Effects of radiation on cutaneous microvascular function in the intact human circulation

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Abstract

Radiation treatment for cancer is associated with an increased risk of cardiovascular disease. Animal studies report decreases in endothelial vasodilator function in radiated arteries, however, there remains limited knowledge about the intact human circulation. Therefore, the aim of the present study was to determine the acute and chronic effects of radiation therapy on cutaneous microvascular reactivity in breast cancer patients. METHODS: The present study utilized a cross-sectional study design in 7 breast cancer patients receiving radiation therapy and 13 survivors > 3 years post-radiation therapy. Cutaneous microvascular reactivity to acetylcholine (ACh) was evaluated following radiation exposure at the site of radiation treatment and at a contralateral control non-radiated site. Red blood cell flux was measured as an index of cutaneous blood flow via laser Doppler flowmetry with ACh-mediated vasodilation determined by iontophoresis drug delivery. Cutaneous vascular conductance (CVC) was calculated by normalizing for mean arterial pressure. RESULTS: %CVC to incremental ACh was significantly attenuated in the radiated compared to non-radiated tissue following a total cumulative dose of 2104±236 cGy within the patients currently receiving radiation. In the survivors (6475±70 cGy) there were no differences between radiated and non-radiated tissue. However, the peak %CVC in the cancer survivors was significantly lower compared to the non-radiated tissue of the cancer patients. CONCLUSION: This study demonstrates that radiation for cancer treatment attenuate cutaneous microvascular function, which could have long-term implications for cardiovascular health.

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Chapter 1 - Introduction

Over 50% of cancer patients receive radiation treatment, which has significantly contributed to increased cancer survival rates (Delaney et. al. 2005). However, secondary to decreasing cancer recurrence, radiation treatment has been shown to increase significantly the risk of adverse cardiovascular outcomes, particularly coronary artery disease, myocardial infarction, and hypertension in patients who received thoracic or chest wall radiation therapy (Paszat et. al. 2007, Darby et.al. 2013, Clarke et. al. 2005). As such, Clarke et. al. (2005) reports that there is a > 25% increase in the risk of cardiac deaths within radiated cancer patients, which highlights the importance of understanding the pathophysiology of radiation-induced toxicity within the cardiovascular system.

Consequent to the increased cardiovascular disease risk, radiation exposure elicits numerous adverse vascular consequences, particularly vascular endothelial injury (Darby et. al. 2013, Seddon et al. 2002), which may potentiate the development of early atherosclerotic cardiovascular disease (Venkatesulu et. al. 2018, Medonca et. al. 2011). Previous in vivo/vitro animal studies have shown a significantly decreased endothelial-dependent relaxation in radiated arteries within days to weeks following exposure (Menendez et. al. 1998, Qi et. al. 1998, Maynard et. al. 1992), with work in human cancer survivors demonstrating decreases in large artery vascular function years after radiation treatment (Beckman et. al. 2001, Sugihara et. al. 1999). Specifically, Beckman et. al. (2001) investigated the flow-mediated endothelial-dependent vasodilation responses in the axillary artery of breast cancer survivors who had a prior history of unilateral radiation therapy. They observed a significant attenuation in vasodilatory responses within radiated arteries when compared with the contralateral, non-radiated arteries.

These findings compliment work from Sugihara et. al. (1999) that demonstrated, in vitro, a decreased endothelial-dependent vasodilation in cervical arteries taken from cancer survivors exposed to radiation. These initial studies in axillary and cervical arteries provide valuable insight into endothelial dysfunction elicited by radiation therapy in cancer patients. However, there is a paucity in our understanding regarding the pathophysiology of radiation-induced endothelial dysfunction in the early radiation treatment process, particularly within the microcirculation. This is a critical knowledge gap given decreases in microvascular function occur early in the progression of numerous cardiovascular and metabolic diseases (Fernlund et. al. 2015, Patik et. al. 2015). As such, the evaluation of microvascular function in patients actively receiving radiation therapy will provide valuable insight into the adverse cardiovascular adaptations that occur with radiation exposure. Given the prolonged effects of radiation therapy mentioned above, it is critical to understand microvascular function years after radiation exposure. Therefore, the primary aim of this study was to determine the acute and chronic effects of localized radiation treatment on cutaneous microvascular function in breast cancer patients. We hypothesized that the endothelial-dependent vasodilatory response to acetylcholine (ACh) would be blunted within radiated tissue when compared to the contralateral, non-radiated tissue in breast cancer patients. We chose to evaluate cutaneous microvascular function as it has previously been used to better understand the pathophysiological role of vascular dysfunction in heart failure, atherosclerosis, coronary artery diseases, peripheral vascular diseases, type 2 diabetes, and chemotherapy-induced cardiotoxicity (Sutterfield et. al. 2018, Cui et. al. 2005, Farkas et. al. 2004, Heitzer et.al. 2001, Rossi et al. 2004, Verma et. al. 2003, Walther et. al. 2015). In addition, the skin receives the highest doses of radiation during treatment and, therefore, is more susceptible to radiation-induced injury (Jaschke et. al. 2017).

