IS THERE ENHANCED LYMPHATIC FUNCTION IN UPPER BODY TRAINED FEMALES?

by

LIANNE BRONAGH DOLAN

B.Sc., The University of Western Ontario, 2003

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIRMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA

December 2006

Abstract

Chronic physical activity has been shown to ameliorate various aspects of human physiology, while specific training can directly influence structural changes. It remains unknown if chronic exercise influences upper extremity lymphatic function in females; thus, the purpose of this cross-sectional study was to compare different exercise stresses on lymphatic function in ten upper body trained females (mean (SD): age= 26.9 (SD 4.4) yrs; ht= 165.0 (SD 11.2) cm; wt= 62.1 (SD 11.8) kg; VO_2= 35.0 (SD 3.2) $ml \cdot kg^{-1} \cdot min^{-1}$) with ten untrained females (age= 31.0 yrs (SD 6.0); ht= 168.1 (SD 6.5); wt= 69.5 (SD 14.7) kg; VO_2= 22.2 (SD 4.8) $ml \cdot kg^{-1} \cdot min^{-1}$). Participants underwent a maximal upper body aerobic test on an arm crank ergometer before undergoing three randomly assigned lymphatic stress tests. Lymphoscintigraphy was used to quantify lymphatic function. ^{99m}Tc-antimony colloid was injected into the third web space of each hand followed by 1 minute spot views taken with a γ-radiation camera. Axillary acquisitions occurred at 18 (SD 5) and 64 minutes. The maximal stress test required individuals to repeat their initial maximal exercise test and then be imaged every 10 minutes until 60 minutes was reached. The submaximal stress test involved arm cranking for 2.5 minutes at 0.6 $W \cdot kg^{-1}$ followed by 2.5 minutes of rest, repeated for 60 minutes. The final stress test was a 60 minute seated resting session. The amounts cleared at the hand (AC) and axillary uptake (Ax) were determined. Four 2X3 ANOVAs were used to test for statistical significance between the two groups and three lymphatic stress tests. Only Ax post maximal exercise was significantly different between trained and untrained, p=0.009. All other measures of lymphatic function between groups were similar. Exercise had a significant impact on lymphatic function: maximal AC was significantly higher at 10 minutes (p=0.000) while submaximal AC was significantly higher at 60 minutes, (p=0.000). Compared to rest, exercise Ax was significantly greater (p=0.000) but the exercise stress, (maximal or submaximal), Ax at 64 minutes did not significantly differ (p=0.426). This study demonstrates no significant difference between upper body trained and untrained females while exercise stress significantly increased Ax and AC.

Table of Contents

Abstract....ii

Table of Contents....iii

List of Tables....v

List of Figures....vi

List of Abbreviations....vii

1. Introduction....1

1.1 The influence of exercise on lymphatic function....1
1.2 Reasoning behind lymphatic research....2
1.3 Measuring lymphatic function....2
1.4 Research Problem....4

2. Methods....4

2.1 Participants....4
2.2 Experimental Design....5
2.3 Experimental Procedures....5
2.4 Imaging....6
2.5 Statistical Analysis....8

3. Results....9

3.1 Baseline Characteristics....9
3.2 Amount Cleared....9
3.3 Axillary Uptake....13
3.4 Heart Rate and Power Output....15

4. Discussion....16

4.1 General Findings....16
4.2 Axillary Uptake....16
4.3 Clearance from the Hand....16
4.4 Untrained vs. Trained....17
4.5 Pressure Gradient....19
4.6 Respiration....19
4.7 Active vs. Passive Transport Mechanisms....20
4.8 Adaptations with Training....20
4.9 Limitations....21

4.10 Future Directions 22
4.11 Conclusion.....23

References.....24

Appendices
1. Literature Review.....29
2. Raw Data 38
3. Clinical Research Ethics Board Approval.....46

List of Tables

Table 1. Participant characteristics.....9

Table 2. Amount of ^{99m} Tc cleared from the hand.....14

Table 3. Axillary Uptake of ^{99m} Tc 14

Table 4. Heart rate and power output.....15

Table 5. Individual descriptive data 39

Table 6. Individual data for power out, heart rate and RPE.....40

Table 7. Individual data for axillary uptake.....41

Table 8. Individual clearance rate equations.....42

Table 9. Amount cleared immediately post-maximal exercise.....45

List of Figures

Figure 1. Scintigraphs acquired of the axilla region and of the web spaces 3

Figure 2. Clearance of ^{99m} Tc from the web space of the hand following maximal exercise.....10

Figure 3. Clearance of ^{99m} Tc from the web space of the hand during submaximal exercise 11

Figure 4. Clearance of ^{99m} Tc from the web space of the hand during rest 12

Figure 5. Difference in AC between 30 and 60 minutes 13

Figure 6. Submaximal heart rate vs. AC 15

Figure 7. Two scintigraphs at initial injection 43

Figure 8. Clearance rates for trained and untrained females 44

List of Abbreviations

^{99m} Tc	Technetium-^{99m} antimony colloid
AC	Amount of radiopharmaceutical cleared from the web space of the hand in %
Ax	Uptake of the radiopharmaceutical in the axillary lymph nodes as a relative % to the initial dose in the webspace
BCRL	Breast Cancer Related Lymphedema
MBq	Megabecquerel
RPE	Rating of perceived exertion; where 0 is no effort and 10 is maximal effort
rpm	Revolutions per minute
$V0_{2max}$	Maximal rate of oxygen consumption

1. Introduction

The lymphatic system plays a key role in maintaining body homeostasis, yet its role in fluid balance is poorly understood (50). The movement of lymph through the system can be influenced by active and passive mechanisms such as: lymphangion contraction, transmural pressure changes, luminal flow, arteriole pulsations, muscle pump activation, neural input, central venous pressure changes and humoral influences (12). There are many known benefits of physical activity; however, whether chronic exercise can improve lymph transport through structural changes remains unknown.

