DEVELOPMENT OF A LYMPHATIC STRESS TEST

by

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ABSTRACT

There is a need for a test that will provide information on the lymphatic system of the upper extremity in women with breast cancer related lymphedema (BCRL) and this forms the basis of this research project. In order to determine an appropriate upper body exercise protocol that could be combined with lymphoscintigraphy to describe lymphatic function in women treated for breast cancer, a series of studies were completed in healthy, college-aged females. Collectively, these studies demonstrated the following: 1) arm cranking was superior to intermittent hand grip exercise in enhancing depot clearance rate (CR), 2) moderate intensity arm cranking exercise was superior to low intensity arm cranking exercise in enhancing CR and uptake of radiopharmaceuticals at the axillary lymph nodes (AX), 3) CR and AX were symmetrical between arms, 4) a lower coefficient of variation was demonstrated with moderate intensity exercise compared to low intensity exercise, and 5) high intra-subject reliability in arm cranking CR. Thus, an intermittent, moderate intensity arm cranking protocol was selected to evaluate lymphatic function in women treated for breast cancer with BCRL (BCRL), breast cancer survivors (no BCRL), and age-matched controls. Main findings of the final project were: 1) contralateral arm lymphatic function was similar between groups at rest or during exercise; 2) AX and uptake of radiopharmaceuticals in the forearm (FORE) in the ipsilateral arm relative to the contralateral arm was compromised in BCRL only; and 3) the addition of exercise increased ipsilateral CR and AX in breast cancer survivors and increased ipsilateral CR and FORE in BCRL subjects (an increase in FORE is not expected with exercise). These results indicate that despite the small sample size, lymphatic function (with the exception of CR) is impaired in BCRL at rest and during exercise compared to breast cancer survivors and controls but not different between breast cancer survivors and controls. Clinical applications of this study are that while breast cancer survivors have similar lymphatic function as controls, there is a highly variable response suggesting that some breast cancer survivors may be at risk for developing BCRL.

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AC	arm cranking
APP	time of first radiopharmaceutical appearance at the axillary lymph nodes
AX	uptake of radiopharmaceuticals at axillary lymph nodes 65 minutes post-injection relative to the initial dose
BC	women treated for breast cancer without lymphedema
BCRL	breast cancer related lymphedema
CONT	control subjects
CR	clearance rate
FORE	uptake of radiopharmaceuticals in the forearm 65 minutes post-injection relative to the initial dose
HG	handgrip exercise
HI	high intensity protocol
HIgG	Human Immunoglobulin G
LO	low intensity protocol
^{99m}-Tc	Technetium-99m
VEGF	vascular endothelial growth factor
W	Watts

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- The thesis author was responsible for developing the research question, performing the literature review, and writing and preparing the manuscript for publication. Dr. Worsley provided expertise on the section related to lymphoscintigraphy. Dr. McKenzie assisted in developing the research question and editing the manuscript.

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CHAPTER ONE Literature Review and Introduction to Thesis

1.1 LITERATURE REVIEW[A]

1.1.1 Introduction

The lymphatic system, as it relates to exercise, is not a physiological system that receives a great deal of attention in the sports medicine literature. It is not often heard that patients are limited by their lymphatic system when performing a job task, recreational activity, or sport. However, breast cancer survivors were once cautioned by healthcare professionals to avoid participating in vigorous, upper-body exercise for fear of causing lymphedema (1). Breast cancer related lymphedema (BCRL) is a chronic swelling that can occur in the ipsilateral hand or arm of breast cancer survivors but has also been reported in the chest wall and breast. As defined by Olszewski (2), lymphedema is an accumulation of water, proteins, migrating immune cells, erythrocytes, biochemical products of immigrating immune cells and resident cells, and debris of apoptotic cells within the expanded intracellular space and lymphatics. It is essentially an incurable condition, although treatments exist to contain swelling. Current research has not supported the notion that there is a relationship between vigorous, upper-body exercise and the occurrence of BCRL (3-8). In fact, there is the possibility that exercise may be useful in the prevention and treatment of BCRL, but further investigation is required to see what physiological changes may be taking place at the tissue level in the affected arm in response to short- and long-term exercise.

This review summarises the current research on the lymphatic system related to exercise and critically evaluates the implications for exercise performance by survivors of breast cancer. A brief synopsis of the relevant anatomy and physiology of the lymphatic system applicable to the topic of exercise and breast cancer is also included. Aukland and Reed (9) and Schmid-Shönbein (10) provide thorough reviews of the anatomy and physiology of the lymphatic system.

[A] Parts of this Chapter have been previously published: **Lane, K.**, Worsley, D., & McKenzie, D. Exercise and the lymphatic system: Implications for breast cancer survivors. *Sports Medicine,* 2005; 35 (6): 461-471

1.1.2 Anatomy and Physiology of the Lymphatic System

The lymphatic system is a one-way transport system composed of lymphatic vessels and lymphoid organs. The lymphatic vessels carry interstitial fluid, plasma proteins, lymphocytes, cytokines, macrophages, and other cellular debris that have leaked into the interstitial space back to the cardiovascular system. The lymphoid organs including the bone marrow, thymus, lymph nodes, spleen, and tonsils each function to produce, maintain and distribute lymphocytes. There are also specialized lymphatic capillaries, called lacteals, in the villi of the intestinal mucosa to assist in fat absorption. Thus, essential functions of the lymphatic system include assisting in the regulation of tissue volume and pressure, aiding immune system function, and contributing to fat absorption in the intestine (11). For the purposes of this article, only the structures involved in lymph transport will be discussed.

Two continuous segments of vessels make up the lymphatic system: (i) the initial lymphatics, also known as lymphatic capillaries; and (ii) the contractile lymphatics. Lymphatic vessels vary between individuals in their exact location and length and this variation may account for why only some breast cancer survivors develop BCRL. The initial lymphatics, which are blind-ending vessels that originate in tissue parenchyma, are found in close proximity to vascular arterioles (12). They have a single layer of endothelial cells with overlapping regions that form one-way valves (9). These one-way endothelial valves, also referred to as primary valves (12), allow interstitial fluid to enter into lymphatic capillaries but prevent lymph from escaping. Evidence for the endothelial valves was provided by Trzewik and colleagues (13) who injected fluorescent microspheres into the cremaster muscle interstitium of rats. Through a variety of experiments, the researchers demonstrated that the microspheres could pass into the lymphatic lumen but could not be forced out. Other anatomical features of the initial lymphatics include anchoring filaments that attach the vessels to collagen fibres in the extracellular matrix, an absence of smooth muscle cells, and intralymphatic (or secondary) valves that may or may not be present (10).

Lymph formation occurs when interstitial substances (i.e., interstitial fluid, proteins, cytokines, lymphocytes, and colloids) enter lymphatic capillaries through the primary valves. Once lymph enters the initial lymphatics, it then travels to the larger, contractile lymphatics that unlike the lymphatic capillaries, are usually present outside of organ parenchyma and have smooth muscle that undergoes spontaneous, peristaltic contractions. Contraction of the smooth muscle lining lymphatic vessels is initiated by pacemaker cells (14) but can also respond to sympathetic activation (9, 15). Contractile lymphatics also have intra-lymphatic valves that prevent the backflow of lymph and ensure lymph moves in the proximal direction (12). It is possible that failure of the intra-lymphatic valves may contribute to the development of BCRL.

Lymph traveling through contractile lymphatic is filtered by clusters of lymph nodes found throughout the body. Lymph nodes relevant to BCRL are found in the axilla and have been classified as levels I–III (16). If any of the lymph nodes throughout the body detect a foreign substance, then specialized lymphoid cells (T-lymphocytes, B-lymphocytes, and macrophages) function to destroy the foreign cells, and if necessary, activate an immune response (17). Damage to lymph nodes and the lymphatic vessels serving the lymph nodes, which can occur as part of breast cancer treatment (i.e. lymph node excision and/or irradiation), contribute to the development of BCRL.

Lymph re-enters the cardiovascular system by way of the central thoracic ducts that drain into the subclavian veins. There is also the possibility that lymph can enter the venous system by way of lymphaticovenous communications (16). These communications are thought to exist in normal, healthy individuals but may not be functional unless there is chronic obstruction of lymphatic vessels or nodes (18). If the lymphatic system were under chronic stress due to obstruction, then the opening of lymphaticovenous communications would allow lymph to bypass the obstructed area. This was demonstrated in early research by Threefoot et al. (19) who used a dog model with the major lymphatic channels obstructed for >5 days to demonstrate the presence of lymphaticovenous communications. The presence and functioning of lymphaticovenous communications may play a role in the development of BCRL.

Mechanisms of Lymph Formation and Transport

Starling's equation describes the forces affecting fluid flux between the microcirculation and the interstitium: $J_{MV} = K_{FC}\ [(P_{MV}-P_{INT}) - \sigma\ (\pi_{MV}-\pi_{INT})]$ – where J_{MV} represents the net microvascular filtration, K_{FC} is the microvascular filtration coefficient, σ is the osmotic pressure of all plasma proteins, P_{MV} and P_{INT} are the hydrostatic pressures of the microvasculature and interstitium, respectively, and π_{MV} and π_{INT} are osmotic pressure of the microvasculature and interstitium, respectively (20). Thus, net microvascular filtration reflects a balance between filtration and absorption. At rest, in individuals without lymphatic dysfunction, the balance lies in favour of absorption, and the accumulation of fluid and substances in the interstitial space is opposed by the osmotic pressure gradient and lymph formation.

Much of what is known about the mechanisms of lymph formation and transport in humans has been derived from animal research. However, caution should be taken when interpreting animal research since variations in the length of lymphatic vessels, tissue volumes, gravitational forces, and hydrostatic pressures between animals and humans have the potential to affect lymph contractility and flow (14, 21).

Unlike the cardiovascular system, the lymphatic system lacks an organ to act as a pump and move lymph from the initial lymphatics to the contractile lymphatics and finally to the cardiovascular system. Pressures within initial lymphatics are near zero and significantly higher in the great veins of the neck/thorax (22); thus, the lymphatic system also has to overcome this pressure gradient. In order to transport lymph from the interstitial space to the venous system, the lymphatic system relies on both intrinsic and extrinsic forces. Extrinsic mechanisms include active and passive limb movements, pressure changes associated with respiration, and the pulse of nearby arteries. Intrinsic mechanisms, on the other hand, involve spontaneous, intermittent contraction of the smooth muscle in the contractile lymphatic vessels. Autoregulation of these spontaneous contractions by nerves and circulating vasocactive substances modulates the frequency and force of contractions (9, 10, 15, 22).

Both lymph formation and lymph propulsion within the initial lymphatics are dependent on extrinsic driving forces since the initial lymphatic vessels do not have smooth muscle media. Mazzoni et al. (23) investigated lymph formation in the spinotrapezius muscle of rats fixed *in situ* in the stretched and contracted states. Stretched skeletal muscle pulled on connective tissue joined to lymphatic vessels and opened the initial lymphatics permitting interstitial fluid to enter. Contracted skeletal muscle, on the other hand, increased muscle fibre cross-sectional area, which compressed both adjoining connective tissue and the lymphatics resulting in lymph being driven in the proximal direction to collecting vessels. Although the work of Mazzoni and colleagues is specific to subfascial lymph formation, skeletal muscle contractions also influence epifascial lymph formation. Similar to the pressure changes associated with respiration and vasomotion of nearby arterioles, skeletal muscle contractions cause fluctuations in tissue pressure that in turn influences interstitial fluid pressure (22, 24). As interstitial fluid pressure rises, the anchoring filaments exert radial tension on the lymphatic capillary, thereby increasing the volume of the lumen and pulling open the endothelial microvalves allowing interstitial fluid and molecules to enter (25).

Lymph propulsion from the initial lymphatics to contractile lymphatics relies on oscillations in tissue pressure from extrinsic forces to expand and compress the initial lymphatics. Also, the presence of both endothelial microvalves and intralymphatic valves ensures that lymph moves proximally to the larger contractile vessels and does not leak out of the initial lymphatic vessel (12). The compression of an initial lymphatic vessel due to a skeletal muscle contraction, for example, forces open the intralymphatic valve and closes the endothelial microvalves resulting in lymph movement in the proximal direction toward the contractile lymphatics.

Once lymph reaches the contractile lymphatics, then intrinsic forces are believed to be the main factor responsible for lymph flow at rest in humans (15). For example, Olszewski and Engeset (21) measured lymph pressure, pulse frequency and lymph flow in the legs of healthy males. Pulse frequency increased during free lymph flow conditions when subjects changed from the recumbent to upright position. This increase was thought to be a result of an increase in pressure exerted on the foot tissues, which would compress the initial lymphatics and force lymph to the larger contractile vessels. As lymph accumulates in the contractile lymphatics, the vessel wall is stretched thereby triggering a reflexive contraction of the smooth muscle endothelium.

Subjects in this study (21) also contracted the foot while in both the recumbent and upright positions. There was no measurable change in flow between pulse waves during foot movement indicating that intrinsic mechanisms were the main factor responsible for propelling lymph through the lymphatic system. Also, neither a significant change in intralymphatic pressures during obstructed flow and free-flow conditions, nor a change in pulse amplitude was recorded. Pulse frequency and mean lymph flow, on the other hand, were observed to significantly rise. The increase in mean lymph flow during foot contractions in both the recumbent and upright positions suggests that the increase in lymph formation occurred due to an increase in capillary filtration and oscillations in tissue pressure as a result of muscle contractions. The increase in lymph formation coupled with repeated foot contractions would also contribute to the higher pulse frequency as described earlier in this section.

The frequency and force of the spontaneous, intermittent contractions of the smooth muscle in the contractile lymphatic vessels is modulated by neural factors and circulating vasoactive substances (15, 22, 26). For example, epinephrine (adrenaline) and norepinephrine (noradrenaline) infusion has been shown to increase the frequency and force of lymphatic pumping in sheep (27, 28). Epinephrine may cause an increase in lymph flow by either acting directly on the lymphatic vessels, reflexively triggering the autonomic nervous system, or increasing blood flow to the region drained by the lymphatic vessels (27). However, not all vascular beds undergo vasodilation in response to sympathetic activation. While capillary filtration can be reduced in some areas, such as the gastrointestinal tract as a result of sympathetic activation, it can be concomitantly increased in other areas, such as active skeletal muscle.

1.1.3 Measurement of Lymphatic Function

The most direct method of measuring lymph flow is direct lymphography which involves infusing oily contrast into cannulated lymphatic collectors in distal limbs (22). This method has been used in animals (29-32), and to a lesser extent in humans (21, 33, 34), to investigate the effects of exercise on lymph flow. However, this procedure in humans is less practical as it is invasive; rather, lymphoscintigraphy is performed to give a semi-quantitative measure of lymph flow. Lymphoscintigraphy typically involves administration of a radiopharmaceutical to a lymphatic drainage source and imaging the limb using a gamma (γ) camera at various time points over one to five hours post-injection. The resulting images can be used to determine objective measures of lymphatic function such as the clearance of radiopharmaceutical from the site of injection (clearance rate, or CR), time taken for the tracer to reach the axillary lymph nodes, and percentage of tracer in the axillary lymph nodes or regions of the upper extremity at a fixed time point post-injection (35-37). The radiopharmaceutical is generally a technetium labeled macromolecule that is of sufficient size (i.e. between 4 and 100nm) to enter the lymphatic capillaries and not blood capillaries (36, 37). Several studies have used massage at the injection site or light exercise such as fist clenches or squeezing a rubber ball with the intent of enhancing radiopharmaceutical clearance (37-42).

Despite lymphoscintigraphy being a standard nuclear medicine technique used to investigate lymphatic obstructions, differences in protocols exist which makes the direct comparison of findings difficult and complicates the understanding of the pathophysiology of BCRL. For example, there are differences in particulate administered (i.e., ^{99m}Tc-antimony colloid, ^{99m}Tc-HIgG, or ^{99m}Tc-human serum albumin (HSA), etc.) which can affect the speed of uptake by lymphatic vessels and also retention within lymph nodes. ^{99m}Tc-HSA is preferred for evaluating lymphatic vessels, whereas colloids such as ^{99m}Tc-antimony colloid are better at assessing lymph nodes due to their longer retention time (36). Other differences in lymphoscintigraphy protocols include intradermal, subcutaneous, or subfascial injections; injections administered in the webspace, forearm, or upper arm; various outcome variables such as depot clearance rate, uptake at the axillary lymph nodes relative to the amount initially injected, systemic blood accumulation rate, and/or lymphatic vessel morphology; and exercise such as fist clenching or arm cranking sometimes being included as part of the lymphoscintigraphy protocol to stimulate lymph flow between imaging scans.

In an attempt to determine an optimal lymphoscintigraphic method to image upper extremity lymphatic vessels, O'Mahoney et al. (43) compared intradermal and subcutaneous injections of ^{99m}Tc-nanocolloid and ^{99m}Tc-HIgG in healthy subjects. When only the injection route was compared (intradermal vs. subcutaneous), clearance rate was significantly faster and image quality superior after intradermal injections compared to subcutaneous injections. When only the radiopharmaceuticals were compared, there was no significant difference in image quality or clearance rate. Slight differences were observed in image quality between the two radiopharmaceuticals for each injection route; however, only three or six subjects were compared in those analyses. Based on better image quality, a faster clearance rate, and several other factors, the authors hypothesized that intradermal injections more closely replicate a direct intralymphatic injection. However, the same lymphatic bed was still accessed after both types of injections which is the ultimate goal of lymphoscintigraphy applied to women with BCRL. It was not stated whether subjects performed any type of exercise in between imaging scans to facilitate lymph flow. O'Mahoney et al. repeated the above study in women with BCRL and found similar results - lymphatic vessels were more clearly visualized in subjects with BCRL after intradermal compared to subcutaneous injections of ^{99m}Tc-HIgG (44). However, CRs were not significantly different between the two radiopharmaceuticals.

Typically, lymphoscintigraphy involves a single isotope, but Pain and colleagues developed a dual-isotope lymphoscintigraphy protocol to provide information on the appearance rate of radiopharmaceuticals in mixed venous blood in addition to depot clearance rate (38-41, 45-47). Dual-isotope lymphoscintigraphy involves administration of Technetium 99m (^{99m}Tc) Human Immunoglobulin G (HIgG) into one hand and Indium-111 (^{111}In)-HIgG into the other hand, repeated imaging over three hours, and bilateral sampling from the medial cubital veins (see ref (38) for full explanation of methods). The appearance rate of tracer in ipsilateral and contralateral mixed-venous blood can be obtained from both arms simultaneously by using two isotopes. Early studies by Pain et al. demonstrated that ^{99m}Tc-HIgG and ^{111}In-HIgG have a similar clearance rate from a subcutaneous injection depot and a similar accumulation rate in contralateral mixed venous blood (40, 47). However, a subsequent investigation found significant quantities of protein-free ^{111}In present in early ipsilateral blood samples (38). The large amount of protein-free ^{111}In in ipsilateral blood indicates that it is less stable than ^{99m}Tc-HIgG and should not be used to calculate the accumulation rate of tracer in ipsilateral blood samples.

Measuring blood accumulation rate of tracer instead of determining storage of tracer within axillary lymph nodes, which is typically quantified, may be advantageous for several reasons. First, lymph is ultimately transported back to the cardiovascular system via lymphaticovenous communications with the subclavian vein. Some lymph will also enter the venous system within lymph nodes. Therefore, measuring the accumulation rate of tracer in mixed venous blood gives an indication of how efficient the lymphatic system transports injected radiopharmaceuticals through the upper extremity. Second, breast cancer treatment often involves lymph node excision and/or irradiation, thereby reducing the number of functional nodes on the treated side. Consequently, assessing the percentage of activity stored in lymph nodes at a specified time-point post-injection may result in an artificially lower percentage on the affected side simply due to fewer lymph nodes. Finally, lymph node retention of tracer is only a 'snapshot' of the amount of tracer present in the axillary lymph nodes at any one time. Despite colloids having good regional lymph node retention (36, 48), these particles will migrate through the lymph nodes and eventually enter the venous circulation. It is unknown how long the radiopharmaceuticals are retained in the lymph nodes and what factors speed or slow their exit from the nodes. A fraction of colloids in the lymph nodes will be phagocitized by the reticuloendothelial system primarily consisting of tissue macrophages (36).

1.1.4 Role of the Lymphatic System During Exercise

Exercise represents an acute disturbance in homeostasis. For example, cardiac output, mean arterial blood pressure, sympathetic nervous system activity, ventilation, skeletal muscle contractions, hydrogen ion concentration, and body temperature increase in response to exercise of increasing intensity. Additionally, strenuous exercise such as eccentric muscular contractions or marathon running provokes an acute inflammatory response marked by increases in neutrophil and lymphocyte concentrations, natural killer cell activity, reactive oxygen species, cytokines, and vasoactive amines (49). The degree of the inflammatory response has been related to the intensity and duration of exercise (50, 51). Starling's forces (20) that govern filtration and absorption in the microvasculature (see Section 1.1.2) favour absorption at rest, but the rise in capillary pressure that occurs in exercising muscle will favour filtration. In healthy individuals, interstitial edema accumulation usually does not persist beyond the exercise bout. This is because normal physiological responses to exercise such as increased sympathetic activity, skeletal muscular contractions, and respiration are also mechanisms that assist with lymph formation and propulsion (9, 22).

The effect of exercise on lymph formation and transport has been measured directly in animals using cannulation of a lymphatic duct (29, 31). In response to exercise of short duration (walking eight steps) in an intact lymphatic preparation in sheep, the frequency of lymphatic contractions increased and lymph flow doubled relative to baseline values in the 1–5 minutes after the beginning of movement (31). Movement was believed to increase lymph formation but not directly affect lymph propulsion since no correlation was found between fluid propulsion and normal walking movements in an isolated preparation.

Exercise of longer duration (2 hours) was measured in the hind limbs of sheep by Coates et al. (29). During the first 15 minutes of steady-state exercise, lymph flow in the hind-limb increased 5-fold from resting values and then gradually decreased to a constant 130% above baseline for the remaining 30 minutes of exercise. The large initial increase in hindlimb lymph flow was thought to be a result of increased pressure in the working muscles and increased sympathetic activation causing increased lymphatic motility. A combination of a greater vascular surface area and a higher hydrostatic pressure likely contributed to the steady-state lymph flow values observed from 90 to 120 minutes of exercise.

Few studies have investigated the role of the lymphatic system during exercise in humans. Havas et al. (52) investigated the effects of dynamic and isometric muscle contractions on lymph clearance using lymphoscintigraphy. Lymph clearance was measured in the legs of four sedentary males and four endurance-trained males (each leg counted as an independent observation; thus, n = 8 per group). The authors did not explain what constituted being endurance trained. The exercises included dynamic knee extensions and two types of isometric contractions (leg flexed at 90° and leg fully extended). In total, 100 submaximal contractions were performed in ten minutes for each condition and all conditions were performed on the same day with 65 minutes rest between each. The results showed that lymphatic clearance rate was highest in both the dynamic and isometric leg-extended conditions compared with the isometric leg-flexed condition. Moreover, the lymphatic clearance rate during the 65 minutes of rest between exercise conditions was nearly two-fold higher in the endurance-trained versus sedentary subjects. The higher clearance rate seen in the endurance-trained group was thought to be a result of an increase in capillary density, which is a known adaptation to long-term endurance exercise (53). A higher capillary density would provide a greater surface area for capillary filtration and endothelial conductance. If the higher capillary density accounts for the difference in lymphatic clearance rate between subject groups during rest, then it might also be expected that the clearance rate should be faster in the endurance-trained subjects during exercise. However, the ten minute exercise bouts used by Havas et al. (52) may not have been of sufficient duration and/or intensity to sufficiently tax the lymphatic system and cause clearance rate to differ between subject groups during exercise. Furthermore, the first 10–15 minutes of exercise has been shown (29, 54) to result in an initial overshoot of lymphatic clearance rate that is approximately 5-fold higher than rest and then declines to a steady-state value for the remainder of the exercise period that is still two- to three-fold higher than rest. It is possible that this initial overshoot response to exercise is similar for all healthy subjects and does not depend on training status. If this is the case, then the ten-minute exercise bouts as used by Havas et al. (52) could reflect the initial overshoot of lymphatic clearance rate and any differences between endurance-trained and sedentary subjects would be masked during this time. A longer exercise bout allowing steady-state conditions to be achieved may have shown differences between endurance-trained and sedentary subjects. More research is needed to clarify the effect of endurance training on lymphatic function during rest, exercise, and recovery from exercise.

Havas et al. (54) again used lymphoscintigraphy to investigate lymph flow dynamics in the lower limbs of eight males during two hours of steady-state exercise at 70% maximum heart rate. Similar to the results found by Coates et al. (29), the first 15 minutes of exercise showed a clearance rate that was five-fold higher than at rest and remained approximately two- to three-fold higher for the remainder of the two-hour exercise bout. A faster lymphatic clearance rate during exercise is expected. Exercise causes an increase in arterial blood pressure and cardiac output resulting in greater capillary filtration. Greater capillary filtration leads to a rise in interstitial pressure, which facilitates the entry of fluid and proteins into lymphatic capillaries (9). Furthermore, during exercise, sympathetic nervous system activity increases, thereby increasing lymph propulsion through autoregulation of smooth muscle in contractile lymphatic vessels.

In summary, the lymphatic clearance rate during exercise is elevated compared to rest. Both studies conducted by Havas et al. (52, 54) used subjects who were college-aged males and the effects of short-term exercise on lymphatic clearance in the lower limbs was investigated. It has not been determined if age or sex alters lymphatic clearance rate during exercise. Also, it is not known if the lymphatic vessels in the upper extremity respond in a similar manner to a given exercise bout as do the lymphatic vessels in the lower extremity. Nor is it known how the intensity or duration of the exercise affects lymphatic function. Although Havas et al. (52) demonstrated a faster lymph clearance rate in endurance-trained males in the recovery period between exercise conditions, more research is needed to elucidate the effects of training status on lymphatic function during short- and long-term exercise.

1.1.5 Breast Cancer Related Lymphedema (BCRL)

BCRL is a chronic swelling that can occur in the ipsilateral hand or arm of women treated for breast cancer (55). Current data suggest that there is a 27% (56, 57) to 49% (58) probability of women developing BCRL over a 20 year period following treatment. BCRL may present months or years after initial treatment and the actual onset may be gradual or rapid (16, 59, 60). Women with BCRL have restricted range of motion in the affected arm, increased risk of infection and impaired limb function. Furthermore, these women report increased anxiety and depression and reduced quality of life (61, 62).

Factors that are known to increase the risk of developing BCRL include dissection of the axillary lymph nodes, radiotherapy to the breast and axilla, pathological nodal status, obesity, and tumour stage (16, 59, 63, 64). There is a greater incidence and severity of subsequent lymphedema when more axillary lymph nodes are excised and/or a larger irradiation field and dose is directed at the axilla (56, 59, 65). A larger radiation dose or field applied to the axillary region may result in damage to lymph nodes, since radiation essentially leaves scar tissue in the area. Older surgical techniques involved radical mastectomy whereby all axillary lymph nodes were removed. Due to advances in technology, specifically the sentinel node biopsy, only potentially cancerous lymph nodes are excised when using this technique. Studies comparing complete axillary lymph node dissection with sentinel lymph node biopsy have shown a lower incidence of BCRL with sentinel lymph node biopsy (66-74). For example, Sener et al. (70) have shown that only 3.0% of subjects who underwent sentinel lymphadenectomy alone were determined to have lymphedema compared with 17.1% who underwent sentinel lymphadenectomy combined with axillary dissection. The possibility also exists that anatomical differences in lymphatic vessel location and length also contributes to development of BCRL. For example, a long variant that bypasses axillary lymphatics has been proposed (75). The existence of this long variant may bypass excised or damaged level I and level II axillary lymph nodes, and thereby potentially reduce the risk of developing BCRL (16).

Pathophysiology of BCRL

Normal lymphatic function reflects a balance between capillary filtration and lymph formation. In BCRL, the excess extracellular fluid volume may be a consequence of an increase in lymph formation relative to what can be accommodated by the initial lymphatics and/or damage to lymphatic vasculature preventing normal lymph transport (60). As the swelling progresses and becomes chronic, cellular changes ensue. Piller (76) demonstrated a reduction in the activity of macrophages and an increase in fibroblast activity in established lymphedema that likely contribute to the process of the swollen tissue becoming fibrotic. Olszewski (2) investigated the composition of lymph in secondary lower limb lymphedema by cannulating an afferent lymphatic trunk in the lower leg and sampling lymph over 24 hrs. Lymph protein and lymph immunoglobulin values were similar between subjects with lymphedema and controls; however, cytokine levels as well as apoptotic DNA were elevated in lymph from lymphedema patients. The elevated lymph levels of cytokines were thought to indicate an intensive inflammatory process while apoptotic DNA may reflect cellular changes in tissues (2).

The original theory regarding the development of BCRL is that the primary insult to the axillary lymphatic system due to axillary dissection and/or irradiation caused global impairment of lymph drainage (16). This traditional view of the pathogenesis of BCRL is analogous to a 'stop-cock' effect. The damage to the axillary lymphatics, primarily lymph nodes and vessels, was thought to interrupt lymph flow and thus cause fluid and plasma proteins to accumulate in the affected arm. For this theory to be true, the following findings should be evident in the literature related to BCRL: (i) all breast cancer survivors who receive identical treatment should develop BCRL; (ii) there should be a similar onset period; (iii) all sections of the arm should be swollen uniformly and the lymph flow rate should be similar throughout the affected arm; and (iv) lymph flow should be slower and protein concentration should be higher in the affected arm compared with the non-affected arm.

As reported previously, the incidence of BCRL is 27% to 49% and the onset period may vary considerably. This suggests that other physiological factors in the affected arm are also contributing to the development of BCRL. In addition, recent studies by Bates et al. (77) and Stanton et al. (42) have challenged the view that BCRL is a result of global impairment of lymph drainage. Specifically, Bates et al. (77) found that protein concentration was lower in the swollen arm compared with the normal arm, while Stanton et al. (42) discovered that lymph flow per unit volume of tracer distribution was faster in the spared hand of the swollen arm compared with the control arm.

The finding of a lower, rather than higher protein concentration in the affected arm, suggests hemodynamic abnormalities may also contribute to BCRL development (77). Lymphedema in any tissue develops when there is an imbalance between capillary filtration and lymphatic drainage rate (55, 78). A lower than expected interstitial protein concentration could result from an increased capillary filtration rate caused by either hyperemia in the affected arm or a rise in capillary pressure (77). In fact, Svenson et al. (79) found evidence of increased blood flow in the affected arm, although there are some methodological concerns with the study. Other factors that could explain the decreased interstitial protein concentration are reduced permeability of the capillary wall to plasma proteins and proteolysis within the interstitial compartment (80).

Regional lymph drainage failure was first proposed by Stanton et al. (42) after unexpectedly finding a faster CR in the spared hand of the swollen arm compared to the contralateral side. In this study, which was one of the first to systematically evaluate lymphatic function in patients with BCRL using lymphoscintigraphy, ^{99m}Tc-HIgG was subcutaneously administered to the swollen forearm or spared hand in patients with BCRL and the corresponding injection site on the contralateral side (the contralateral arm served as controls for all comparisons). As expected, lymph drainage of the radiopharmaceutical was 25% slower in the swollen forearm, but lymph drainage was 18% faster in the spared hand of the swollen arm compared to the contralateral hand. Stanton et al. (81) repeated the study in BCRL subjects with swollen hands and found a significantly slower CR in the ipsilateral hand compared to the contralateral hand. When CR values in BCRL subjects with swollen hands were compared to those with spared hands, CR in the ipsilateral hands was similar, but CR in the contralateral hands was significantly different (CR for the contralateral hand of the swollen hand group was 66% greater than CR for the contralateral hand of the spared hand group). It should be noted that subjects in the swollen hand group had a larger arm volume (50% increased arm volume) than the spared hand group (27% increased arm volume). Pain and colleagues, in a series of studies described above, also found evidence of regional lymph drainage failure. Specifically, the subcutaneous CR was slower in swollen hands but similar in spared hands of swollen arms when compared to both the contralateral arm and control data (39, 41). These findings collectively indicate a slower CR with subcutaneous injections to the swollen forearm or swollen hand compared to a similar injection site on the contralateral arm. Yet when subcutaneous injections are administered to the spared region of an affected arm, the clearance rate is similar (Pain), or even faster (Stanton) when compared to the contralateral arm. However, if the contralateral arm is not 'normal' then comparisons between arms in women with BCRL may not be appropriate.

Stanton and coworkers have continued to investigate regional differences in lymph drainage rates (81-83). Previous studies (38, 39, 41, 42) used subcutaneous injections of radiopharmaceuticals and did not find correlations between CR and arm volume. Consequently, Stanton et al. (83) measured subfascial drainage rates by administering ^{99m}Tc-HIgG intramuscularly to the forearms of women with established BCRL in order to test the hypothesis that epifascial swelling involves an overflow phenomenon or impaired superficial-to-deep drainage. Similar to earlier work using subcutaneous injections (38, 39, 41, 42), a significantly slower CR was found in the swollen forearm compared to the normal forearm (83) even though it has been shown that the edema is primarily restricted to the epifascial compartment (skin and subcutis) (84). Previous work did not find subcutaneous CRs to be correlated to arm volume; however, a significant negative correlation was found between subfascial CR and arm volume. Dermal backflow, which has been reported with subcutaneous injections to the hand (42), was not evident when radiopharmaceuticals were administered to the subfascial component of the swollen forearm. The lack of dermal backflow could mean that either the radioactivity is able to move in the proximal direction or any backflow that is taking place is in a deep area of the forearm and not visible on images taken with the gamma camera.

