to postmenopausal women. Whilst, the greater VO_{2peak} levels in breast cancer patients is surprising given that VO_{2peak} has been shown to be 24% lower in comparison to age- and sex-matched healthy controls (Jones et al, 2007); it is possible the breast cancer patients were indeed fitter or age/menopausal status was an influencing factor. The mean age of participants in both groups in the current study was similar, but there was a greater age range and both pre and post-menopausal women in the breast cancer group compared to postmenopausal women. Both fitness (Jackson et al, 2009) and cerebral blood flow (Stoquart-ElSankari et al, 2007) decline with age with large decrement after the menopause. It is possible that increased cardiorespiratory fitness levels found in breast cancer patients undergoing endocrine therapy in this study may be responsible for higher MCA*v* at rest compared to postmenopausal women as well as blunted physiological changes during hot flushes. However, it is not possible to separate the impact of the menopause on the current data set.

4.4.1 Conclusion

This study found that despite comparable subjective hot flush frequency and severity data in breast cancer patients undergoing endocrine therapy and postmenopausal women; breast cancer patients demonstrated smaller changes in sweat rate, skin and cerebral blood flow during an objectively measured hot flush. Thus, indicating that the physiological severity of endocrine therapy mediated hot flushes are less severe than postmenopausal hot flushes.

5 Can exercise training reduce the frequency and severity of hot flushes in breast cancer patients undergoing endocrine therapy?

5.1 Introduction

Endocrine therapy is prescribed to ER+ breast cancer patients after completion of 'active' cancer treatment. This type of breast cancer accounts for 80% of all cases. Endocrine therapy reduces the risk of breast cancer recurrence (Burstein et al, 2010). At least five years of endocrine therapy, with either tamoxifen or AIs, are recommended to ER+ breast cancer patients. Full compliance to tamoxifen reduces breast cancer mortality rate during the first 15-years post cancer treatment by at least one third (Early Breast Cancer Trialists' Collaborative, 2011). Likewise, AIs reduce breast cancer mortality by ~40% in the first decade of treatment (Early Breast Cancer Trialists' Collaborative, 2015).

Despite the importance of endocrine therapy on morbidity and mortality (Burstein et al, 2010), up to 90% of patients prescribed endocrine therapy discontinue or reduce their dosage due to the side effect of hot flushes (Murphy et al, 2012; Makubate et al, 2013). A hot flush is a sudden, intense sensation of heat causing skin reddening, flushing and profuse sweating (Low et al, 2008) corresponding to elevations in skin blood flow, sweating and reductions in cerebral blood flow. Hot flushes have a direct negative impact on a breast cancer patient's daily life and can cause anxiety, depression, sleep disturbances and chronic fatigue (Gupta et al, 2006). Targeting hot flushes in breast cancer patients undergoing endocrine therapy is essential to ensure women adhere to treatment guidelines as outlined by the NCRI Breast Cancer Symptom Management group and NICE guidelines for menopause (Julious, 2004; Morgan and Fenlon, 2013)

The usual pharmacological treatment for hot flushes is hormone replacement therapy (HRT) but this is contraindicated in breast cancer. Therefore, there is a clear requirement to identify alternative treatments to target hot flushes. Alternative non-pharmacological

treatments such as cognitive behavioural therapy (CBT) and exercise have been proposed to alleviate hot flushes in breast cancer. Duijts and colleagues (2012) found that 12 weeks of combined CBT and exercise had a positive effect on hot flush frequency in breast cancer patients. Exercise alone, was found to decrease hot flush frequency, though not significantly. This lack of change could be due to no participant exercise supervision and the intervention may not have been of a sufficient exercise intensity. Exercise training in post-menopausal women, without breast cancer, led to a decrease in subjective and objective hot flushes (Bailey et al, 2016a; Bailey et al, 2016b). The changes in hot flushes coincided with improved thermoregulatory efficiency and improved vascular function. Taken together, the impact exercise training in breast cancer patients undergoing endocrine therapy warrants further investigation.

The aim of the current study was to investigate if exercise training could reduce the subjective and objective frequency and severity of hot flushes in breast cancer patients undergoing endocrine therapy. In addition, a secondary aim was to assess changes in thermoregulatory and vascular function. It was hypothesised that 16-weeks of exercise training would reduce the subjective and physiological frequency and severity of hot flushes.

5.2 Methods

5.2.1 Participants

Eight breast cancer patients at least 3 months post active treatment were recruited from the Royal Liverpool and Broadgreen University Hospital Trust (Table 5.1). Individuals with uncontrolled or severe hypertension or diabetes were excluded from the study. Participants were taking tamoxifen (n=5) or AI (n=3) and were experiencing >4 hot flushes

per day. The study was approved by the local NHS research ethics committee and adhered to the Declaration of Helsinki (2000).