1.1 The influence of exercise on lymphatic function

Exercise has a positive influence on lymph flow (16, 18, 32). Physical activity may stimulate various mechanisms that encourage lymph flow, such as: activating the muscle pump, increasing blood flow, changing various pressures, and augmenting sympathetic nervous activation (22). With exercise-induced increases in arterial blood pressure and vascular surface area in the working muscles, an increase in interstitial pressure occurs, increasing the pressure gradient, which promotes fluid uptake into the lymphatic capillaries. With greater lymph formation occurring in the lymphatics, the increase in fluid causes an increase in wall tension, triggering a stronger contraction in the lymphangions, which increases lymph propulsion (12, 47). There is a continuous drive from the working muscles to aid lymph propulsion by compressing upon the surrounding lymphatics and centrally guiding the lymph towards the ducts to return it to systemic circulation.

Exercise influences lymph flow in both animals and humans. Changes in cardiac output alters lymph flow in sheep (8, 9), while contractions of different muscle groups have been reported to influence lymphatic clearance rates in humans (16) and in rats (24). Lane et al., (23) studied the acute differences between rest, handgrip exercise, and arm cranking exercise on upper body lymphatic clearance and demonstrated that arm cranking induced the greatest clearance rates (23). It was speculated that the faster clearance rate with dynamic exercise occurred due to increased use of the muscle pump, and a rise in interstitial pressure. Male trained runners were shown to have an overall greater

lymphatic clearance rate in their legs than untrained runners, which was attributed to the increase in vascularization in trained individuals. These trained runners had increased resting lymph flow (16), leading to a possibility of regional differences within the lymphatic system, both functional and in contractile elements (5, 12).

Chronic exercise training leads to various changes in human physiology, but whether adaptations occur within the lymphatic system remains unknown. Due to improvements in cardiac function with exercise, there is a potential link that the lymph transport mechanisms of vasomotion and pressure changes could increase the efficiency of lymph formation and transport, thus improve lymphatic function. This information leads us to believe regular exercise programs may have an effect on systemic lymph flow, which begs the question: does chronic exercise result in improved lymphatic function in the upper extremities?

1.2 Reasoning behind lymphatic research

One in nine women is at risk for developing breast cancer and of those women, up to 50% are at risk for developing breast cancer related lymphedema (BCRL). With the rise in breast cancer diagnosis, combined with the increase in survival rate, there will likely be an increase in the occurrence of lymphedema (44). Unfortunately there is no cure for women diagnosed with this debilitating condition. Understanding the mechanisms that influence lymphatic function may help prevent or treat lymphedema. Exercise can increase lymph flow 2-3 times by altering interstitial pressure and activating the muscle pump (15). Other mechanisms such as transmural pressure, luminal flow, neural input, and humoral influences all affect lymph contractility, which in turn can alter lymph flow (5). Habitual exercise may influence acute and chronic changes in these factors, consequently altering the effectiveness of the pumping action and the pressure-flow relationship (5), potentially improving lymphatic function.

1.3 Measuring lymphatic function

Historically, BCRL was monitored by measuring changes in arm circumference or changes in arm volume using a water displacement method. These methods do not relay

information on functional changes within the lymphatic system. Lymphoscintigraphy, radiopharmaceutical labeling of a lymphatic drainage source, is considered the leading technique in primary diagnosis of lymphedema. It monitors the rate of clearance, or removal of a radiolabelled tracer from an injection site, and is considered one of the best ways to estimate local tissue lymph flow, characterizing lymphatic function (2, 42). It is considered a highly sensitive, reproducible technique capable of measuring the transport capacity of the lymphatic vessels (44). There are a range of techniques used for lymphoscintigraphy, from the type of radiopharmaceutical to the location and depth of the injection. In Canada, Technetium-99m (^{99m}-Tc) antimony colloid has been selected to indirectly quantify lymphatic flow. Antimony colloid is preferentially absorbed by the lymphatic system (19); the radioisotope is injected subcutaneously into the web spaces and then a gamma camera is used to capture sequential images throughout the testing period, as seen in Figure 1.