Another approach to understanding the regional distribution of swelling has been to administer subcutaneous injections to the maximally and minimally swollen region of the swollen forearm and corresponding sites on the contralateral forearm (82). Using this approach, no significant difference between CR at the site of maximal and minimal swelling was found. Rather, proximal CR was faster than distal CR, irrespective of the site of swelling, on both the ipsilateral and contralateral arms. The greater muscle component in the proximal region of the forearm was thought to account for the difference in CR seen between the proximal and distal portion of the forearm. The skeletal muscle pump is an extrinsic mechanism known to facilitate lymph formation and propulsion. In fact, subfascial CR has been shown to be two times faster than epifascial CR (85).

The idea of lymph drainage failure being regional rather than uniform throughout the arm suggests that there may be re-routing of lymphatic drainage within the swollen arm, which could account for the higher clearance rate seen in the non-swollen hand. Re-routing of lymphatic drainage is also supported by the appearance of 'dermal backflow' and the finding of a higher density of lymphatic vessels in the dermis of the swollen arm of women with BCRL. The greater lymphatic network in the forearms of women with BCRL was demonstrated by Mellor and colleagues (86) who also showed that breast cancer survivors, who did not present with BCRL, showed none of the changes in lymphatic density seen in women with BCRL. Thus, the increased lymphatic network appeared to be a result of the edema and not a result of breast cancer treatment. Furthermore, when the increased skin area due to lymphedema was taken into account, the total length of lymphatic capillaries in a 1-cm annulus of skin was significantly greater in swollen arms compared to control arms. This enhanced lymphatic density may indicate lymphangiogenesis or re-routing of lymph flow both of which could facilitate lymph drainage around regions where lymphatic vessels are failing. Angiogenesis has also been documented in the swollen arm during chronic limb swelling and, again, the increased microvascular density appeared to be due to edema and not breast cancer treatment (87). Mellor et al. (87) propose that the angiogenesis may contribute slightly to preserving swollen arm volume because an increased microvascular density increases capillary surface area, which should thereby increase the fluid filtered into the dermal interstitium of the whole arm per unit time per unit net Starling force. However, one could also consider that as long as angiogenesis occurs at the same relative rate as lymphangiogenesis, then the augmented lymphatic density should negate the increased fluid load on the lymphatic system from angiogenesis.

Collectively, these studies indicate that regardless of whether the injection is subfascial or epifascial (subcutaneous), CR appears to be impaired when radiopharmaceuticals are administered to swollen tissue. Yet the degree of swelling is only correlated to CR when the injection is subfascial. Caution should also be taken when comparing studies when CR is from different regions of the forearm, as proximal CR measures faster than distal CR in spite of differences in areas of maximal and minimal swelling. Stanton et al. hypothesize that the strong correlation between subfascial CR and arm swelling indicates that subfascial lymphatic drainage in some way must influence the degree of swelling that occurs in the epifascial compartment (83). For example, muscle has a large capillary network that may contribute more to edema fluid than does the skin and subcutis. As well, the fascia surrounding the muscle may lead to low compliance and prevent edema from occurring in this compartment (83). While lymphatic connections between epifascial and subfascial compartments have been documented in the normal wrist and elbow (75), and leg (88, 89), it is unknown if the direction of flow is one-way (and in what direction) or if it is bi-directional. Theoretically, impaired subfascial to epifascial lymph drainage would show some evidence of dermal backflow, but Stanton et al. did not observe dermal backflow with subfascial administration of radiopharmaceuticals.

Another factor that may contribute to the development of BCRL is the presence of functioning lymphaticovenous communications (16). Aboul-Enein et al. (90) evaluated women treated for breast cancer with and without BCRL, as well as healthy subjects, for the presence of lymphaticovenous communications in the upper extremity. Healthy subjects and breast cancer patients with lymphedema showed little lymphaticovenous transfer of the iodinated human serum albumin, while breast cancer patients who did not present with lymphedema demonstrated greater radiopharmaceutical activity in the venous blood within an hour of injection. Furthermore, radiological detection of lymphaticovenous shunts was assessed in two subjects without BCRL. This study indicates that functioning lymphaticovenous communications in women treated for breast cancer may help prevent the occurrence of BCRL.

In summary, the exact etiology and pathophysiology of BCRL appears to be multi-factorial and not fully understood. Recent investigations question the view that simple axillary obstruction is the primary determinant of BCRL and instead suggest that only regional lymph drainage failure may be occurring. If only some lymph vessels of the arm are compromised, then a higher interstitial pressure would help facilitate lymph flow through these failing vessels. Edema could provide this driving interstitial pressure. There is also evidence that hemodynamic factors, such as increased capillary filtration, may also be contributing to the development of BCRL. Further research is needed to determine the pathophysiological mechanisms that underlie BCRL so that appropriate treatments can be developed.

Diagnosis

The diagnosis of BCRL typically involves volumetric or circumference measurements as well as symptoms of heaviness and tightness in the affected arm. Additionally, bioelectric impedance (BIA), magnetic resonance imaging (MR), and tissue tonometry are also used to reflect a change in status. There is no consistent operational definition in the literature of what describes clinically significant BCRL; therefore, research varies in the method used to identify cases of BCRL. Currently, the gold standard for limb volume determination is water displacement volumetry. Megens et al. (91) investigated whether two methods of calculating upper extremity volume (using arm circumferences) could substitute for water displacement volumetry. Direct and calculated measurements of arm volume were both found to be related and reliable; however, they were not determined to be interchangeable since truncated cone calculations of arm volumes exaggerated actual arm volume.

Despite water displacement volumetry being the gold standard, it is neither a portable nor simple method to perform. As such, the clinical practitioner often uses circumferential measurements. The Steering Committee for Clinical Practice Guidelines for the Care and Treatment of Breast Cancer in Canada (92) reviewed the relevant literature and recommended four points for measuring arm circumference: (i) the metacarpal-phalangeal joints; (ii) the wrists; (iii) 10cm distal to the lateral epicondyles; and (iv) 15cm proximal to the lateral epicondyles. If a difference of >2.0cm between arms at any of the measurement points is found, treatment for lymphedema may be warranted.

Bioelectrical impedance (BIA) is also used in the diagnosis of BCRL as it reflects changes in extracellular fluid volume (93-95). Cornish (93) describe the principles of BIA, and briefly, it involves passing a small alternating current through the body segment and measuring the impedance to the flow. In the application of BCRL, the impedance to current flow can be used to determine extracellular fluid volume of the limbs. The increase in lymph volume is described by the ratio of impedance measures from the affected and unaffected limbs, which reflect extracellular fluid content of the respective limbs. An index greater than 1.00 indicates clinical lymphedema (93). As reviewed by Ward (96), BIA has shown to be a non-invasive, consistent, and valid measure of BCRL that can be used to measure a change in volume in clinical trials.

Tissue tonometry measures the amount of pressure necessary to depress the skin a specified amount, and thus, provides a measure of tissue resistance (97). Piller and Clodius (98) showed a correlation between arm circumference and tissue resistance measured by tonometry. A number of studies have used this method to measure a change in lymphedema status as a result of a therapeutic intervention (98-105).

Although water displacement volumetry, arm circumferences, bioelectrical impedance, and tonometry are non-invasive methods used to quantify lymphedema, they do not indicate changes in lymphatic function. Lymphoscintigraphy was developed to assess lymphatic status in patients with a variety of conditions that cause lymphatic obstruction as it provides semi-quantitative measures of lymphatic function (36, 106). Now this technique is being used both in the investigation of the pathology of BCRL (38-42, 45-47, 81-83, 107) and in the examination of various therapeutic interventions prescribed to manage BCRL (108-112).

<u>*Current Treatment Methods Prescribed for BCRL*</u>

BCRL is essentially an incurable condition, although treatments such as skin care, compression sleeves, pneumatic pumping, manual lymphatic drainage, and low-level laser therapy exist to contain swelling (16, 61, 113). There are other treatments available to patients with lymphedema including medications and surgery, but at present, these play a very small role in the management of patients with this condition. Current research investigating the efficacy of treatments prescribed for BCRL has had equivocal results as discussed below.

Compression Sleeves

After education, compression sleeves represent the first step in the initial management of patients with lymphedema despite limited objective data supporting their use. Graded compression garments deliver pressures of 20 to 60 mm Hg to facilitate lymph transport (92). Suitable compression garments can be custom-made or prefabricated, and ideally, trained personnel should fit them. Some sleeves start at the wrist and end at the upper arm, while other sleeves incorporate the shoulder. Some clinicians recommend the use of a compression garment for up to 24 hours per day, while others recommend its use only during waking hours or while exercising. Collins and colleagues (84) used CT scanning to assess the effect of compression garment therapy in 27 women with BCRL. They found significant decreases in the cross-sectional area of subcutaneous compartments with a larger decrease found in the distal portion of the limb (26%) compared to the proximal portion (9%). Bertelli et al. conducted a randomized control trial investigating the use of a compression sleeve plus electrically stimulated lymphatic drainage compared to the use of a compression sleeve alone. Both modalities reduced limb girth by 17%, which suggested that compression sleeve therapy alone is effective (114).

Intermittent Pneumatic Compression

There has been only one randomized trial that has evaluated pneumatic compression pumps for the treatment of lymphedema. Dini and colleagues (115) assigned 80 women with BCRL to either intermittent pneumatic compression or no treatment. Women in the treatment group completed 10 pump sessions over 2 weeks, followed by a 5 week break, and then another 10 sessions. The mean decrease in arm circumference in the treatment group was nearly four times that in the control group (1.9 cm and 0.5 cm, respectively); however, the differences between the two groups failed to reach statistical significance at the end of the experimental protocol. There are a number of other studies that have used compression pumps to treat lymphedema and the results have been mixed (116-118). Rockson (60) suggests that this treatment modality can play an important role in the management of patients with BCRL, citing results from several studies that are consistent in showing a reduction in arm volume following treatment with any of the different pumps tested.

Manual Lymphatic Drainage

Manual lymph drainage is a massage technique that involves the skin surface only and follows the anatomic lymphatic pathways of the body. A session of manual lymph drainage starts centrally in the neck and trunk to clear out the main lymphatic pathways with the intent of facilitating drainage from the arm (92). Anderson et al. (119), conducted a randomized trial involving 42 women with BCRL and compared standard therapy alone with standard therapy plus manual lymph drainage and training in self-massage. Standard therapy included use of a custom-made sleeve-and-glove compression garment worn during the day, instruction in physical exercises, education in skin care, and information and recommendations about lymphedema. Both groups obtained a significant reduction in limb volume, a decrease in discomfort and increased joint mobility over time; however, no significant differences in were found between the two groups.

Prospective investigations of complete decongestive lymphatic physiotherapy, including manual lymphatic drainage, have validated the efficacy of these interventions for the initial reduction of edema and long-term maintenance of limb volume in BCRL (120, 121). In one of the few studies to use lymphoscintigraphy to evaluate the efficacy of therapeutic interventions for BCRL, Szuba et al. (109), measured lymphatic function before and after manual lymphatic therapy in 19 women with BCRL. A correlation was found between the ratio of radioactivity within the affected to normal axillae and the percentage reduction in edema volume. However, the study was limited by the variability in radiopharmaceutical uptake in the affected axilla relative to the normal axilla pre- and post-treatment. Some of the variability in response could be due to the exercise protocol employed by Szuba et al.

which consisted of squeezing a ball intermittently for 1 minute every 5 minutes during the first 30 minutes of imaging with the intent of stimulating lymph flow. The amount of work competed with this type of exercise cannot be accurately quantified and if the effort, or work, differs among subjects or between conditions, this can potentially lead to varying amounts of radiopharmaceutical uptake at the axilla. Kligman et al. (113) conclude that there is enough evidence to suggest that compression therapy and manual lymphatic drainage may improve established lymphedema, but further studies are needed. Anecdotally, manual lymphatic drainage is well tolerated by patients and appears to offer a reasonable treatment option for many.

Low-Level Laser Therapy

Low-level laser therapy has been purported to have several therapeutic benefits including the treatment of lymphedema. Carati and colleagues (101) examined the treatment of patients with BCRL with low-level laser therapy using a double blind, placebo controlled trial. One or two cycles of laser treatment was delivered in blocks of nine sessions (active laser or placebo) to the axillary region of the treated arm with one block comprised of treatment three times per week for three weeks. The mean affected limb volume at 2-3 months post-treatment when two cycles of laser therapy was administered was significantly less than after placebo treatment. However, the mean affected limb volume was not significantly different than pre-treatment values. Immediately post-treatment, extracellular fluid volume, as measured by BIA, was significantly lower than pre-treatment values for all conditions (one cycle of laser, two cycles of laser, or placebo). At 2-3 months post-treatment, only the laser conditions demonstrated significantly lower extracellular fluid volume compared to pre-treatment values. Piller and Thelander (103) administered 16 treatment sessions with low-level laser therapy over 10 weeks. Similar to the results of Carati et al., arm volume decreased post-treatment.

1.1.6 Effects of Exercise on BCRL

Until recently, physicians, physiotherapists or health professionals erred on the side of caution and recommended survivors of breast cancer to avoid vigorous upper-body exercise for fear of promoting or worsening BCRL (1). This view stemmed from the belief that simple axillary obstruction of lymph flow was the sole cause of BCRL. Thus, if a woman who survived breast cancer engaged in vigorous exercise on a regular basis, then lymph production would increase, corresponding to an eventual increase in arm volume. All recent studies (3, 5, 7, 8, 122, 123) except one (6) have shown no significant change in arm volume with resistance training or upper body aerobic exercise. Although we demonstrated a significant increase in arm volume in breast cancer survivors with and without BCRL from a 20 week exercise program consisting of aerobic training, resistance training, and dragonboat paddling, this was not believed to be construed as reason for concern (6). First, the increase in arm volume was consistent for both arms. Second, there was a significant gain in upper body muscular strength over the course of the study period. Although changes in muscle volume were not directly measured in our study, Abe et al. (124) demonstrated a significant increase in muscle thickness with resistance training in men and women over a 12 week program. Thus, muscle hypertrophy likely accounted for the increase in arm volume seen in our subjects.

All of the above studies investigating the effect of exercise on limb volume used volumetry or circumferences to measure arm volume. Johansson et al. (7) measured both volumetry and BIA in women with BCRL in response to a controlled, low intensity, resistance training session. At 24 hours post-exercise, no increase was found in arm volume regardless of whether a sleeve was worn. Further research is needed to determine the short- and long-term changes in extracellular fluid volume measured by BIA in response to moderate intensity resistance and/or aerobic exercise.

The fact that exercise has not been shown to increase arm volume is promising but not conclusive. A limitation of these studies performed to date is that the measurement techniques used do not indicate changes in lymphatic function. Lymphoscintigraphy was developed to assess lymphatic status in patients with a variety of conditions that cause lymphatic obstruction as it provides semi-quantitative measures of lymphatic function (36, 106). Now this technique is being used both in the investigation of the pathology of BCRL (38-42, 45-47, 81-83, 107) and in the examination of various therapeutic interventions prescribed to manage BCRL (108-112).

As part of the study conducted by Stanton et al. (42), lymphoscintigraphy was used to investigate the effects of exercise on BCRL. Five subjects performed a total of five minutes of intermittent exercise consisting of squeezing a ball in both hands simultaneously at 20 contractions per minute. Subjects did not wear compression sleeves during the experimental protocol. Lymph clearance was measured over five hours post-exercise and no significant change in lymph clearance was found with the intermittent ball squeezing protocol. However, exercise of this type may not be of sufficient duration or intensity to result in a noticeable change in lymph clearance over the course of a five-hour measurement period. For example, Havas et al. (52) demonstrated significant increases in lymphatic clearance in males performing 100 submaximal contractions in 10 minutes of either dynamic knee extensions or isometric contractions with the leg extended. Perhaps, Stanton et al. would have achieved different results with subjects wearing compression sleeves and/or performing a more challenging exercise protocol.

There is the potential that long-term exercise performed by survivors of breast cancer could lead to improved lymph flow; however, this hypothesis needs further research. The propulsion of lymph within initial lymphatics is dependent on extrinsic mechanisms (muscular contractions, pulse of nearby arteries, and pressure changes with ventilation), while the propulsion of lymph within contractile lymphatic is dependent on intrinsic mechanisms (sympathetic activation of the spontaneously contracting smooth muscle) (9). Consequently, physiological changes associated with exercise training such as increased muscular contractions, increased sympathetic outflow, and increased ventilation could facilitate lymph return. For example, contraction of the smooth muscle in contractile lymphatic vessels is initiated by pacemaker cells (14) but can also be modulated by sympathetic activation (28, 125). Given that exercise results in an increase in sympathetic activation, then a corresponding increase in the frequency and force of lymphatic pumping would also be expected.

The effect of a larger muscle mass may also influence lymph flow even at rest. Modi et al. (82) found faster depot clearance of human serum immunoglobulin in women with BCRL when injected subcutaneously into the proximal forearm compared to the distal forearm, irrespective of the site of swelling. They hypothesized that the larger muscle mass (and thus, larger influence of the skeletal muscle pump) in the proximal forearm region may have accounted for the difference in CR. Perhaps, increasing muscle mass can influence lymph flow in the same manner at other sites in the arm.

The possibility of lymphangiogenesis, recruitment of dormant lymphatic vessels, and opening of lymphaticovenous communications in response to exercise training may also occur. For example, Yoon et al. (126) demonstrated in an animal model of lymphedema that vascular endothelial growth factor-C (VEGF-C) therapy reversed swelling through the generation of new lymphatic vessels (lymphangiogenesis). As habitual exercise in a healthy population is known to induce angiogenesis through up-regulation of other isoforms of VEGF (127), it is reasonable to expect concurrent lymphangiogenesis through up-regulation of VEGF-C. Lymphangiogenesis and recruitment of dormant vessels could help to reduce the stress put on the lymphatic vasculature damaged by breast cancer treatment, namely axillary lymph node dissection and radiation to the axilla.

Perhaps, breast cancer survivors who were cautioned against performing vigorous, upper-body exercise with the affected arm were fearful of doing even low- to moderate-intensity upper-body exercise. If light to moderate activity is not performed regularly, then the lymphatic vessels of the affected arm may become compromised as a result of the lack of stress put on mechanisms to assist in lymph return. Studies (56-58, 61, 70) investigating the incidence of BCRL have not differentiated between those survivors who regularly engaged in moderate, upper-body physical activity and those who have abstained from upper-body exercise altogether.

Although exercise has not been shown to cause or exacerbate BCRL (3, 5, 7, 8, 122, 123), caution should still be taken by breast cancer survivors with and without BCRL when performing moderate-intensity, upper-body exercise until research can conclusively determine that exercising at these intensities will not lead to the development or worsening of BCRL. Theoretically, wearing a compression sleeve, lifting the arm above the head after exercise, and performing a light, active recovery (i.e., low intensity exercise) should help to alleviate the edema accumulation that may normally occur after exercise.

1.1.7 Conclusions and Future Directions for Research

The lymphatic system is a one-way transport system that functions to produce, maintain and distribute lymphocytes, as well as to assist in the regulation of tissue volume and pressure. Lymphatic vessels have similar structural features to the venous system and rely primarily on extrinsic mechanisms such as the skeletal muscle pump, the respiratory pump and the pulse of nearby blood vessels to facilitate lymph return. Women with BCRL were originally cautioned against performing vigorous, upper-body exercise for fear of promoting lymphedema. It was originally thought that the primary insult to the axillary lymphatic system (i.e., dissection and/or irradiation) caused global impairment of lymph drainage similar to a 'stop-cock' effect. Current research suggests that hemodynamic factors may also contribute to the chronic swelling and lymph vessel failure may only be regional. Thus, the exact etiology and pathophysiology of BCRL appears to be multi-factorial and not fully understood. Both resistance and upper-body exercise have not been shown to lead to significant changes in arm volume; however, further research is needed to better understand the effects of short- and long-term exercise on lymphatic function using lymphoscintigraphy.

1.2 STATEMENT OF THE RESEARCH PROBLEM

The gold standard for the diagnosis of BCRL remains volumetric displacement of the arm yet this technique does not measure lymphatic function. Consequently, there is a need for a test that will provide information on lymphatic function of the upper extremity in women with BCRL and this forms the basis of this research project. A simple, safe lymphatic challenge test using lymphoscintigraphy to evaluate lymphatic function will serve as a definitive diagnostic tool, and thus, will allow formal assessment of treatment methods currently prescribed. Furthermore, this test could eventually be used to determine the effects of a chronic exercise program on the adaptive response of the lymphatic system. Knowing how exercise affects lymphatic function would allow appropriate exercise prescription guidelines to be developed to prevent and treat BCRL.

1.3 PURPOSE AND HYPOTHESES OF THE THESIS

The overall purpose of the study was to design a standardized exercise test that could be combined with lymphoscintigraphy to measure changes in lymphatic function in women treated for breast cancer with and without BCRL. Lymphoscintigraphy was used to assess lymphatic function and upper body exercise was used to stress the lymphatic system. In total, three projects were completed. The purpose and hypotheses of each were as follows:

Project 1

Purpose: To determine a useful protocol to challenge the lymphatic system in breast cancer survivors by evaluating the effects of arm crank ergometry and isometric handgrip contractions on radiopharmaceutical clearance from the hands of healthy females.

Hypothesis: Arm crank ergometry would result in faster radiopharmaceutical clearance from the injection site as measured by lymphoscintigraphy than the intermittent handgrip protocol.

Project 2

Purpose: To determine if varying the intensity of the arm cranking protocol affects radiopharmaceutical clearance rate from the hands of healthy females. A new primary outcome measure - percent uptake of the radiopharmaceuticals at the axillary lymph nodes one-hour post injection relative to the initial dose - was also introduced in this study.

Hypothesis: The arm crank protocol of higher intensity will result in faster radiopharmaceutical clearance from the depot and greater uptake at the axillary lymph nodes one-hour post injection than the arm crank protocol of lower intensity.

Project 3

Purpose: Projects 1 and 2 determined that an intermittent, moderate intensity, arm crank ergometry protocol may be useful to challenge the lymphatic system in women treated for breast cancer. Thus, the purpose of Project 3 was to evaluate lymphatic function both at rest and in response to this intermittent, arm cranking protocol in women treated for breast cancer with lymphedema (BCRL), breast cancer survivors with no lymphedema (BC), and healthy, age-matched control subjects (CONT). The primary outcome measure was clearance rate (CR), while secondary outcome measures of lymphatic function include radiopharmaceutical uptake at the axilla at 65 min post-injection relative to the initial dose (AX), radiopharmaceutical uptake in the forearm 65 min post-injection relative to the initial dose (FORE), and time of first appearance of radiopharmaceuticals at the axilla (APP).

Hypotheses:

1. CR, AX, FORE, and APP on the non-affected side (i.e., contralateral arm) will be equivalent in all subject groups both at rest and during exercise.
2. Lymphatic function relative to the contralateral arm (assuming contralateral arm lymphatic function is equivalent across groups) will differ between subject groups. Specifically, lymphatic function will be poorest (i.e., slowest CR, slowest APP, lowest AX, and highest FORE) in BCRL while lymphatic function will be highest in CONT (i.e., fastest CR, fastest APP, highest AX, and lowest FORE). The lymphatic function of BC will fall between BCRL and CONT.
3. Lymphatic function will improve from rest to exercise.

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CHAPTER TWO Lymphoscintigraphy to Evaluate the Effects of Upper Body Dynamic Exercise and Handgrip Exercise on Radiopharmaceutical Clearance from Hands of Healthy Females[B]

2.1 INTRODUCTION

Breast cancer related lymphedema (BCRL) is a poorly understood complication of breast cancer treatment. The chronic swelling occurs in the ipsilateral hand or arm of women treated for breast cancer (1) and can result in numerous physiological and psychological sequelae, including restricted range of motion in the affected arm, increased risk of infection, impaired limb function, increased depression, and reduced quality of life (2, 3). Current data suggest the prevalence of this condition to be from 27% (4, 5) to 49% (6). Factors that increase the risk of developing BCRL are dissection of axillary lymph nodes, radiotherapy to the breast and axilla, pathological nodal status, obesity, and tumor stage (7-10). BCRL is essentially an incurable condition although treatments such as skin care, manual lymphatic drainage, pneumatic pumping, and compression sleeves exist to contain swelling (7, 11). Exercise was originally thought to exacerbate BCRL; however, recent studies show no significant change in arm volume with either resistance training or upper body exercise in women treated for breast cancer with and without BCRL (12-17).

The diagnosis of BCRL usually involves volumetric, circumference, or bioelectrical impedance measurements as well as symptoms of heaviness and tightness in the affected arm (2, 18-21). In clinical practice, a difference of more than 200 mL (20) measured by water displacement or 2 cm measured by arm circumference at various landmarks (19, 20) may signify mild to moderate lymphedema. Alternatively, a bioelectrical impedance ratio greater than 1.00 may also indicate BCRL (22). Although arm circumference and volume measurements are noninvasive and relatively simple to perform, they do not indicate changes in lymphatic function. Conversely, lymphoscintigraphy does give an indication of lymphatic function. This technique involves radiopharmaceutical labeling of a lymphatic drainage source and was developed to assess lymphatic status in patients with a variety of

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conditions that cause lymphatic obstruction. It is now being used to investigate the pathophysiology of BCRL (23-29) and to evaluate the efficacy of certain treatment methods prescribed for BCRL (30, 31).

Several reviews have suggested the use of a standardized lymphoscintigraphy stress protocol when investigating lymphatic dysfunction (28, 32, 33). Although all studies examining the lymphatic function of women treated for breast cancer include forearm exercise, such as clenching the fists or squeezing a rubber ball, (23-27, 29, 31) it is unknown if this type of exercise significantly increases lymph flow compared to rest in normal subjects or subjects with lymphatic disease. Therefore, the purpose of the study was to evaluate the effects of arm crank ergometry (AC) and handgrip contractions (HG) on radiopharmaceutical clearance from the hands of healthy females. Lymphoscintigraphy was used to assess lymphatic function and upper body exercise was used to stress the lymphatic system. Healthy, college-aged females were recruited so that a normal response of the lymphatic system to exercise of different modes could be investigated.

2.2 MATERIALS AND METHODS

2.2.1 Subjects

Six healthy females between the ages of 18–30 years participated in the study. Prior to testing, informed consent was obtained from each subject and the study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects at the University of British Columbia.

2.2.2 Experimental design

A repeated-measures design was used to compare the effect of two exercise conditions on lymphatic clearance rate (CR) from each hand. The independent variable was the exercise condition, arm cranking (AC) or handgrip contractions (HG), while the dependent variable was the lymphatic CR from each hand during exercise. The subjects were designated to treatment order by a randomized draw to prevent any sequencing effects from occurring. At least 48 hours separated each test to prevent fatigue from biasing data and to allow decay of the radiopharmaceutical.

2.2.3 Experimental procedures

Imaging

Lymphoscintigraphy was used to quantify lymphatic function and was performed at Vancouver General Hospital, Department of Nuclear Medicine. Subjects were asked to arrive hydrated and rested. The same technician prepared and administered subcutnaeous injections of the radiopharmaceutical technetium-99m (^{99m}Tc)-antimony colloid to all of the subjects on each testing day. During early pilot work prior to the start of this study, filtered ^{99m}Tc sulfur colloid was being used as the radiopharmaceutical for the lymphoscintigraphy procedure. However, clearance rate at rest showed little movement of this particle. Consequently, ^{99m}Tc- antimony colloid was chosen, as it is a smaller particle that is preferentially taken up by the lymphatic system. Filtered sulfur colloid yields particles 10-100 nm in size whereas antimony colloid yields particles between 3 and 30 nm (34).

A total of 18 MBq of $^{99m-}$Tc antimony colloid in 0.05 mL was administered subcutaneously to the first and fourth web spaces of each hand prior to the start of the exercise protocol. After administration, subjects placed both hands prone on a gamma camera equipped with a low energy, ultra high resolution collimator (Sopha Medical Vision, Waukesha, Wisconsin). Only the forearms and the hands were in the field of view of the camera and a single camera head was used. One minute static acquisitions with a 20% energy window was centered on a 140 keV peak. The initial image was acquired within 1 minute of the injection and sequential images were acquired every 10 minutes post-injection over a total period of 60 minutes.

The images were processed using Siemens Icon software (Version 8.5, Siemens Medical Systems Inc., Hoffman Estates IL). A region of interest (ROI) was drawn around each of the injection sites to give the number of activity counts. The counts from the first and fourth web spaces of each hand were added together and corrected for physical decay of ^{99m}Tc using the following equation:

$$A_t = A_o e^{-\lambda t}$$

whereby A_t = activity after an elapsed time (min), A_o = activity in original sample, λ = 0.693/363 min physical half-life of ^{99m}Tc, and t = elapsed time from the original image. The corrected count at each time point was divided by the corrected count measured immediately after injection and was plotted against time. CR from the injection site was linear, and so, the equation given by Havas et al. (35) was used to calculate CR:

$$CR = [((A_1 - A_0)A_0^{-1})\ 100]\ (T_1 - T_0)^{-1}$$

whereby A_0 = the radioactivity (counts·min^{-1}) at the beginning of the period of interest, A_1 = the radioactivity at the end of the period of interest, and T_0 and T_1 are the times (min) at A_0 and A_1, respectively. CR is expressed as the percent of activity initially administered per minute (%·min^{-1}).

Exercise Protocols.

On separate days, subjects did one of two exercise conditions that began immediately after the first imaging scan and continued from 0 to 60 minutes (Fig 2.1). The exercise conditions for AC were six repeated bouts of 5 minutes at 0.6 W·kg^{-1} on a calibrated arm crank ergometer (model 881e, Monark, Sweden) followed by 5 minutes of rest, and for HG were twelve repeated bouts of 75 contractions in 2.5 minutes at 50% maximum voluntary contraction (MVC) followed by 2.5 minutes of rest. HG was performed with the right hand only using an isometric dynamometer (Almedic, Quebec City, Quebec) at a duty cycle of one contraction every 2 seconds, while the left hand served as the control (CONT). For both AC and HG, all imaging scans were performed during rest periods. During early pilot work prior to the start of this study, a handgrip protocol consisting of 30 contractions at 30% MVC in 1 minute followed by 9 minutes of rest was used. This protocol was not found to differ substantially from resting data and it was thought that the exercise stimulus was too conservative. Thus, the handgrip protocol was adjusted by increasing the intensity and the number of contractions and investigated in the current study.

2.2.4 Statistical Analysis

A 2 x 2 (hand x exercise) within-subjects, repeated measures analysis of variance (ANOVA) was used to determine if there were differences in CR between the right and left hands for each condition (AC and HG) during exercise. Statisica version 5.1 was used and

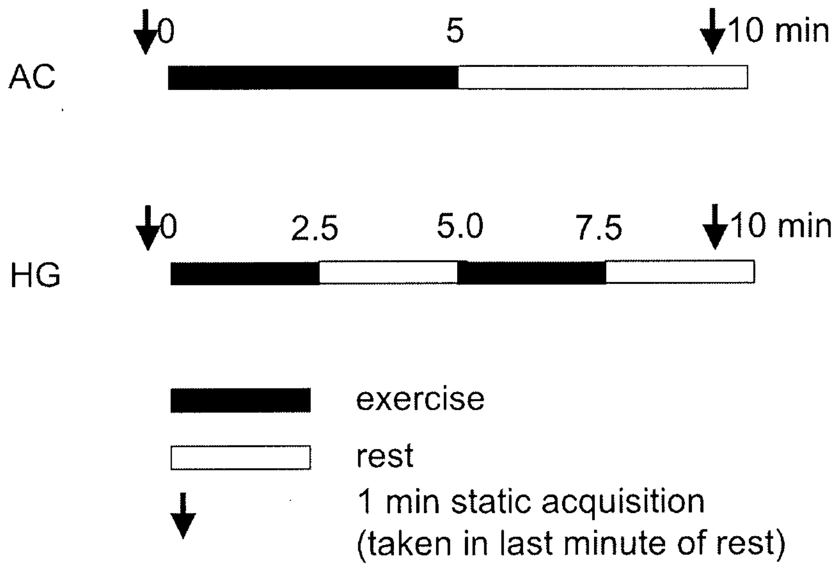

Figure 2.1 Graphical representation of the arm cranking (AC) and intermittent handgrip (HG) protocols. The 10 min segment of the protocol outlined above was repeated six times.

significance was set at $p \leq 0.05$. Post-hoc analysis for any main effects was performed using Tukey's test of honest significant differences. Data is reported as mean ± standard deviation.