5.2.2 Research Design

Participants arrived at the laboratory following a 6-hour fast not having consumed alcohol for 24 hours and caffeine for 12 hours or performed strenuous exercise for 24 hours before each visit. Participants completed a Physical Activity Readiness Questionnaire (PAR-Q) and an ECG was performed. Flow mediated dilation and VO_{2peak} was assessed. Participants completed a subjective hot flush questionnaire for 7 days between visits. Six hours prior to visit 2, participants to consumed a thermometric pill to measure gut temperature as an index of core body temperature. Thermoregulatory responses were measured throughout a heat stress challenge. All measurements took place in a temperature-controlled laboratory (24 ± 1 °C).

Supervised exercise training intervention: Participants completed a 16-week supervised exercise-training program. Exercise training was performed at a moderate intensity (~45-70% HR_{max}), calculated through maximal HR reached during the participant's cardiorespiratory fitness test. Exercise training involved 30 minutes of moderate intensity exercise 3 times per week for the first 4 weeks. Exercise training included treadmill walking/running, cycling, cross-training and rowing. Every four weeks, the intensity and duration of the exercise increased gradually so that by week 12, the participant was exercising for 60 minutes per session 5 times per week. At least one exercise training session per week was under supervision at the university gym. A moderate-intensity program was used in line with previous studies that have shown improvements in cardiorespiratory fitness (Hodges et al, 2010; Pugh et al, 2013; Sprung et al, 2013; Bailey et

al, 2016a; Bailey et al, 2016b). Participants wore a heart rate monitor (Polar Fitness, Polar Electro Oy, Finland) during all exercise sessions to track HR and ensure that they are reaching their target HR. To track compliance, participants were provided with a Bluetooth HR monitor (Polar H10, Polar Electro, Warwick, England) to record HR data from each training session (PolarBeat v: 2.5.0, Polar Electro, Warwick, England).

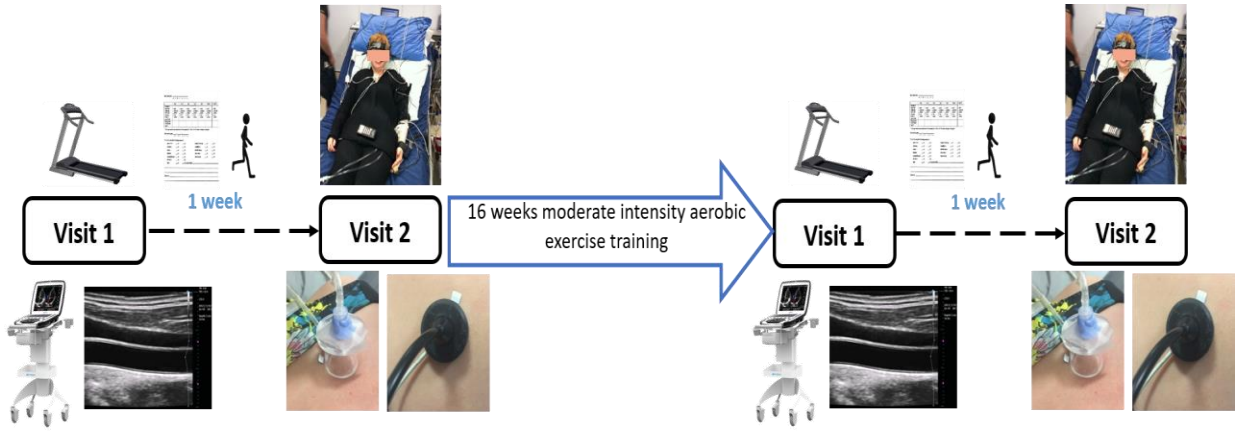


Figure 5.1 Schematic of research design.

5.2.3 Measurements

During visit one, anthropometric measurements were obtained along with brachial FMD, carotid IMT and VO_{2peak}, all described in chapter 3 (sections 3.2.2 and 3.2.3). A pre VO_{2peak} screening 12-lead ECG (Riding et al, 2014) was completed to ensure participants were safe to perform exercise. A subjective hot flush questionnaire (Sloan et al, 2001) was completed during this 7-day period between visits. Participants wore a tube-lined suit (Med-Eng, Ottawa, Canada) which covered the entire body except for the head, feet and right forearm. During a thermal heating test, MCAv, BP, HR, SV, CO, $P_{ET}CO_2$, LDF, CVC, SR, skin temperature and core temperature were measured as described previously (Chapter 3, section 3.2.4). Following whole body passive heating, local cutaneous heating was

performed at the chest and forearm using laser Doppler flowmetry, outlined in Chapter 3 (section 3.2.5).

5.2.4 Data Reduction

Data were extracted from LabChart during normothermia, passive heating and during a hot flush. In total 14 hot flushes were objectively measured in the laboratory. Onset thresholds for local sweating and cutaneous vasodilation and local sweating: mean body temperature and cutaneous vasodilation: core temperature slopes were established from this data (See general methods Chapter 3 section 3.3).

5.2.5 Statistical analysis

Analysis was conducted using the Statistical Package for Social Sciences (SPSS 23; Chicago, IL). Statistical significance was defined at $p < 0.05$ and exact p values are reported (p-values of "0.000" are reported as "< 0.001"). For comparison of all data from pre- to post-intervention a paired sample t-test was employed. Thermoregulatory and haemodynamic responses before, during and after a hot flush were evaluated using a two-way general linear model with main effects of time (16 levels) and pre/post intervention (2 levels). Data in the text are expressed as means and 95% confidence intervals, unless stated otherwise.