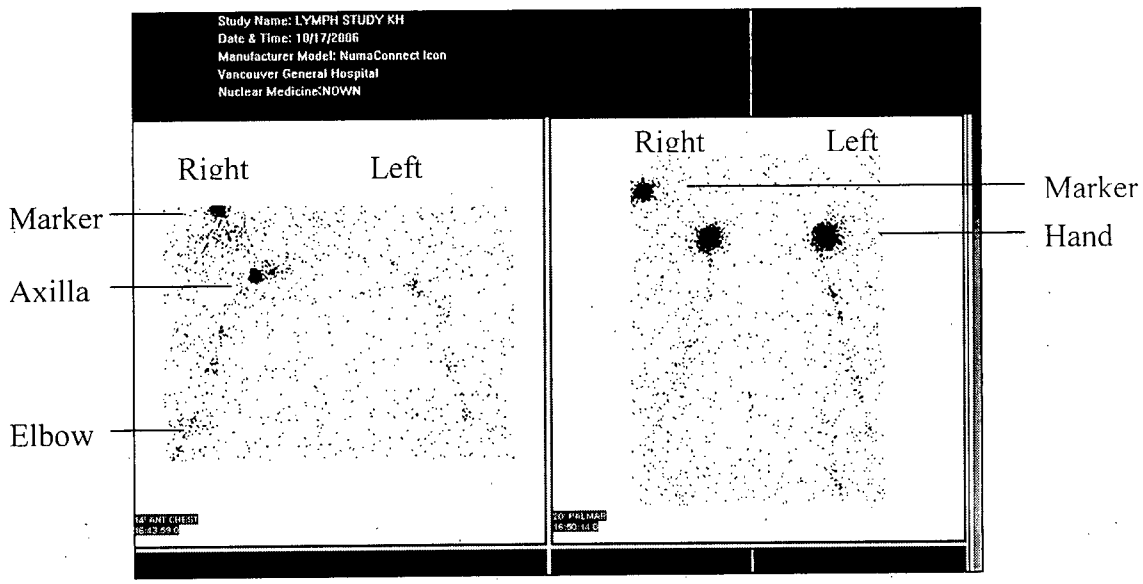

Figure. 1 Scintigraphs acquired of the axilla region and of the web spaces. The lines leaving the two web spaces in the image on the right display lymphatic clearance in the vessels. In the left scintigraph the lines leading up to the axilla region display flow in the lymphatic vessels through the arm, accumulating in the lymphatic nodes. The circular image in the top left corner represents a marker designating the right side.

1.4 Research Problem

It is acknowledged that exercise increases lymph flow (32), but it still remains unclear if there is a general improvement in lymphatic function following exercise training. This is an important question that needs to be addressed due to its potential relationship in aiding individuals with BCRL. If chronic exercise in healthy individuals improves lymphatic function, then it is logical to implement an exercise program for women who are at risk for BCRL. Ideally, a training study looking at the effect of an upper body exercise program on lymphatic function is preferred, however to explore this concept, we started with a cross-sectional study. Therefore, the purpose of this study was to determine if upper body trained females have enhanced lymphatic function. The secondary purpose of this study was to investigate the effect of maximal exercise on lymphatic function.

Hypotheses:

- Upper body trained females will have increased lymphatic clearance rates and axillary uptake in comparison to untrained females.
- Maximal exercise will induce greater clearance rates and axillary uptake than submaximal exercise.

2. Methods

2.1 Participants

Twenty healthy females between the ages of 20-40 were assigned to two equal groups based on maximal aerobic capacity ($VO_{2\ max}$) and upper body training history. Females were considered trained who participated in upper body activities at a competitive level, but were only accepted into the study if their upper body $VO_{2\ max}$ was greater than 30 $ml \cdot kg^{-1} \cdot min^{-1}$ during an arm ergometer maximal exercise test. Females were considered untrained if they did not participate in regular physical activity (less than twice a week). Exclusion criteria included history of upper extremity axillary surgery, and history of breast cancer or lymphedema. Informed consent was obtained prior to testing and the

study procedures were approved by the Clinical Research Ethics Board of the University of British Columbia.

2.2 Experimental Design

A cross-sectional, exploratory, mixed model design was used to compare the effect of three lymphatic stress protocols on lymphatic function, between both groups at two different time points. The independent variables were the lymphatic stress protocols (submaximal, maximal, and rest) and the training status, while the dependent variables, measuring lymphatic function, were the amount of radiopharmaceutical cleared (AC) and the amount of radiopharmaceutical arriving in the axillary region, axillary uptake (Ax).

The subjects were randomly designated to treatment order by draw, preventing any sequencing effects from occurring. A minimum of 48 hours separated each test in order to prevent fatigue from biasing data and to allow full decay of the radioisotope.

2.3 Experimental Procedures

Each volunteer was subjected to four different tests on four different days consisting of one maximal aerobic exercise test and three lymphatic stress tests using lymphoscintigraphy to quantify lymphatic function. The same technician prepared and administered subcutaneous injections of the radiopharmaceutical Technetium^{-99m} antimony colloid to each subject for each lymphatic stress test.

Test 1- Upper body aerobic maximal stress test

Day one of testing consisted of a maximal aerobic stress test on an arm ergometer to determine upper body $VO_{2\ max}$. Individuals performed the $VO_{2\ max}$ test on a calibrated Lode arm ergometer (Angio single set model, Netherlands), following a step protocol that started at 10 watts and increased by 10 watts per minute until volitional fatigue. Pulmonary ventilation and expired gas concentrations were measured with a TrueOne 2400 metabolic cart (Parvo Medics Inc, Sandy, UT), and values were reported as the average of 15 second intervals. Rpm, heart rate (using a Polar T31 heart rate monitor (Polar Electro Inc, Lake Success, NY)) and rating of perceived exertion (using the Borg

scale (4); 0 representing no effort to 10 representing maximal effort), were noted toward the end of every minute.

Test 2- Submaximal intermittent lymphatic stress test

After the first static image was acquired, participants began twelve repeated bouts of arm cranking, on a calibrated Lode ergometer, for 2.5 minutes at a resistance of 0.6 watts per kilogram of body weight ($W \cdot kg^{-1}$) followed by 2.5 minutes of rest. Heart rate was obtained during the last thirty seconds of each interval bout, and then averaged to give an overall submaximal heart rate.