Chapter 2 - Methods

Participants

Seven women currently receiving unilateral chest adjunctive radiation therapy for breast cancer and thirteen women whom were cancer survivors that received similar unilateral adjunctive radiation therapy for breast cancer participated in the study. Cancer diagnosis, site of radiation treatment, dosage, and duration were confirmed by each patient's treating oncologist and radiologist. Patients were excluded from this study if they had known atherosclerotic cardiovascular disease. All procedures were approved by the Institutional Review Board of Kansas State University and conformed to the standards set by the Declaration of Helsinki. Written informed consent was obtained from all patients prior to participation in this study.

Experimental Procedure

All tests were performed in a controlled temperature room (21-23°C) after a 5-10 minute period of supine rest. Patients remained in the supine position throughout all testing procedures. Cutaneous microvascular reactivity of the upper portion of both the radiated and contralateral non-radiated chest was determined with integrated laser Doppler flowmeter (PeriFlux 5010 laser-Doppler perfusion monitor; Perimed, Jarfalla, Sweden), combined with iontophoresis of ACh. This system allows for continuous measurements of cutaneous red blood cell flux, which was used as an index of cutaneous blood flow (CBF) and was calibrated according to the manufacturers' speciation with Brownian motility standard solution (Perimed). In each patient, the iontophoresis drug delivery probe (Perimed: PF 383) with an integrated laser Doppler probe

and temperature regulator was placed over the 3rd or 4th intercostal space along the mid-clavicular line of both the radiated tissue and contralateral tissue, approximately 15cm away from the conductive hydrogel drug dispersive electrode (Perimed: PF 384). The temperature regulator located around the perimeter of the probe maintained local skin temperature of 33°C throughout the duration of the test. Following a 1-minute baseline, a 200 µL of a 2% ACh solution was delivered in seven successive 20-second iontophoresis doses within a 60 second interval. A 5-minute recovery was recorded after the seventh dose to ensure the peak vasodilatory response was reached. This delivery protocol is consistent with our previous work and has been shown to elicit no detectable current-induced axon-mediated vasodilation (Loader et. al. 2017). Intensity, duration, and intervals of the current delivery were controlled by a USB power supply (PF 751, Perimed, Järfälla, Sweden) connected to both the drug dispersive and drug delivery probes was managed and confirmed with the systems software (PeriIont Software; Perimed). Throughout the test beat-by-beat blood pressure was continuously measured throughout each visit via photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands). Unless restricted by lymph node dissection or lymphedema, blood pressure was performed on the right side at heart level.

Data Analysis

Cutaneous blood flow was recorded at 100 Hz by data acquisition software (DI-720, DATAQ Instruments, Akron, OH, USA) and reported in arbitrary perfusion units (PU). Baseline averages were calculated over a 1-minute rest interval for CBF and MAP. Cutaneous blood flow responses to ACh were binned in 10-second averages, from which peak values were identified

for each ACh iontophoresis delivery dose. To normalize for MAP, cutaneous vascular conductance (CVC, PU/mmHg) was calculated as: (PU/MAP) x 100. The relative change in CVC from baseline to each peak dose response was calculated as: [(peak-baseline CVC)/baseline CVC] x 100.

Statistical Analysis

Statistical analyses were performed using a commercially available software package (Prism 8, GraphPad Software, San Diego, CA, USA). Pair differences between radiated and non-radiated tissues were determined by independent samples t-tests. Peak CVC responses were compared between patients with 2-way repeated ANOVA. All data are presented as mean ± SE, unless otherwise stated. Statistical significance was declared at $P < 0.05$.