5.3 Results

5.3.1 Participants

Three of the women who were recruited dropped out of the study. Five participants were therefore measured pre and post intervention and adherence to exercise was 68%. Among the three women who did not continue, one dropped out due to having to discontinue endocrine therapy and two declined to return follow-up phone calls to ascertain the reasons for withdrawing.

Table 5.1 Participant characteristics (n=8)

Characteristic	Group
Age (years)	53 ± 8
Height (cm)	162.9 ± 5.2
Weight (kg)	77.0 ± 16.1
BMI (kg/m^2)	29.0 ± 5.6
Resting SBP (mmHg)	125 ± 18
Resting DBP (mmHg)	69 ± 8
Mean Arterial Pressure (mmHg)	89 ± 9
Medication type (#):	
Tamoxifen	5
Letrozole	2
Anastrozole	1

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MCAv, middle cerebral artery velocity; FMD, flow mediated dilation. * Significant difference between groups.

5.3.2 Hot flushes

5.3.2.1 Subjective hot flushes:

Subjective hot flush frequency was 40 (-21, 102) per week lower post exercise training compared to pre (*P* = 0.14, Figure 5.2). Similarly, subjective severity was 64 (-39, 166) AU lower post exercise training (*P* = 0.16, Figure 5.2).

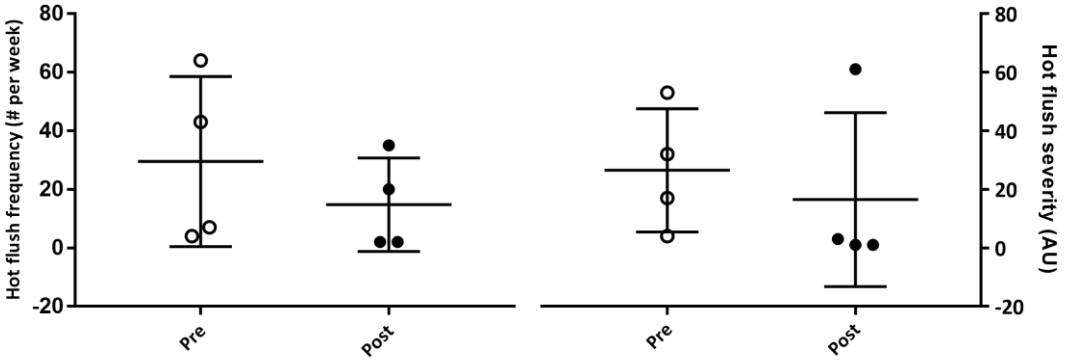


Figure 5.2 Subjective hot flush frequency (# per week) and severity (AU) pre and post exercise training (n=4, due to one participant failing to return post exercise intervention questionnaires). Thick line indicates mean, solid lines are SD, empty circle indicates individual pre data whilst filled circles indicate individual post data.

5.3.2.2 Objective hot flushes:

In total 8 hot flushes were objectively measured in the laboratory (5 pre and 3 post).

Hot flush duration: Hot flushes were 308 (-690, 74) seconds shorter pre training (103 ± 83 seconds) compared to post training (411 ± 176 seconds; $P = 0.08$).

Haemodynamics during hot flushes: HR increased by 7 beats min^{-1} during a hot flush ($P = 0.01$; Table 3.2). There was no significant main effect of intervention ($P = 0.60$) or intervention x time interaction ($P = 0.43$). There was no main effects of intervention, time or intervention x time interaction in MAP ($P > 0.05$, Table 5.2).

Table 5.2 Haemodynamic responses during hot flushes in breast cancer patients pre and post exercise training (n=5).

	Pre					Post					Group main effect	Time main effect	Group x time interaction
Variable	Baseline (2 min)	0%	50%	100%	Post (+2 min)	Baseline (2 min)	0%	50%	100%	Post (+2 min)			
MAP (mmHg)	90 (12)	85 (9)	83 (9)	88 (14)	89 (18)	95 (17)	91 (11)	88 (10)	95 (15)	92 (21)	*P* = 0.69	*P* = 0.20	*P* = 1.00
HR (beat.min^{-1})	66 (15)	69 (16)	70 (14)	71 (20)	75 (13)	77 (16)	79 (15)	83 (18)	84 (20)	79 (17)	*P* = 0.60	***P* = 0.01**	*P* = 0.43
CBVC (cm s^{-1} mmHg^{-1})	0.74 (0.06)	0.71 (0.07)	0.69 (0.10)	0.70 (0.11)	0.62 (0.30)	0.53 (0.02)	0.55 (0.10)	0.56 (0.09)	0.54 (0.11)	0.56 (0.06)	*P* = 0.11	*P* = 0.81	*P* = 0.99
Absolute chest SR (mg.cm^2.min^{-1})	0.19 (0.07)	0.19 (0.07)	0.22 (0.08)	0.20 (0.07)	0.19 (0.07)	0.26 (0.19)	0.27 (0.21)	0.41 (0.19)	0.34 (0.19)	0.30 (0.21)	*P* = 0.26	***P* < 0.001**	*P* = 0.06
Absolute forearm SR (mg.cm^2.min^{-1})	0.20 (0.07)	0.19 (0.08)	0.20 (0.09)	0.19 (0.09)	0.20 (0.12)	0.30 (0.37)	0.31 (0.29)	0.42 (0.30)	0.35 (0.31)	0.34 (0.32)	*P* = 0.17	***P* < 0.001**	***P* < 0.001**