Test 3- Maximal lymphatic stress test

A three to five minute warm up occurred on the same Lode arm ergometer, at 10 watts prior to the initial injection. After the initial injection, the first static image was acquired and then the maximal protocol began. The maximal lymphatic stress test mimicked Test 1, except no ventilatory parameters were measured. Rating of perceived exertion, rpm and heart rate were used to verify if maximum was reached. A two minute cool down at 10 watts occurred followed by a second static image. The individuals then rested until sixty minutes was reached.

Test 4 – Resting lymphatic stress test

The third lymphatic stress protocol was a resting session, where the subjects were asked to remain seated, with their arms resting on a pillow. Heart rate was obtained three times throughout the sixty minute period and averaged to give an overall resting rate.

2.4 Imaging

Lymphoscintigraphy occurred at Vancouver General Hospital, in the Department of Nuclear Medicine and Imaging. A total of 18 MBq of ^{99m}Tc antimony colloid in 0.05 mL was administered subcutaneously to the third web space of each hand prior to the start of the exercise protocol. The radiopharmaceutical purity of the injected preparation was between 92-96%. 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. 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.

During the stress tests, sequential images were taken approximately every ten minutes over a period of sixty minutes, allowing for the calculation of depot clearance rate. Images were not acquired during the maximal protocol, but were acquired prior to the onset of exercise and then immediately post-maximal exercise and throughout the recovery phase. The clearance rate equation was used to calculate the amount of radiopharmaceutical cleared from the hand at various time points. Two, one minute static images were acquired at the designated Test 1 end point time and at sixty-four minutes post-injection to determine the radiopharmaceutical uptake in the axilla relative to the initial activity in the hand. This image was taken with subjects lying in a supine position. The reliability of lymphoscintigraphy during intermittent submaximal exercise is r = 0.939, while variability between arms is 30.4% for rest and 14.4% during exercise (20).

The images were processed using Siemens Icon software (Version 8.5, Siemens Medical Systems Inc., Illinois, USA). The region of interest (144 pixels) was drawn around the injection site of each hand to give the number of activity counts per hand. It was corrected for physical decay of ^{99m}Tc using the following equation:

$$A_t = A_0 e^{-\lambda t}$$

Whereby A_t = activity after an elapsed time (min), A_0 = 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. Right and left arm clearance rates are symmetrical during rest (36), and during submaximal exercise (17), accordingly right and left data were averaged to produce one clearance rate per subject. Depot clearance rate from each hand is linear during rest and submaximal exercise; therefore it is expressed as a linear constant (% administered activity·min^{-1}). It was unknown how maximal exercise would affect clearance rate, thus clearance rates for dominant and non-dominant arms were compared before averaging the two to determine the overall slope.

Maximal exercise (with recovery time) percentages were graphed against time to determine the line of best fit. A logarithmic slope was determined to represent maximal clearance rate. Due to the mathematical differences in slopes between maximal, submaximal and resting clearance rates, the determined clearance rate equations were used to compare the AC in percentages at two selected time points: 10 and 60 minutes.

Region of interests were drawn around axillary lymph node regions and were corrected for physical decay of ^{99m}Tc. The corrected axillary counts were made relative to the palmar region of interest boxes of 144 pixels. There was an anterior and posterior axillary acquisition taken in the supine position, hence the geometrical mean was determined with the corrected counts then compared to the initial amount of radioactivity at the injection site which determined percent of axillary uptake. Axillary uptake has been shown to be equivalent between dominant and non-dominant arms (17) but it was unknown if that held true for maximal exercise. Thus, Ax between dominant and non-dominant arms was compared, and if differences were found, comparisons were made using the dominant arm.

2.5 Statistical Analysis

SPSS version 14.0 (SPPSS Inc, Chicago, IL) was used for statistical analysis. For this exploratory study, paired t-tests were run between dominant and non-dominant arms to determine if AC and Ax were equivalent between arms during maximal exercise. Separate 2x3 analyses of variance (ANOVAs) were used to determine if there were significant differences in AC at 10 and at 60 minutes. As well, separate 2x3 ANOVAs were used for Ax at 18 and 64 minutes. A paired t-test was used to determine if AC and Ax at 60 minutes was greater than at 10 minutes. An alpha level of 0.05 was used for all tests of significance. Contrasts were determined and bonferroni adjustments were made for multiple comparisons. Results are given as means (SD) if not otherwise stated.

3. Results

3.1 Baseline Characteristics

There was no significant difference in anthropometric data between the trained and untrained females, but there was a significantly higher $VO_{2\ max}$ in the trained group, p= 0.000 (Table 1). All subjects except for two in the trained group attained similar maximal values during Test 3 as in Test 1, based on their initial maximal time, power output, heart rate, perceived exertion, and rpms.

Table 1. Participant characteristics (n=10)

	Age (yrs)	Ht (cm)	Wt (kg)	BMI (kg/m^2)	VO_2 $ml \cdot kg^{-1} \cdot min^{-1}$	VO_2 $L \cdot min^{-1}$
Trained	26.9 (4.4)	165.0 (11.2)	62.1 (11.8)	22.7 (2.9)	35.0 (3.2)*	2.17 (0.4)*
Untrained	31.0 (6.0)	168.1 (6.5)	69.5 (14.7)	24.6 (5.2)	22.2 (4.8)	1.48 (0.2)

Values are means (SD). * $p < 0.05$ compared with untrained values.