Chapter 3 - Results

Seven postmenopausal women scheduled to receive daily, unilateral radiation treatment for breast cancer were enrolled in the study. Participants were aged 65.7 ± 5.1 years, had a resting MAP of 99.4 ± 8.2 mmHg, and body mass index of 28.4 ± 5.7 kg/m^2. The average fractional dose of radiation received was 209 ± 9 cGy, with the average cumulative dose of radiation at the time of the study was 2104 ± 236 cGy. Five patients received radiation to the left breast. Three patients had received chemotherapy prior to the initiation of radiation treatment. No patients were currently taking Tamoxifen, one was taking Anastrozole, two were on statins and three were on hypertension medication at the time of the study.

Resting CVC in the cancer patients was 11.0 ± 4.1 PU/mmHg and 10.7 ± 2.9 PU/mmHg for radiated and non-radiated tissue, respectively (P = 0.96). A significantly lower endothelial-dependent vasodilation was observed at each dose of ACh administration within the radiated tissue when compared to non-radiated contralateral tissue in breast cancer patients currently receiving radiation therapy (P=0.03) (Figure 1), with the peak %CVC in response to ACh being significantly lower in radiated tissue compared to non-radiated (radiated: 506 ± 167 %, non-radiated: 983 ± 223 %; P = 0.02).

A second cohort of thirteen postmenopausal women with a history of unilateral radiation therapy for breast cancer were also enrolled. Participants were aged 55.9 ± 10.7 years, had a resting MAP of 94.0 ± 7.8 mmHg, and body mass index of 31.0 ± 6.7 kg/m^2. The average length of time between last radiation treatment and enrollment was 6.9 ± 1.2 years. The average

cumulative dose of radiation in the cancer survivors was 6475 ± 70 cGy. Nine cancer survivors had received radiation to the left breast. Twelve survivors had received chemotherapy. Seven survivors were currently taking Tamoxifen, three were taking Anastrozole, three on statins and six on hypertension medication at the time of the study. Three survivors' radiation total dosage and drug information were unavailable.

Resting CVC in the cancer survivors was 9.2 ± 1.5 PU/mmHg and 11.4 ± 3.4 PU/mmHg for radiated and non-radiated tissue, respectively (P = 0.50). No difference in endothelial-dependent vasodilation to ACh was observed within the radiated tissue when compared to non-radiated contralateral tissue (P = 0.4) (Figure 2). Similarly, with the peak %CVC in response to ACh was similar between radiated tissue and non-radiated tissue (radiated: 506 ± 76 %, non-radiated: 558 ± 81 %; P = 0.25). To evaluate the acute and chronic effects of radiation treatment, the peak %CVC response for the cancer patients and cancer survivors were compared (Figure 3). The peak %CVC was lower in the radiated and non-radiated tissue of the cancer survivors compared to the non-radiated tissue of the current cancer patients. There were no differences between peak %CVC in the radiated tissue of the current cancer patients and that observed in either the radiated or non-radiated tissues of the cancer survivors.