Data are presented as mean (SD). Hot flushes were statistically analysed over eight segments, but are represented over three time segments (0, 50, 100%) above. MAP, mean arterial pressure; HR, heart rate; CBVC, cerebrovascular conductance; SR, sweat rate.

Cutaneous vascular conductance: There was a significant main effect of time for chest skin blood flow (CVC_{max}) compared to pre hot flush baseline (P = 0.002). Similarly, there was a significant main effect of time in forearm CVC_{max} (P < 0.001). Yet, there was no main effect of intervention or intervention x time interaction in chest or forearm CVC_{max} during hot flushes (P > 0.05, Figure 5.3).

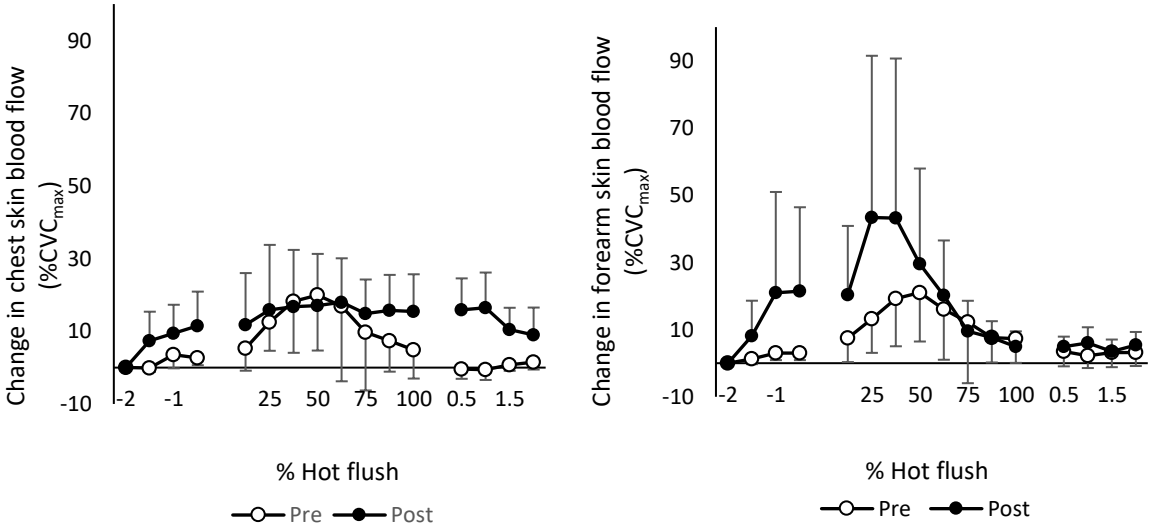


Figure 5.3 Changes in chest and forearm cutaneous vascular conductance during hot flushes in breast cancer patients pre (n=5 hot flushes) and post (n=3 hot flushes) exercise training. Error bars are SD.

Sweat rate: There was a significant main effect of time in the change in chest and forearm SR compared to prior to a hot flush ($P < 0.001$). The change in chest and forearm SR increased by 0.91 ± 0.04 and 0.64 ± 0.01 mg.cm^2.min^{-1} respectively during a hot flush. There was an intervention x time interaction in the change in forearm SR ($P < 0.001$), but not at the chest ($P = 0.23$). The change in forearm SR was greater post exercise training (0.09 mg.cm^2.min^{-1}) compared to pre (0.02 mg.cm^2.min^{-1}). There was no main effect of intervention in the change in chest or forearm SR during hot flushes ($P = 0.13$, Figure 5.4).