3.2 Amount Cleared

AC, inferring lymph flow, showed equivalence between dominant and non dominant arms in trained and untrained females, p= 0.211. Accordingly, data between extremities was collapsed for further statistical analysis. AC was not significantly different between trained and untrained females at 10 or 60 minutes, p= 0.813 and p= 0.804 respectively, but exercise stress, at both time points, had a significant affect on the amount cleared, p= 0.000 (Table 2). At 10 minutes, maximal exercise AC was significantly greater than submaximal AC, p=0.000. Approximately seven percent of the radiopharmaceutical was cleared from the web space immediately post-maximal exercise, but during recovery, the clearance rate slowed generating a logarithmic slope (Fig. 2). AC between 30 and 60 minutes post-maximal exercise was equivalent to rest AC, 1.95% (SD 0.5) and 1.74% (SD 1.6) respectively, p= 0.52 (Fig. 5).

Submaximal and rest stress tests demonstrated a linear clearance rate (Figures 3 & 4 respectively), and at 60 minutes, there was significantly higher AC produced with submaximal exercise than with the other lymphatic stress tests, p= 0.000 (Table 2).

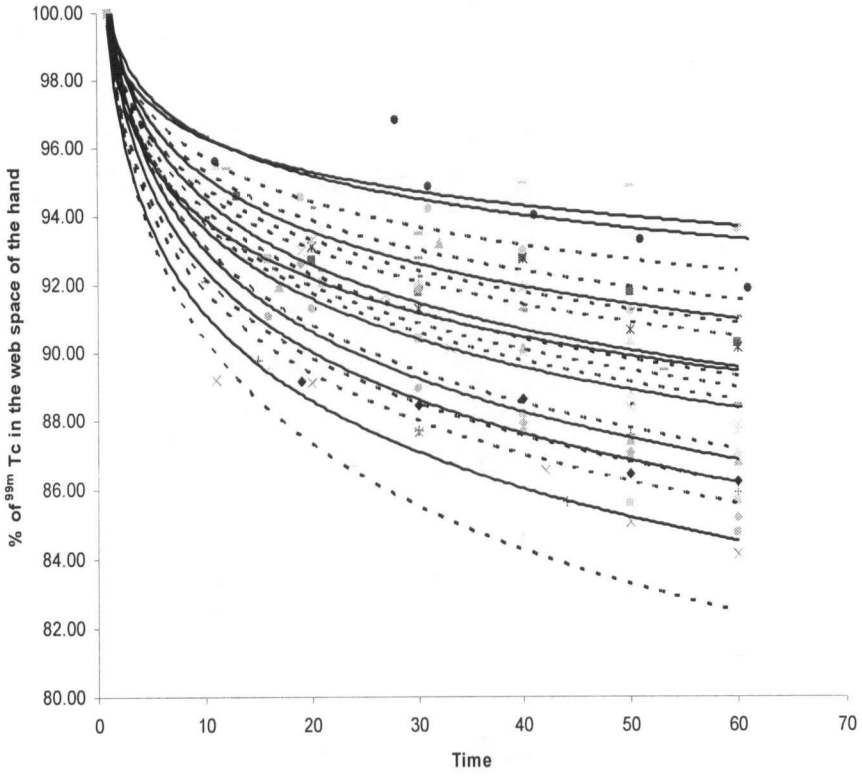

Figure 2. Clearance of ^{99m}-Tc from the web space of the hand following maximal exercise of trained females (---) and untrained females (–).

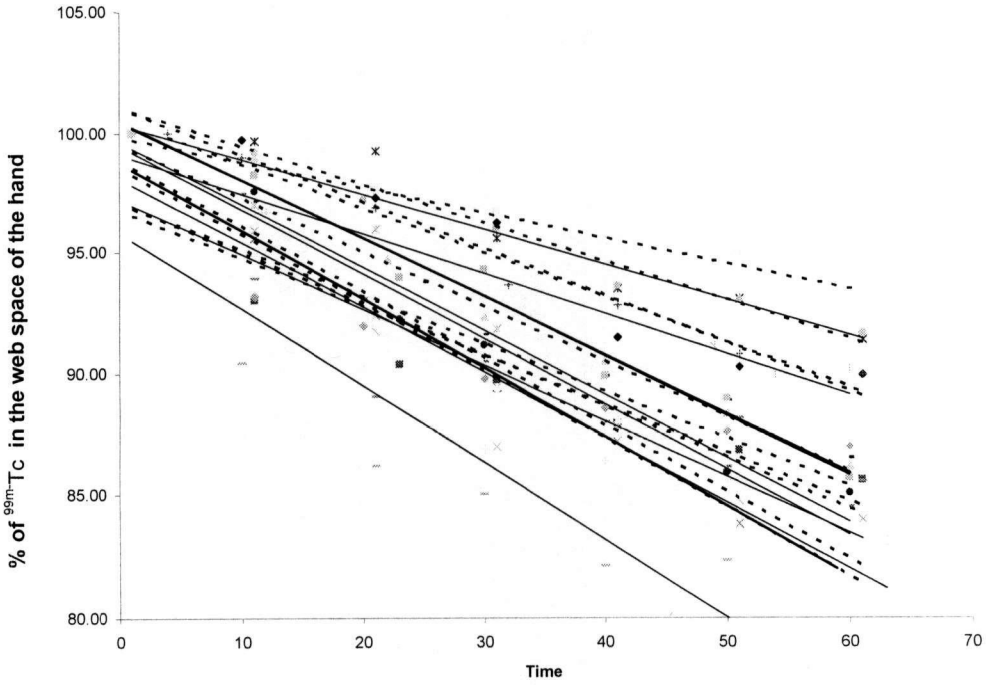

Figure 3. Clearance of ^{99m}-Tc from the web space of the hand during submaximal, intermittent exercise at 0.6 W·Kg^{-1} of trained females (---) and untrained females (–).