Figure 1

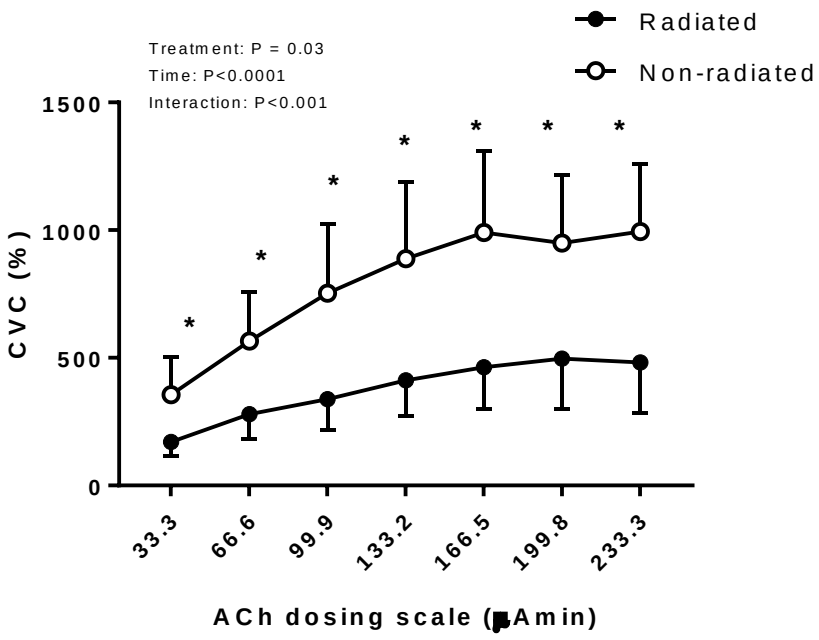

Figure 1 - Relative increase in cutaneous vascular conductance (%CVC) in response to incremental ACh iontophoresis doses in cancer patients currently receiving radiation therapy. %CVC was significantly lower in the radiated tissue compared to non-radiated tissue. * Denotes significantly different vs. non-radiated. Values are mean±SE.

Figure 2

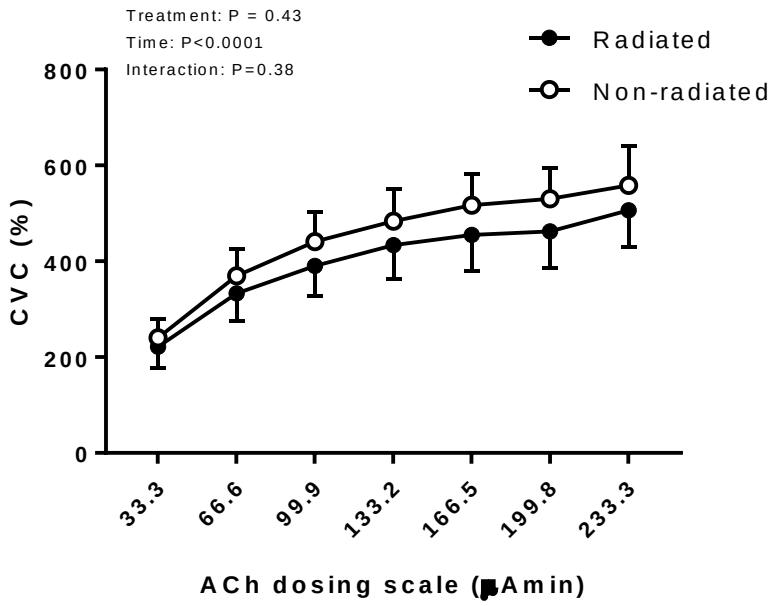

Figure 2 - Relative increase in cutaneous vascular conductance (%CVC) in response to incremental ACh iontophoresis doses in cancer survivors with a history of radiation therapy. %CVC was not significantly different between radiated and non-radiated tissue. Values are mean±SE.

Figure 3

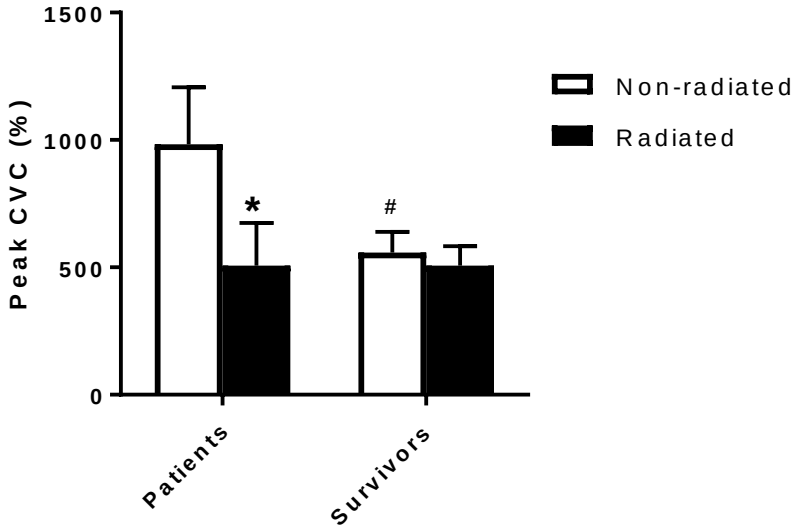

Figure 3 - Peak increase in cutaneous vascular conductance (%CVC) in response to acetylcholine. Note the significantly lower response in the non-radiated and radiated tissue of the cancer survivors compared to the non-radiated tissue in cancer patients. # denotes significantly different compared to patients. * Denotes significantly different vs. non-radiated. Values are mean±SE.