2.3 RESULTS

Six healthy females completed the study (age = 29.2 ±1.9 years; height = 165.4 ±4.5 cm; body weight = 55.6 ±7.1 kg; BMI = 20.3 ±2.1 $kg \cdot m^{-2}$; MVC = 32.7 ±2.7 kg). Figure 2.1 shows that a significantly higher clearance rate was observed with the right and left hands for AC (right -0.27 ±0.03% per min; left -0.29 ±0.06% per min) compared to both HG (-0.18 ±0.03% per min) and CONT (-0.14 ±0.05% per min; p=0.000). The left hand on Fig. 2.2 for HG is equivalent to the control hand or CONT. No significant difference was found during AC between the right and left hands (p=0.503) nor was a significant difference found between HG and CONT (p=0.091).

Figure 2.3 shows individual CR as a function of time for AC (counts for the right and left hands averaged at each time point), HG (right hand only), and CONT (left hand only for HG protocol).

2.4 DISCUSSION

The primary finding of this study is that significantly quicker clearance of ^{99m}Tc-antimony colloid from the hands of healthy females was evident during upper body dynamic exercise using an arm crank ergometer compared to intermittent handgrip contractions using a handgrip dynamometer. In fact, the handgrip exercise resulted in a clearance rate that was equivalent to the resting or control arm. This study is unique in that it is the first to report if performing exercise with the upper limb between imaging scans can stimulate lymph flow as measured by clearance of ^{99m}Tc-antimony colloid from the injection site.

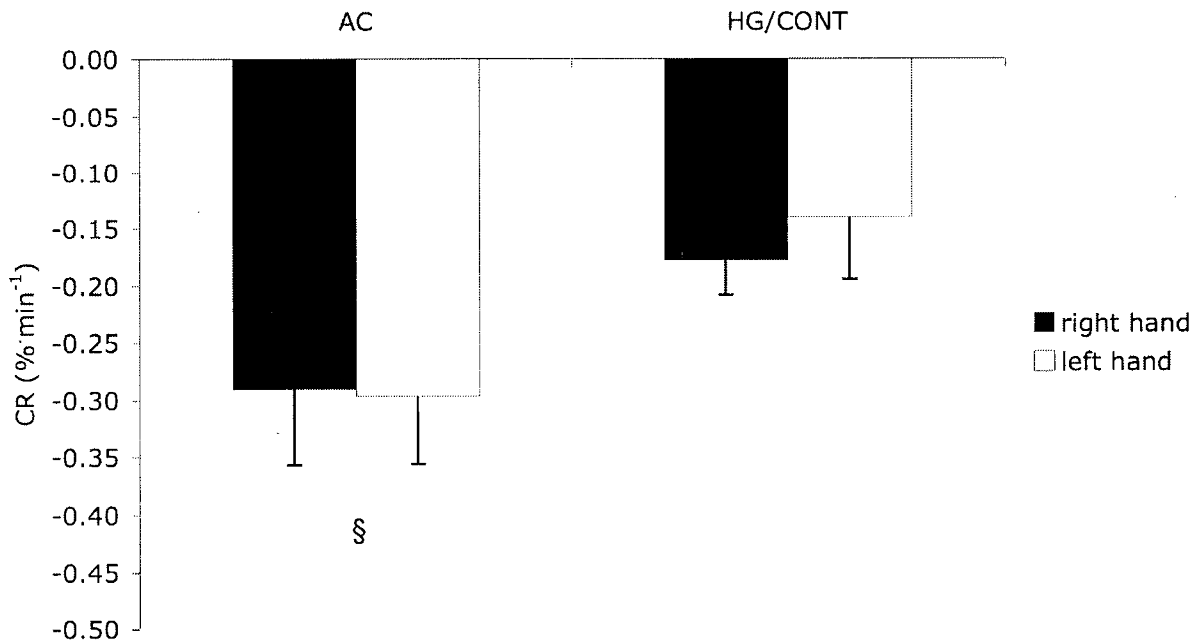

Figure 2.2 The effects of arm cranking (AC) and intermittent handgrip (HG) on clearance rate (CR) from the hands of healthy females (n=6). § denotes a significantly greater ($p<0.05$) clearance rate for AC (right and left arms) compared to both HG and CONT. NOTE: The left hand is a control (CONT), and thus, did not do the exercise protocol.

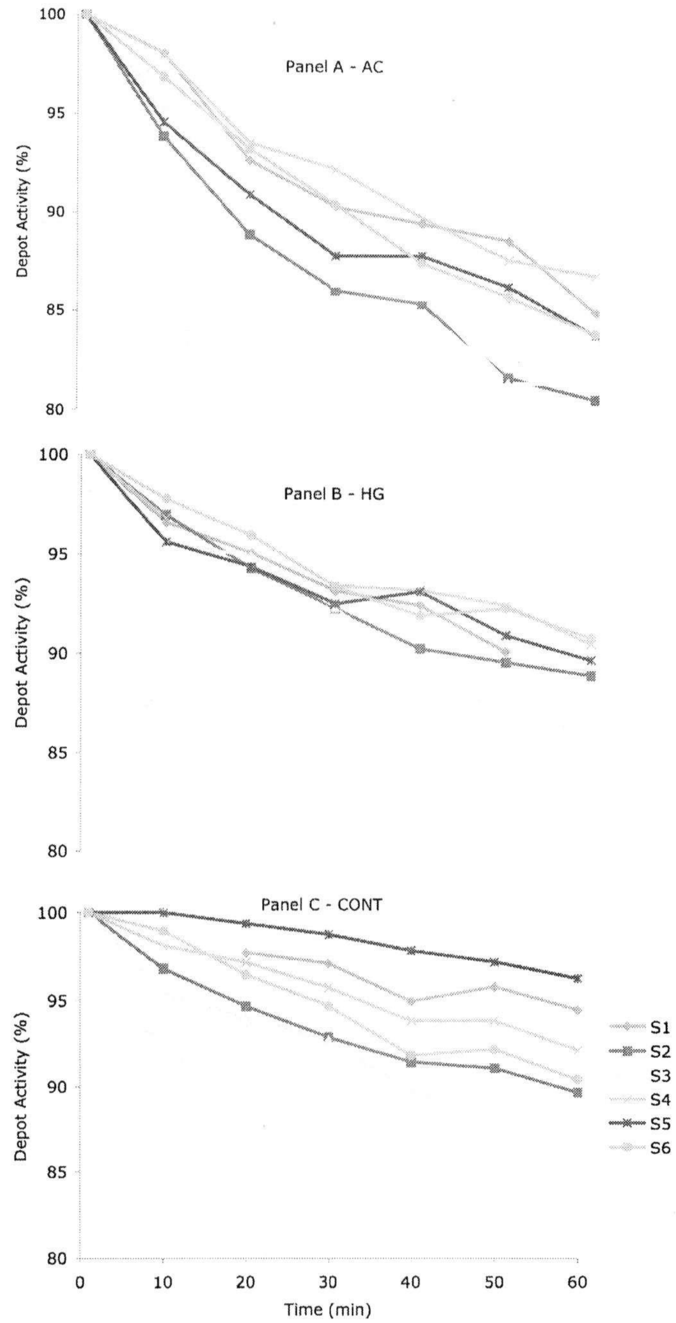

Figure 2.3 Individual depot activity (%) time (min) curves for arm cranking with the counts averaged for the right and left hand at each time point (Panel A - AC), intermittent handgrip exercise (Panel B - HG), and a control arm (Panel C - CONT) from the hands of healthy females (n=6).

Typically, when lymphoscintigraphy is used to investigate the pathophysiology of BCRL, the protocol has subjects performing intermittent forearm exercise, such as clenching the fists or squeezing a rubber ball, between imaging scans (23-27, 29, 31). Although repeated forearm muscle contractions should theoretically stimulate lymph flow by way of the skeletal muscle pump, this has not been investigated to date. Other studies (35-38) have examined the effects of muscle activity on lymph clearance using lymphoscintigraphy, but each one has used a leg model and male subjects. Thus, it is unclear if the lymphatic vessels in the upper extremity respond in a similar manner to a given exercise bout as do the lymphatic vessels in the lower extremity or if there is a gender effect. Further, clenching the fist or squeezing a rubber ball does not allow the amount of work completed to be precisely quantified. If the effort, or work, differs among subjects, then this can potentially lead to varying rates of radiopharmaceutical clearance from the injection sites. Consequently, handgrip exercise was used in the present study as it closely mimics the muscle groups activated during fist clenching and it allows the amount of work to be precisely quantified. Upper body dynamic exercise using an arm crank ergometer was also included in order to see how a different type of exercise condition affects clearance rate.

In the present study, the CR observed during HG (-0.18% per min) was similar to that observed in the lower limb of males performing isometric knee extension exercise (-0.20% per min) but higher than the CR observed for isometric knee flexion exercise (0.06% per min) (35). Further, the upper limb CR during AC (-0.28% per min, average CR for right and left hands) was higher than that reported for males during submaximal dynamic knee extensions (-0.16% per min) (35) or 2 hours of steady-state running (-0.18% per min) (36). As Table 2.1 shows, the present study and those of Havas et al. (35, 36) vary in terms of muscle groups activated, type and intensity of exercise performed, body position of subjects, gender of subjects, and location and type of radiopharmaceutical injection. The relative contribution of each of the factors listed in Table 2.1 to lymph function during acute dynamic exercise warrants further investigation.

It is expected that radiopharmaceutical clearance rate will increase in response to acute exercise. The lymphatic system must rely on both intrinsic and extrinsic mechanisms to propel lymph through the vessels against gravity. Intrinsic mechanisms involve spontaneous, intermittent contraction of smooth muscle lining the contractile lymphatics (37, 39), whereas extrinsic mechanisms include active and passive limb movements, breathing,

Table 2.1 Comparison of methods used in present study and those of Havas et al. (35, 36).

	Present Study	Havas et al. (35)	Havas et al. (36)
Clearance Rate (%·min^{-1})	AC = -0.29 HG = -0.20 CONT = -0.13	Dynamic knee extensions = -0.16 Isometric knee extensions = -0.20 Isometric knee flexion = -0.06	-0.18 (averaged over entire exercise bout)
Type of Exercise	AC = Arm cranking (70 RPM) using an upper body ergometer HG = handgrip exercise using a dynamometer	Dynamic knee extensions Isometric knee extensions Isometric knee flexion	Treadmill running (average running speed not reported)
Intensity of Exercise	AC = 0.6 W·kg^{-1} HG = 50% maximum voluntary effort	100 submaximal contractions at 10 contractions / min at 30% maximal voluntary effort	70% maximum heart rate
Duration of Exercise	AC = 5 min work followed by 5 min rest repeated 6 times (30 min total duration) HG = 2.5 min work followed by 2.5 min rest repeated 12 times (30 min total duration)	10 minutes	120 minutes
Body Position during Exercise	Upright	Supine	Upright
Gender of Subjects	Female	Male	Male
Training Status of Subjects	Recreationally active	Sedentary and endurance trained	Endurance trained
Type of Radioisotope	^{99m}Tc antimony colloid	^{99m}Tc human serum albumin	^{99m}Tc human serum albumin
Location of Injection	Subcutaneous depot in 1st & 4th web space	Vastus lateralis muscle	Vastus lateralis muscle

and the pulse of nearby arteries (40). Thus, the theoretical reason for performing exercise during lymphoscintigraphy is to take advantage of several of the physiological changes associated with exercise, such as increased skeletal muscle contractions, ventilation, sympathetic nervous system activity, and blood flow that influence lymph formation and propulsion. For example, dynamic exercise results in vasodilation in the active musculature and an increase in cardiac output which both serve to increase muscle blood flow, and thus, capillary filtration. An increase in capillary filtration leads to a higher interstitial pressure which facilitates lymph formation (40, 41).

The skeletal muscle pump is another example of how dynamic exercise affects lymph formation, and thus, lymph transport. For instance, an increase in mean lymph flow was shown in males performing foot contractions (37). It is likely that foot contractions caused an increase in pressure exerted on the foot tissues, thereby compressing the initial lymphatics and forcing lymph in the proximal direction to contractile lymphatics. When the lymph vessel wall of contractile lymphatics is stretched by the accumulating lymph, a reflexive contraction of the smooth muscle endothelium ensues (41).

A normal physiological response to dynamic exercise is an increase in sympathetic nervous system activity. In the context of the lymphatic system, an increase in sympathetic nervous system activity would increase the frequency and force of the spontaneous peristaltic smooth muscle contractions that occur in the larger contractile lymphatics (40). Modulation of the pacemaker cells by sympathetic activation has been demonstrated in an animal model (42, 43). Furthermore, the increase in lymph formation by way of increased capillary filtration or increased skeletal muscle contractions or both should also increase the contraction frequency of the smooth muscle (40, 41). The effect of movement causing a rise in lymph formation and a concomitant increase in contraction frequency of lymphatic smooth muscle has been verified in both human (37) and animal (44) research.

The finding that AC resulted in a faster lymphatic clearance rate than HG may be explained by the fact that arm cranking utilizes a larger muscle mass, and thus, larger muscle pump than fist clenching. AC involves the forearm flexors and extensors, biceps brachii, triceps brachii, and the muscles of the upper back and shoulders, whereas HG only involves the forearm flexor muscle group. Consequently, the skeletal muscle pump may not have been activated to the same degree during HG and this may have affected the clearance rate observed. In addition, there are hemodynamic differences between arm cranking and handgrip exercise that may have contributed to the variation in clearance rates. Intermittent handgrip exercise sporadically occludes the artery, slowing capillary filtration, whereas dynamic exercise enhances capillary filtration.

The lymph clearance rate during HG did not statistically differ from CONT and the lymph clearance during CONT (-0.15% per min) was higher than other resting data previously reported (0.09% per min (35)). Two explanations may account for this finding. First, the discrepancy in results may be due to differences between the present study and that by Havas et al. (35) including the muscle group used, body position, gender, and type and location of radiopharmaceutical injection (Table 1). Second, the control arm during HG may not in fact reflect a true resting condition. Muscle activation in the contralateral limb has been shown to occur when performing a muscle contraction with the ipsilateral limb (45). If contralateral muscle activation were occurring when subjects were completing the HG protocol, then this would have artificially enhanced radiopharmaceutical clearance in the control arm.

In summary, significantly faster clearance of ^{99m}Tc-antimony colloid from the hands of healthy females was evident during intermittent upper body dynamic exercise using an arm crank ergometer compared to intermittent isometric exercise using a handgrip dynamometer. The results indicate that AC may be more effective in promoting lymphatic clearance from the hand and may be a useful protocol to challenge the lymphatic system in breast cancer survivors when one wishes to examine the functional status of the lymphatic system.

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CHAPTER THREE Lymphoscintigraphy to Evaluate the Effect of High Versus Low Intensity Upper Body Dynamic Exercise on Lymphatic Function in Healthy Females[C]

3.1 INTRODUCTION

Quantitative lymphoscintigraphy is developing into an important tool for investigating lymphatic dysfunction, particularly in women with breast cancer related lymphedema (BCRL) (1-9). This technique typically involves administering a radiopharmaceutical into a subcutaneous depot and imaging the limb using a gamma (γ) camera at various time points from 0 minutes to 6 hours post-injection. The radiopharmaceutical is generally a technetium labeled macromolecule or colloid that is of sufficient size (i.e., between 4 and 100 nm) to enter the lymphatic capillaries and not blood capillaries (5, 10).

Most studies examining lymphatic function in women treated for breast cancer have subjects perform intermittent forearm exercise such as clenching the fists or squeezing a rubber ball between imaging scans to stimulate lymph flow (1-4, 7, 8, 11). We have previously shown arm crank exercise to be more effective than intermittent handgrip exercise in enhancing depot clearance rate (CR) of ^{99m}Tc-antimony colloid from the hands of healthy females (12). Although there was a trend towards faster CR during handgrip exercise (-0.18 $\%\cdot min^{-1}$ vs. -0.14 $\%\cdot min^{-1}$, respectively), the intermittent handgrip protocol used by our lab resulted in CR values that were not statistically different than the control arm.

Little is known about the effects of arm cranking exercise on lymphatic function and what constitutes a normal response to acute exercise. This information is needed before using quantitative lymphoscintigraphy to evaluate lymphatic function in women with BCRL. Therefore, the purpose was to determine the effect of low intensity versus high intensity upper body exercise on lymphatic function in healthy females. Lymphatic function was assessed by two outcome variables: CR from the injection site and uptake of tracer in the

[C] Parts of this chapter have been previously published: **Lane, K.,** Dolan, D., Worsley, D., & McKenzie, D. Lymphoscintigraphy to evaluate the effect of high versus low intensity upper body dynamic exercise on lymphatic function in healthy females. *Lymphatic Research and Biology*. 2006: 4(3): 159-65.

axillary lymph nodes 65 minutes post-injection (AX). Healthy, college-aged females were recruited so that a normal response of the lymphatic system to arm cranking exercise of different intensities could be investigated.

3.2 MATERIALS AND METHODS

3.2.1 Subjects

Eight healthy females (all right hand dominant) between the ages of 18-32 years participated in the study (age = 27.2 years ±2.6; body mass = 59.7 kg ±9.6; height = 169.1 cm ±8.7; BMI = 20.8 $kg{\cdot}m^2$ ±2.3). Prior to testing, informed consent was obtained from each subject and the study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects at the University of British Columbia.

3.2.2 Experimental Design

A within-subjects design was used to compare differences in lymphatic function during arm cranking of high (HI) and (LO) intensity. The independent variable was the exercise protocol (HI and LO) while the dependent variable was depot clearance rate (CR) and percent uptake of the radiopharmaceutical at the axillary lymph nodes 65 min post-injection (AX). The subjects were designated to treatment order by a randomized draw to prevent any sequencing effects from occurring. At least 24 hours separated each test in order to prevent fatigue from biasing data and to allow full decay of the radioisotope.

3.2.3 Experimental Procedures

Imaging

Lymphoscintigraphy was used to quantify lymphatic function and was performed at Vancouver General Hospital, Department of Nuclear Medicine. Subjects were asked to arrive hydrated and rested. The same technician prepared and administered subcutaneous injections of the radiopharmaceutical Technetium-99m (^{99m}Tc)-antimony colloid to all of the subjects on each testing day. ^{99m}Tc-antimony colloid is 3-50 nanometers in size.

Four injections with a total activity of 18 MBq, each dose in 0.05 mL, were introduced into the first and fourth web spaces of each hand prior to the start of the exercise protocol. Subjects placed both hands prone on a gamma camera equipped with a low energy, ultra high resolution collimator (Sopha Medical Vision, Waukesha, Wisconsin). Only the forearms and the hands were in the field of view of the camera and a single camera head was used. One minute static acquisitions with a 20% energy window centered on a 140 kV peak were taken within one minute of the injection and again every 10 minutes post-injection over a total period of 60 minutes. In addition, at 65 minutes post-injection a whole body scan was taken in order to calculate AX. Subjects were in the supine position for the whole body scan and the camera head was centered on the axilla. The images were stored on a hard drive as a 256 x 256 Word frame format for later analysis.

The images were processed using Siemens Icon software (Version 8.5, Siemens Medical Systems Inc., Hoffman Estates, Illinois). The region of interest (ROI), or the selection of pixels for analysis, was drawn around each of the injection sites to give the number of activity counts. The two counts from each hand were added together and corrected for physical decay of ^{99m}Tc using the following equation:

$$A_t = A_o e^{-\lambda t}$$

whereby A_t = activity after an elapsed time (min), A_o = activity in original sample, λ = 0.693/363 min physical half-life of ^{99m}Tc, and t = elapsed time from the original image. The corrected count at each time point was divided by the corrected count measured immediately after injection and was plotted against time. CR from the injection site was linear and expressed as a slope (% administered activity·min^{-1}). We have shown symmetry in CR and AX between arms during arm cranking exercise in normal subjects (Chapter Four); therefore, an average value between the right and left arms was used in the present study.

ROIs were also drawn around both axillary lymph node regions from the images generated from the two whole-body scans. These were corrected for physical decay of ^{99m}Tc and compared to the initial amount of radioactivity at the injection site to determine AX.

Exercise Protocols

On separate days, subjects did one of two AC protocols that began immediately after the first imaging scan and continued until 60 min post-injection. All exercise protocols were performed on a calibrated Lode arm ergometer (Angio single set model, The Netherlands). The AC protocols were as follows: HI - twelve repeated sets of arm cranking for 2.5 minutes at 0.6 $W \cdot kg^{-1}$ followed by 2.5 minutes of rest, and LO - twelve repeated sets of arm cranking for 2.5 minutes at 0.3 $W \cdot kg^{-1}$ followed by 2.5 minutes of rest. Heart rate was recorded every 10 minutes throughout both conditions using a Polar T31 heart rate monitor (Polar Electro Inc, Lake Success, NY).

3.2.4 Statistical Analyses

A paired t-test was used to determine differences in CR, AX, and HR between high and low intensity arm cranking exercise. SPSS version 11 was used to analyze the data and significance was set at $p \leq 0.05$. Data is reported as mean ± standard deviation (SD).

3.3 RESULTS

3.3.1 Lymphatic Function

An average value between the right and left arms was used in the present study since there was no significant difference between arms at either intensity and we have shown symmetry in CR and AX between arms during arm cranking exercise in normal subjects (Chapter Four).

Figure 3.1 shows a significant difference in CR between HI (-0.235%$\cdot min^{-1}$ ±0.059) and LO (-0.191%$\cdot min^{-1}$ ±0.051; p=0.003). The coefficient of variation for CR between measurements from the right and left arms of the same subject for the high intensity and low intensity conditions was 11.7% and 25.0%, respectively.

Significantly greater amounts of tracer were evident in the axilla 65 min post-injection (HI: 6.3% ±1.6, LO: 4.8% ±1.1, p=0.004, Fig 3.2). Moderate correlations were found between CR and AX for both conditions (HI: $r = -0.78$; LO: $r = -0.84$).

Figure 3.3 shows individual CR as a function of time for both exercise conditions (HI - panel A; LO - panel B).

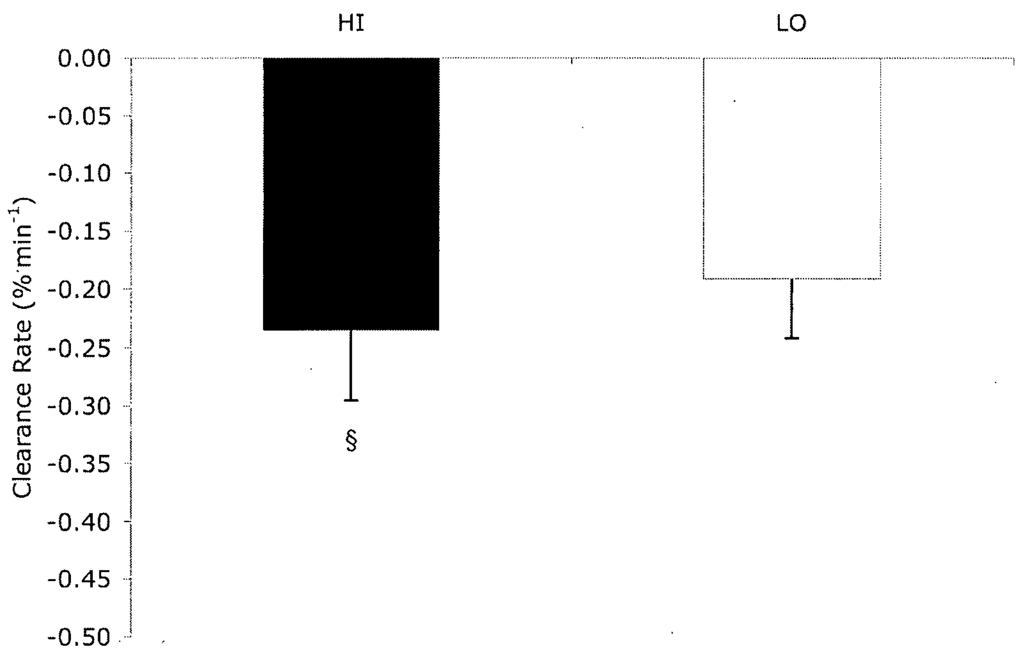

Figure 3.1 The effects of high (HI) versus low (LO) intensity arm cranking on clearance rate from the hands of healthy females (n=8). § denotes a significantly greater ($p<0.05$) value.

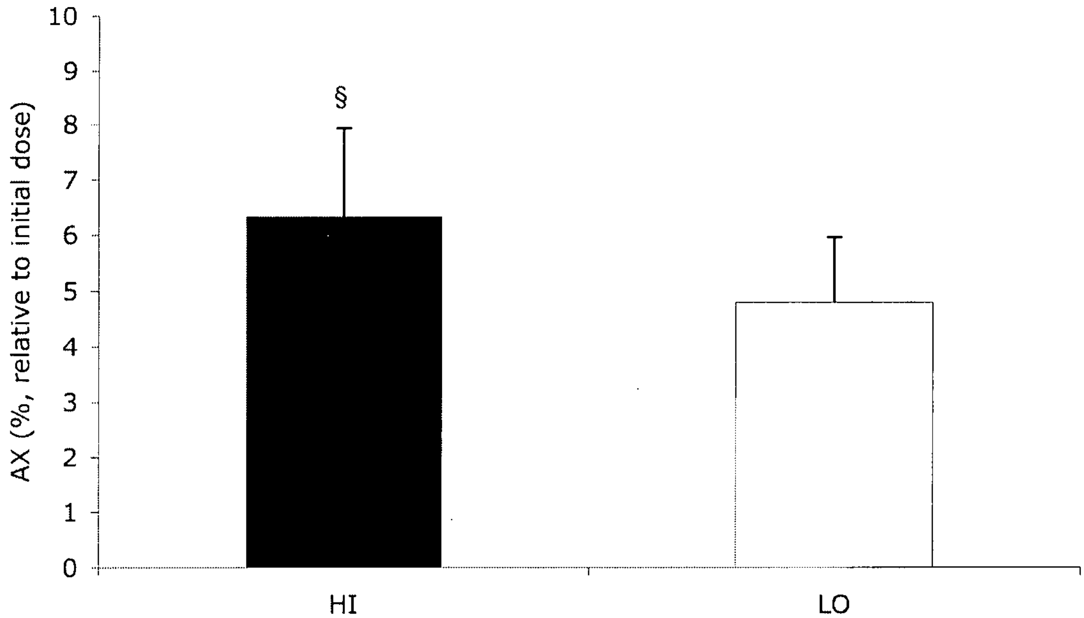

Figure 3.2 The effects of high (HI) versus low (LO) intensity arm cranking on radiopharmaceutical uptake at the axilla 65 min post-injection relative to the initial dose (AX) in healthy females (n=8). § denotes a significantly greater ($p<0.05$) value.

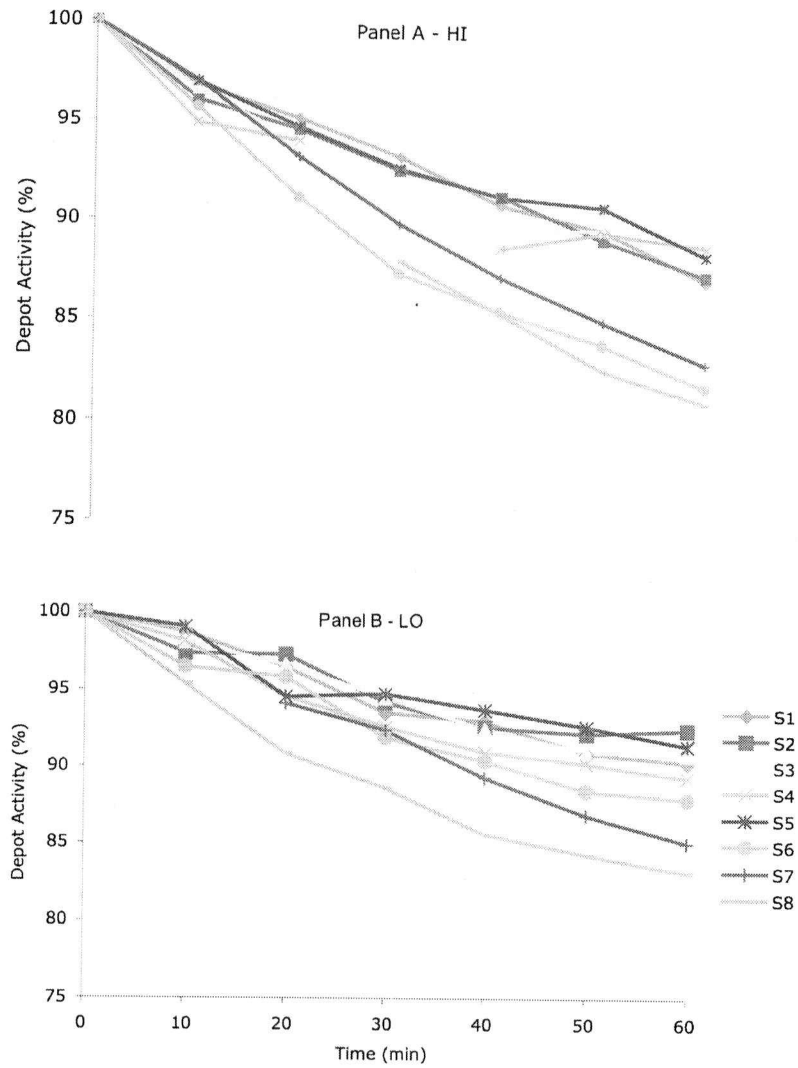

Figure 3.3 Individual depot activity (%)-time (min) curves for high intensity (HI - panel A) and low intensity (LO – panel B) arm cranking with the counts averaged for the right and left hand at each time point.

3.3.2 Heart Rate Data

High intensity arm cranking exercise resulted in a significantly greater heart rate averaged over the 60 min intermittent protocol (115 beats per minute (bpm) ±10.1) than did low intensity arm cranking exercise (94 bpm ±10.8; $p=0.000$).

3.4 DISCUSSION

This study demonstrated significantly faster clearance of ^{99m}Tc-antimony colloid from the hands of healthy females during intermittent arm crank exercise of high intensity (HI) compared to low intensity (LO). As well, a significantly greater amount of tracer was present in the axilla 65 min post-injection after HI.

Studies using lymphoscintigraphy to assess lymphatic function in women with BCRL often include intermittent forearm exercise between imaging scans such as squeezing a rubber ball or clenching the fist to stimulate lymph flow. There are several concerns with doing this type of exercise. First, the amount of work performed when fist clenching cannot be precisely quantified. Thus, if each subject performs the task at a different intensity, then lymph flow rate could be affected thereby introducing artificial variation into the data. Second, fist clenching recruits a small muscle mass and induces local muscle fatigue without a significant effect on regional blood flow. We have shown (12) that intermittent handgrip exercise (HG: twelve repeated bouts of 75 contractions in 2.5 minutes at 50% maximum voluntary contraction (MVC) followed by 2.5 minutes rest) resulted in CR values that were significantly slower than arm cranking exercise (5.0HI: six repeated bouts of 5.0 min arm cranking exercise at 0.6 $W{\cdot}kg^{-1}$ followed by 5.0 minutes rest). Moreover, the handgrip protocol yielded CR values that were similar to the control or resting arm. This indicates that an intermittent handgrip protocol may not be the most effective means of stimulating lymph flow between imaging scans.

The arm cranking protocol used by our lab previously (5.0HI, described above) only differed from HI in the present study in terms of the duty cycle (i.e., 5.0 minutes or 2.5 minutes of exercise followed by equal rest repeated over 60 minutes). Both the intensity and total volume of exercise completed over 60 minutes were identical between the two arm cranking protocols. 5.0HI resulted in a faster CR compared to the high intensity protocol used in the present study (-0.28 %$\cdot$min^{-1} vs. -0.24 %$\cdot$min^{-1}, respectively). The slight discrepancy in CR data could be due to different subjects taking part in each study and simply reflect normal variation. Or, possibly, intermittent exercise bouts of longer duration may have a more prominent effect on enhancing CR when the intensity of exercise is held constant.

Comparable CR values were found between the low intensity arm cranking task used in the present study and the intermittent handgrip protocol used previously (-0.19 %$\cdot$min^{-1} and -0.18 %$\cdot$min^{-1}, respectively) (12). Several mechanisms such as an increase in skeletal muscle contractions, sympathetic nervous system activity, ventilation, and changes in interstitial pressure are thought to stimulate lymph formation and lymph propulsion (13, 14). The extent to which each mechanism influences lymph transport in the upper extremity during different modalities of exercise is unknown. Likely, arm cranking exercise increases lymph flow by a combination of each of the aforementioned mechanisms, while rhythmic handgrip contractions does so predominantly by way of activation of the skeletal muscle pump and increased sympathetic nervous activity. Regardless of the mechanism employed to facilitate lymph transport, it appears that both moderate intensity handgrip contractions and low intensity arm cranking exercise similarly affect lymphatic function in the upper extremity.

Faster radiopharmaceutical clearance from the injection site is expected during high intensity opposed to low intensity arm cranking exercise. Typical physiological responses to exercise of increasing intensity include augmented blood flow, sympathetic nervous system activity, ventilation, and skeletal muscle contractions. As each of these physiological responses to exercise are also thought to be mechanisms that assist with lymph formation and propulsion (13, 14), then a concomitant increase in lymph flow with increased exertion would be expected. Increased exertion by the subjects was corroborated by the significantly higher heart rate during HI compared to LO.