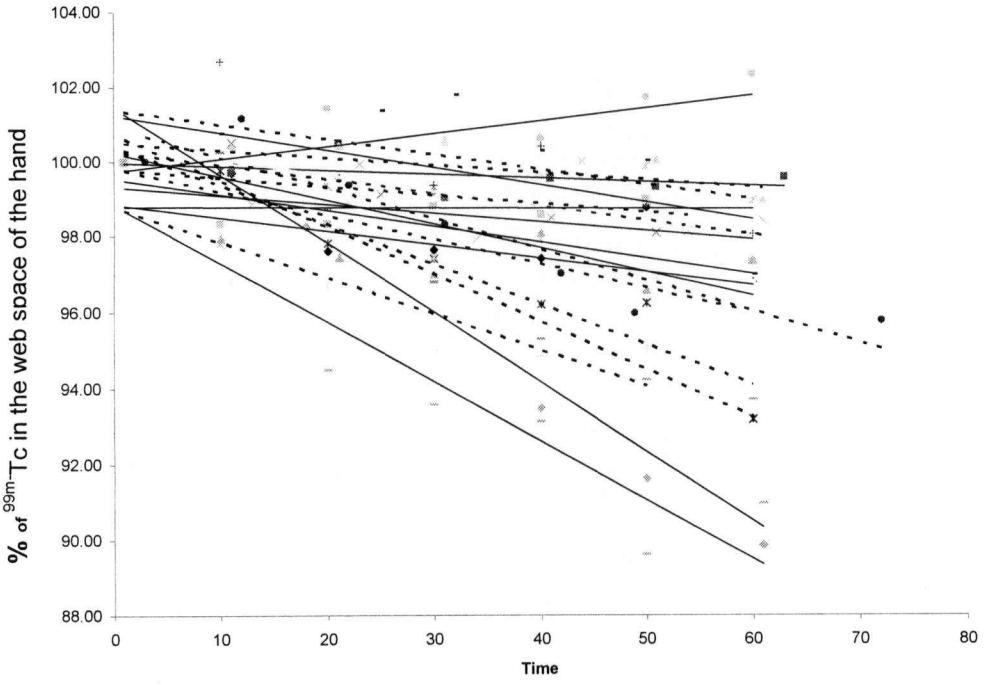

Figure 4. Clearance of ^{99m}-Tc from the web space of the hand during rest of trained females (---) and untrained females (–).

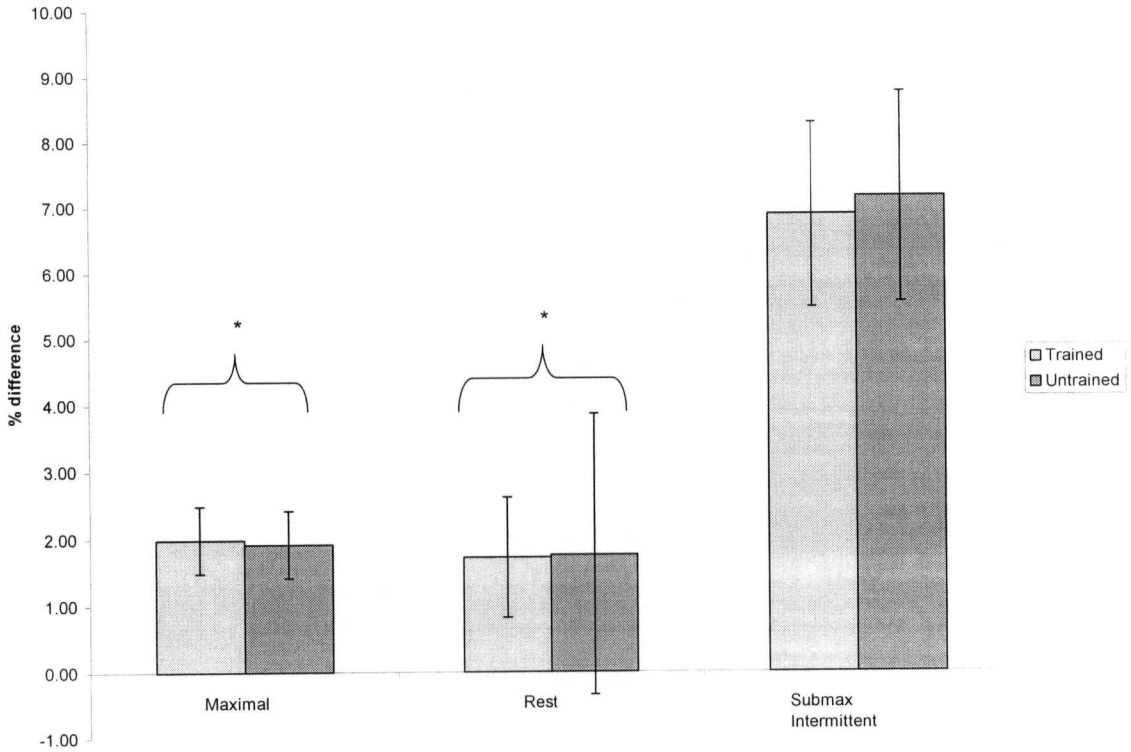

Figure 5. Difference in AC between 30 and 60 minutes in trained and untrained females during three modes of lymphatic stress tests. *p< 0.05 compared to submaximal intermittent exercise.