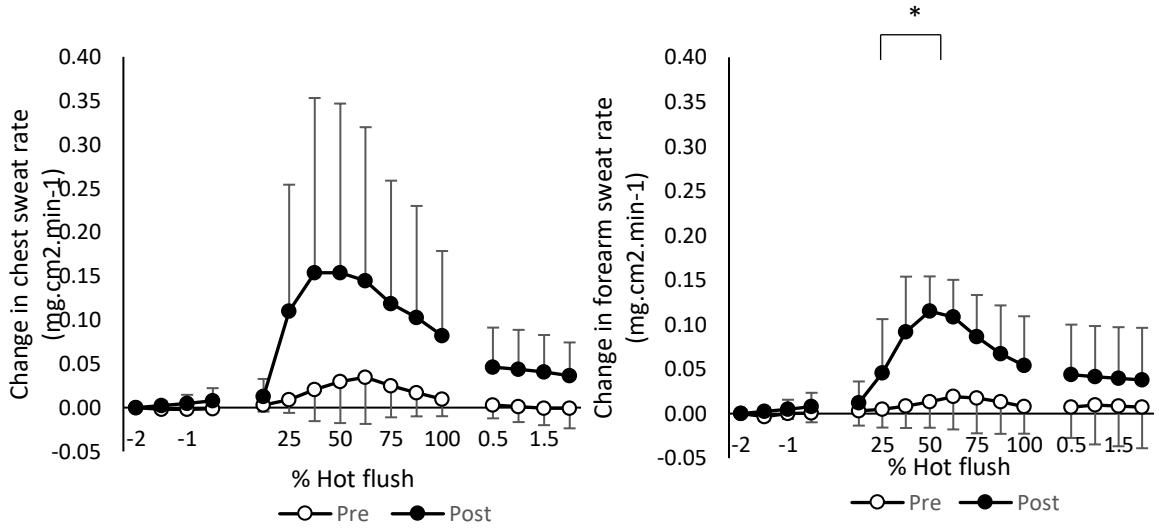


Figure 5.4 Changes in chest and forearm sweat rate during hot flushes in breast cancer patients pre (n=5 hot flushes) and post (n=3 hot flushes) exercise training. Error bars are SD.

Cerebral blood flow: There was no main effect for either intervention (*P* = 0.31) or time (*P* = 0.53) and no intervention x time interaction (*P* = 0.91) in MCA*v* during hot flushes (Figure 5.5). When expressed as CBVC, similar findings were evident (*P* > 0.05; Table 5.2).

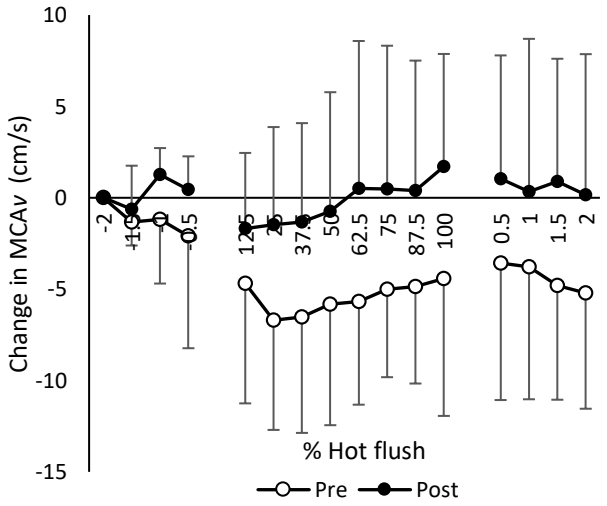


Figure 5.5 Changes in MCA*v* during hot flushes in breast cancer patients pre (n=5 hot flushes) and post (n=3 hot flushes) exercise training. MCA*v*, middle cerebral artery velocity. Error bars are SD.

5.3.3 Measurements during the heat stress challenge

Thermoregulation: Basal core body temperature (Table 5.3), resting skin blood flow (Table 5.3) or CVC_{max} at the chest and forearm did not change pre to post exercise training (*P* > 0.05).

Cutaneous blood flow: There were negligible changes in the core temperature onset for chest and forearm cutaneous vasodilation and the rate of change in chest and forearm cutaneous vasodilation with exercise training (*P* > 0.05, Table 5.3).

Sweating: There were negligible changes in mean body temperature onset for chest and forearm sweating and the rate of change in chest and forearm sweating with exercise training (*P* > 0.05, Table 5.3).

Table 5.3 Resting baseline data (mean ± SD) before and after exercise training (n=5).

Variable	Pre	Post	Change from pretraining	Main effect of intervention
Basal core body temperature (°C)	36.68 ± 0.69	36.84 ± 0.63	0.16	*P* = 0.72
Basal chest CVC (AU/mmHg)	0.49 ± 0.43	0.37 ± 0.28	-0.12	*P* = 0.62
Basal forearm CVC (AU/mmHg)	0.30 ± 0.23	0.30 ± 0.19	0.00	*P* = 1.00
Absolute chest vasodilatory threshold (°C)	36.79 ± 0.56	36.99 ± 0.53	0.20	*P* = 0.61
Absolute forearm vasodilatory threshold (°C)	36.87 ± 0.52	37.01 ± 0.53	0.12	*P* = 0.72
Rate of change in chest vasodilation (CVC.°C)	2.20 ± 0.96	3.62 ± 2.44	1.42	*P* = 0.45
Rate of change in forearm vasodilation (CVC.°C)	4.08 ± 1.04	2.96 ± 1.90	-1.12	*P* = 0.27
Absolute chest sweating threshold (°C)	36.92 ± 0.60	37.03 ± 0.39	0.18	*P* = 0.53
Absolute forearm sweating threshold (°C)	36.87 ± 0.63	37.00 ± 0.44	0.14	*P* = 0.74
Rate of change in chest sweating ($mg.min^{-1}.cm^2.°C$)	0.88 ± 0.40	0.36 ± 0.27	-0.52	*P* = 0.31
Rate of change in forearm sweating ($mg.min^{-1}.cm^2.°C$)	1.20 ± 0.23	0.51 ± 0.47	-0.69	*P* = 0.23

Data are presented as mean (SD). T_{core}, core temperature.