Chapter 4 - Discussion

The major finding in the present study was that cutaneous microvascular endothelial-dependent vasoreactivity was lower in radiated tissue when compared to non-radiated tissue in breast cancer patients. Specifically, dose-dependent decreases in ACh-mediated vasodilation were observed within radiated tissue compared with contralateral, non-radiated tissue. These finding suggest that radiation has a negative impact on microvascular endothelial-dependent vasoreactivity, which may contribute to an increased risk of adverse cardiovascular events as previously suggested (Darby et. al. 2013, Suddon et. al. 2002, Hooning et. al. 2007). To the best of our knowledge, this is the first study investigating the effects of radiation on cutaneous microvasculature within human cancer patients currently undergoing radiation therapy.

The present study demonstrates significant decreases in cutaneous endothelial function during radiation therapy. Given the established early role for microvascular dysfunction in the progression of numerous cardiovascular and metabolic disease (Fernlund et. al. 2015, Patik et. al. 2015, and Verma et. al. 2003), these findings provided added insight into the changes in cardiovascular health associated with radiation therapy. While the regulation of the cutaneous circulation is somewhat different compared to other vascular bed, previous work has proposed it as a model of generalized microvascular function (Holowatz et. al. 2008), given that it is correlated with coronary vascular function (Khan et. al. 2008) and has been used to predict cardiac events in coronary artery disease patients (Heitzer et. al. 2001). This work, therefore, is clinically significant given reports of accelerated atherosclerosis, leading to severe coronary artery disease, following chest radiotherapy (Cheng et. al. 2017; Lee et. al. 2013; Hull et. al. 2003).

In addition to the development of arterial disease, radiation therapy significantly compromises wound healing, which may be due, in part, to changes in endothelial function (Haubner et. al. 2012; Jacobson et. al. 2017). Lee et al. (1999) has previously demonstrated a key role for eNOS in wound repair. In eNOS knockout mice, excisional wound closure and incisional wound strength were significantly impaired compared to wild-type mice. In addition, they demonstrated that eNOS facilitated growth factor-stimulated angiogenesis. Given, that ACh-mediated vasodilation in the cutaneous circulation is mediated, in part, by eNOS production of NO, the findings of the present study suggest that impaired wound healing may occur early in radiation treatment. These findings are consistent with reports of decreased NO expression in wounds of animals exposed to 12 and 24 Gy radiation (Schaffer et. al. 2002). As such, assessment of the cutaneous microcirculation during and following radiation treatment may provide valuable insight into a patient's risk of wound healing complications.

Our group and others have used laser Doppler flowmetry with iontophoretic administration of ACh to evaluate microvascular endothelial reactivity in response to cancer therapies and a variety of cardiovascular disease (Medow et. al. 2005, Rodriguez-Miguelez et. al. 2016, Walther et. al. 2015). Administration of exogenous ACh causes an endothelial-dependent vasodilation in the skin primarily through the actions of nitric oxide (NO) and prostaglandins (Kellogg et. al. 2005, Medow et. al. 2008, Khan et. al. 1997, Noon et. al. 1998, Holowatz et. al. 2005), with reports of some contributions of endothelial-derived hyperpolarizing factor (EDHF) (Brunt et. al. 2015). Kellogg et al. (2005) demonstrated that both NO- and prostaglandin-dependent pathways are used in the vasodilatory response to ACh. Conversely, Holowatz et. al.

(2005) suggest that ACh mediates cutaneous vasodilation via primarily prostaglandin and non-NO-dependent pathways. These authors observed no differences in peak vasodilation during ACh administration following administration of eNOS inhibitors, but was significantly reduced when cyclooxygenase (COX) was inhibited. Thus, when ACh binds to muscarinic receptors on the surface of endothelial cells it activates not only G-proteins, resulting in the NOS pathway, in which L-arginine is converted to NO, but also stimulates the production of prostaglandins. While the relative contribution of these factors remains somewhat contested, the primary mechanism for ACh- mediated cutaneous vasodilation is via endothelial-dependent factors. Therefore, our data demonstrates a significant decrease in endothelial-dependent cutaneous microvascular vasodilation, with further work needed to more completely elucidate the exact signaling pathways impacted.