3.3 Axillary Uptake

During each lymphatic stress test Ax was measured at two different time points: immediately post maximal exercise at 18 (SD 5) minutes and at 64 minutes. As expected, there was significantly greater Ax at 64 minutes than at 18 minutes within each lymphatic stress test. Ax was equivalent at 64 minutes between the dominant and non dominant arm yet there was a significant difference in Ax between arms at 18 (SD 5) minutes, p= 0.03.

Immediately post-maximal exercise the trained females had significantly higher Ax than untrained, p= 0.009. At 64 minutes, there was no difference in Ax between groups p= 0.531, while there was a significant difference within the lymphatic stresses, p= 0.000 (Table 3). Exercise stresses had higher uptake compared to resting values p= 0.000, but there was no significant difference between maximal and submaximal Ax, p= 0.426 (Table 3).

Table 2. Amount of ^{99m}Tc cleared from the hand

	Maximal		Submaximal		Rest	
	10 min	60 min	10 min	60 min	10 min	60 min
Trained	6.41 (SD 1.73)	11.54 (SD 3.10)	3.37 (SD 2.25)	14.82 (SD 4.28)	0.46 (SD 0.67)	3.17 (SD 2.09)
Untrained	5.97 (SD 1.64)	10.85 (SD 2.95)	3.80 (SD 1.79)	15.72 (SD 4.05)	0.80 (SD 0.84)	3.86 (SD 3.73)
T&UT	6.19 (SD 1.66)*	11.19 (SD 2.97)*	3.59 (SD 2.98)	15.27 (SD 4.08)	0.64 (SD 0.76) *	3.51 (SD 2.97) *

Values are means (SD) in % of the initial amount injected at time zero. Due to lack of significant differences between groups, means were pooled, n= 20 for trained and untrained females, T&UT. * $p < 0.05$ when compared to submaximal stress

Table 3. Axillary uptake of ^{99m}Tc

	Maximal		Submaximal		Rest	
	18 (SD 5) min	64 min	18 (SD 5) min	64 min	18 (SD 5) min	64 min
Trained	0.70 (SD 0.30)	1.56 (SD 0.76)	0.61 (SD 0.82)	1.77 (SD 0.78)	0.05 (SD 0.04)	0.71 (SD 0.86)
Untrained	0.28 (SD 0.34)	1.31 (SD 0.76)	0.23 (SD 0.20)	1.62 (SD 1.05)	0.04 (SD 0.08)	0.52 (SD 0.55)
T&UT	n/a	1.44 (SD 0.75)	0.42 (SD 0.61)	1.69 (SD 0.91)	0.05 (SD 0.06) *	0.61 (SD 0.71) *

Values are means (SD) in % relative to the initial amount injected at time zero in the web space. Due to lack of significant differences between groups, means were pooled, n= 20 for trained and untrained females (T&UT) except immediately post maximal exercise. * $p < 0.05$ compared to submaximal stress.

3.4 Heart Rate and Power Output

Heart rates in the trained females were significantly lower throughout interval and resting protocols, p= 0.000, while maximal heart rate did not significantly differ, p= 0.140 (Table 4). As expected, maximal wattage was significantly higher in the trained group p= 0.000, but there was no group difference in wattage during the submaximal exercise p= 0.240.

Table 4. Heart rate and Power Output (n=10)

	HR max	HR submax	HR rest	PO max	PO submax
Trained	180 (9)	103 (14)*	58 (6)*	129 (22)*	37.0 (7.1)
Untrained	173 (2)	123 (11)	76 (7)	79 (9)	40.3 (7.5)

Values are means (SD) in beats per minute for heart rate (HR), and watts for power output (PO). Results are presented between trained and untrained during maximal, submaximal and resting protocols. * $p < 0.05$ compared with untrained values.

In Figure 6, average heart rates for each 10 minute interval during submaximal stress were calculated for the trained and untrained groups and plotted against AC for each time point. The generated trend demonstrates that the trained group required a lower, steady heart rate to clear the equivalent amount as the untrained group.

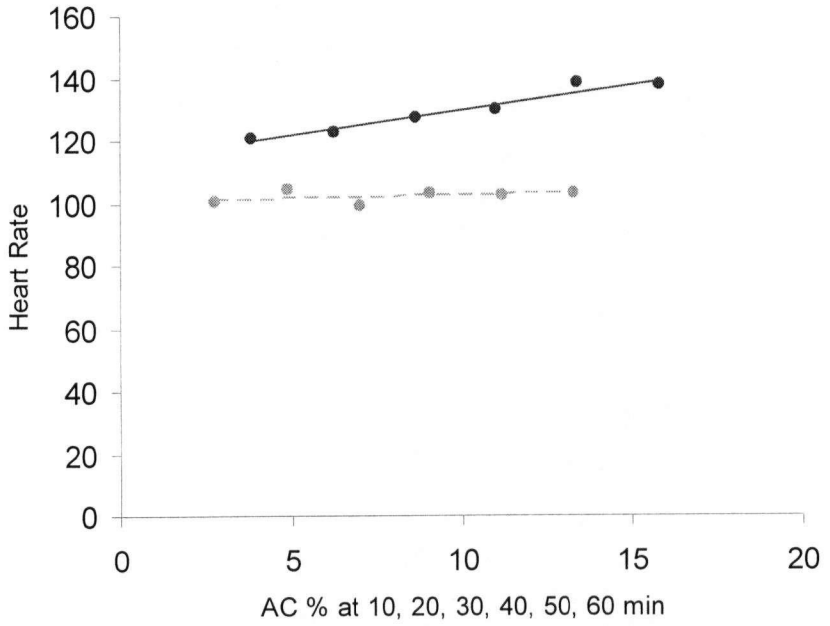

Figure 6. Submaximal heart rate vs. AC. Average heart rate (bpm) was calculated for each time point at 10, 20, 30, 40, 50 and 60 minutes for trained (---) and untrained females (–) and graphed against AC of ^{99m}Tc as a percent.