5.3.4 Measurements during local heating

Maximal skin blood flow at the chest and forearm did not change post exercise training ($P > 0.05$). Likewise, maximal CVC during local heating at the chest and forearm were unchanged after exercise training ($P > 0.05$).

5.3.5 Cardiovascular Health

Cerebral blood flow: Resting MCA*v* did not change with exercise training ($P = 0.27$). This finding remained after accounting for BP ($P = 0.31$).

Cardiorespiratory fitness and blood pressure: There were negligible differences in resting heart rate, systolic and diastolic BP ($P > 0.05$). VO_{2peak} increased by 0.9 (-4.32, 2.52) $ml.kg.min^{-1}$ after exercise training, yet this did not reach statistical significance ($P = 0.46$; Figure 5.6).

Flow mediated dilation: There were no changes in conduit brachial artery endothelial function FMD%, shear rate area under curve, brachial artery diameter and FMD peak diameter with exercise training ($P > 0.05$). No change in FMD% remained after allometric scaling was performed ($P = 0.57$). There was a reduction of 29 (17.64, 39.60) seconds in time to peak dilation with exercise training ($P = 0.002$).

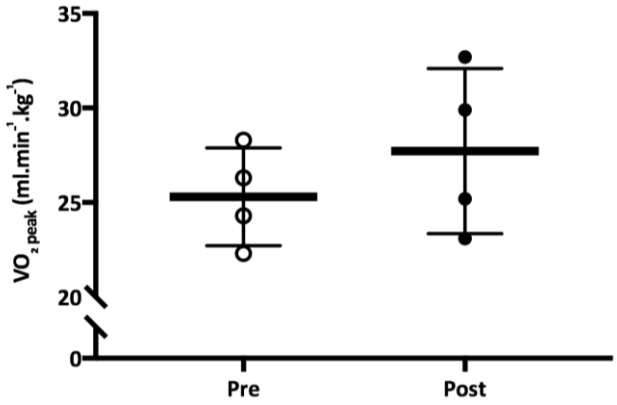


Figure 5.6 Cardiorespiratory fitness (VO_{2peak}) before and after exercise training (n=4, due to technical issues with one pre exercise intervention test). Thick line indicates mean, solid lines are SD, empty circle indicates individual pre data whilst filled circles indicate individual post data.

5.4 Discussion

The aim of the current study was to compare subjective and physiological frequency and severity of hot flushes in breast cancer patients undergoing endocrine therapy following a 16-week moderate-intensity exercise training intervention. The results of the study show that whilst exercise training reduced subjective hot flush frequency by 40 hot flushes per week and severity by 64 AU, this did not reach statistical significance. Moreover, the physiological responses during a hot flush including sweat rate, skin and cerebral blood flow were largely unchanged after exercise training. Taken together, these data suggest that exercise training in breast cancer patients undergoing endocrine therapy may not influence the frequency and severity of hot flushes.

The results suggest that the exercise training intervention caused a reduction in subjective hot flush frequency and severity, although not statistically significant, that could be practically meaningful (~ 40 flushes per week), suggesting that exercise may have some

benefit. Nevertheless, a physiological marker of a hot flush, skin blood flow, did not show meaningful reductions following exercise training. Yet, the increase in sweat rate after the exercise intervention may suggest an improved ability to dissipate heat and therefore reduce the severity of hot flushes. Likewise, there was some evidence of a positive cerebral blood flow change suggesting individuals experience less dizziness during hot flushes. These positive physiological changes in sweat rate and cerebral blood flow could potentially support the subjective data. Intriguingly, these findings in physiological measures in breast cancer patients undergoing endocrine therapy are in contrast to postmenopausal women (Bailey et al, 2016a), who demonstrated that not only did 16 weeks of moderate intensity exercise training mediate reductions in subjective hot flush severity but also mediated reductions in sweating, skin and cerebral blood flow during a hot flush. One potential explanation for the disparity in the objective markers and/or the findings from post-menopausal women is that breast cancer patients do not have as severe hot flushes and some hot flushes do not have a sweating response, potentially due to peripheral neuropathy (*study 1*). The increased sweat rate during a hot flush post exercise training in breast cancer patients may suggest an improvement in peripheral neuropathy symptoms. It is acknowledged that the findings of the current study are based on a small number of women and further research with a larger sample size is required to support these preliminary observations.