The percentage of radiopharmaceuticals in the axillary lymph nodes relative to the initial dose may be a potential indicator of lymphatic drainage in individuals with lymphatic dysfunction (5, 10, 15, 16). Although symmetry in AX between upper extremities has been demonstrated during arm cranking exercise (Chapter Four), it is unknown if AX is sensitive enough to discriminate between changes in exercise intensity. HI resulted in significantly greater amount of radiopharmaceuticals measured in the axilla 65 min post-injection compared to LO. This would be expected considering CR was also faster during HI arm cranking protocol and, in fact, CR and AX were correlated (HI: r = -0.78; LO: r = -0.84). There are some limitations, however, to considering AX as an outcome measure. Despite colloid particles having good regional lymph node retention (10, 17), AX is only a 'snapshot' of the amount of tracer present in the axillary lymph nodes at any one time. Colloids will migrate through the lymph nodes and eventually enter the systemic circulation which can be confirmed in our subjects by visualization of activity in the liver at 65 min post-injection. It is unknown how long the radiopharmaceuticals are retained in the lymph nodes and if exercise affects retention time.

In conclusion, high intensity arm cranking exercise performed by healthy females resulted in significantly faster clearance of ^{99m}Tc-antimony colloid from the hands and significantly greater uptake of tracer in the axilla post-exercise compared to low intensity exercise. Further, the coefficient of variation for CR was 11.7% during high intensity exercise and 25.0% during low intensity exercise. Thus, the lymphatic system of healthy females has a predicable response to higher intensity exercise – function is increased and the variability in data is reduced.

A clinical application of this study is that a standardized lymphoscintigraphy exercise protocol has been developed that can now be applied to breast cancer survivors with and without BCRL in order to evaluate lymphatic function under a 'stressed' situation. This approach is similar to the way exercise is used by the cardiologist or respirologist to diagnose pathologies that are latent at rest.

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CHAPTER FOUR Symmetry in Lymphatic Function between the Right and Left Upper Extremities during Intermittent Arm Crank Exercise in Healthy Subjects

4.1 INTRODUCTION

Traditionally, quantification of lymphatic function has been confined to the measurement of circumferences or volume displacement to reflect change in status. Lymphoscintigraphy has added another dimension of lymphatic function and recently, Pain et al. (1) used quantitative lymphoscintigraphy to demonstrate that there is symmetry in lymphatic function between the upper extremities in normal subjects. This validates the common use of expressing the volume or lymphatic function of the affected arm relative to the normal arm, when investigating unilateral lymphatic disease such as breast cancer related lymphedema (BCRL). Although Pain et al. instructed subjects to perform a standardized exercise protocol consisting of 30 fist clenches between each set of readings, we have previously shown arm crank exercise to be more effective in enhancing depot clearance rate (CR) from the hands compared to hand grip exercise (2). In fact, we demonstrated that handgrip exercise yields CR values that were not statistically different than rest. As several reviews have suggested the use of a standardized stress protocol to investigate lymphatic dysfunction using lymphoscintigraphy (3-5), it is important to ensure there is symmetry in lymphatic function during exercise. Therefore, the purpose of the study was to evaluate the effects of arm crank ergometry on two measures of lymphatic function (radiopharmaceutical clearance rate and percent uptake of tracer in the axillary lymph nodes 65 minutes post-injection) in healthy subjects. A secondary purpose was to evaluate the reliability of the standardized stress protocol. As this study was designed to investigate symmetry in lymphatic function between arms during arm cranking exercise, both males and females were recruited. There is no theoretical reason to suggest that lymphatic function between arms is modified by sex.

4.2 MATERIALS AND METHODS

4.2.1 Subjects

Fifteen healthy females and six healthy males between the ages of 23-35 years participated in the study (age = 25.9 years ± 3.4; body weight = 65.7 kg ± 11.8; height = 173.1 cm ± 9.3; BMI = 21.8 $kg{\cdot}m^2$ ± 2.3). Seven subjects also participated in a reliability study and performed the exercise protocol on two separate days separated by a minimum of 48 hours. Prior to testing, informed consent was obtained from each subject and the study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects at the University of British Columbia.

4.2.2 Lymphoscintigraphy

Lymphoscintigraphy was used to quantify lymphatic function and was performed at Vancouver General Hospital, Division of Nuclear Medicine. Subjects were asked to arrive hydrated and rested. The same technician prepared and administered subcutaneous injections of the radiopharmaceutical Technetium-99m (^{99m}Tc)-antimony colloid to each subject on each testing day. The radiochemical purity of the radiopharmaceutical was greater then 95%. ^{99m}Tc-antimony colloid is 3-50 nanometers in size.

A total of 18 MBq of ^{99m}Tc-antimony colloid in 0.05 mL was administered to the first and fourth web spaces of each hand prior to the start of the exercise protocol. Subjects placed both hands prone on a gamma camera equipped with a low energy, ultra high resolution collimator (Sopha Medical Vision, Waukesha, Wisconsin, USA). Only the forearms and the hands were in the field of view of the camera and a single camera head was used. An initial one minute static acquisition with a 20% energy window centered on a 140 keV peak was acquired within one minute of the injection. Sequential images were taken every 10 minutes thereafter over a period of 60 minutes to allow for the calculation of depot clearance rate (CR). As well, a one minute static acquisition of the axilla was taken immediately post-exercise (approximately 65 minutes post-injection) to determine the radiopharmaceutical uptake in the axilla relative to the initial activity at the depot (AX). This image was taken with subjects lying supine.

4.2.3 Exercise Protocol

The exercise protocol started immediately after the first static image was acquired and involved repeated bouts of arm cranking for 2.5 minutes at 0.6 Watts per kilogram body weight ($W \cdot kg^{-1}$) followed by 2.5 min of rest on a calibrated LODE arm ergometer (Angio single set model, The Netherlands). This protocol was demonstrated to result in greater depot CR compared to hand grip exercise in a previous study conducted by our lab (2).

4.2.4 Data Analysis

The images were processed using Siemens Icon software (Version 8.5, Siemens Medical Systems Inc., Hoffman Estates IL, USA). The region of interest (ROI) was drawn around the injection sites of each hand to give the number of activity counts per hand. This was corrected for physical decay of ^{99m}-Tc using the following equation:

$$A_t = A_o e^{-\lambda t}$$

whereby A_t = activity after an elapsed time (min), A_o = activity in original sample, λ = 0.693/363 min physical half-life of ^{99m}-Tc, and t = elapsed time from the original image. The corrected count at each time point was divided by the corrected count measured immediately after injection and was plotted against time. Depot CR from each hand was linear and expressed as a linear constant (% administered activity$\cdot min^{-1}$).

4.2.5 Statistical Analysis

Linear regression using Statistica version 5.1 was used to assess symmetry in CR and AX. Significance was set at $p \leq 0.05$ and data is reported as mean ± standard deviation.

4.3 RESULTS

Fifteen healthy females and 6 healthy males completed the study. All 21 subjects were included in the analysis of CR while 12 subjects had AX measured. All data was analyzed for a curvilinear versus a linear fit and a linear fit was found to best represent all data sets.

There was a strong correlation between the right and left upper extremities for both CR ($y = 0.95x-0.02$; $r = 0.83$; $p = 0.001$; $n = 21$; Fig. 4.1) and AX ($y = 0.90x+0.56$; $r = 0.81$; $p = 0.001$;

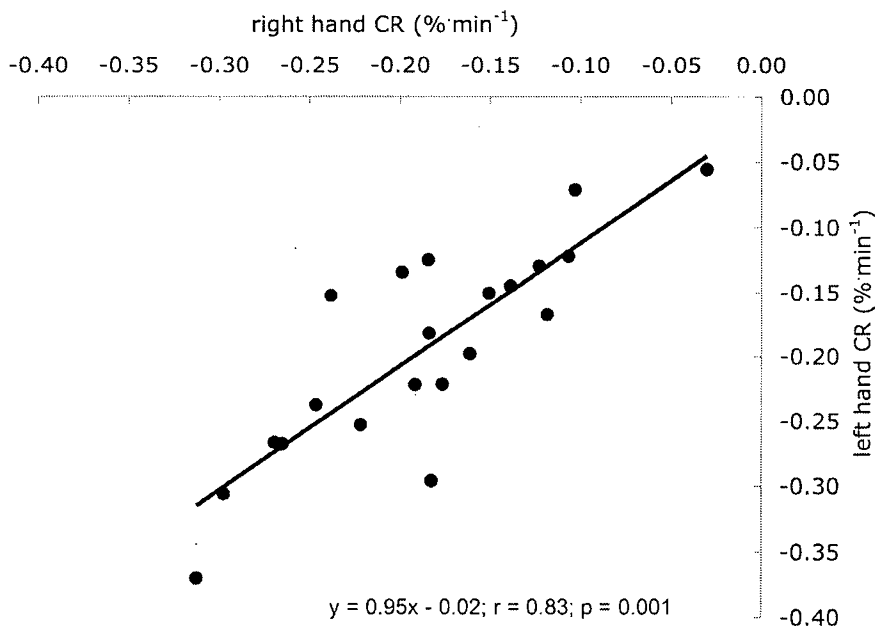

Figure 4.1 Correlation in depot clearance rate (CR) between right and left upper extremities in healthy subjects (n=21).

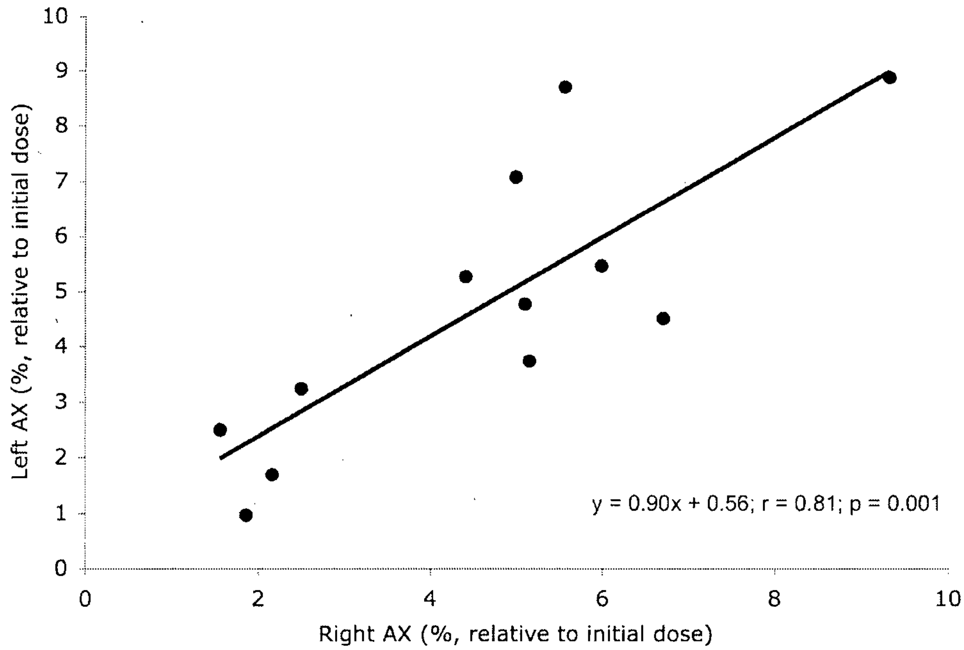

Figure 4.2 Correlation in tracer uptake (AX) between right and left axillary lymph nodes 65 minutes post-injection relative to initial dose in healthy subjects (n=12).

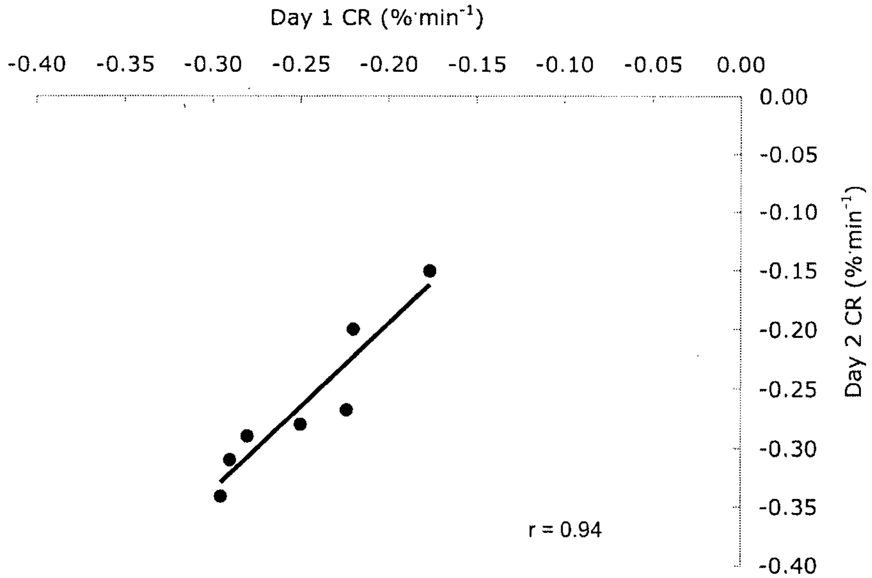

Figure 4.3 Reliability of depot clearance rate (CR) during moderate intensity arm cranking exercise performed on two separate days (n=7).

n = 12; Fig. 4.2). All subjects except one were right handed; thus, there was symmetry in dominant versus non-dominant arms as well. The correlations were essentially the same for both CR and AX whether the data was analyzed as right versus left upper extremity or dominant hand versus non-dominant hand.

Figure 4.3 shows intra-subject reliability of CR (r = 0.94, n = 7).

4.4 DISCUSSION

The present study demonstrated symmetry in depot clearance rate (CR) and tracer uptake at the axillary lymph nodes 65 minutes post-injection (AX) between upper extremities during arm cranking exercise in normal subjects. This extends the findings of Pain et al. (1) who found side-to-side symmetry in depot clearance rate and blood appearance rate at rest. Additionally, this study demonstrates high intra-subject reliability in CR (r = 0.94).

Only one other study has presented information on symmetry between limbs during exercise. Havas et al. (6) investigated lymphatic clearance rate in the legs of eight males during two types of isometric leg contractions (knees flexed and knees extended) and also dynamic knee extensions. The authors treated each leg as an independent observation due to the weak and non-significant correlation found in clearance rates between the legs. However, there are several factors that may explain the difference in findings amongst the present study and those by Havas et al. First, the weak correlation found by Havas et al. included clearance rate data pooled together for all three exercise conditions (i.e., n=24). Consequently, the reader does not know the variability for each type of muscular contraction. Perhaps, isometric leg contractions produce greater variability between the legs than did dynamic knee extensions. This scenario could produce an overall weak correlation despite one exercise condition showing symmetry between limbs. Second, the type of radiopharmaceutical used and location of injection differed between the studies. Havas et al. injected ^{99m}Tc human serum albumin directly into the vastus lateralis muscle of subjects, whereas subcutaneous injections of ^{99m}Tc-antimony colloid were administered in the present study. Other variations that may account for the discrepancy in findings may be the type and intensity of exercise performed, body position of subjects, and limbs used. The relative contribution of these factors to lymph function during acute dynamic exercise warrants further investigation.

It was expected that CR and AX would be comparable between upper extremities in normal subjects during upper body exercise. Bilateral exercise such as arm cranking involves the same muscle groups in each extremity - the forearm flexors and extensors, biceps brachii, triceps brachii, and the muscles of the upper back and shoulders. Subjects were also instructed not to favour their dominant arm during the arm cranking exercise protocol. Considering that the frequency and intensity of muscle contractions were similar between arms, the effect of the muscle pump on lymph flow should also be comparable.

Other factors that may affect the formation and propulsion of lymph are interstitial pressure, capillary filtration, muscle blood flow, blood pressure, and sympathetic nervous system activity (7). Again, these factors should be similarly influenced by arm cranking exercise in each extremity in normal subjects. For example, arm cranking exercise results in an increase in muscle blood flow in both arms due to vasodilation in the active musculature and an overall increase in cardiac output. An increase in muscle blood flow leads to greater capillary filtration, and correspondingly, higher interstitial pressure. This increase in interstitial pressure facilitates the entry of fluid and proteins into the initial lymphatic vessels through endothelial microvalves (7).

In conclusion, side-to-side symmetry in both clearance of ^{99m}Tc-antimony colloid from the hands of healthy subjects (CR) and uptake of tracer at the axillary lymph nodes 65 min post-injection (AX) was evident during intermittent upper body dynamic exercise. The results indicate that when investigating the effect of exercise on lymphatic function in women with breast cancer related lymphedema, the untreated arm can be used as each subject's own control during exercise conditions.

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CHAPTER FIVE Upper Extremity Lymphatic Function at Rest and during Exercise in Breast Cancer Survivors with and without Lymphedema

5.1 INTRODUCTION

Over the past five years there has been a significant increase in research investigating the pathophysiology of breast cancer related lymphedema (BCRL) using lymphoscintigraphy (1-12). Despite the increase in active research, BCRL is still a poorly understood complication of breast cancer treatment. Current data suggest 28% of women treated for breast cancer will develop BCRL (13, 14), although the incidence can vary from 13% (15) to 49% (16) depending on the operational definition of BCRL. Women with BCRL have restricted range of motion in the affected arm, increased risk of infection, and impaired limb function. Furthermore, these women report increased anxiety and depression and reduced quality of life (17). BCRL is essentially an incurable condition although treatments such as compression sleeves (18-20), pneumatic pumping (21-25), manual lymphatic drainage (26-30), and low-level laser therapy (31, 32) exist to contain swelling.

Exercise was originally thought to exacerbate BCRL; however, all recent studies (33-38) except one (39) have shown no significant change in arm volume with resistance training or upper body aerobic exercise. Our finding of a significant increase in arm volume in breast cancer survivors with and without BCRL after a 20 week exercise program consisting of aerobic training, resistance training, and dragonboat paddling was not construed to be reason for concern (39). The changes in arm volume were consistent for both arms and there was a significant gain in upper body muscular strength over the course of the study period. Abe et al. (40) demonstrated a significant increase in muscle thickness with resistance training in men and women over a 12 week program. Although we did not measure muscle volume directly, the training programs were comparable. Thus, muscle hypertrophy likely accounted for the increase in arm volume seen in our subjects.

A limitation of research investigating the effect of regular exercise on the development or exacerbation of lymphedema is that measures of arm circumference or volume have been used to indicate a change in status. While arm volume is a simple and non-invasive measurement to perform, it does not provide information on changes in lymphatic function.

Lymphoscintigraphy, on the other hand, does give an indication of lymphatic function. This technique was developed to assess lymphatic status in patients with a variety of conditions that cause lymphatic obstruction and is now being used to investigate the pathophysiology of BCRL. Typically, lymphoscintigraphy applied to BCRL involves administering a radiopharmaceutical to the finger web-space or forearm and imaging the upper extremity using a gamma camera at various time points post-injection. The radiopharmaceutical is generally a technetium labeled macromolecule or colloid that is of sufficient size (i.e., between 4 and 100 nm) to enter the lymphatic capillaries and not blood capillaries (41-43).

To date, all studies using lymphoscintigraphy to examine lymphatic function in women treated for breast cancer have been essentially performed at rest. Intermittent forearm exercise, such as clenching the fists or squeezing a rubber ball between imaging scans, has been used in several studies to stimulate lymph flow (4, 6, 7, 10, 44-46). However, we have previously shown depot clearance rate (CR) in healthy females to be similar whether rest or hand grip exercise was performed (47). There are several advantages to measuring lymphatic function in breast cancer survivors with and without BCRL during moderate intensity exercise. First, a standardized exercise protocol can help to decrease variability in the data especially when the intensity and duration of the activity is quantified. For example, we have demonstrated a lower coefficient of variation (CV) for depot CR during moderate intensity arm cranking exercise versus low intensity exercise (48). Second, evaluating the lymphatic system in a 'stressed' situation can help to diagnose pathologies that may be latent at rest similar to the way exercise is used by a cardiologist or respirologist.

In order to determine an appropriate upper body exercise protocol that could be combined with lymphoscintigraphy to describe lymphatic function in women treated for breast cancer, we have completed a series of studies in healthy, college-aged females (Chapters 2-4). Collectively, these studies demonstrated the following: 1) arm cranking was superior to intermittent hand grip exercise in enhancing CR (Chapter 2), 2) moderate intensity arm cranking exercise (same protocol used in the present study) was superior to low intensity arm cranking exercise in enhancing CR and uptake of radiopharmaceuticals in the axilla (Chapter 3), 3) CR and uptake of radiopharmaceuticals in the axilla were symmetrical between arms (Chapter Four), 4) moderate intensity arm cranking exercise results in a lower CV than low intensity arm cranking exercise (Chapter 3), and 5) there is a high intra-subject reliability in arm cranking CR (Chapter Four).

Thus, the purpose of this exploratory study was to evaluate upper extremity lymphatic function at rest and during intermittent, moderate intensity exercise in breast cancer survivors (BC), breast cancer survivors with BCRL (BCRL), and age-matched controls (CONT). The primary outcome measure of lymphatic function is defined as the radiopharmaceutical clearance rate of Technetium-99m (^{99m}Tc)-antimony colloid from a subcutaneous injection site in the hand (CR). Secondary outcome measures of lymphatic function include uptake of radiopharmaceuticals at the axilla 65 min post-injection relative to the initial dose (AX); uptake of radiopharmaceuticals in the forearm 65 min post-injection relative to the initial dose (FORE); and, time of first appearance of radiopharmaceuticals at the axilla (APP).

The hypotheses to be tested were:

1) Lymphatic function will be similar between groups in the contralateral arm,
2) Lymphatic function relative to the contralateral arm will be poorest in women with BCRL (i.e., slowest CR and APP, lowest AX, and highest FORE) and highest in control subjects (i.e., fastest CR and APP, highest AX, and lowest FORE). Women treated for breast cancer without lymphedema will have lymphatic function that falls between those with BCRL and healthy controls.
3) Lymphatic function will increase from rest to exercise in all subject groups.

5.2 MATERIALS AND METHODS

5.2.1 Subjects

Three subject groups (breast cancer survivors with lymphedema, BCRL, n=10; breast cancer survivors no lymphedema, BC, n=22; healthy age-matched females with no history of breast cancer, CONT n=17) were included in the prospective, cross-sectional analysis. Both BC and BCRL included women diagnosed in the past 25 years with Stage I-III breast cancer and treatment. Those with BCRL had clinically significant lymphedema defined as a difference of greater than 2.0 cm at either of the following measuring points (20) - 10 cm distal to the lateral epicondyles or 15 cm proximal to the lateral epicondyles. CONT included healthy females with no history of breast cancer that were matched by age to BC and BCRL. For control subjects, the right or left arm was randomly designated as the ipsilateral arm using a random numbers generator. Descriptive data is provided in Table 5.1 while details of breast cancer treatment is provided in Table 5.2.

Prior to testing, informed consent was obtained from each subject and the study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects at the University of British Columbia.

Sample size was determined using a webpage developed by the Department of Math at York University to calculate power and sample size for between-group ANOVA designs (http://www.math.yorku.ca/SCS/Online/power). The primary outcome measure, CR, was used to calculate the sample size. Based on CR values observed in college-aged females performing the same exercise protocol (48) and pilot work testing several subjects with BCRL, the anticipated difference between means was 0.07 % min^{-1} and the anticipated standard deviation was set at 0.06 % min^{-1}. Power and type I error was selected to be 80% and 5%, respectively. Using these parameters, a minimum of 16 subjects was required per group.

5.2.2 Experimental Design

A prospective, exploratory, between-groups design was used to compare differences in lymphatic function in the three subject groups. The independent variables were the intermittent exercise protocol and rest, while the dependent variable was lymphatic function. The primary outcome measure of lymphatic function was depot clearance rate (CR), while secondary outcomes measures included time of first appearance of radiopharmaceuticals at the axilla (APP), and uptake of the radiopharmaceuticals at 65 minutes post-injection relative to the initial dose at the axilla (AX) and forearm (FORE).

5.2.3 Experimental Procedures

Imaging

Lymphoscintigraphy was used to quantify lymphatic function and was performed at Vancouver General Hospital, Department of Nuclear Medicine. Subjects were asked to arrive hydrated and rested. They were also asked to avoid caffeine in the six hours before the start of the study. The same technician prepared and administered subcutaneous

Table 5.1 Sample characteristics (mean ± standard deviation) of subjects with breast cancer related lymphedema (BCRL), breast cancer (BC), and controls (CONT).

	BCRL	BC	CONT
n	10	22	17
Age (yrs)	61.6 ±11.0	55.4 ±7.2	56.8 ±9.6
Weight (kg)	80.7 ±15.6§	70.3 ±9.9	68.9 ±13.4
Height (m)	1.63 ±0.04	1.65 ±0.06	1.63 ±0.03
BMI ($kg \cdot m^2$)	30.4 ±5.8§	25.7 ±3.3	26.0 ±4.6
Exercise Power Output (Watts)	39.9 ±10.3	41.0 ±4.8	38.1 ±4.5
Exercise Heart Rate ($beats \cdot min^{-1}$)	129.8 ±16.0	128.6 ±17.2	126.5 ±18.4
Arm Circumference (%, ipsilateral arm relative to contralateral arm)			
Forearm	115.8 ±10.8§	100.6 ±3.4	99.1 ±3.9
Upper arm	110.5 ±12.9§	100.5 ±2.6	99.6 ±2.1

§ significantly different from BC and CONT ($p<0.05$)

Table 5.2 Details of patients treated for breast cancer with (BCRL) and without (BC) breast cancer related lymphedema.

Subject	BCRL or BC	Age (yrs)	BMI (kg·m^2)	Ipsilateral Arm	Year of Diagnosis	Type of Surgery	Nodes Excised	Nodes Positive	RT to Axilla	CT	BCRL Duration	Increase in volume (%) Forearm	Upper Arm
1	BCRL	50	28.2	left	1996	m	19	0	y	y	10 yr	125	100
2	BCRL	52	37.5	rt	2000	m	20	1	y	y	5 yr	108	107
3	BCRL	46	33.0	left	2005	m	16	2	y	y	1 yr	115	100
4	BCRL	59	40.6	left	2004	m	14	1	n	y	2 yr	102	109
5	BCRL	65	25.0	left	2003	m	7	0	y	y	3 yr	117	103
6	BCRL	74	28.8	left	2000	l	20	3	y	y	6 yr	133	116
7	BCRL	74	29.7	rt	1984	l	4	0	y	n	18 yr	127	142
8	BCRL	77	29.2	rt	1993	m	16	7	y	y	12 yr	123	119
9	BCRL	64	20.4	rt	2001	l	25	2	y	y	1 yr	111	102
10	BCRL	55	31.9	rt	2004	m	21	4	y	y	1 yr	102	107
11	BC	50	27.5	rt	2004	m	n/a	0	y	y		98	102
12	BC	53	31.0	left	2001	l	10	0	y	y		102	101
13	BC	55	21.1	rt	1994	l	10	2	y	y		100	100
14	BC	59	26.3	left	2000	l	1	1	y	y		100	98
15	BC	59	23.3	rt	2000	m	13	0	n	y		99	101
16	BC	53	22.9	left	2005	m	7	1	n	y		92	108
17	BC	59	31.8	left	1995	m	13	0	n	n		104	99
18	BC	59	24.9	rt	2000	m	40**	2	n	y		103	102
19	BC	66	22.4	rt	1998	l	14	3	y	n		105	98
20	BC	44	26.7	rt	2005	m	13	0	y	y			
21	BC	45	23.5	rt	2001	l	9	1	y	y		100	102
22	BC	47	26.8	rt	2000	m	0	0	n	n		98	101
23	BC	54	30.8	rt	1991	m	10	0	n	n		105	103
24	BC	58	25.9	left	1996	l	13	2	y	y		99	100
25	BC	58	28.2	left	1999	m	6	0	n	n		100	100
26	BC	58	32.0	left	1990	m	4	4	y	y		98	95
27	BC	60	23.2	left	2005	l	19**	3	y	y		98	96
28	BC	72	21.3	left	1995	m	16**	0	y	n		103	101
29	BC	54	24.0	rt	2004	m	19**	2	y	y		102	99
30	BC	63	25.5	rt	1983	m	0	0	n	n		98	101
31	BC	44	22.3	rt	2005	m	0	0	y	y		101	102
32	BC	48	25.0	rt	2005	m	12	2	y	y		109	103

*l=lumpectomy, m=mastectomy, RT=radiation therapy, CT=chemotherapy, increase in volume=increase is relative to circumference on contralateral side, **BC subjects with similar number of axillary lymph nodes dissected as all BCRL subjects.

injections of the radiopharmaceutical ^{99m}Tc-antimony colloid to all of the subjects on each testing day. ^{99m}Tc-antimony colloid is 3-50 nanometers in size.

Four injections with a total activity of 18 MBq, each dose in 0.05 mL, were introduced into the first and fourth web spaces of each hand prior to the start of the exercise protocol. Subjects placed both hands prone on a gamma camera equipped with a low energy, ultra high resolution collimator (Sopha Medical Vision, Wisconsin, USA). Only the forearms and the hands were in the field of view of the camera and a single camera head was used. One minute static acquisitions with a 20% energy window centered on a 140 kV peak were taken within one minute of the injection and again every ten minutes post-injection over a total period of 60 minutes to allow for the calculation of CR. APP was determined from a one minute static acquisition taken of the axilla every ten minutes until radiopharmaceuticals were visualized in axillary lymph nodes on both sides (NOTE: this variable was introduced after the first seven subjects had completed the study). Subjects were seated with the camera heads in a vertical orientation for APP. At 65 minutes post-injection, a whole body scan was taken in order to calculate AX and FORE. Subjects were in the supine position for the upper body scan and the camera head was centered on the axilla. The images were stored on a hard drive as a 256 x 256 Word frame format for later analysis.

The images were processed using Siemens Icon software (Version 8.5, Siemens Medical Systems Inc., Illinois, USA). The region of interest (ROI), or the selection of pixels for analysis, was drawn around each of the injection sites to give the number of activity counts and each ROI was corrected for physical decay of $^{99m-}$Tc using the following equation:

$$A_t = A_o e^{-\lambda t}$$

whereby A_t = activity after an elapsed time (min), A_o = activity in original sample, $\lambda = 0.693/363$ min physical half-life of $^{99m-}$Tc, and t = elapsed time from the original image. All ROIs for CR and AX were normalized to an area of 1500 pixels while all ROIs for FORE were normalized to an area of 3000 pixels.

CR was calculated from images of the depot sites. The two counts from each hand were included in the same ROI and the corrected count at each time point was divided by the corrected count measured immediately after injection and was plotted against time. CR from the injection site was linear ($R^2 = 0.95$ during exercise for all subject groups) and, therefore, expressed as a slope (% administered activity$\cdot$min^{-1}).

ROIs were also drawn around the axilla and forearms from the images generated from the upper body scan taken at 65 min post-injection. These were expressed relative to the initial amount of radioactivity at the injection site to determine AX and FORE. Since no markers were used to indicate the position of the wrist, elbow, and axilla for each image, then the ROI for the forearm had to be estimated. This was done by measuring the distance from the proximal portion of the activity at the injection site to the distal portion of activity in the axillary lymph nodes in the normal, or contralateral arm, and dividing it into two symmetrical, rectangular ROIs with the most distal rectangular ROI representing the forearm. Future research should use markers to identify anatomical landmarks on the upper extremity.

APP was determined from images of the axilla taken every 10 minutes throughout the 60 minute protocol. Background activity was determined for each set of images and ROIs were drawn around the axilla on each side. Once activity in the ROI of the axilla doubled background activity, APP was noted. Experimenters were not blinded to the subjects or experimental condition when doing ROI.

Exercise & Rest Conditions

On separate days, subjects did either the intermittent exercise protocol (twelve repeated sets of arm cranking for 2.5 min at 0.6 $W \cdot kg^{-1}$ followed by 2.5 min of rest) or seated rest. The exercise began immediately after the first imaging scan and continued to 60 min post-injection and was performed on a calibrated Lode arm ergometer (Angio single set model, The Netherlands). Heart rate (HR) was recorded every 10 minutes throughout using a Polar T31 heart rate monitor (Polar Electro Inc, Lake Success, NY). Not all subjects were able to complete the prescribed power output for EX (five BCRL subjects, one BC subject, and one CONT subject). For these subjects, power output was decreased by 10-15 Watts so they could complete the 60 minute intermittent, exercise challenge.

5.2.4 Statistical Analyses

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Inc., Chicago, IL) and significance was set at $p \leq 0.05$. Descriptive statistics (mean ± standard deviation) were calculated for subject characteristics. Differences in height, weight, BMI, age, exercise power output, exercise heart rate, and forearm and upper arm circumferences (ipsilateral arm relative to contralateral arm) were compared using one-way analysis of variance (ANOVA).