Radiation is believed to decrease vasoreactivity due to the increased superoxide (O_2^-) production that is caused from the radiation induced cellular damage, and thus attenuates the eNOS pathway by O_2^- blinding to NO, creating peroxynitrate ($ONOO^-$), decreasing NO bioavailability, and ultimately decreasing the vasoreactivity response (Baselet et.al. 2018). A decrease in ACh-induced NO-mediated vasodilation has been shown in rats receiving radiation (Hatoum et. al. 2006). Additionally, a decrease in eNOS expression in radiated arteries has been observed by Sugihara et. al. (1999) within human cervical arteries radiated with an average of 4790 cGy when compared to non-radiated control vessels. Similarly, Qi et. al. (1998) observed a significantly decreased, but not eliminated, expression of eNOS in rabbit ear arteries following 4500 cGy irradiation when compared to non-radiated arteries at both 1 and 4 weeks post radiation (~69 % and ~70% decreased relative to control, respectively). Complimenting these

finding, Holler et. al. (2009) observed a decreases in eNOS expression following both 1000 cGy and 4500 cGy doses of radiation in mice. These findings support a significant decreases in eNOS at both low and high therapeutic radiation doses, which may contribute to the decreased ACh-mediated vasodilation observed in the present study. There is, however, a potential to restore eNOS function as Holler et. al. (2009) investigated the effects of pravastatin on endothelial function. They observed a restoration in eNOS levels within 4500 cGy radiated tissue when administered pravastatin compared to 4500 cGy radiation alone. The expression of eNOS within the radiated tissue combined with pravastatin expressed an average of 11.8 positive vessels per field, whereas radiation only expressed 5.6 positive vessels per field. Additionally, they found a decrease in eNOS levels at 3, 7, and 14 days post 1000 cGY radiation when compared to radiated treated with pravastatin using western blot analysis. This suggests the potential of restoring eNOS expression in radiated tissues. In addition to reductions in eNOS expression, prostacyclin (PGI_2) expression has been shown to be blunted within radiated cells when compared to non-radiated, bystander cells (Allen et. al. 1981). However, EDHF pathway in cutaneous tissue is thought to be relatively resistant to radiation (Soloviev et. al. 2003). These findings in total suggest a that the reduction in vascular vasodilator capacity is a consequence of decreases in NO and PGI_2 dependent pathways, and that the EDHF allows for some maintenance of vasodilator function following radiation exposure.

Previous research in animals exposed to high and low radiation levels have demonstrated significant decreases in endothelial dependent vascular function (Qi et. al. 1998, Soloviev et. al. 2003, Maynard et. al. 1992, Hatoum et. al. 2006, On et. al. 1998, Menendez et. al. 1998). Hatoum et al. (2006) removed submucosal intestinal arteries from radiated rats, and

demonstrated an attenuated response to ACh-mediated vasodilation following the second fractional dose of 250 cGy. Importantly, they also observed high levels of superoxide within the radiated vessels when compared to those of the controls. These increased superoxide levels were observed at each radiation dose. In agreement with these findings, On et. al. (1998) found ACh-induced relaxation of the thoracic aorta was significantly decreased in radiated rats (1000 cGy) when compared to healthy controls, but was prevented when rats were supplemented with the antioxidant vitamin C. This prevention of radiation induced endothelial dysfunction by pretreatment with vitamin C supports the role of oxidative damage as a potential underlying mechanism.

Currently there is limited information of the effects of therapeutic levels of radiation on endothelial dependent vascular health in the human population. Sugihara et. al. (1999) examined ACh-mediated relaxation in cervical arteries taken from the neck region of cancer patients who received on average 4790 cGy of radiation therapy. The maximum ACh-mediated vasodilatory response was significantly diminished (~70%) in radiated arteries compared with controls. In addition, Beckman et. al. (2001) evaluated the effects of radiation treatment on the flow-mediated vasodilator response in radiated axillary arteries in breast cancer survivors who received radiation therapy at least 3 years prior to the study. They showed a diminished flow-mediated dilation when compared to contralateral non-radiated axillary arteries and arteries of healthy age-matched controls. Similar to the work of Beckman et.al. (2001) we compared radiated and non-radiated vasculature within the same patient, to minimize the effects of variable confounders between patients. Our findings expand on this early work by demonstrating an attenuated ACh-mediated endothelial-dependent vasodilation within the radiated cutaneous

microvasculature. Thus, our work in combination with the previous findings of Beckman et. al. (2001) and Sugihara et. al. (1999), demonstrate that radiation induces significant decreases in endothelial-dependent micro- and macrovascular function, that based on the present study occurs very early in the radiation treatment process.