Another novel aspect of the current study was to investigate the thermoregulatory and vascular systems in response to exercise, given that these systems have been implicated in causal pathway for hot flushes (Bechlioulis et al, 2010; Freedman, 2014) and been shown to change with exercise training alongside alleviations in hot flushes in post-menopausal women (Bailey et al, 2016b). Nevertheless, there were negligible changes in sweat rate and

skin blood flow thresholds and sensitivities after 16 weeks of moderate intensity exercise training in breast cancer patients undergoing endocrine therapy. This could be linked to the lack of change in cardiorespiratory fitness in the current study; generally improvements in heat loss responses require an increase in cardiorespiratory fitness by 15-20% (Pandolf, Burse and Goldman, 1977). Breast cancer patients typically exhibit exercise intolerance as active cancer treatment can impact oxygen delivery, and utilisation (Jones et al, 2009). Nonetheless, results from *study 1* demonstrate that VO_{2peak} was similar in breast cancer patients compared to postmenopausal women and multiple studies have demonstrated increases in cardiorespiratory fitness in breast cancer patients after exercise training interventions (Nieman et al, 1995; Kolden et al, 2002; Courneya et al, 2003; Pinto et al, 2005). Although participants attended exercise training sessions at the university gym most weeks, adherence to sessions on own accord was low which might explain the lack of change in fitness in the current study.

Alternative explanations for exercise training not mediating improvements in skin blood flow during hot flushes, sweating and cutaneous vasodilatory thresholds or cardiorespiratory fitness could be related to time since beginning tamoxifen or AI. Mortimer *et al* (2008) found that hot flushes were more common in women who were closer to diagnosis and time since beginning tamoxifen treatment, whilst age and chemotherapy possessed no link. As no association between age and hot flush occurrence was reported in breast cancer patients, presumably menopausal status may also not be a key factor. On the other hand, the type of endocrine treatment may be associated with hot flushes. Breast cancer patients taking anastrozole have reported reduced hot flush occurrence compared to those taking tamoxifen (Howell et al, 2005). Thus, these differing mechanisms of action may influence hot flushes.

5.4.1 Conclusion

In summary, this study found some preliminary evidence of a positive impact on subjective severity of hot flushes with exercise training. Nevertheless, there is little evidence that a 16-week moderate intensity aerobic exercise training intervention reduces objective frequency and severity of hot flushes in breast cancer patients undergoing endocrine therapy. Whether these findings are explained by the endocrine therapy directly requires further investigation.

6 Research Synthesis

6.1 Aims and objectives

Prior to the research work completed within this thesis, there was little insight into the physiology of hot flushes in breast cancer patients undergoing endocrine therapy and/or non-pharmacological alternatives to alleviate symptoms. The studies provide new data regarding differences between postmenopausal and endocrine mediated hot flushes as well as contribute to understanding of the effect of exercise training on endocrine mediated hot flushes.

6.2 Major findings

- Endocrine therapy mediated hot flushes are physiologically less severe than postmenopausal hot flushes
- Exercise training had little impact of the frequency and severity of hot flushes in breast cancer patients undergoing endocrine therapy
- Thermoregulatory and vascular control, alongside cardiorespiratory fitness, may be improved after exercise training in breast cancer patients

6.3 Implications and future directions

6.3.1 Breast cancer patients undergoing endocrine therapy demonstrate smaller changes in sweat rate, skin and cerebral blood flow, thus physiological severity, during a hot flush compared to postmenopausal women.

The reduced physiological severity of hot flushes in breast cancer patients compared to postmenopausal women found in *study 1* suggests that endocrine therapy may not prompt more severe hot flushes in these individuals. Although speculative, it could be possible that the breast cancer patients recruited in this thesis may possess a reduced sensitivity to a hot flush stimulus and therefore they may perceive hot flushes as less severe compared to

postmenopausal women. Further research recording subjective severity of physiologically measured hot flushes should be conducted. Education should be provided to both healthcare professionals and patients regarding the differences in endocrine mediated and menopausal hot flushes. How to deal with the differences needs to be explored in more detail.

6.3.2 A number of hot flushes demonstrated no change in sweating

Intriguingly, a number of endocrine therapy mediated hot flushes in this thesis demonstrated no change in sweat rate. Typically, a hot flush is defined as an increase in skin blood flow prior to an increase in sweating (Low et al, 2008). Figure 6.1 depicts an example of a hot flush in a breast cancer patient undergoing endocrine therapy with no change in sweat rate. This hot flush was identified via a transient increase in skin blood flow, decrease in cerebral blood flow, change in blood pressure and disruption to the ECG signal. The absence of sweating could suggest that peripheral mechanisms that initiate sweating are incapable of responding to stimuli in these individuals and therefore limit heat dissipation and/or sweat glands function differently in breast cancer patients in comparison to postmenopausal women. Participants could potentially report these hot flushes as more severe as they are unable to dissipate the heat. The subjective severity of hot flushes was not recorded during physiological measurements in this study so this hypothesis cannot be examined with the current data set. In accordance, data found in *chapter 4* may suggest that hot flushes in breast cancer patients undergoing endocrine therapy may be mediated differently compared to postmenopausal women. This could suggest that different interventions may be required to demonstrate improvements in hot flushes in the differing population (discussed below).