To determine if lymphatic function in the unaffected, or contralateral arm, of women treated for breast cancer is normal (i.e., similar to control data), a 3x2 mixed model ANOVA was used to determine differences in lymphatic function (CR, AX, FORE, and APP) between groups at rest and during exercise. As contralateral arm function in both breast cancer groups was determined to be normal, then the contralateral arm served as each subject's own control and was used for all subsequent analyses.

3x2 mixed model ANOVA on ipsilateral relative to contralateral arm lymphatic function was used to determine changes between subject groups at rest and during exercise.
Post-hoc analysis for any main effects was performed using Tukey's test of honest significant differences.

5.3 RESULTS

Recruitment began in October 2003 and ended in June 2006. Seventeen women with BCRL expressed interest in the study; however, only ten subjects decided to take part. Of those, two did not want to undergo a second test which was the resting protocol in both cases. Thirty-five women with breast cancer (no BCRL) expressed interest in the study and 22 subjects took part. Of those, one did not want to undergo a second test (the rest protocol) and one subject has no exercise data due to technical reasons. Subjects who expressed interest but declined to participate were asked their reason and every woman who declined sited the administration of radiopharmaceuticals with a needle as their reason for not participating. (Women treated for breast cancer are often told by their health care professionals to avoid needles on the treated side to minimize risk of lymphedema development). The APP measure was added after the first seven subjects were tested, and so, was not available for every subject. Finally, a number of data points were unavailable due to technical problems.

All data was checked for normality of distribution. The Shapiro-Wilk normality tests indicated that each variable was normally distributed for BC and CONT at rest and during exercise. For

BCRL subjects, AX and APP were not normally distributed; however, this may be due to the small sample size. Data compared by ANOVAs were checked to ensure the assumption of sphericity was not violated. Although data for FORE had unequal variance between groups, the assumption of sphericity, as calculated by SPSS, was not violated.

Other research using lymphoscintigraphy with breast cancer patients show mono-exponential time-activity curves (4, 7, 9-11, 44); however, we have demonstrated linear relationships between CR and time (47, 48). This difference in time-activity relationships is explained by the use of different radiopharmaceuticals. For example, a smaller radiopharmaceutical such as ^{99m}Tc-antimony colloid would likely have a faster CR than a larger radiopharmaceutical such as human serum albumin (41). In the present study, R^2 values between CR and time during exercise for the three subject groups - BCRL, BC, and CONT - were 0.92, 0.94, and 0.92 for the contralateral arm, respectively, and 0.94, 0.95, and 0.95 for the ipsilateral arm, respectively.

5.3.1 Subject Characteristics

Characteristics of the study participants are summarized in Table 5.1. Age, height, exercise power output, and exercise heart rate were similar between groups. Subjects with BCRL had a significantly greater weight (p=0.050), BMI (p=0.018), forearm circumference (p=0.000), and upper arm circumference (p=0.000) compared to both BC and CONT. Details of breast cancer treatment are provided in Table 5.2.

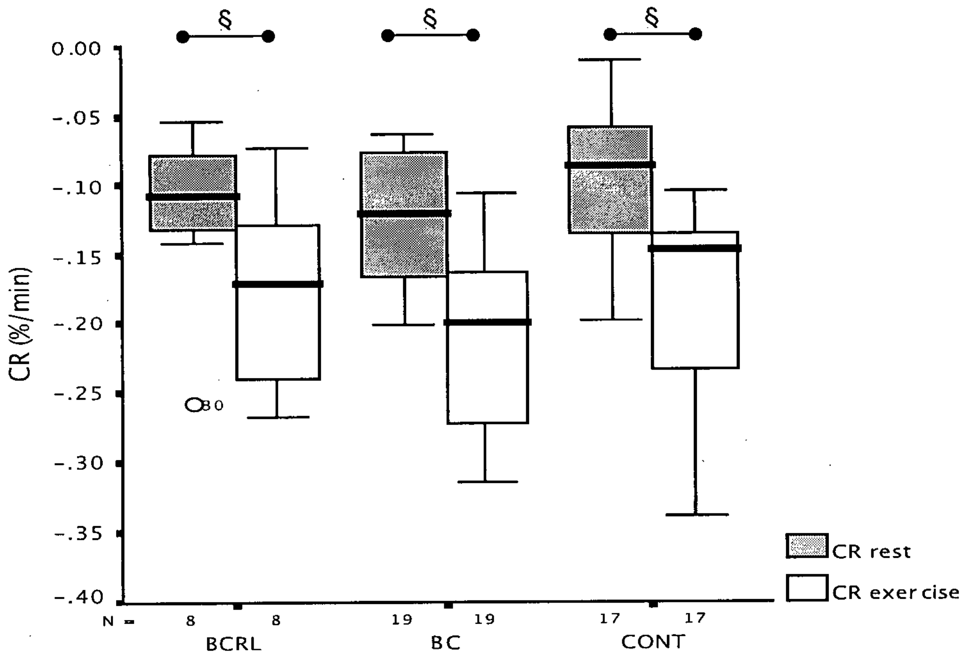
5.3.2 Contralateral Arm Lymphatic Function

Contralateral arm lymphatic function (CR, AX, and FORE) in women treated for breast cancer (BC and BCRL) was not significantly different than results obtained for the control group (Fig. 5.1). Thus, the contralateral arm served as each subject's own control for subsequent analyses.

The addition of exercise caused CR and AX to significantly increase (CR: p=0.000; AX: p=0.003). As expected, FORE did not change from rest to exercise (p=0.553). Post-hoc analysis revealed that BC and CONT displayed significant increases in CR and AX from rest to exercise, but BCRL only achieved significant increases in CR. BCRL subjects showed a trend towards an increase from rest to exercise in AX (6.4% ±3.1 vs. 7.4% ±3.0, p=0.249); however, statistical significance was not achieved.

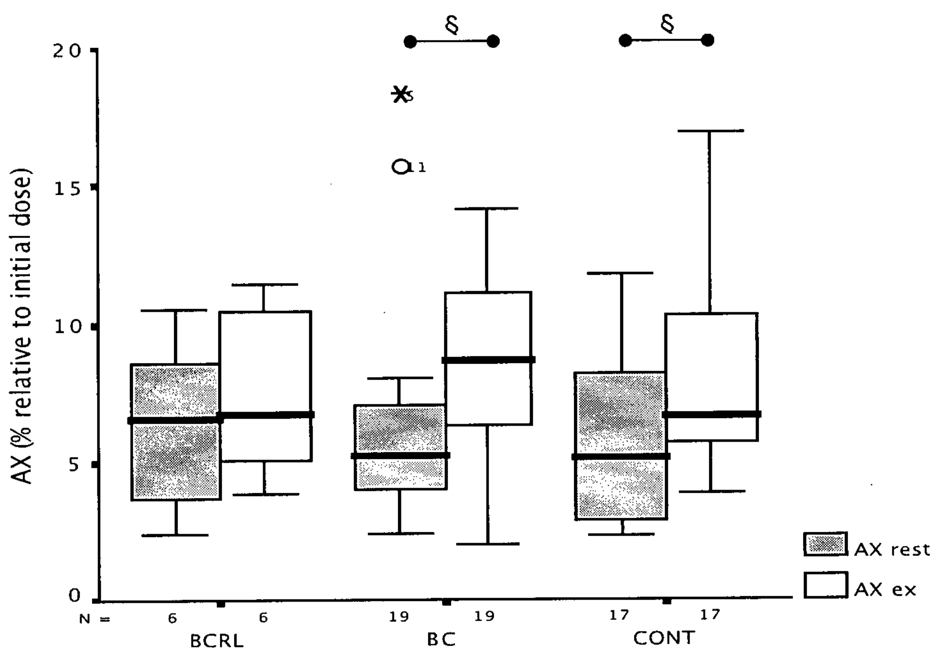
Figure 5.1 Lymphatic function as represented by depot clearance rate (CR, Panel A), uptake in the axillary lymph nodes at 65 min post-injection (AX, Panel B), and uptake in the forearm at 65 post-injection (FORE, Panel C) at rest and during exercise in the normal, or contralateral arm, of women treated for breast cancer with lymphedema (BCRL), women treated for breast cancer with no lymphedema (BC), and age-matched controls (CONT). Resting data is indicated in grey boxes while exercise data is in white boxes. The box plot indicates the distance between the 75th percentile and 25th percentile (box), the median value (solid back line), and the highest and lowest values (whiskers), excluding outliers (circles) and extreme outliers (asterisks). § denotes a significant difference between resting and exercise CR values for all subject groups. A significant difference (§) between resting and exercise AX values was found for BC and CONT as well. As expected, no difference was found between rest and exercise FORE values for any group.

Panel A - CR



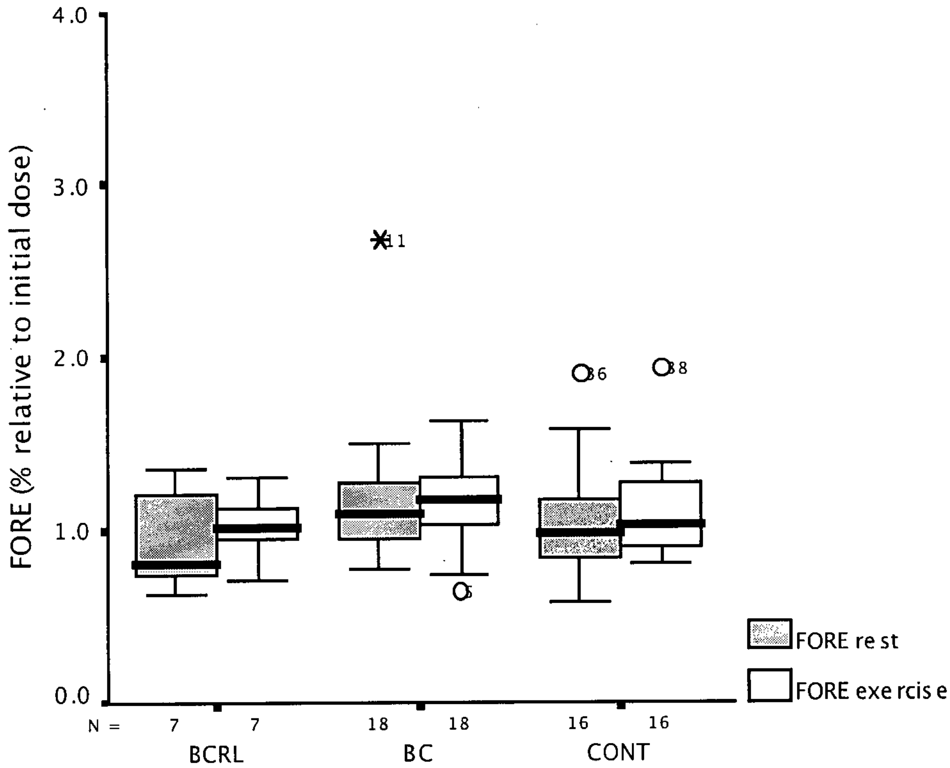
§ Indicates significant difference (p<0.05) between conditions

Panel B - AX



§ Indicates significant difference (p<0.05) between conditions

Panel C - FORE



4.0
3.0
2.0
1.0
0.0
FORE (% relative to initial dose)
11
36
38
5
FORE rest
FORE exercise
N =
7
7
18
18
16
16
BCRL
BC
CONT

Appendix D shows the relationship between CR and AX for the contralateral arm for resting (Panel A) and exercise conditions (Panel B). A significant relationship was found for at rest (contralateral CR vs. contralatereral AX: r = -0.439, p=0.004, n=44) and during exercise (contralateral CR vs. contralateral AX: r = -0.309, p=0.049, n=44).

5.3.3 Ipsilateral Relative to Contralateral Arm CR

Fractional removal rate of ^{99m}Tc-antimony colloid from the subcutaneous depot over the 60 min exercise and rest protocol in ipsilateral and contralateral arms for each subject group are presented in Figure 5.2. Box plots showing ipsilateral relative to contralateral CR at rest and during exercise are shown in Figure 5.3. When lymphatic function in the ipsilateral arm is stated relative to the contralateral arm, then a value of 100% indicates similar lymphatic function between arms.

There was no difference in ipsilateral relative to contralateral CR between groups at rest or during exercise (p=0.703). Further, this ratio did not change from rest to exercise (p=0.648). Individual CR data for women with BCRL and BC is in Figure 5.4. The coefficient of variation (CV) for CR between measurements from the right and left arms of the same CONT subject during rest and exercise was 30.4% and 14.4%, respectively.

For BCRL, there was a non-significant inverse relationship between exercise CR and forearm circumference (r = -0.457, p=0.184, n=10) and between exercise CR and upper arm circumference (r = -0.200, p=0.580, n=10). This was also true for BC (exercise CR and forearm circumference: r = -0.038, p=0.879, n=19; exercise CR and upper arm circumference: r = -0.190, p=0.437, n=19). Correlations between exercise CR and forearm/upper arm circumference for BCRL are presented in Figure 5.5.

5.3.4 Ipsilateral Relative to Contralateral AX

Figure 5.6 shows uptake at the axilla 65 min P.I. relative to the initial dose (AX) for the ipsilateral relative to the contralateral arm. There was a significant difference in AX between groups at rest (p=0.001) and during exercise (p=0.002). Specifically, BCRL at rest demonstrated significantly lower AX (27.2% ±24.1) compared to both BC (74.8% ±39.8) and CONT (104.9% ±52.1). During exercise, BCRL subjects also had significantly lower AX (26.5% ±33.6)

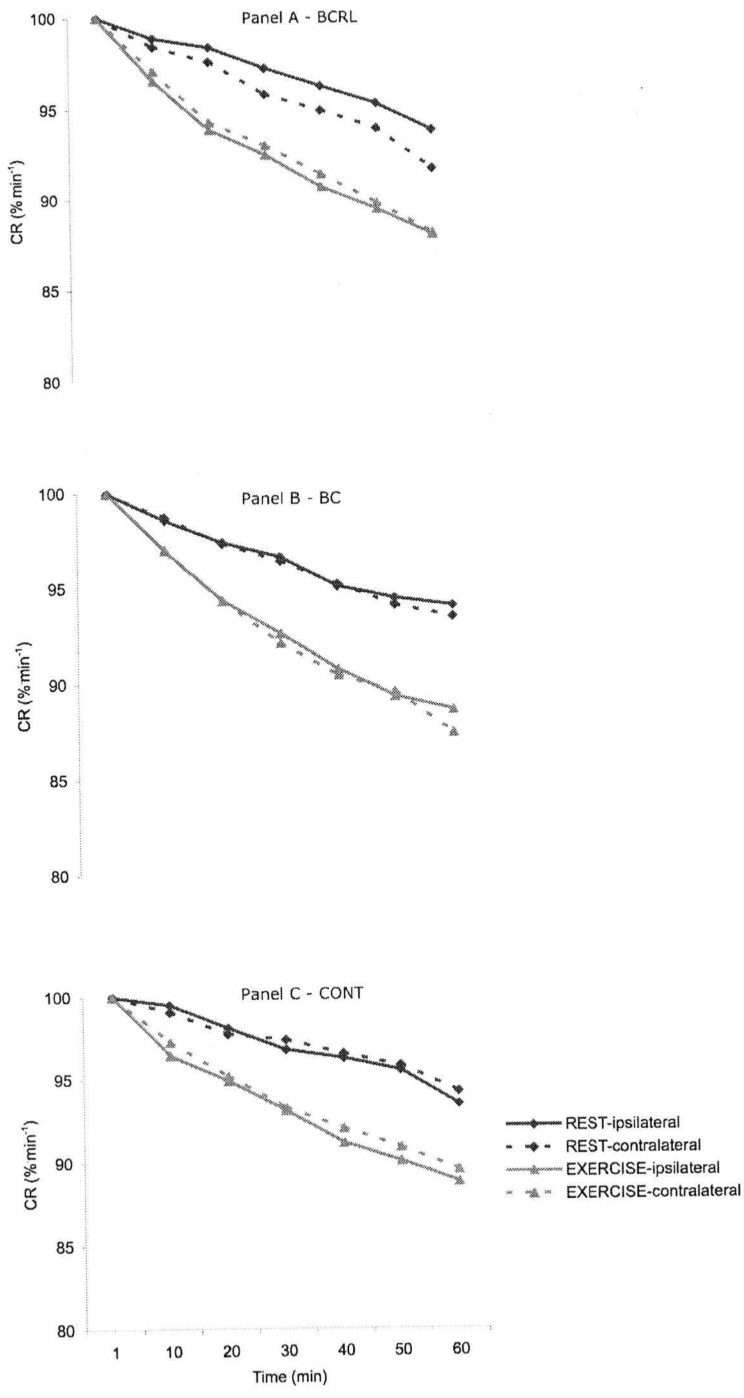


Figure 5.2 Plot of fractional removal rates of ^{99m}-Tc antimony colloid from the subcutaneous depot of the affected, or ipsilateral arm (solid line) and normal, or contralateral arm (dashed line) of women treated for breast cancer with lymphedema (Panel A - BCRL), women treated for breast cancer with no lymphedema (Panel B - BC), and age-matched controls (Panel C - CONT) at rest and during exercise.

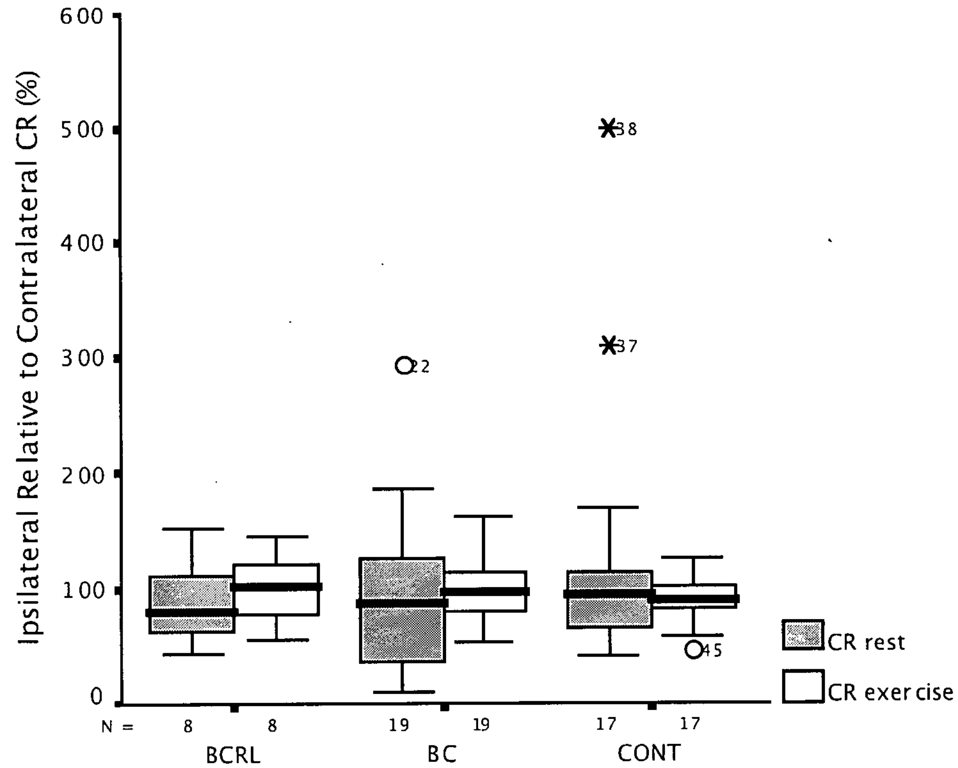


Figure 5.3 Ratio of ipsilateral to contralateral arm depot clearance rate (CR) in women treated for breast cancer with lymphedema (BCRL), women treated for breast cancer (BC), and age-matched controls (CONT) at rest and during exercise. Resting data is indicated in grey boxes while exercise data is in white boxes. A ratio of 100% indicates symmetry between arms. A ratio greater than 100% indicates faster CR on the ipsilateral compared to contralateral side while a ratio less than 100% indicates slower CR on the ipsilateral compared to contralateral side. The box plot indicates the distance between the 75th percentile and 25th percentile (box), the median value (solid back line), and the highest and lowest values (whiskers), excluding outliers (circles), and extreme outliers (asterisks). No significant difference was found between groups at rest or during exercise.

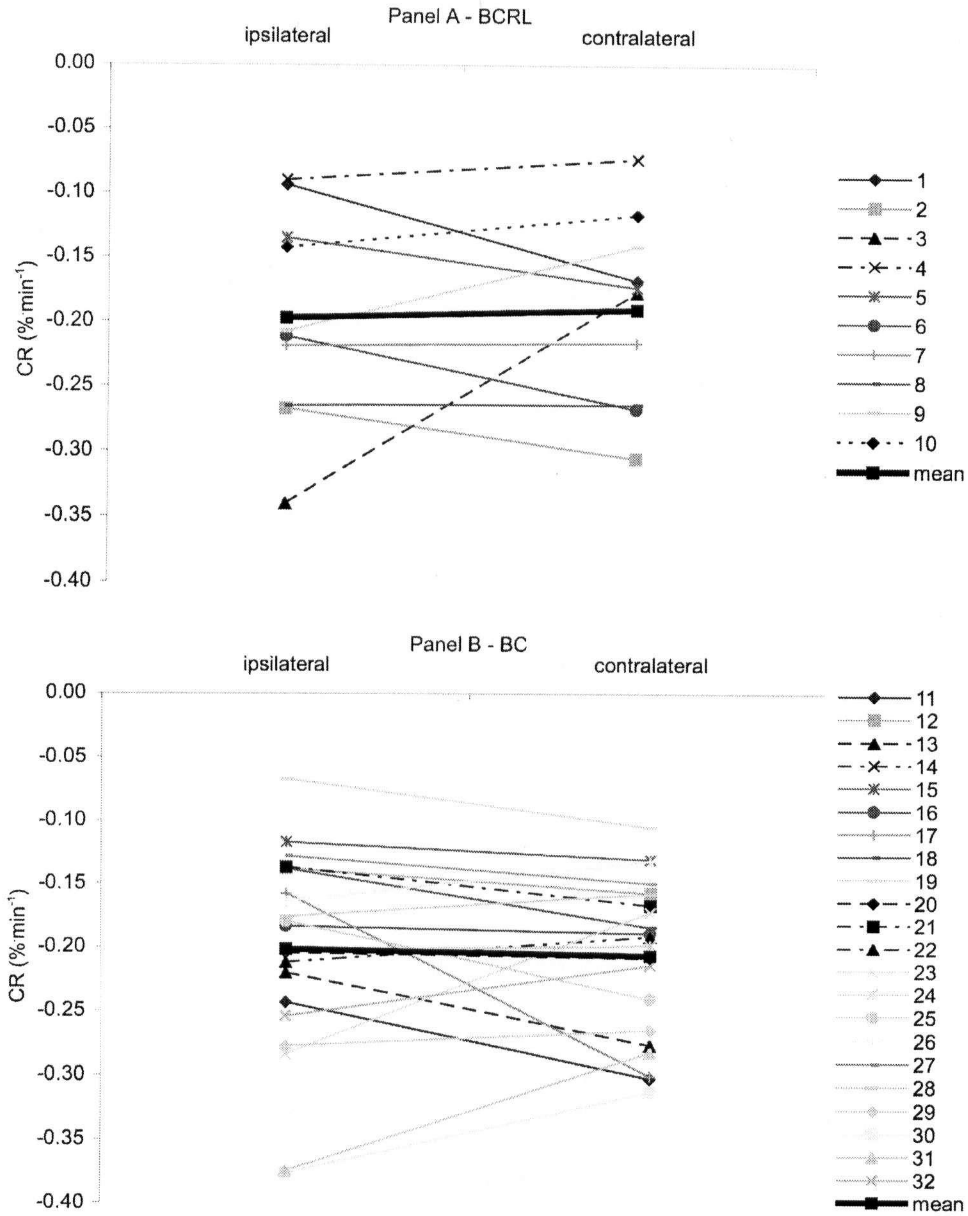


Figure 5.4 Clearance rate (CR) during exercise for all pairs of contralateral and ipsilateral arms of women treated for breast cancer with lymphedema (Panel A - BCRL) and women treated for breast cancer (Panel B - BC). The numbers indicate the patient represented by each pair of points (same subject number as in Table 1).

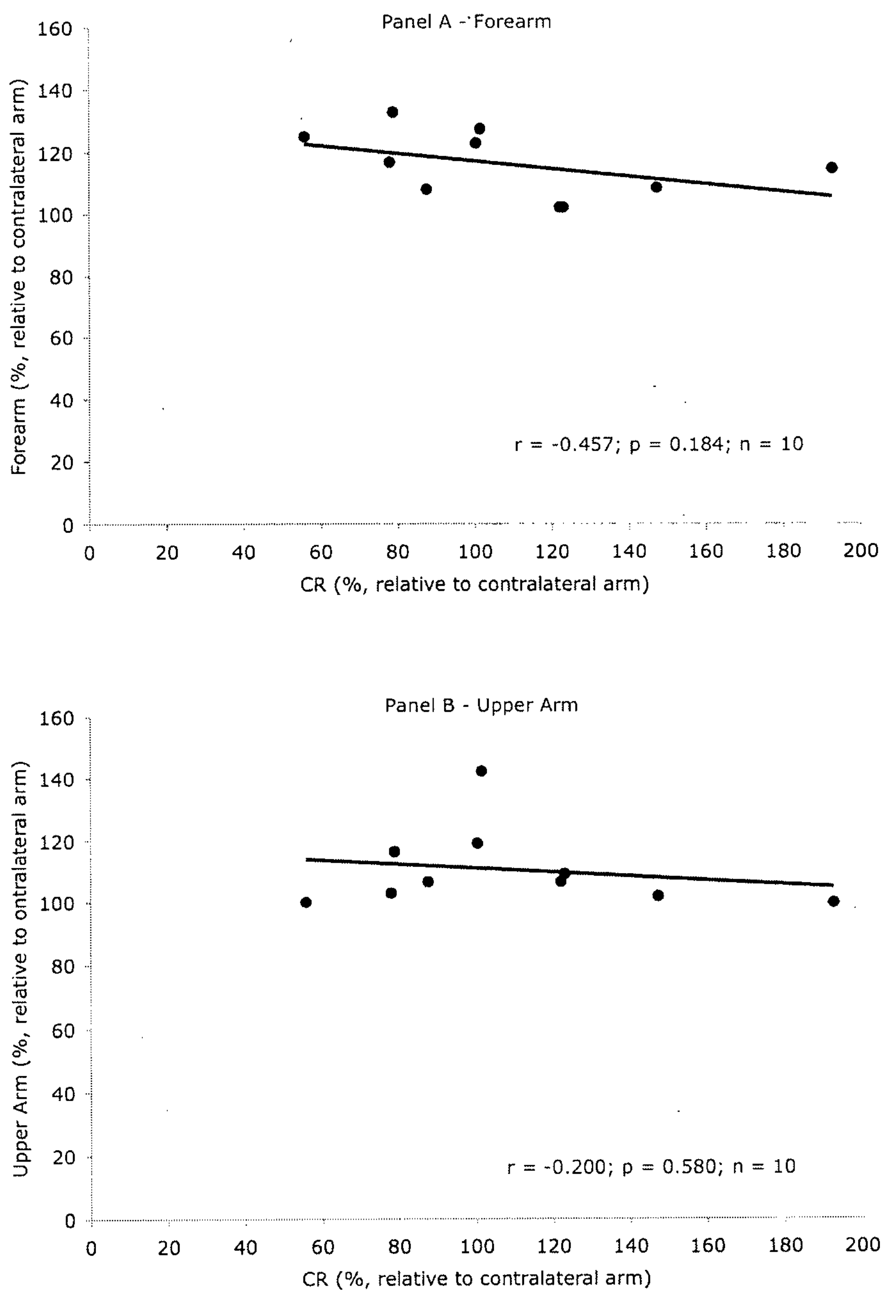


Figure 5.5 Scattergram of clearance rate (CR) plotted against arm circumference (forearm, Panel A; upper arm, Panel B) for ipsilateral relative to contralateral arms in patients with BCRL during exercise (n=10).

compared to both BC (70.4% ±45.4) and CONT (98.4% ±43.5). There was no difference between BC and CONT at rest or during exercise.

The ratio of ipsilateral relative to contralateral AX did not change from rest to exercise (p=0.773). Individual AX data for women with BCRL and BC is in Figure 5.7.

A non-significant inverse relationship was observed between exercise AX and arm circumference in subjects with BCRL (AX vs. forearm circumference: r = -0.479, p=0.230, n=8; AX vs. upper arm circumference: r = -0.354, p=0.390, n=8). The same relationship was seen in BC (AX vs. forearm circumference: r = -0.399, p=0.090, n=19; AX vs. upper arm circumference: r = -0.238, p=0.326, n=19). Correlations for BCRL between exercise AX and forearm/upper arm circumference are presented in Figure 5.8.

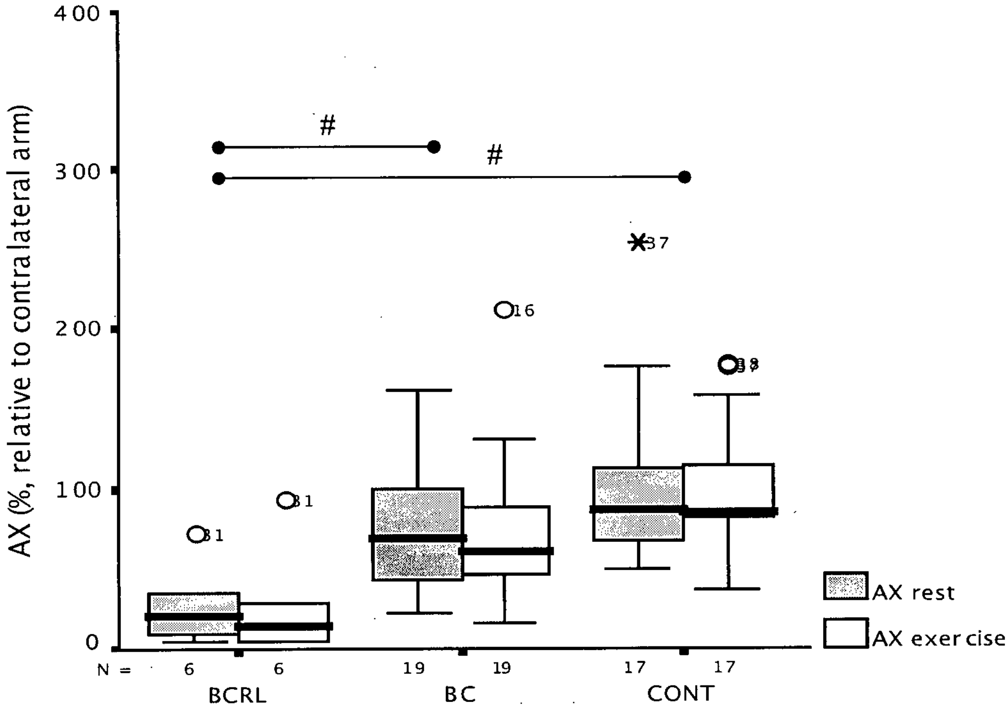
5.3.5 Ipsilateral Relative to Contralateral FORE

Uptake in the forearm 65 min P.I. relative to the initial dose (FORE) for the ipsilateral relative to contralateral arm is presented in Figure 5.9. A significant main effect for groups was found (p=0.000). Post-hoc analysis revealed that at rest, BCRL had significantly greater FORE (247.3% ±136.6) than both BC (90.9% ±21.2) and CONT (92.4% ±20.4). Similarly, during exercise BCRL had significantly greater FORE (470.1% ±294.0) than both BC (93.5% ±26.8) and CONT (95.1% ±13.6). There was no difference between BC and CONT at rest or during exercise.

BCRL demonstrated a significant increase in FORE from rest to exercise (p=0.027). FORE in the ipsilateral relative to contralateral arm did not change from rest to exercise in either BC (p=0.854) or CONT (p=0.711).

5.3.6 Time of Radiopharmaceutical Appearance at the Axillary Lymph Nodes (APP)

One-minute scans of the axillary lymph nodes were taken at 10-minute intervals until radioactivity was visualized in the axillary lymph nodes (Fig. 5.10). If radiopharmaceuticals were not visualized in the axillary nodes by the end of the protocol, then 60 min was entered as the value for that arm. This was particularly evident in subjects with BCRL. For example, at rest,



# denotes significant difference (p<0.05) between groups

Figure 5.6 Ratio of ipsilateral to contralateral arm radiopharmaceuticals uptake in the axilla 65 min post-injection (AX) women treated for breast cancer with lymphedema (BCRL), women treated for breast cancer with no lymphedema (BC), and age-matched controls (CONT) at rest and during exercise. Resting data is indicated in grey boxes while exercise data is in white boxes. A ratio close to 100% indicates symmetry between arms. A ratio greater than 100% indicates greater AX on the ipsilateral compared to contralateral side while a ratio less than 100% indicates lower AX on the ipsilateral compared to contralateral side. The box plot indicates the distance between the 75th percentile and 25th percentile (box), the median value (solid back line), and the highest and lowest values (whiskers), excluding outliers (circles) and extreme outliers (asterisks). # denotes a significantly lower AX both at rest and during exercise in BCRL subjects compared to both BC and CONT subjects.