Long term effects of radiation treatment on endothelial-dependent vasoreactivity have previously been demonstrated in animal models. Maynard et. al. (1992) investigated the relaxant responses within rabbit ear arteries at 6 weeks post a single dose of 4500 cGy radiation, which is equivalent to ~3.75 human years (Andreollo et. al. 2012). These authors demonstrated that ACh-mediated endothelial-dependent relaxation remained significantly decreased when compared to age-matched controls despite the extended duration post-radiation. Similarly, Qi et. al. (1998) found that percent relaxation to ACh was significantly blunted in rabbit ear arteries after 6 and 10 weeks post 4500 cGy irradiation. Complementing these findings, Menendez et. al. (1998) explored the acute and chronic effects of radiation on ACh mediated dilation in the rodent abdominal artery. They observed that abdominal vessels extracted at 6 months post radiation showed significant decreases in ACh mediated relaxation compared to nonradiated thoracic vessels of the same animal. This response was similar to that observed in animals investigated 72 hr post radiation, highlighting the potential long-term effects of radiation on endothelial-dependent relaxation. In humans receiving radiation, Beckman et. al. (2001) is the only study to our knowledge that has investigated the effects of radiation therapy on the axillary artery in breast cancer survivors with an average length of 12 ± 6 years post radiation. Using flow mediated dilation of the axillary artery, radiated arteries showed a significantly decreased percent vasodilation when compared to contralateral, non-radiated axillary arteries and matched healthy

controls, ultimately decreasing endothelial function. Important to the interpretation of their study, only nine of the sixteen (~56%) of the breast cancer patients studied had a history of cancer chemotherapy treatment. This is an important point, given that previous studies have shown decreased vasoreactivity in patients undergoing chemotherapy. Patients currently treated with Paclitaxel chemotherapy demonstrated a reduction in flow mediated dilation when compared to controls (Vassilakopoulou et. al. 2010). Similarly, Sutterfield et. al. (2018) observed a decreased response in flow mediated dilation within the cancer patients receiving chemotherapy when compared to matched controls. Additionally, cutaneous vascular conductance was attenuated within the cancer patients when compared to controls, demonstrating an impairment in microvascular function. Previous work in cancer survivors 24.6 ± 4.8 years post chemotherapy demonstrated a significantly lower flow mediated dilation response when compared to healthy controls (Dengel et. al. 2008). In the present study, twelve of the thirteen cancer survivors (~92%) had previously received chemotherapy. Therefore, the overall decreased response in the cancer survivors at the radiated and non-radiated tissues is likely attributed to the systemic vascular effects of chemotherapy.

There are several experimental considerations regarding this study. First, some of the patients in the present study also received chemotherapy, which has previously been shown to decrease cutaneous microvascular function (Sutterfield et. al. 2018). Since the effects of chemotherapy are systemic and patients served as their own control, this allowed these patients to be included in the study. Second, the population measured in this study was strictly female breast cancer patients, therefore these results are not representative of cancer patient population in its entirety. Radiation treatment is the most commonly used therapeutic intervention for men

diagnosed with prostate cancer and therefore, may experience similar decreases in cutaneous microvascular function (Michaelson et. al. 2008, Arcangeli et. al. 2012). Third, iontophoresis of ACh, can elicit a nonspecific axon reflex vasodilation. However, we utilized previously reported low current density delivery protocols, which eliminates the impact of nonspecific axon reflexes (Sutterfield et. al. 2018, Rodriguez-Miguelez et. al. 2016, Walther et. al. 2015 Hamdy et. al. 2001). Lastly, we were unable to heat the skin to evaluate cutaneous microvascular vasodilation, which is primarily NO dependent (Kellogg et. al. 2005), due to the increased risk of injury associated with heating the radiated skin.

In conclusion, the present study demonstrates that endothelial-dependent vasoreactivity within microvascular beds is attenuated in breast cancer patients currently receiving radiation therapy. There was no change between the radiated and non-radiated tissues in the cancer survivors, however an attenuated response was observed within the non-radiated tissue of the cancer survivors when compared to patients currently receiving radiation. Taken together these findings suggest that patients undergoing radiation therapy experience decreased vasoreactivity within radiated tissue which may contribute to the increased long-term risk of cardiovascular disease morbidity and mortality.

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