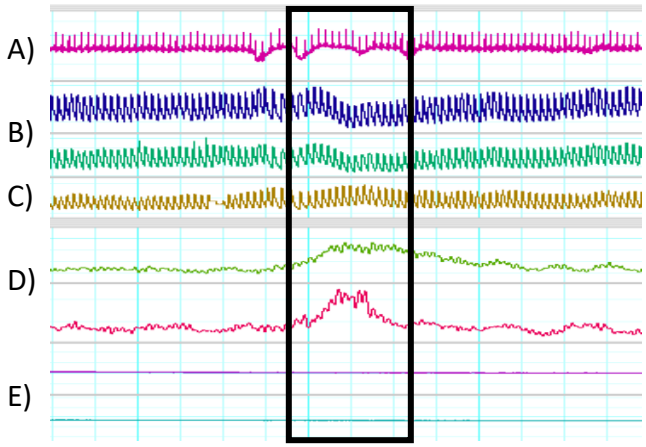


Figure 6.1 LabChart output during a hot flush A) pink trace is ECG B) blue and dark green trace is cerebral blood flow C) gold trace is blood pressure D) light green and red trace are chest and forearm skin blood flow, respectively E) purple and blue trace are chest and forearm sweat rate, respectively. Black box specifies the hot flush.

6.3.3 Hot flushes in breast cancer patients undergoing endocrine therapy and postmenopausal women may be mechanistically different.

Endocrine therapy *per se* could alter mechanisms mediating hot flushes, and thus be responsible for the physiological differences between endocrine mediated and menopausal hot flushes seen in *chapter 4*. Due to postmenopausal women experiencing more subjectively and physiologically severe hot flushes and possessing a reduced cardiorespiratory fitness in *chapter 4*, it could be postulated that this group would exhibit an increased amount of vascular and thermoregulatory dysfunction in comparison to breast cancer patients undergoing endocrine therapy. Yet, no differences in these variables were present. Endocrine therapy may therefore mediate hot flushes via alternative mechanisms, such as altering neural drive and autonomic function. Studies investigating these mechanisms require further attention including alternative interventions for example warm water immersion and/or sauna training may mediate thermoregulatory and

vascular benefits via different mechanisms than exercise training (Green et al, 2010; Bailey et al, 2016c).

6.3.4 Exercise training demonstrated little impact on physiological hot flush severity in breast cancer patients undergoing endocrine therapy

This could be due to the lack of change in cardiorespiratory fitness. Postmenopausal women increased VO_{2peak} by 4.5 $ml.kg^{-1}.min^{-1}$ after 16 weeks of aerobic exercise training (Bailey et al, 2016a; Bailey et al, 2016b) whilst breast cancer patients showed a 0.9 $ml.kg^{-1}.min^{-1}$ increase in *chapter 5*. These results could suggest that an increased cardiorespiratory fitness is beneficial for thermoregulatory function and consequently reduces the severity of hot flushes. It is proposed that an improvement in VO_{2peak} of 15-20% is required to mediate beneficial thermoregulatory adaptations in premenopausal women (Pandolf, 1979; Ichinose et al, 2009). Adherence to the exercise training intervention in *chapter 5* was poor in breast cancer patients (68%). Higher adherence to exercise training by participants could be encouraged by supervision of all exercise sessions and/or group exercise sessions. It is plausible to assume that greater changes in cardiorespiratory fitness could occur by higher adherence to exercise which in turn may induce thermoregulatory adaptations in breast cancer patients undergoing endocrine therapy. Nevertheless, *chapter 5* demonstrated that the subjective hot flush frequency and severity was reduced post intervention. Hot flush frequency and severity was 77% and 83% respectively, lower after exercise training compared to pre-exercise. Although not statistically significant such a reduction could significantly improve quality of life, consequently be clinically relevant, and provide evidence for exercise training as a non-

pharmacological intervention recommended for management of hot flushes in breast cancer patients undergoing endocrine therapy. Conversely, the subjective changes could be a result of a 'placebo effect' that is common for hot flush studies (Cancer Research UK, 2015), whereby patients feel better after performing an intervention that they think will help. A larger randomised control trial is required to confirm these findings.

6.4 Methodological considerations

Strengths: Subjective hot flush severity was rated using a questionnaire and objective physiological measurements of hot flush severity were recorded that were independent of bias and perceptions. The physiological measures utilised in the current study may provide a more accurate representation of objective hot flushes in breast cancer patients. Nevertheless, it is recognised that physiologically measured hot flushes in a laboratory may not provide comparable results to hot flushes in everyday life.

Limitations: During acute hot flush assessment using physiological measures in a laboratory, subjective severity of hot flushes was not reported. Additionally, in *chapter 5,* a small number of participants was likely insufficient to generalise findings to all breast cancer patients undergoing endocrine therapy. *Chapter 5* also lacked a no-exercise control group. Thus, confirmation of these findings is required using a larger randomised controlled trial is warranted.

6.5 Recommendations for future research

Multiple potential areas of future research have emerged from the data reported in this thesis.

1. A fully powered randomised control trial of exercise training compared to a no-exercise control in breast cancer patients undergoing endocrine therapy.

2. An investigation into the impact of autonomic function and hot flush perception in breast cancer patients undergoing endocrine therapy compared to post-menopausal women.
3. A randomised control trial of water immersion/sauna training compared to conventional care in breast cancer patients undergoing endocrine therapy.

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