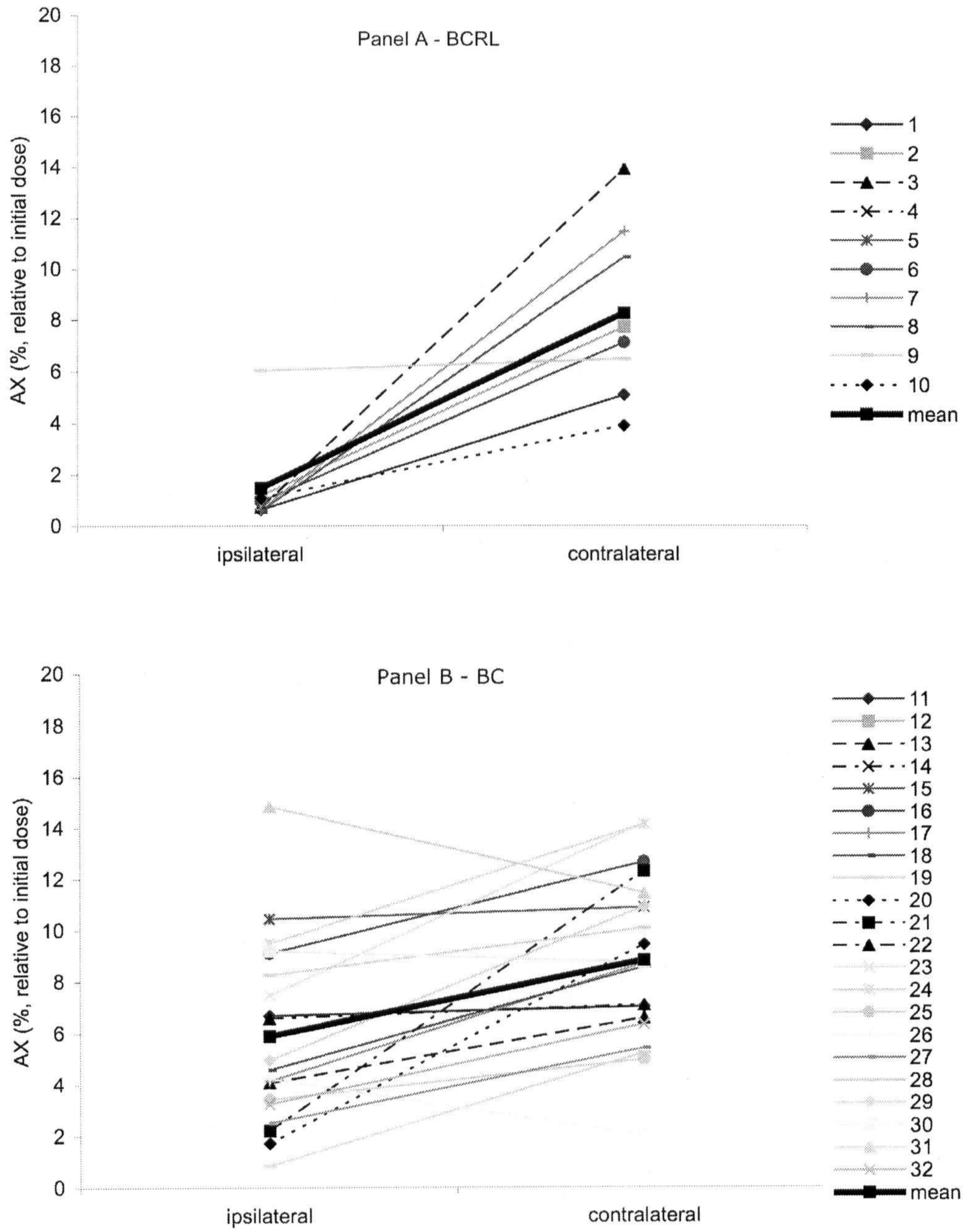


Figure 5.7 Radiopharmaceutical uptake in the axilla 65 min post-injection (AX) during exercise for all pairs of contralateral and ipsilateral arms of women treated for breast cancer with lymphedema (Panel A - BCRL) and women treated for breast cancer (Panel B - BC). The numbers indicate the patient represented by each pair of points (same subject number as in Table 1).

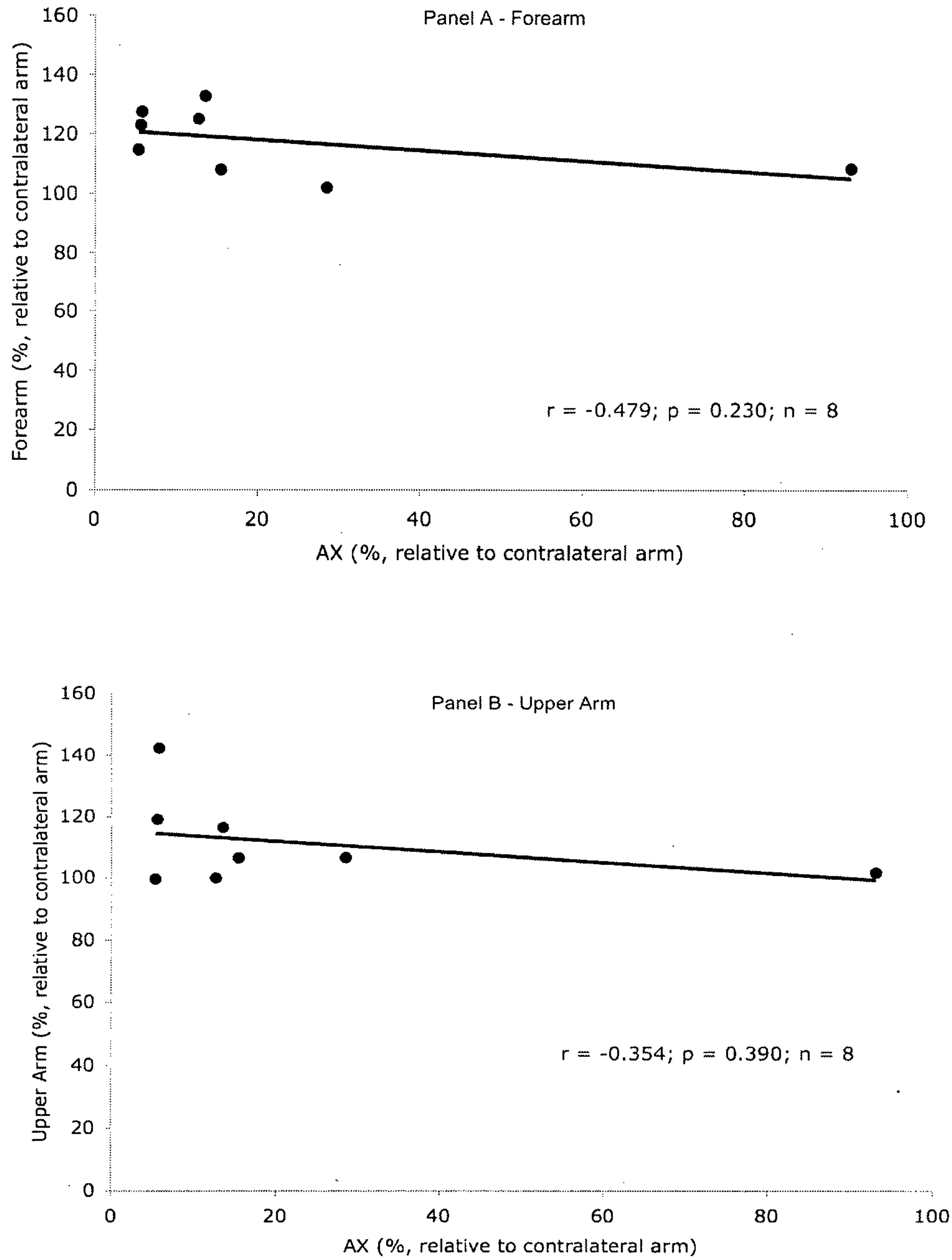
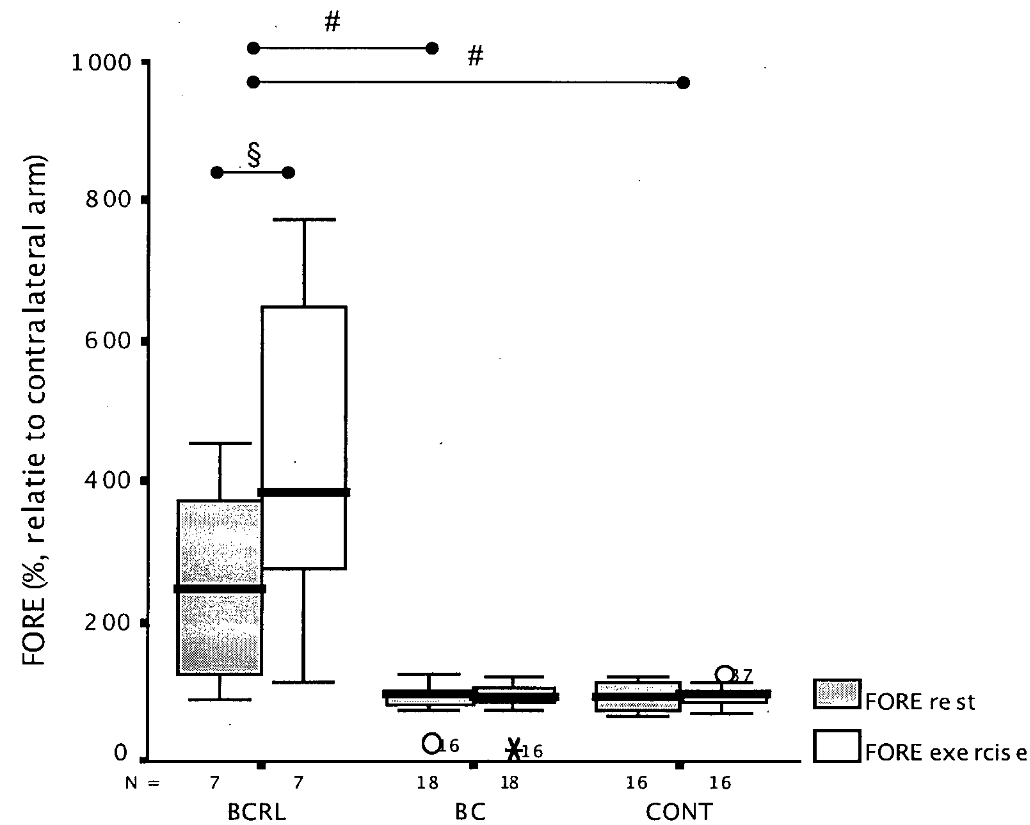


Figure 5.8 Scattergram of radiopharmaceuticals uptake in the axilla 65 min post-injection (AX) plotted against arm circumference (Panel A – forearm; Panel B - upper arm) with circumferences expressed relative to contralateral arm in patients with breast cancer related lymphedema (BCRL) during exercise (n=10).



§ Indicates significant difference (p<0.05) between conditions
# denotes significant difference (p<0.05) between groups

Figure 5.9 Ratio of ipsilateral to contralateral arm radiopharmaceuticals uptake in the forearm 65 min post-injection (FORE) in women treated for breast cancer with lymphedema (BCRL), women treated for breast cancer (BC), and age-matched controls (CONT) at rest and during exercise. Resting data is indicated in grey boxes while exercise data is in white boxes. A ratio close to 100% indicates symmetry between arms. A ratio greater than 100% indicates greater FORE on the ipsilateral compared to contralateral side while a ratio less than 100% indicates less FORE on the ipsilateral compared to contralateral side. The box plot indicates the distance between the 75th percentile and 25th percentile (box), the median value (solid back line), and the highest and lowest values (whiskers), excluding outliers (circles) and extreme outliers (asterisks). § denotes a significant increase in FORE from rest to exercise in BCRL only. # denotes a significantly higher FORE in BCRL at rest and during exercise compared to both BC and CONT.

five of eight subjects with BCRL had no radiopharmaceuticals observable in the ipsilateral axillary lymph nodes at 60 minutes. The addition of exercise resulted in one of the aforementioned subjects showing activity in ipsilateral axillary lymph nodes (data was not available for the other two subjects during exercise due to a change in data acquisition protocol). Radiopharmaceuticals were visualized in all other subjects except one BC subject at rest. This BC subject who did not show activity in the lymph nodes during rest, did show activity in the lymph nodes at 25 minutes during exercise.

Ipsilateral APP was significantly different between groups (p=0.000). At rest, APP was significantly slower in women with BCRL (52.1 min ±11.9) compared to both BC (25.8 min ±13.7) and CONT (21.5 min ±9.4). Again, APP was significantly slower in women with BCRL during exercise (44.3 min ±16.2) versus both BC (12.7 min ±6.0) and CONT (13.5 min ±5.5). There were no differences between BC and CONT for both exercise and rest conditions.

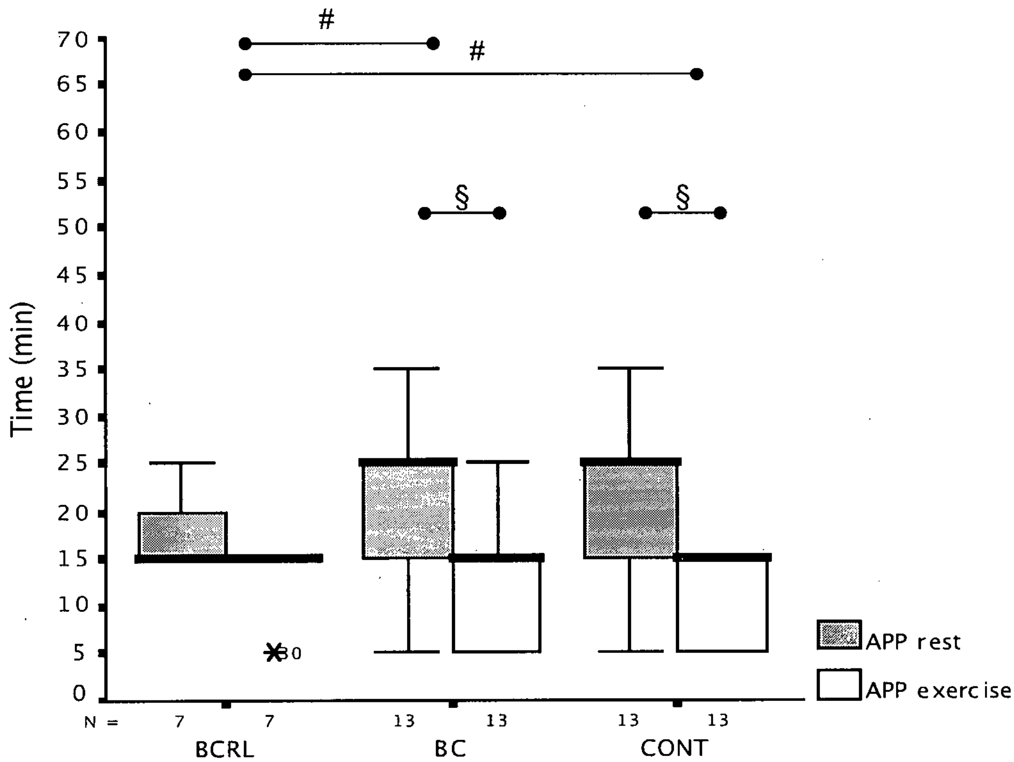
Contralateral APP was not significantly different between groups. The addition of exercise caused APP to significantly increase (p=0.000). Post-hoc analysis revealed that only BC and CONT displayed significant increases in APP from rest to exercise. BCRL showed a trend towards an increase from rest to exercise (17.9 min ±4.9 vs. 13.6 min ±3.8 min, p=0.078); however, statistical significance was not achieved.

5.3.7 Effect of Exercise on Ipsilateral Lymphatic Function

The effect of exercise on the ratio of ipsilateral to contralateral lymphatic function was included in all analyses described above; however, changes in the contralateral arm could potentially mask changes observed in the ipsilateral arm. Consequently, paired t-tests were performed between ipsilateral lymphatic function at rest and ipsilateral lymphatic function during exercise in BCRL and BC. Ipsilateral CR significantly increased in both BCRL (p=0.002) and BC (p=0.000). AX did not significantly increase in BCRL (p=0.531) while significance was just reached in BC (p=0.050). FORE was not expected to change from rest to exercise and this trend was observed in BC (p=0.881). However, ipsilateral FORE did significantly increase in BCRL from rest to exercise (p=0.004). Finally, all groups demonstrated significantly faster APP in the ipsilateral arm from rest to exercise (p=0.000).

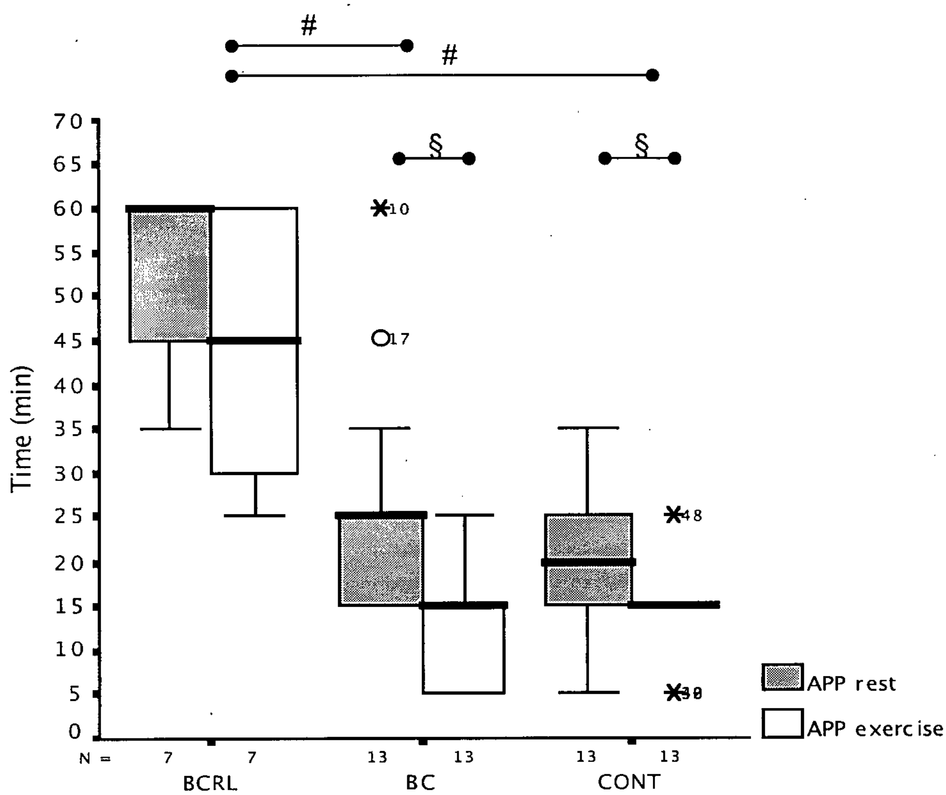
Figure 5.10 Time of first radiopharmaceutical appearance (APP) at rest and during exercise in the normal, or contralateral arm (Panel A), and the affected, or ipsilateral arm (Panel B), of women treated for breast cancer with lymphedema (BCRL), women treated for breast cancer with no lymphedema (BC), and age-matched controls (CONT). Resting data is indicated in grey boxes while exercise data is in white boxes. A lower APP appearance of radiopharmaceuticals at the axilla was visualized on the images faster. Note on Panel B, the median for BCRL is 60 minutes. This indicates that radiopharmaceuticals were not visualized in the axilla on the affected (ipsilateral) side by the time the last image was taken. The box plot indicates the distance between the 75th percentile and 25th percentile (box), the median value (solid back line), and the highest and lowest values (whiskers), excluding outliers (circles), and extreme outliers (asterisks). § denotes a significantly faster APP during exercise versus rest in BC and CONT. # denotes a significantly slower APP in BCRL subjects at rest and during exercise compared to both BC and CONT.

Panel A - Contralateral Arm



§ denotes significant difference (p<0.05) between conditions
# denotes significant difference (p<0.05) between groups

Panel B - Ipsilateral Arm



5.3.8 Effect of the Number of Axillary Lymph Nodes Dissected on Lymphatic Function

Correlations were performed between the number of axillary lymph nodes dissected (ALND) and lymphatic function during exercise (Appendix D). When all women treated for breast cancer (BC and BCRL, n=32) were included, no relationship was evident between ALND and CR (r = -0.109, p = 0.565, n=30) but a significant inverse relationship was found between ALND and AX (r = -0.408, p=0.031, n=28). When this relationship was analyzed in BCRL only, still no relationship was observed between either ALND and CR (r = 0.168, p=0.643, n=10) or ALND and AX (*r* = 0.602, p=0.114, n=8). In BC only, no significant relationship was seen between ALND and CR (r = -0.355, p=0.125, n= 20) although significance was reached between ALND and AX (r = -0.448, p=0.047, n=19). All BC and BCRL subjects were stratified according to whether radiation therapy was performed, and the correlations were repeated. For those who had radiation therapy, no significant relationship was observed between exercise CR and ALND (r = 0.049, p=0.828, n=22), but a significant inverse relationship was evident between exercise AX and ALND (r = -0.450, p=0.046, n=20). For those who did not have radiation therapy, no significant relationships were observed between either CR and ALND (r = -0.324, p=0.434, n=8) or AX and ALND (r = -0.560, p=0.148, n=8).

5.4 DISCUSSION

This is the first study to measure lymphatic function during intermittent, moderate exercise in women treated for breast cancer with and without BCRL and age-matched, healthy controls. The principal findings of this prospective, exploratory study are the following: 1) contralateral arm lymphatic function was similar between groups; 2) CR in the ipsilateral arm relative to the contralateral arm did not differ between subject groups at rest or during exercise; 3) AX was significantly lower in women with BCRL compared to both BC and CONT during rest and exercise conditions; 4) FORE was significantly higher in women with BCRL compared to both BC and CONT during rest and exercise conditions; and, 5) the addition of exercise increased ipsilateral CR, AX, and APP in BC subjects and increased ipsilateral CR, FORE, and APP in BCRL subjects (an increase in FORE is not expected with exercise)..

5.4.1 Ipsilateral Relative to Contralateral Arm Clearance Rate

Contrary to the hypothesis, lymphatic function, as measured by depot CR, was not impaired in women treated for breast cancer with and without BCRL at rest nor during exercise. In fact, the mean ratio between the affected, or ipsilateral arm, and the normal, or contralateral arm, during exercise was 101% for BCRL and 98% for BC subjects indicating symmetry between arms. It is difficult to make direct comparisons between the findings in the present study and other research primarily due to differences in lymphoscintigraphic protocols (i.e., particulate administered, location of injection, type, intensity, and duration of exercise such as fist clenching or ball squeezing performed intermittently between imaging scans, etc.). Moreover, no study has investigated lymphatic function during moderate intensity exercise in women treated for breast cancer. Although several investigations had subjects with BCRL perform intermittent forearm exercise, such as clenching the fists or squeezing a rubber ball between imaging scans to stimulate lymph flow (4, 6, 7, 10, 44-46), we have previously shown depot clearance rate (CR) in healthy females to be similar whether rest or hand grip exercise was performed (47).

Despite differences in methodology between the present study and those published in the literature, current research has indicated that resting CR in women with BCRL may depend on whether radiopharmaceuticals are administered to swollen or spared tissue of the affected arm. For example, administering radiopharmaceuticals subcutaneously (2-4, 7, 10, 11) or subfascially (9) to swollen tissue has demonstrated slower lymph drainage compared to a similar injection site on the contralateral arm. Yet when injections are administered to the spared region of the swollen arm, CR is similar (4, 7) or even faster (10). Although hand volume was not measured in the present study, three of ten subjects with BCRL had noticeably slower CR during exercise in the ipsilateral arm compared to the contralateral arm, while another three had noticeably faster CR in the ipsilateral arm during exercise. The remaining four subjects had comparable CR between arms. There is the possibility that the BCRL subjects with a slower CR were also the ones with swollen hands, while subjects who displayed a similar or faster CR had spared hands. Future research investigating the effects of exercise on lymphatic function should ensure that hand volume, as well as arm volume, is measured and considered as a confounding variable.

The use of CR in understanding lymphatic function is further complicated by the finding that proximal forearm CR is faster than distal CR, irrespective of the site of swelling on both the ipsilateral and contralateral arms (2). This was shown by Modi et al. who administered subcutaneous injections to the forearm at sites of maximal and minimal swelling and corresponding sites on the contralateral forearm in 11 women with established BCRL. No difference was found between CR at the site of maximal and minimal forearm swelling, rather the proximal forearm CR was faster than distal CR. The authors hypothesized that the greater muscle component in the proximal region of the forearm likely contributed to the faster CR seen in this portion of the forearm. The skeletal muscle pump is an extrinsic mechanism known to facilitate lymph formation and propulsion (49). In fact, subfascial CR has been shown to be two times faster than epifascial CR (1).

Other reasons, such as differences in breast cancer treatment, may also account for the lack of statistical difference in CR between groups. For example, as listed in Table 5.2, the number of axillary lymph nodes dissected (ALND), inclusion of radiotherapy, and type of breast surgery differed between groups. The mean ALND for BCRL was 16 (range: 4 to 25) and 11 for BC (range: 0 to 40). Further, nine of ten women with BCRL had radiotherapy that included the axilla, while 14 of 22 BC subjects reported radiation therapy to the same region. Both radiotherapy to the axilla and extent of ALND are risk factors for the development of lymphedema (16); consequently, these confounding variables may need to be controlled when analyzing CR. However, a significant relationship was not observed between exercise CR (ipsilateral relative to contralateral arm) and ALND when all subjects treated for breast cancer (BC and BCRL) were included in the analysis. There was still no significant relationship between exercise CR and ALND when women treated for breast cancer were stratified according to whether radiation therapy was performed. There has been no report of similar analyses performed on CR and ALND/radiation therapy in previous research.

The Effect of Exercise on Clearance Rate

This is the first study to investigate the ability of the lymphatic system to respond to an exercise stimulus in women treated for breast cancer with and without BCRL. In the present study, CR approximately doubled in all groups from rest to exercise. This response to exercise is comparable to data we have collected in healthy, college-aged females (Lane, unpublished comparison of data from (47) and (48)).

It is expected that CR will increase from rest to exercise in those with uncompromised lymphatic function. Typical physiological responses to exercise of increasing intensity include augmented blood flow, sympathetic nervous system activity, ventilation, and skeletal muscle contractions.

As each of these physiological responses to exercise are also thought to be mechanisms that assist with lymph formation and propulsion (49), then a concomitant increase in lymph flow with increased exertion is likely. Although subjects with BCRL had a comparable increase in CR as control subjects, this did not result in more radiopharmaceuticals reaching the axillary lymph nodes on the ipsilateral side (as will be discussed later). The results of this study indicate that when radiopharmaceuticals are administered to a subcutaneous hand depot in those with compromised lymphatic function (i.e., subjects with BCRL), comparable increases in CR are observed between those with compromised and uncompromised lymphatic function. Individual BCRL results demonstrated that seven of eight subjects tested had a noticeably faster CR with exercise.

In summary, the rate at which radiopharmaceuticals clear from the injection depot (i.e., CR) is a typical outcome variable used to describe lymphatic function in women treated for breast cancer. However, CR on its own may not be a suitable measure to evaluate lymphatic function in this population. We demonstrated no differences in CR at rest or during exercise in breast cancer survivors with and without BCRL compared to age-matched control subjects. Even when other factors were considered (i.e., radiotherapy to axilla and the number of lymph nodes excised), CR did not differ between groups. This would indicate that the lymphatic obstruction resulting in the development of BCRL does not affect subcutaneous CR from the hand. However, the small sample of subjects with BCRL and the variability in ipsilateral relative to contralateral CR may have reduced the power necessary to detect differences between groups.

5.4.2 Ipsilateral Relative to Contralateral AX and APP

In the absence of taking blood samples, the time of first appearance of radiopharmaceuticals at the axilla (APP) and the percentage of radiopharmaceuticals in the axillary lymph nodes relative to the initial dose at a set time-point post-injection (AX) may be potential indicators of the efficiency of the lymphatic drainage system (41, 42, 50, 51). There are some limitations, however, to considering AX and APP as outcome measures. For example, lymph node retention of tracer is only a 'snapshot' of the amount of tracer present in the axillary lymph nodes at any one time. Despite colloids having good regional lymph node retention (41, 52), these particles will migrate through the lymph nodes and eventually enter the systemic circulation. It is unknown how long the radiopharmaceuticals are retained in the lymph nodes and what factors speed or slow tracer exit from a lymph node. Another limitation of AX and APP is that breast cancer treatment often involves lymph node excision and/or radiation therapy, thereby reducing the number of functional nodes on the treated (ipsilateral) side. Consequently, analyzing the axillary lymph nodes may result in an artificially lower percentage on the ipsilateral side simply due to fewer functional lymph nodes.

Pain and colleagues have published several papers using dual-isotope lymphoscintigraphy to measure the rate of accumulation of tracer in contralateral venous samples (3-7, 44). Briefly, this technique involves administration of ^{99m}Tc- Human Immunoglobulin G (HIgG) into one hand and ^{111}In-HIgG into the other hand, repeated imaging over three hours, and bilateral sampling from the medial cubital veins (see ref (3) for full explanation of methods). Because two isotopes are used and blood is sampled at various time points post-injection, the appearance rate of tracer in mixed-venous blood can be obtained from both arms simultaneously. The appearance rate can then be used to indicate how efficient the lymphatic system transports injected radiopharmaceuticals through the upper extremity. While there are apparent advantages to using dual isotope lymphoscintigraphy, the disadvantages are that it is more invasive than using AX / APP and still assumes (as with any lymphoscintigraphy protocol) that the majority of particulate administered reaches mixed venous blood via the lymphatic system.

Despite the limitations in using AX and APP, we have demonstrated a moderate but significant inverse relationship between contralateral CR and contralateral AX (rest: $r = -0.439$, exercise: $r = -0.309$). Thus, the faster the radiopharmaceutical clears from the injection depot, the greater the percentage of activity stored at the axillary lymph nodes 65 minutes post-injection and this observation provides a basis for using AX as an outcome variable. The observation of a stronger relationship at rest compared to exercise may be due to the differences in transit time of radiopharmaceuticals through axillary lymph nodes. The specific factors that affect transit time and storage are unknown; however, it is likely that exercise speeds tracer exit from lymph nodes to central circulation.

As hypothesized, our results demonstrated the lowest AX values and the slowest APP times in BCRL compared to both BC and CONT. Although AX did not significantly differ between BC and CONT, there was a trend towards a lower AX in BC. As mentioned previously, a breast cancer survivor will likely have fewer functional lymph nodes on the ipsilateral side than a control subject due to axillary lymph node dissection and/or radiation therapy. In fact, as a group, BCRL had the highest mean number of ALND (16 for BCRL and 11 for BC). However, ALND does not solely explain the lower AX seen in BCRL compared to BC. First, a significant relationship between AX and ALND (r = -0.41) was only evident when all subjects treated for breast cancer (BC and BCRL) were included in the analysis. Sub-analysis revealed that BCRL subjects had a positive relationship between AX and ALND (r = 0.60, non-significant), but BC showed an inverse relationship (r = -0.45, p=0.05). An inverse relationship would be expected between AX and ALND. Second, when comparing the BCRL group to BC subjects with a similar number of axillary lymph nodes excised (n=4, denoted by * on Table 5.2), a lower AX in BCRL subjects was still observed (23% vs. 56%, respectively). Further, these same four BC subjects at rest had a faster mean APP compared to BCRL subjects (33 minutes and 54 minutes, respectively). During exercise, mean APP in the selected BC subjects and BCRL subjects was 15 and 44 minutes, respectively. (It should be noted that exercise APP values were not available in three BCRL subjects and two of the four BC subjects due to APP being introduced after the first seven subjects were tested). Thus, evidence that the number of functional lymph nodes is not the only variable contributing to the development of BCRL is provided by the following: 1) both AX and APP are impaired in BCRL compared to BC subjects with similar numbers of lymph nodes excised, and, 2) the lack of a strong relationship between AX and ALND in BCRL.

<u>*The Effect of Exercise on AX*</u>

Age-matched control subjects had a 1.8% increase in ipsilateral AX from rest to exercise, while BCRL had a 0.3% increase and BC had a 1.4% increase. Although we have not measured AX at rest in healthy, college-aged females, a significant increase was seen from low to moderate intensity arm cranking exercise (48). The increase in AX from rest to moderate intensity exercise indicates the capacity of the axillary lymph nodes to store greater amounts of radiopharmaceuticals and increased transport through lymphatic vessels. It is unknown if lymph nodes have a maximum capacity for radiopharmaceutical storage. Despite the limitations of using AX as an outcome variable as described earlier, a normal response to exercise appears to be an increase in AX by ~2% as demonstrated by CONT subjects. However, BCRL as a group only showed a negligible change in AX on the ipsilateral side from rest to exercise. Moreover, there were other individual BC and CONT subjects that did not demonstrate a noticeable change from rest to exercise. For example, considering the average increase for ipsilateral AX in BC subjects from rest to exercise was 1.5%, five (of six) BCRL, ten (of 19) BC, and four (of 17) CONT did not increase AX by this value. Perhaps, the inability of lymph nodes at the axilla to increase storage of radiopharmaceuticals during exercise may indicate a latent pathology. Further research is needed to evaluate the suitability of comparing rest and exercise AX to identify latent pathologies.

5.4.3 Ipsilateral Relative to Contralateral FORE

FORE was investigated to determine the presence of dermal backflow in the arms of women treated for breast cancer. Stanton et al. (10) describe dermal backflow as a rerouting of lymph towards skin that is represented by diffuse activity in the ipsilateral arm with intense amounts of activity at the periphery of the image. Only the forearm was included in the region of interest for this measure due to a large number of subjects displaying localized foci of activity in the upper arm (likely epitrochlear lymph nodes). As hypothesized, women with BCRL had significantly greater FORE compared to both BC and CONT. Further, dermal backflow was observed in each BCRL subject at rest and during exercise. Other studies administering subcutaneous injections of radiopharmaceuticals to the hand of women with BCRL have also observed dermal backflow regardless if the hand of the affected arm was swollen or spared (10, 11). However, dermal backflow was not seen with subfascial injections to the forearm (9). Considering that dermal backflow has been observed in both swollen and spared hands, Stanton and colleagues hypothesize that the access point for radiopharmaceuticals to dermal lymphatic routes is likely near the hand or the wrist.

The Effect of Exercise on FORE

As expected, FORE did not change from rest to exercise in BC and CONT; however, there was a significant increase in FORE in women with BCRL. Taken with the CR and AX data, this would indicate the following: In women with BCRL, radiopharmaceuticals are able to clear from the hand at the same rate as other subject groups, but instead of reaching the axilla, the activity gets trapped in the dermis as evidenced by the significantly lower AX and significantly higher FORE. Theoretically then, the obstruction to lymphatic system drainage in our subjects with BCRL does not impair CR from the hand depot, but does impair the ability for radiopharmaceuticals to travel through deeper lymphatic vessels to the axillary lymph nodes. The exercise protocol in the present study does not appear to overcome the resistance to the obstruction in those with BCRL, as FORE was shown to increase from rest to exercise.

5.4.4 Contralateral Arm Lymphatic Function

The majority of research using lymphoscintigraphy has focused on the evaluation of lymphatic function in patients after completion of breast cancer treatment. However, there is the possibility that pre-operative differences in lymphatic function exist and the unaffected, or contralateral arm, may not be normal (7, 11). For example, Stanton et al. (11) compared BCRL subjects with a swollen hand to BCRL subjects with a spared hand. A significant difference in contralateral arm CR was observed between subjects (swollen hand = -0.157 $\%\cdot min^{-1}$ vs. spared hand = -0.095 $\%\cdot min^{-1}$) while ipsilateral arm CR was similar. Although both subjects were alike in terms of age and treatment, they differed substantially in terms of arm volume. Specifically, the swollen hand group had a 50% increase in ipsilateral arm volume, while those with a spared hand had a 27% increase in ipsilateral arm volume. Perhaps, differences that contribute to the severity of BCRL affect contralateral arm lymphatic function.

Pain et al. (7) has also observed variations in contralateral arm function. They observed a lower, but non-significant, contralateral arm CR and contralateral arm blood gain rate in BCRL subjects with a swollen hand compared to BCRL subjects with a spared hand - opposite to what was found by Stanton et al. (10). It should be noted that the BCRL subjects included in the study by Pain et al. (7) included four subjects with a smaller ipsilateral arm volume at the time of the study, so it can be argued that these subjects did not have clinically significant BCRL.

Hand volume was not measured in the present study, so contralateral lymphatic function cannot be compared between BCRL subjects with a spared or swollen hand. Rather, contralateral lymphatic function was compared between subjects with BCRL, breast cancer survivors with no BCRL, and age-matched controls. Comparing lymphatic function in women with BCRL to other females who have undergone similar treatment, but without the presentation of lymphedema, may be a more appropriate comparison to evaluate contralateral arm lymphatic function. Moreover, we have shown the coefficient of variation with resting CR in CONT subjects to be higher than that calculated for exercise CR (30.4% and 14.4%, respectively). Considering that both Pain et al. (7) and Stanton et al. (10) obtained their data at rest, the observed CR values may have greater variability reflected in the equivocal findings.

The results of this study indicate no significant differences in contralateral lymphatic function at rest or during exercise as assessed by CR, AX, APP, and FORE. However, the contralateral response to exercise did differ between subject groups. For example, a significant increase in lymphatic function was observed in BC and CONT for each variable (except FORE which was not expected to change with exercise). But BCRL did not show a significant increase in AX and APP from rest to exercise, although a trend towards an increase was observed for both variables (AX: 6.4% vs. 7.4%; APP: 17.9 min vs. 13.6 min). Again, the small sample size of BCRL subjects reduced the power to detect a significant difference in both AX and APP data between resting and exercising conditions to 20%. It must also be considered that systemic changes in lymphatic function occurred due to the development of lymphedema and that contralateral arm lymphatic function was not normal in subjects with BCRL.

5.4.5 Clinical Implications

Factors that are known to increase the risk of developing BCRL include dissection of the axillary lymph nodes, radiotherapy to the breast and axilla, pathological nodal status, obesity, and tumour stage (53-56). Overall, BCRL subjects in this study presented with more risk factors than BC subjects. For example, as shown in Table 5.2, eight of 10 subjects with BCRL had greater than 14 lymph nodes excised. This compares to five of 22 BC subjects. Further, all BCRL subjects except one and 14 of 22 BC subjects had radiotherapy to the axilla. Using BMI to screen for obesity revealed 10% of BCRL subjects were normal weight, 50% were overweight, 20% have Class I obesity, and 20% have Class II obesity. On the other hand, 45% of BC subjects were normal weight, 36% of BC subjects were overweight, 18% have Class I obesity, and none were identified as having Class II obesity. Future research should investigate whether a weight loss intervention can help to reduce arm swelling in subjects with BCRL.

Another important finding of this study is that not all subjects treated for breast cancer have similar lymphatic function during exercise. To give one example, mean (±SD) ipsilateral AX in BCRL during exercise was 1.5% ±2.4; however, one subject with BCRL (Subj. #9, Fig. 5.11) had an AX value of 6.0% in the ipsilateral arm despite having the most lymph nodes excised in the BCRL group (25 vs BCRL mean of 16). Subject #9, compared to others in the BCRL group, was also unique in other aspects: she had the lowest BMI (20.4 vs. BCRL mean of 30.4 $kg \cdot m^2$), was only one of two BCRL subjects who reported performing moderate intensity exercise more than 4 days per week, and had a region of swelling confined to the distal portion of the forearm. Interestingly, Subject #9's lymph nodes on the ipsilateral side appear to be closer to the neck than the axilla. This is likely not a genetic abnormality as her identical twin sister (CONT, Fig. 5.11) was also investigated and has symmetrical lymph nodes in the axillary region.

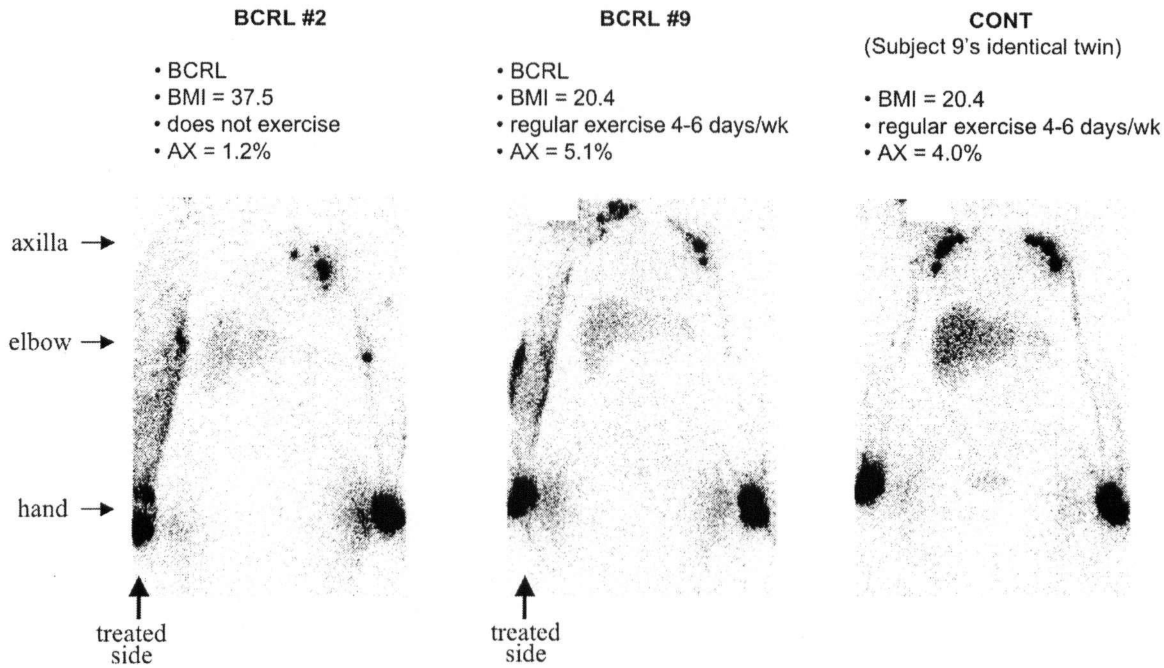


Figure 5.11 Individual lymphoscintigrams taken at 65 min post-injection from two subjects with breast cancer related lymphedema (BCRL, Subject #2 and #9) and Subject #9's identical twin (control subject).

Although aerobic fitness was not assessed in this study, there is the possibility that the regular exercise performed by Subject #9 resulted in adaptations to the lymphatic system, such as the generation of new lymphatic vessels (lymphangiogenesis) around the damaged axillary lymphatics. Yoon et al. (57) demonstrated in an animal model of lymphedema that vascular endothelial growth factor-C (VEGF-C) therapy reversed swelling through lymphangiogenesis. As habitual exercise in a healthy population is known to induce angiogenesis through up-regulation of other isoforms of VEGF (58), it is reasonable to expect concurrent lymphangiogenesis through up-regulation of VEGF-C. Lymphangiogenesis could help to reduce the stress put on the lymphatic vasculature damaged by axillary lymph node dissection and radiation to the axilla. Perhaps, the regular exercise performed by Subject #9 resulted in the creation of new lymphatic vessels draining into lymph nodes found near the neck on the ipsilateral side. This speculation requires further research.

Some of the individual variability in lymphatic function, both between arms and in response to exercise, may indicate latent lymphatic pathology and could potentially account for the finding that only ~29% of breast cancer patients develop lymphedema (13, 14). Conceivably, a small proportion of the population may have atypical lymphatic function that goes undiagnosed until treatment for a disease, such as breast cancer, compromises and challenges the normal performance of the lymphatic system. Those with normal lymphatic function may be able to compensate for the increased resistance faced by the axillary lymphatic vasculature when lymph nodes are excised and/or irradiated as part of breast cancer treatment by a number of potential mechanisms. First, lymphaticovenous communications may open allowing fluid and plasma proteins to bypass obstructed lymphatic vessels and instead access local blood vessels. Lymphaticovenous communications are thought to exist in normal, healthy individuals but may not be functional unless there is chronic obstruction of lymphatic vessels or nodes (59). If the lymphatic system were under chronic stress due to obstruction, then the opening of lymphaticovenous communications would allow lymph to bypass the obstructed area. This was demonstrated in early research by Threefoot et al. (60) who used a dog model with the major lymphatic channels obstructed for over five days to demonstrate the presence of lymphaticovenous communications. In humans, lymphatic-venous microsurgery has resulted in an average reduction in excess volume of 69% (61). Second, there is the possibility of anatomical differences in lymphatic vessel location and length. For example, a long variant that bypasses axillary lymphatics has been proposed (62). The existence of this long variant may bypass excised or damaged level I and level II axillary lymph nodes, and thereby, potentially reduce the risk of developing BCRL (54).

The majority of research using lymphoscintigraphy has focused on the evaluation of lymphatic function in patients after completion of breast cancer treatment. However, one study has used lymphoscintigraphy to evaluate pre-operative changes in lymphatic function. In order to determine early pathophysiological responses to surgery, Pain et al. (5) used dual-isotope lymphoscintigraphy to evaluate lymphatic function in sixteen women diagnosed with breast cancer before, and three months after, axillary clearance surgery. Clearance rate and blood appearance rate did not change as a result of surgery; however, the variability in data increased from pre- to post-surgery (CR coefficient of variation: 24% and 44%, respectively; blood appearance rate coefficient of variation: 49% and 73%, respectively). This study indicates that there are no early changes in lymphatic function as a result of axillary surgery. However, the variability in data may be due to the measurement of CR and blood appearance rate during rest. Additionally, future studies should follow breast cancer patients over a longer period post-surgery as Armer et al. (63) found that lymphedema incidence rates were higher one-year post-diagnosis versus six months post-diagnosis, regardless of the method of defining clinically significant lymphedema.

5.4.6 Using Exercise to Identify Latent Lymphatic Pathologies

Exercise is often used in clinical research to reduce variability in data and determine if the physiological system in question has a latent pathology that is only uncovered during a stressed situation. The coefficient of variation (CV) for the primary outcome measure, CR, between the right and left arms of the same CONT subjects during both exercise and rest was 14.4% and 30.4%, respectively. The exercise CV for CONT (14.4%) is similar to what we have found previously for healthy, college-age, females performing the same exercise stimulus (CV = 11.7%). BC subjects and controls, on average, had improved lymphatic function compared to subjects with BCRL. However, there was considerable variability between ipsilateral and contralateral arms for each measure of lymphatic function as shown in the figures presenting individual data. The variability in data may be in part due to limitations to the study described in the following section; however, it is also possible that early stage lymphatic dysfunction was developing. Although identifying latent lymphatic pathologies with exercise was not a main objective of this thesis, we have retrospectively analyzed the data to determine if evaluating lymphatic function during a stressed situation, such as exercise, has the potential to identify latent lymphatic pathologies.

Overall, our results demonstrate that in women with BCRL, radiopharmaceuticals are able to clear from the hand at the same rate as other subject groups, but instead of reaching the axilla, the activity gets trapped in the dermis. As shown by the variance in data, especially the outliers and extreme outliers, some BC subjects and controls also score values of lymphatic function similar to those with BCRL. If we considered only one measure of lymphatic function and a subject scored an atypical value, then we would not know if this atypical value simply reflects normal variance in the data or if early stage development of BCRL is taking place. However, if we consider several measures of lymphatic function and a subject has more than one atypical measure, then we may feel more confident in identifying this subject as having early stage development of BCRL. One study to date has developed an empirical scoring system based on visual inspection of lymphoscintigrams to quantify changes in lymphatic function in subjects with BCRL as a result of manual lymphatic drainage treatment (46). This scoring system, developed by Szuba and colleagues and presented in Appendix D, gives a score from zero to five for visualization of lymph nodes on the ipsilateral side (0 points = symmetrical bilateral lymph node visualization; 5 points = no lymph node visible on the affected side). Additionally, a score of zero to three is used for visualization of dermal backflow on the affected side (0 points = no dermal backflow; 3 points = circumferential dermal backflow involving more than one limb segment). The two scores are summed together to give a total score out of eight. Thus, the scoring system developed by Szuba et al. considers visualization of axillary lymph nodes and visualization of dermal backflow in one score of lymphatic function.

We have attempted to modify this scoring system by using objective measures rather than visual interpretation of the data. Specifically, we use AX (%, relative to contralateral arm) to represent axillary lymph nodes and FORE (%, relative to contralateral arm) to represent dermal backflow. The same range in scores (0-5 points for AX; 0-3 points for FORE) is used as to Szuba and colleagues. For our scoring system, we used the 95% confidence interval of control subjects as a score of zero for both AX and FORE, while the remaining scores were empirically determined. To illustrate the applicability of our scoring system, consider a subject who presents with an AX value of 10% in the ipsilateral arm relative to the contralateral arm and a FORE value of 200% in the ipsilateral arm relative to the contralateral arm. This subject essentially has a very small amount of radiopharmaceutical reaching the axilla and instead it appears to be getting trapped in the forearm region. Using our empirically derived scoring system, the subject would receive 5/5 points for AX and 2/3 points for FORE giving a total score of 7/8. It should be noted that both the scoring system developed by Szuba and the one used in the present study have not been validated as tools for identifying breast cancer related lymphedema.

Applying our empirically derived scoring system to subjects in the present study shows the mean score during rest and exercise conditions in subjects with BCRL was 5.4 ±2.3 and 6.0 ±1.9, respectively, out of a total possible score of eight (Appendix D, Table D-14). The mean BCRL score minus one standard deviation (i.e., score of 3) was used to identify individuals who may be at risk for developing lymphedema. Using this criterion, all but one subject with BCRL (Subject #10) was correctly classified as having lymphedema at rest and all BCRL subjects were classified as having lymphedema during exercise. With rest and exercise, there were four BC subjects (rest: Subjects #20, #21, #27, #28; exercise: #17, #19, #20, #21) identified as having a similar score to those with BCRL. However, only two were subjects identified both at rest and during exercise. Three CONT subjects at rest (#34, #37, #38) and during exercise (Subject #37, #38, #41) were identified to be at risk for developing BCRL and two were the same subjects. Again, not all CONT subjects identified at rest were also identified during exercise.

Our modified scoring system may have clinical implications. Perhaps, BC subjects identified at rest and during exercise are in the early phases of developing BCRL and should be monitored closely (i.e., regular arm circumference measurements and regular physiotherapy specific to preventing BCRL). Those CONT subjects who were identified may have lymphatic pathologies that will remain latent until there is some disruption of the lymphatic system (i.e., due to breast cancer treatment). Although our modified scoring system did not correctly predict the same subjects both at rest and during exercise to be at risk for developing lymphedema, this would not be unexpected. The variability in the rest condition may have incorrectly identified BC and CONT subjects as resulted in false positives. Further, there may have been other subjects who were only identified with exercise because their lymphatic system was not able to respond to the demands of exercise. Additional research is needed to validate the use of this scoring system.

5.4.7 Limitations

Efforts were made to control extraneous variables that may influence lymphatic function. All subjects arrived at the testing location at the same time of day and were asked to drink normal amounts of water in the three days prior. They were also asked to avoid caffeine and exercise during the day of measurement. However, other factors may also have an influence on lymphatic function such as medications, menopausal status (anecdotally, one control subject reported having a hot flash during the rest protocol and rest CR was similar to exercise CR), hypertension, and location of arm swelling in women with BCRL. A brief medical history was taken from each subject, but neither blood pressure nor hand swelling was recorded.

Not all subjects could complete the exercise bout at the prescribed load of 0.6 $W \cdot kg^{-1}$. This included five BCRL subjects, one BC subject, and one CONT subject. Intensity was dropped by 10-15 Watts when the subjects complained of volitional fatigue or when exercise heart rate reached 85% of their age predicted maximum. Intensity was dropped to ensure the subject could complete the 60 minute intermittent, exercise protocol.

Another limitation of this study was the small number of subjects with BCRL who volunteered. Women treated for breast cancer are told by health professionals to avoid repeated injections in the ipsilateral arm. As this study involved two injections per hand on two separate occasions, this was a cause for concern for many potential women treated for breast cancer (both BC and, particularly, BCRL). Often the only subjects with BCRL who volunteered were referred by their oncologist or were involved in previous studies conducted by our lab.

5.4.8 Conclusions

The main objectives of this study were: 1) to investigate whether contralateral lymphatic function in women treated for breast cancer with and without BCRL is equivalent to controls; 2) to examine lymphatic function relative to the contralateral arm between groups at rest and in response to exercise; and, 3) to determine if the lymphatic system can respond to an exercise stimulus when lymphatic function has been compromised by breast cancer treatment.

We found contralateral lymphatic function to be similar between groups; however, the ability of the lymphatic system to respond to an exercise stimulus by increasing AX and speeding APP was impaired in the contralateral arm of those with BCRL. The inability of AX and APP to change with the addition of exercise may have been a result of the small sample of BCRL subjects or that systemic changes in lymphatic function due to the development of lymphedema resulted in an abnormal AX and APP response to exercise.

Despite subjects with BCRL having similar clearance of ^{99m}Tc-antimony colloid from the hand depot during exercise, we identified impairment to radiopharmaceuticals reaching ipsilateral axillary lymph nodes. Rather, we observed significantly greater activity in the forearm region of women with BCRL. Additionally, we found that the number of axillary lymph nodes dissected (both when all women treated for breast cancer were included and when only breast cancer patients who were treated with radiation therapy were included) was inversely related to AX, but had no relationship to CR. Axillary lymph node dissection and radiation therapy was not all that explained the significantly lower AX in BCRL compared to controls since subjects treated for breast cancer with similar numbers of axillary lymph nodes dissected had a large variation in AX.

Not all aspects of the lymphatic system appear to respond normally to an exercise stimulus in subjects with BCRL. Although ipsilateral CR and APP improved from rest to exercise in BCRL, ipsilateral AX did not change and FORE increased (a higher percentage of FORE in the ipsilateral arm relative to the contralateral arm - i.e., a ratio greater than 100% - is not a normal response and indicates dermal backflow).

In conclusion, BCRL is still a poorly understood complication of breast cancer treatment. As shown in the present study, subjects with BCRL had, on average, a greater number of axillary lymph nodes dissected, more instances of radiation therapy to the axilla, and more cases of overweight and obesity. Compression sleeves, intermittent pneumatic compression, manual lymphatic drainage, and low-level laser therapy are recommended as treatment options for BCRL. However, the majority of studies evaluating the efficacy of these treatment options have used measures of arm volume or circumference to indicate a change in lymphedema status. This study demonstrates the usefulness of a lymphatic stress test which combines moderate intensity upper body exercise with lymphoscintigraphy. The lower co-efficient of variation shown during exercise compared to rest makes it a sensitive and useful clinical tool to investigate the efficacy of common treatment modalities for BCRL. Furthermore, exercise has the potential to be a useful clinical tool in identifying breast cancer survivors at risk for developing lymphedema. Although identifying latent lymphatic pathologies with exercise was not a main objective of this thesis, we modified a scoring system developed by Szuba and colleagues (28) to complement the clinical expression of this disease. Further research is needed to develop a validated scoring system for identifying women treated for breast cancer at risk for developing lymphedema.

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CHAPTER SIX General Summary and Conclusions

Approximately 28% of women treated for breast cancer are at risk for developing breast cancer related lymphedema (BCRL) yet it remains a poorly understood complication of this disease. Treatment variables commonly associated with increased risk (mastectomy, extent of axillary surgery, axillary radiotherapy, radiotherapy to the breast, nodal disease status), do not, on their own, accurately distinguish the at risk population (1). Furthermore, little is known about how to manage BCRL. Although compression sleeves (2, 3), pneumatic pumping (4-7), manual lymphatic drainage (8-11), and low-level laser therapy (12, 13) are often recommended to contain swelling, research investigating their efficacy is often limited by the use of anthropometric measurements, such as arm volume and arm circumference, to reflect a change in lymphedema status. While anthropometric measurements are simple and non-invasive, they do not provide information on changes in the physiology of the lymphatic system. It is possible that studies showing no change in arm volume with a therapeutic intervention have, in fact, made positive adaptations to the lymphatic system not reflected in an arm volume measurement. Considering that most interventions are either acute or relatively short in duration (i.e., 4 – 12 weeks), adaptations to the lymphatic system may precede changes in arm swelling unless the intervention is continued over a long duration. Consequently, there is a need for a better clinical tool to evaluate the effectiveness of various therapeutic interventions prescribed for BCRL.

Lymphoscintigraphy has added another dimension to the evaluation of lymphatic function. This technique was developed to assess lymphatic status in patients with a variety of conditions that cause lymphatic obstruction (14, 15). Lymphoscintigraphy is being used increasingly in the investigation of the pathology of BCRL (16-27) and in the examination of various therapeutic interventions prescribed to manage BCRL (9, 28-31). While these studies provide some new insight into the pathophysiology of BCRL and potentially useful therapeutic interventions, they are limited in the application of the lymphoscintigraphy protocol. Specifically, lymphoscintigraphy is usually performed at rest and when exercise is used to stimulate lymph flow, subjects perform intermittent forearm exercise between imaging scans, such as clenching the fists or squeezing a rubber ball (20, 21, 24-26, 32, 33). While exercise can facilitate lymph clearance of the radiopharmaceutical from the injection depot and help to reduce variability in data, the intensity and duration of the exercise protocol must be precisely quantified. Otherwise the effort, or work, will differ among subjects or between conditions and compromise the research design. Currently, a clinical test involving a standardized exercise protocol combined with lymphoscintigraphy that will provide information on lymphatic function of the upper extremity in women with BCRL does not exist. Therefore, the purpose of this thesis was to design a lymphatic stress test that could be used to measure lymphatic function in women treated for breast cancer with and without BCRL. Initial studies were performed on healthy, college-aged females in order to determine a suitable exercise protocol and to appreciate a normal response of the lymphatic system to exercise.

The purpose of the first study (Chapter 2) was to evaluate the effects of arm crank ergometry and handgrip contractions on radiopharmaceutical clearance from the hands of six healthy females. On separate days, subjects performed arm cranking (6 repeated bouts of arm cranking for 5 minutes at 0.6 $W \cdot kg^{-1}$ followed by 5 minutes rest) or handgrip exercise (12 repeated bouts of 75 contractions in 2.5 minutes at 50% MVC followed by 2.5 minutes of rest). The handgrip protocol was done with the right hand only while the left hand served as a control. Prior to the start of exercise, ^{99m}Tc-antimony colloid was injected into the first and fourth finger-web of each hand and one minute spot views were taken immediately after the injection and every 10 minutes thereafter over 60 minutes. Clearance from the injection sites was linear and expressed as a slope (% administered activity$\cdot min^{-1}$). Significantly faster clearance was observed with arm cranking (rt = -0.27 ± 0.03 %$\cdot min^{-1}$; left = -0.29 ± 0.06 %$\cdot min^{-1}$) compared to both handgrip (-0.18 ± 0.03 %$\cdot min^{-1}$) and the control condition (-0.14 ± 0.05 %$\cdot min^{-1}$; $p < 0.000$). The results of this study indicated that arm cranking was more effective in promoting lymphatic clearance from the hand and may be a useful protocol to challenge the lymphatic system in breast cancer survivors.

Although the first study demonstrated that arm cranking was superior to intermittent handgrip exercise in promoting lymphatic clearance from the hand, it was not clear if changing exercise intensity would have an appreciable effect on lymphatic function. Thus, the purpose of the second study (Chapter 3) was to determine the effect of low versus high intensity upper body exercise on lymphatic function in healthy females. On separate days, eight females performed either high intensity arm cranking (12 repeated sets of arm cranking for 2.5 minutes at 0.6 $W \cdot kg^{-1}$ followed by 2.5 minutes of rest) or low intensity arm cranking (12 repeated sets of arm cranking for 2.5 minutes at 0.3 $W \cdot kg^{-1}$ followed by 2.5 minutes of rest). The same lymphoscintigraphy protocol described in the first study was used for this project except one additional outcome variable, radiopharmaceutical uptake in the axillary regions (AX) at 65 min post-injection, was also measured. High intensity arm cranking exercise resulted in significantly greater CR ($-0.24\%\cdot min^{-1} \pm 0.06$) than did low intensity exercise ($-0.19\%\cdot min^{-1} \pm 0.05$; $p=0.003$). A similar trend was seen in AX (high intensity protocol: 6.3% ±1.6 versus low intensity protocol: 4.8% ±1.1, $p=0.004$). The results of the second study indicated that an arm cranking protocol of higher intensity is more effective in promoting both lymphatic clearance from the hand and radiopharmaceutical uptake at the axillary lymph nodes. Furthermore, AX appeared to be a useful outcome variable in the assessment of lymphatic function. The high intensity protocol was selected to be used as the lymphatic stress test.

Before applying the lymphatic stress test to breast cancer survivors with and without BCRL, another study using healthy females was needed for two reasons – to determine reliability of the lymphatic stress test and to establish if symmetry in lymphatic function between upper extremities occurs while performing the standardized exercise protocol. Thus, the purpose of the third study (Chapter 4) was to determine the reliability of the lymphatic stress test and to evaluate if lymphatic function is similar between the arms. The lymphatic stress test protocol was as described in the preceding paragraph. Symmetry was seen in both CR ($y=0.95x-0.02$; $r=0.83$; $p<0.001$; $n=21$) and AX ($y=0.90x+0.56$; $r=0.81$; $p=0.001$; $n=12$). Additionally, the lymphatic stress test was shown to be reliable ($r=0.94$, $n = 7$). The results of this study indicated that the lymphatic stress test is reliable and lymphatic function as measured by CR and AX is similar between arms during exercise. Thus, when investigating the effect of exercise on lymphatic function in women with breast cancer related lymphedema the affected arm can be expressed relative to the normal arm.

As a result of the initial pilot work, an intermittent, moderate intensity arm cranking protocol was selected to investigate lymphatic function in women treated for breast cancer with BCRL (BCRL, n=10), breast cancer survivors with no BCRL (BC, n=22), and age-matched controls (CONT, n=17). This exploratory study (Chapter 5) was the first to investigate the response of the lymphatic system to exercise in women treated for breast cancer with and without BCRL using a standardized exercise test. Three main questions were identified: 1) Is contralateral lymphatic function in women treated for breast cancer with and without BCRL equivalent to controls, 2) Is lymphatic function in the ipsilateral arm relative to the contralateral arm (assuming the contralateral arm is normal) different between groups in response to exercise, and 3) Can the lymphatic system respond to an exercise stimulus if lymphatic function has been compromised by breast cancer treatment? Originally, the primary outcome measure was CR as used by

Stanton et al. (23) and the secondary outcome measure was AX as used by Szuba et al. (33). Early in data collection, radiopharmaceutical uptake in the forearm region relative to the initial dose (FORE) and time of first radiopharmaceutical appearance at the axilla (APP) were added as secondary outcome measures.

Overall, this study revealed the following: 1) contralateral lymphatic function is similar between groups, 2) BCRL have similar clearance to BC and CONT of ^{99m}Tc antimony colloid from the hand depot during exercise; however, the amount stored in ipsilateral axillary lymph nodes relative to the contralateral side is impaired and the radiopharmaceuticals get trapped in the forearm, 3) on average, BC demonstrated a normal response to exercise (faster CR, greater AX, no change in FORE, and faster APP) yet in BCRL only CR and APP improved from rest to exercise (AX did not change and FORE increased), 4) breast cancer survivors have similar lymphatic function as CONT; however, there is a highly variable response which may suggest that some BC subjects may be at risk for developing lymphedema, and 5) the coefficient of variation was lower with exercise compared to rest.

There were several limitations to these studies. We chose to use a relative exercise load (based on body weight) rather than using an absolute intensity. As a result, several subjects (primarily those with BCRL) could not complete the prescribed intensity. Relative exercise loads based on body mass are generally used in exercise physiology because those with a greater mass usually also have more lean muscle tissue. However, when the greater body mass is in the form of adipose tissue, then an exercise load based on body weight may not be appropriate. It would have been desirable to use a relative load based on a percentage of maximal aerobic power or maximal heart rate, but this would have necessitated the subjects performing an upper body maximal exercise test on a separate day. For future studies, if 0.6 $Watts \cdot kg^{-1}$ cannot be tolerated, then 0.3 $Watts \cdot kg^{-1}$ can be used. Our previous work in healthy, college-aged females has demonstrated that 0.3 $Watts \cdot kg^{-1}$ results in CR that are still greater than rest (34). Hand volume should be measured to ensure this potential confounding variable is controlled in data analyses. Markers should be used to identify the wrist, elbow, and axilla to allow for exact regions of interest around the forearm. This would also allow the upper arm segment to be evaluated. Although we consider AX and APP as appropriate outcome measures to assess the efficacy of the lymphatic drainage system, there are limitations to these measures. For example, lymph node retention of tracer in axillary lymph nodes is only a 'snapshot' of the amount of tracer present in the axillary lymph nodes at any one time. Another limitation is that breast cancer treatment often requires lymph node excision and/or radiation therapy, which reduces the number of functional nodes on the treated (ipsilateral) side. Consequently, dual isotope lymphoscintigraphy should be considered to measure the efficacy of the lymphatic drainage system. However, dual isotope lymphoscintigraphy requires additional injections for blood sampling; moreover, it still assumes (as with any lymphoscintigraphy protocol) that the majority of particulate administered reaches mixed venous blood via the lymphatic system.

There are several directions for future research that stem from the findings of this thesis. First, exercise has the potential to be a useful clinical tool in identifying breast cancer survivors at risk for developing lymphedema similar to the way exercise is used by the cardiologist or respirologist. Further research is needed to develop a validated scoring system for identifying women treated for breast cancer at risk for developing lymphedema. We modified a scoring system empirically developed by Szuba et al. (33) and identified several BC and CONT subjects with scores similar to the BCRL group. Women diagnosed with breast cancer should be evaluated longitudinally using our exercise protocol combined with lymphoscintigraphy. For example, measuring lymphatic function in breast cancer patients before treatment (i.e., axillary lymph node dissection and/or radiation therapy), after treatment, and thereafter at six month intervals over several years would determine the ability of our scoring system to predict development of lymphedema. Second, the efficacy of various treatment options recommended for BCRL should be re-evaluated using our lymphatic stress test. As we have demonstrated, our lymphatic stress test reduced variability in data; thus, there is improved power to detect changes in lymphatic function due to an intervention. Third, regular upper body exercise should be investigated to determine if it can lead to adaptations in the lymphatic system.

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APPENDIX A. INDIVIDUAL DATA FROM CHAPTER TWO

Table A-1 Individual descriptive data

Subject	Weight (kg)	MVC (kg)	Dominant Arm
1	41.7	30	right
2	56.7	30	right
3	56.7	30	right
4	63.3	30	left
5	61.7	36	right
6	56.7	33	right
7	60.0	30	right
8	56.7	33	right

Table A-2 Individual clearance rate data.

Subject	Arm Cranking Right Arm ($\%\cdot min^{-1}$)	Arm Cranking Left Arm ($\%\cdot min^{-1}$)	Hand Grip Right Arm ($\%\cdot min^{-1}$)	Control Left Arm ($\%\cdot min^{-1}$)
1	-0.24	-0.26	-0.11	-0.06
2	-0.26	-0.26	-0.19	-0.15
3	-0.44	-0.35	-0.16	-0.15
4	-0.31	-0.38	-0.17	-0.14
5	-0.30	-0.34	-0.21	-0.19
6	-0.25	-0.19	-0.15	-0.13
7	-0.25	-0.31	-0.09	-0.13
8	-0.27	-0.28	-0.09	-0.17

APPENDIX B. INDIVIDUAL DATA FROM CHAPTER THREE

Table B-1 Individual descriptive data.

	Age (yr)	Weight (kg)	Height (cm)	Body Mass Index ($kg \cdot m^2$)	Dominant Arm
1	28	45.0	162	17.1	rt
2	31	55.0	165	20.2	rt
3	24	64.0	183	19.1	rt
4	30	63.0	165	23.1	rt
5	29	50.5	164	18.8	rt
6	25	62.0	167	22.2	rt
7	25	76.5	183	22.8	rt
8	26	61.5	164	22.9	rt

Table B-2 Individual data for clearance rate (CR), axillary uptake (AX), and heart rate (HR) during the high and low intensity arm cranking conditions.

	CR-high	CR-low	AX-high	AX-low	HR-high	HR-low
1	-0.207	-0.172	7.13	5.50	127	102
2	-0.199	-0.134	4.84	3.74		95
3	-0.195	-0.163	4.45	4.28	101	83
4	-0.180	-0.184	4.93	3.53	111	94
5	-0.183	-0.141	5.73	3.58	117	99
6	-0.302	-0.207	9.10	5.58	103	77
7	-0.291	-0.250	7.41	5.95	120	92
8	-0.325	-0.279	7.07	6.32	124	108

APPENDIX C. INDIVIDUAL DATA FROM CHAPTER FOUR

Table C-1 Individual descriptive data.

Subject	Age (yr)	Weight (kg)	Height (cm)	BMI ($kg \cdot m^2$)	Dominant Arm
1	27.0	41.7	160.0	16.3	right
2	30.0	56.7	165.0	20.8	right
3	24.0	64.0	183.0	19.1	right
4	29.0	56.7	165.0	20.8	right
5	29.0	50.5	164.0	18.8	right
6	24.0	63.5	169.0	22.2	right
7	25.0	91.5	188.0	25.9	left
8	33.0	77.0	188.0	21.8	right
9	34.0	78.0	178.0	24.6	right
10	24.0	73.3	181.0	22.4	right
11	23.0	66.7	182.0	20.1	right
12	28.0	78.3	183.0	23.4	right
13	25.0	75.5	181.9	22.8	right
14	24.0	63.5	169.0	22.2	right
15	24.0	70.0	175.0	22.9	right
16	24.0	59.0	162.0	22.5	right
17	24.0	70.0	170.2	24.2	right
18	22.0	57.0	168.5	20.1	right
19	22.0	59.0	161.5	22.6	right
20	24.0	76.5	178.0	24.1	right
21	24.0	51.0	162.0	19.4	right

Table C-2 Individual clearance rate (CR) and axillary uptake (AX) data for the right and left upper extremities.

Subject	CR-right ($\% \cdot min^{-1}$)	CR-left ($\% \cdot min^{-1}$)	AX-right (%)	AX-left (%)
1	-0.192	-0.222	5.56	8.70
2	-0.176	-0.221	4.42	5.27
3	-0.238	-0.152	5.15	3.74
4	-0.161	-0.198	5.10	4.77
5	-0.184	-0.182	5.99	5.47
6	-0.297	-0.306	9.32	8.88
7	-0.184	-0.125	1.86	0.96
8	-0.222	-0.253	2.50	3.24
9	-0.199	-0.134	2.16	1.69
10	-0.312	-0.370	4.99	7.08
11	-0.269	-0.266	6.71	4.50
12	-0.151	-0.151	1.56	2.49
13	-0.139	-0.145		
14	-0.107	-0.122		
15	-0.030	-0.056		
16	-0.103	-0.071		
17	-0.182	-0.296		
18	-0.265	-0.267		
19	-0.118	-0.167		
20	-0.246	-0.237		
21	-0.123	-0.130		

APPENDIX D. INDIVIDUAL DATA FROM CHAPTER FIVE

Table D-1 Individual clearance rate data for breast cancer related lymphedema subjects.

Subject	Rest Ipsilat. ($\%\cdot min^{-1}$)	Rest Contralat. ($\%\cdot min^{-1}$)	Exercise Ipsilat. ($\%\cdot min^{-1}$)	Exercise Contralat. ($\%\cdot min^{-1}$)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	-0.039	-0.092	-0.093	-0.167	42.0	55.8
2			-0.267	-0.306		87.5
3			-0.340	-0.176		192.7
4	-0.048	-0.068	-0.090	-0.073	70.6	123.0
5	-0.134	-0.121	-0.135	-0.173	110.4	78.0
6	-0.085	-0.121	-0.211	-0.268	70.4	78.8
7	-0.123	-0.142	-0.219	-0.216	86.7	101.4
8	-0.141	-0.260	-0.265	-0.264	54.2	100.3
9	-0.097	-0.086	-0.208	-0.141	11.9	147.3
10	-0.082	-0.053	-0.142	-0.117	154.8	122.0

Table D-2 Individual clearance rate data for breast cancer subjects.

Subject	Rest Ipsilat. ($\%\cdot min^{-1}$)	Rest Contralat. ($\%\cdot min^{-1}$)	Exercise Ipsilat. ($\%\cdot min^{-1}$)	Exercise Contralat. ($\%\cdot min^{-1}$)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	-0.185	-0.106	-0.243	-0.303	174.7	80.4
2	-0.044	-0.167	-0.138	-0.157	26.2	87.8
3	-0.195	-0.200	-0.220	-0.277	97.7	79.5
4	-0.019	-0.009			205.9	
5	-0.179	-0.240	-0.117	-0.131	74.4	89.5
6	-0.062	-0.066	-0.183	-0.189	94.1	97.0
7	-0.106	-0.121	-0.158	-0.301	87.6	52.4
8	-0.097	-0.064	-0.138	-0.184	150.7	75.0
9	-0.031	-0.082	-0.067	-0.105	37.4	63.8
10	-0.040	-0.141	-0.205	-0.209	28.1	98.1
11	-0.083	-0.143	-0.137	-0.167	58.3	81.8
12			-0.212	-0.192		110.7
13	-0.133	-0.071	-0.284	-0.173	187.7	164.6
14	-0.100	-0.098	-0.203	-0.199	102.5	102.1
15	-0.137	-0.194	-0.179	-0.241	70.5	74.4
16	-0.065	-0.190	-0.164	-0.132	34.3	123.7
17	-0.013	-0.118	-0.128	-0.149	10.9	85.6
18	-0.015	-0.071	-0.176	-0.157	20.9	111.7
19	-0.111	-0.161	-0.277	-0.265	68.6	104.5
20	-0.215	-0.160	-0.376	-0.313	134.4	120.0
21	-0.238	-0.200	-0.374	-0.282	118.8	132.6
22	-0.183	-0.063	-0.254	-0.215	291.5	118.5

Table D-3 Individual clearance rate data for control subjects.

Subject	Rest Ipsilat. (%·min^{-1})	Rest Contralat. (%·min^{-1})	Exercise Ipsilat. (%·min^{-1})	Exercise Contralat. (%·min^{-1})	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	-0.067	-0.059	-0.103	-0.128	113.8	80.7
2	-0.095	-0.198	-0.194	-0.339	48.2	57.2
3	-0.062	-0.058	-0.077	-0.108	107.4	71.2
4	-0.054	-0.098	-0.193	-0.233	55.5	82.7
5	-0.102	-0.033	-0.098	-0.111	308.9	88.5
6	-0.054	-0.011	-0.117	-0.138	499.6	84.4
7	-0.104	-0.110	-0.122	-0.142	94.4	86.0
8	-0.065	-0.084	-0.227	-0.254	77.9	89.1
9	-0.109	-0.064	-0.154	-0.138	169.9	111.3
10	-0.078	-0.142	-0.211	-0.167	54.8	125.9
11	-0.097	-0.086	-0.166	-0.135	113.5	122.2
12	-0.019	-0.046	-0.153	-0.166	41.5	92.4
13	-0.112	-0.135	-0.149	-0.338	83.2	44.1
14	-0.151	-0.135	-0.229	-0.226	112.1	101.6
15	-0.130	-0.175	-0.259	-0.244	74.2	106.2
16	-0.053	-0.080	-0.099	-0.104	66.1	95.4
17	-0.125	-0.109	-0.132	-0.146	113.8	90.3

Table D-4 Individual axillary uptake data for breast cancer related lymphedema subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	0.637	2.378	0.647	5.096	26.8	12.7
2			1.194	7.729		15.5
3			0.745	13.966		
4	0.306	1.155			26.5	
5	1.007	15.593			6.45	
6	0.918	8.612	0.966	7.120	10.65	13.6
7	1.070	7.930	0.665	11.508	13.5	5.8
8	0.687	10.607	0.589	10.500	6.5	5.6
9	3.786	5.287	6.032	6.481	71.6	93.1
10	1.296	3.764	1.113	3.904	34.4	28.5

Table D-5 Individual axillary uptake data for breast cancer subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	11.476	7.101	6.685	7.008	161.6	95.4
2						
3	4.072	5.259	4.071	6.576	77.4	61.9
4	2.334	3.242			72.0	
5	12.878	18.284	10.450	10.886	70.4	96.0
6	2.667	2.956	9.107	12.662	90.2	71.9
7	2.891	4.096	4.143	8.698	70.6	47.6
8	4.636	4.286	4.570	8.562	108.2	53.4
9	1.086	2.728	0.833	5.218	39.8	16.0
10	1.048	4.827	1.710	9.444	21.7	18.1
11	4.328	15.693	2.210	12.313	27.6	17.9
12			6.575	7.073		93.0
13	4.173	7.092	7.452	14.169	58.8	52.6
14	5.010	3.944	9.485	14.146	127.0	67.0
15	2.482	4.508	3.421	4.958	55.1	69.0
16	2.243	3.323	4.223	2.002	67.5	211.0
17	1.355	5.544	2.512	5.409	24.4	46.4
18	2.193	6.113	8.243	10.080	35.9	81.8
19	3.677	8.093	4.950	10.922	45.4	45.3
20	6.520	6.942	9.194	8.714	93.9	105.5
21	9.539	7.871	14.844	11.449	121.2	129.6
22	2.965	2.390	3.247	6.342	124.1	51.2

Table D-6 Individual axillary uptake data for control subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	11.332	9.914	7.436	8.040	114.3	92.5
2	1.980	3.975	2.568	6.645	49.8	38.6
3	6.110	5.218	7.491	6.497	117.1	115.3
4	1.869	2.895	4.735	5.756	64.6	82.3
5	5.945	2.356	12.763	7.273	252.3	175.5
6	4.566	2.643	11.794	6.657	172.8	177.2
7	6.549	8.156	12.654	15.348	80.3	82.4
8	1.869	2.895	4.735	5.756	64.6	82.3
9	4.060	3.854	5.946	5.133	105.3	115.8
10	4.559	5.236	5.339	6.234	87.1	85.6
11	8.151	7.189	12.929	9.094	113.4	142.2
12	2.797	2.808	9.121	10.710	99.6	85.2
13	6.562	9.912	6.363	16.895	66.2	37.7
14	8.071	10.266	5.795	10.378	78.6	55.8
15	8.091	11.808	11.496	12.697	68.5	90.5
16	2.576	3.504	2.201	3.926	73.5	56.1
17	14.488	8.232	7.137	4.519	176.0	157.9

Table D-7 Individual forearm uptake data for breast cancer related lymphedema subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	1.689	0.968	2.771	1.043	174.5	265.7
2			1.571	0.933		168.4
3			11.296	1.191		948.3
4	0.862	0.776			111.1	
5	3.187	0.807	5.912	1.028	395.1	574.9
6	2.194	0.63	4.746	1.23	349.7	385.0
7	1.938	1.269	3.739	1.297	152.8	288.3
8	3.352	1.345	6.761	0.944	249.2	716.2
9	3.174	0.702	5.532	0.717	452.3	771.1
10	1.094	1.164	1.099	0.971	93.9	113.1

Table D-8 Individual forearm uptake data for breast cancer subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	0.721	0.991	1.040	1.349	72.8	77.1
2						
3	1.204	0.966	0.772	1.080	124.6	71.5
4						
5	1.146	1.193	0.599	0.641	96.1	93.5
6	0.944	0.887	0.951	0.908	106.4	104.7
7	1.014	1.045	1.081	0.930	97.0	116.2
8	0.822	0.869	1.016	1.043	94.6	97.4
9			0.962	0.601		160.0
10	0.915	1.047	1.363	1.138	87.4	119.7
11	2.120	2.679	1.221	1.468	79.1	83.2
12			0.878	1.015		86.5
13	1.424	1.396	1.161	1.238	102.0	93.8
14	0.940	0.962	1.681	1.625	97.8	103.5
15	1.119	1.166	0.897	1.155	96.0	77.6
16	0.769	0.782	0.884	0.742	25.0	15.0
17	0.691	0.890	1.047	1.053	77.7	99.5
18	1.452	1.269	1.455	1.309	114.4	111.1
19	1.286	1.292	1.199	1.441	99.6	83.2
20	0.964	1.286	1.133	1.308	75.0	86.6
21	1.260	1.250	1.232	1.254	100.8	98.2
22	1.367	1.497	1.177	1.289	91.3	91.3

Table D-9 Individual forearm uptake data for control subjects.

Subject	Rest Ipsilat. (%)	Rest Contralat. (%)	Exercise Ipsilat. (%)	Exercise Contralat. (%)	Rest (Ipsilat. Vs. Contralat.) (%)	Exercise (Ipsilat. Vs. Contralat.) (%)
1	0.705	0.772	0.787	0.928	91.4	84.7
2	0.917	0.747	0.856	1.008	122.8	84.9
3	0.948	0.847	1.005	0.879	111.9	114.4
4	1.200	1.900	1.229	1.283	63.2	95.8
5	0.651	0.586	1.126	0.903	110.9	124.6
6	0.562	0.845	1.323	1.930	66.5	68.5
7	0.639	0.926	0.685	0.811	69.0	84.4
8	1.178	1.108	1.229	1.283	106.3	95.8
9	0.837	1.306	0.882	1.046	64.1	84.4
10	1.067	0.964	0.785	0.894	110.7	87.8
11	0.899	1.043	1.231	1.288	86.2	95.5
12	1.123	1.022	1.016	1.043	109.9	97.4
13	1.190	1.046	1.295	1.385	113.8	93.5
14	0.823	0.912	0.915	0.928	90.2	98.6
15	1.424	1.588	1.072	1.224	89.7	87.6
16	0.921	1.273	1.182	1.057	72.3	111.8
17			1.205	1.117		107.9

Table D-10 Individual time of first appearance data for breast cancer related lymphedema subjects.

Subject	Rest Ipsilat. (min)	Rest Contralat. (min)	Exercise Ipsilat. (min)	Exercise Contralat. (min)
1	No app	15	No app	15
2				
3	No app	25		
4	No app	25	No app	15
5	No app	25		
6	No app	15	No app	15
7	35	15	25	15
8	No app	15	45	5
9	35	15	25	15
10	55	25	35	15

Table D-11 Individual time of first appearance data for breast cancer subjects.

Subject	Rest Ipsilat. (min)	Rest Contralat. (min)	Exercise Ipsilat. (min)	Exercise Contralat. (min)
1	15	5	5	5
2	35	15		
3	15	15	15	15
4	25	25		
5	25	15		
6	35	35	15	15
7	25	25	15	15
8	35	25		
9	55	35		
10	No app	25	25	15
11	25	15		
12	25	5		
13	20	20	5	5
14	25	35		
15	15	25	5	5
16	25	25	15	15
17	45	25	15	25
18	25	35		
19	25	15	15	15
20	15	25	5	15
21	15	15	15	15
22	25	25	15	5

Table D-12 Individual time of first appearance data for control subjects.

Subject	Rest Ipsilat. (min)	Rest Contralat. (min)	Exercise Ipsilat. (min)	Exercise Contralat. (min)
1	5	25	15	15
2	15	5	15	5
3	20	20	15	15
4	25	25	5	5
5	35	35		
6	25	25		
7	15	15		
8	25	25	5	5
9				
10	15	15	5	5
11	35	35	15	15
12	35	25	15	15
13	15	15	15	5
14	25	25	15	15
15	15	15	15	15
16	35	35	25	15
17	15	15	15	15

Table D-13. Comparison of scoring system for evaluating breast cancer related lymphedema developed by Szuba et al. and modified by Lane et al.

Scoring System Proposed by Szuba (103)		Modified Scoring System by Lane
Visualization of lymph nodes on the ipsilateral side	Score	Axillary uptake (%, relative to contralateral arm)
▪ Symmetrical bilateral lymph node visualization	0	78-120% (based on 95% CI of CONT)
▪ Asymmetrical axillary lymph node clearly visible after exercise	1	56-77%
▪ Other lymph node visible after exercise	2	34-55%
▪ Any lymph node on the affected side, visible only on the late images	3	12-33%
▪ No lymph node visible on the affected side	5	<11%
Dermal backflow	Score	FORE (%, relative to contralateral arm)
▪ No dermal backflow	0	90-105% (based on 95% CI of CONT)
▪ Small, localized, dermal backflow	1	106-160%
▪ Circumferential dermal backflow involving one segment of the limb	2	161-238%
▪ Circumferential dermal backflow involving more than one limb segment	3	>239%
Total	**8**	**Total**

Table D-14. Application of the modified scoring system for evaluating breast cancer related lymphedema applied to subjects with breast cancer related lymphedema (BCRL), breast cancer (BC), and controls (CONT) at rest and during exercise.

Subj.	REST				Exercise			
	AX /5	FORE /3	TOTAL /8	At risk? (3 or higher)	AX /5	FORE /3	TOTAL /8	At risk? (3 or higher)
BCRL								
1	3	2	5	At risk?	3	3	6	At risk?
2					3	2	5	At risk?
3					5	3	8	At risk?
4	3	1	4	At risk?				
5	5	3	8	At risk?				
6	5	3	8	At risk?	3	3	6	At risk?
7	3	1	4	At risk?	5	3	8	At risk?
8	5	3	8	At risk?	5	3	8	At risk?
9	1	3	4	At risk?	0	3	3	At risk?
10	2	0	2		3	1	4	At risk?
BC								
11	0	0	0		0	0	0	
12								
13	0	1	1		1	0	1	
14								
15	1	0	1		0	0	0	
16	0	1	1		1	0	1	
17	1	0	1		2	1	3	At risk?
18	0	0	0		2	0	2	
19					3	2	5	At risk?
20	3	0	3	At risk?	3	1	4	At risk?
21	3	0	3	At risk?	3	0	3	At risk?
22					0	0	0	
23	1	0	1		2	0	2	
24	0	0	0		1	0	1	
25	1	0	1		0	0	0	
26	1				2	0	2	
27	3	0	3	At risk?	2	0	2	
28	2	1	3	At risk?	0	1	1	
29	2	0	2		2	0	2	
30	0	0	0		0	0	0	
31	0	0	0		0	0	0	
32	0	0	0		2	0	2	

CONT								
33	0	0	0		0	0	0	
34	2	1	3	At risk?	2	0	2	
35	0	1	1		0	1	1	
36	1	1	2		0	0	0	
37	2	1	3	At risk?	3	1	4	At risk?
38	3	1	4	At risk?	3	0	3	At risk?
39	0	1	1		0	0	0	
40	1	1	2		0	0	0	
41	0	1	1		0	0	0	At risk?
42	0	1	1		0	0	0	
43	0	0	0		1	0	1	
44	0	1	1		0	0	0	
45	1	1	2		2	0	2	
46	0	0	0		1	0	1	
47	1	0	1		0	0	0	
48	1	1	2		1	1	2	
49	0	0	0		2	0	2	

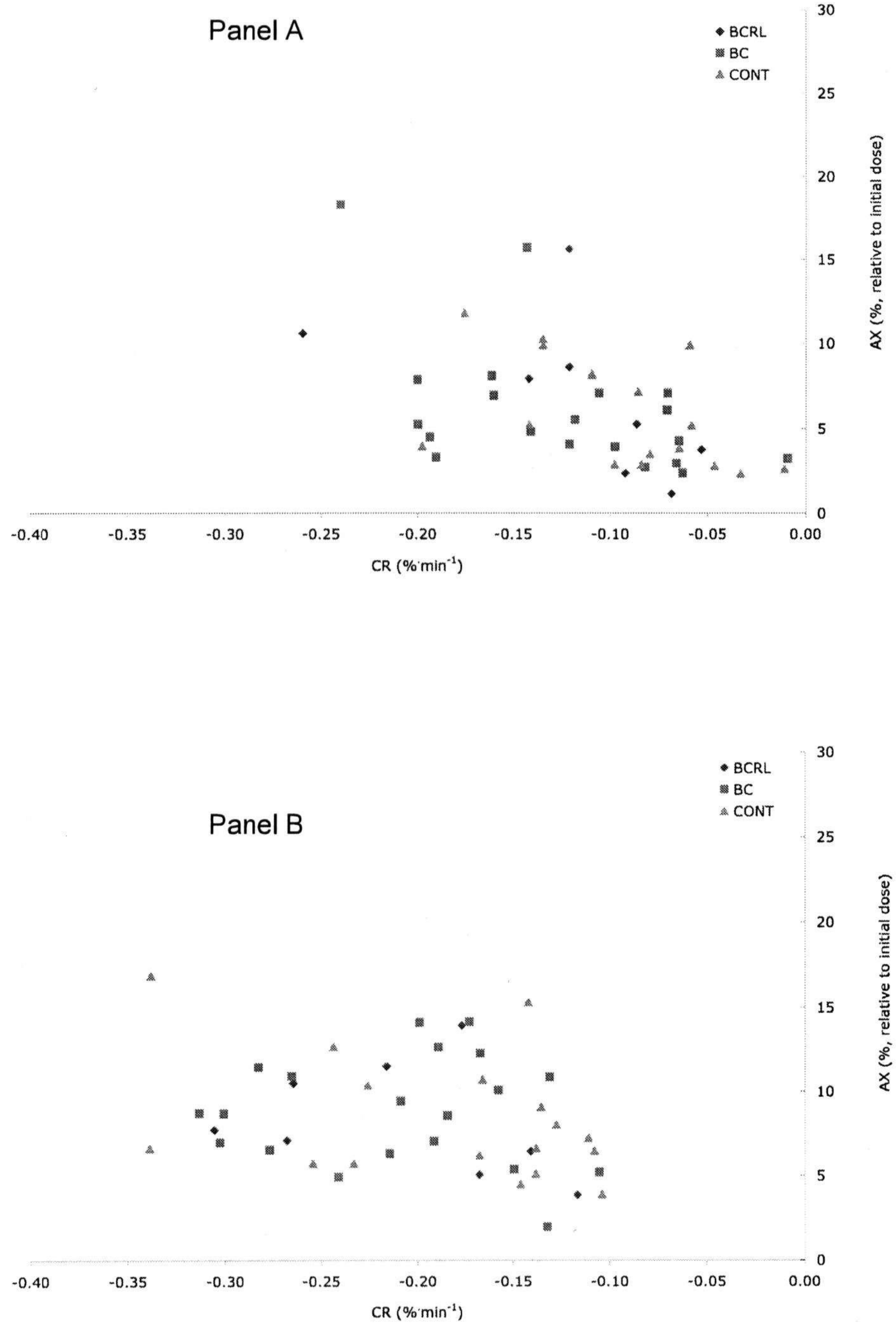

Figure D-1 Scattergram of contralateral clearance rate (CR) plotted against contralateral axillary uptake (AX) at rest (Panel A) and during exercise (Panel B) in all subjects (n=44). BCRL – diamond, BC – square, CONT – triangle.

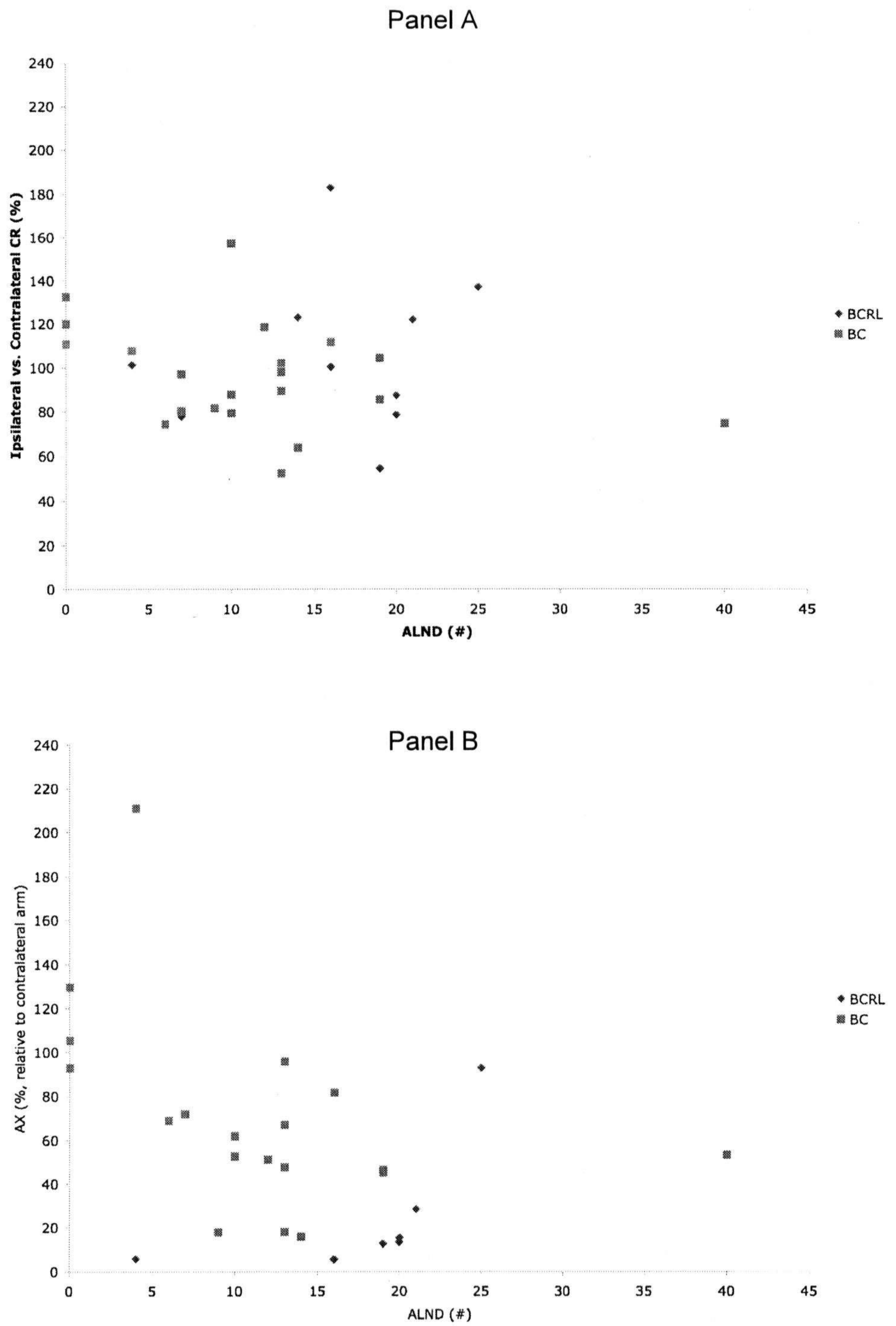

Figure D-2 Scattergram of axillary lymph nodes dissected (ALND) plotted against clearance rate (CR, Panel A) and axillary uptake (AX, Panel B) during exercise in women treated for breast cancer (BC – diamond, BCRL - square).

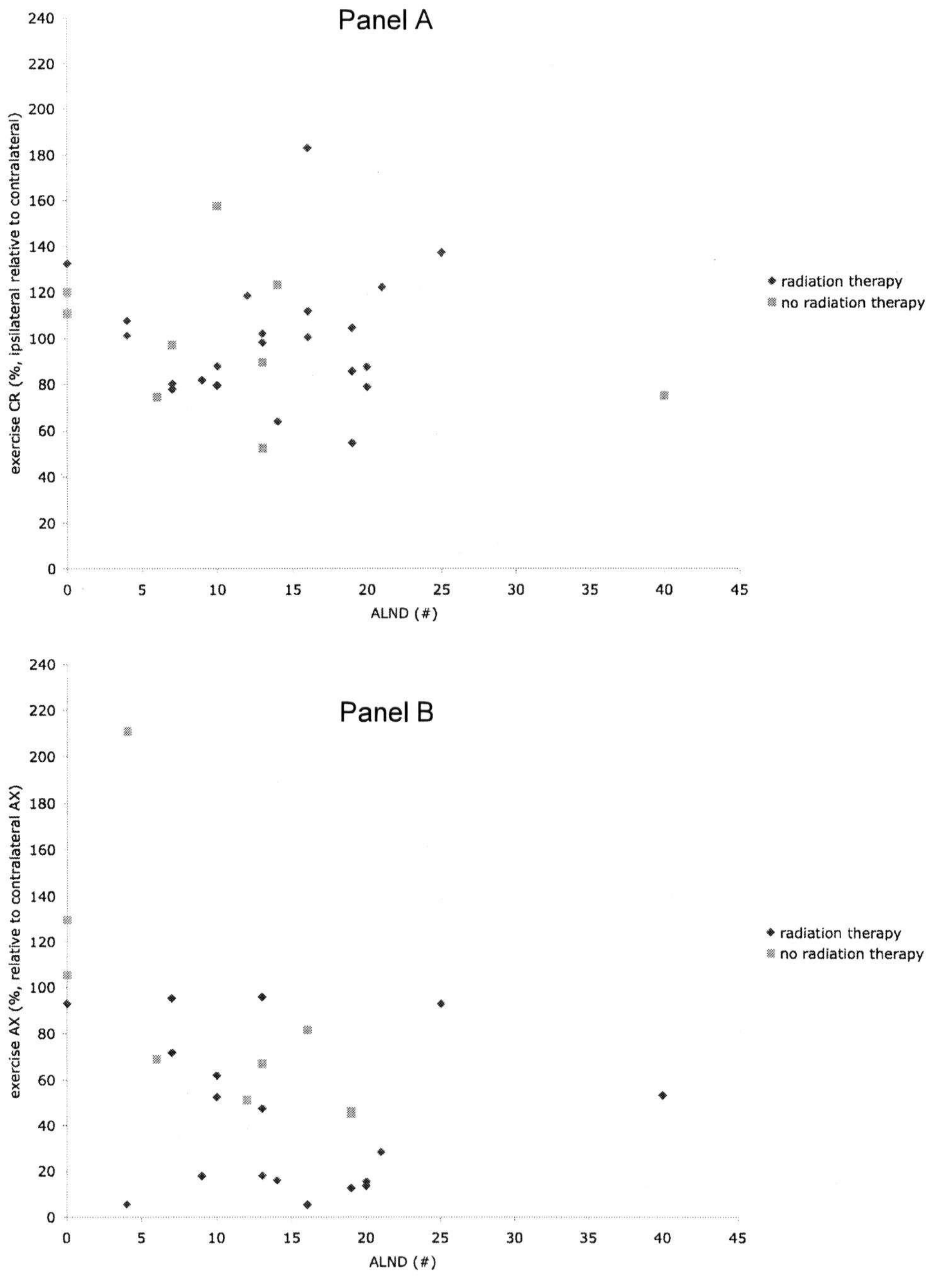

Figure D-3 Scattergram of clearance rate (CR, Panel A) and axillary uptake (AX, Panel B) during exercise in women treated for breast cancer (BC and BCRL) plotted against number of axillary lymph nodes dissected (ALND) and stratified by whether radiation therapy was performed (radiation therapy – diamond, no radiation therapy - square).