4. Discussion

4.1 General Findings

This exploratory study provides new information concerning the differences in lymphatic function between upper body trained and untrained females. Further, this study compares different exercise stresses on axillary uptake and the amount of radiopharmaceutical cleared in the upper limb of healthy females.

The principal findings of this study are:

1) No significant difference was found between groups except Ax immediately post-maximal exercise; 2) Maximal AC was significantly greater at 10 minutes, whereas submaximal AC was significantly higher at 60 minutes; and 3) Maximal and submaximal Ax did not differ.

Our results confirm other findings that exercise increases clearance rates, inferring lymph flow, due to exercise induced activation of lymph transport mechanisms enhancing lymph propulsion (15, 16, 23). We also demonstrated an increase in axillary uptake of ^{99m}Tc during exercise, an alternate measurement to infer lymph flow in the upper limb.

4.2 Axillary Uptake

It remains unknown what influences retention time of a radiopharmaceutical within the lymphatic nodes and what physiological changes promote its return into the lymphatic vessels. With the inability to monitor the exact amount of radiopharmaceutical entering, staying and exiting a lymph node, we are unable to state if axillary uptake, at these time points, is an accurate view of total lymph transport within the upper limb. However, axillary acquisitions give an estimation of lymph flow from the hand and its movement through the upper limb to the axilla region. With this estimation, this study demonstrates the importance of exercise in promoting lymph flow in the upper limbs.

4.3 Clearance from the Hand

High variability occurred within the resting data (see Fig 4), which is not unexpected considering previous results found in other studies (20). The initial injection of the

radiopharmaceutical into the web space may cause high variability due to the immediate change in resting fluid homeostasis triggering an increase in lymph formation and flow, to prevent this instant edema (49). This natural response of increasing flow may differ between individuals, thus producing excessive variability within the resting values, promoting the concept of using an exercise stress for greater consistency to investigate lymphatic function. Another reason for the variability could be attributed to the lack of compliance of the subjects to stay still. Previous studies have shown that slight changes in body posture can alter resting lymph flow (10, 49). Cooke et al. demonstrated that a slight 15 degree change in supine body position altered resting lymph flow from 104±36μl/h to 341±126μl/h (10). During the resting period, the majority of women altered their posture so they could go to the bathroom, make a phone call or they simply forgot to stay still.

4.4 Untrained vs. Trained

Baseline physical characteristics of the two groups were not significantly different in age, weight or height, while physiological differences in VO_{2max} were shown (Table 1). Females were defined as upper body trained who participated in upper body sports such as swimming, rock climbing, rowing or outrigger paddling. The subjects in this study participated in their sport at least four times a week with a minimum of 3 years (up to 18 years) of experience and all used other modes of cross training such as running and weight lifting. The untrained females did not participate in regular exercise, but monthly activity consisted of a yoga class or casual walks.

Contrary to the hypotheses, lymphatic function was similar between groups through all stresses, except immediately post-maximal exercise. Specifically, Ax for the trained group was significantly higher than that of the untrained subjects' Ax immediately post-maximal exercise. A viable explanation is the time difference and the total amount of work completed during the maximal condition when comparing the two groups. Trained individuals exercised approximately four minutes longer with a power output of approximately forty extra watts; meanwhile, maximal heart rate was not significantly different between the groups. Even with greater work attained by the trained, Ax was

similar between the trained and untrained group at the 64 minute mark, which introduces the possibility of a maximal flow limitation.

The trained females had a lower resting heart rate, and a lower submaximal heart rate even though the average power output required was equivalent between the two groups (see table 4). These results imply that the trained required less cardiac effort to clear an equal amount of radiopharmaceutical from the hands and transport an equal amount to the axilla region than the untrained (Fig. 6). The lower heart rate in the trained, yet equivalent AC and Ax between groups insinuates the possibility of an effective lymph transport mechanism such as enhanced arteriole pulsation in the trained. Magnetic resonance imaging has recently demonstrated vascular remodeling in both peripheral and central conduit arteries in upper body trained athletes (38). Enhanced diameters in the brachial arteries have been observed with training (29), and chronic changes were found in the vascular system: greater pulse pressure and stroke volume with a decreased heart rate (38). Perhaps trained individuals transport the same amount of lymph with a lower heart rate due to this increased pulse pressure, and greater alterations in artery and arteriole diameters, enhancing the lymphatic transport mechanism of vasomotion. This interpretation encourages the concept of greater efficiency within the lymphatic system in trained females.

Only one other study has looked at differences in training status in humans and lymph flow (16). The lower extremity lymphatic clearance rates in four runners and four untrained males were compared. No differences were found in clearance rates during 10 minute exercise bouts or during the 65 minute recovery phase between each exercise condition. When the three recovery phases were combined, the overall resting mean lymphatic clearance rate in the trained was significantly higher than in the untrained, (0.06% (SD 05) and 0.03% (SD 0.03) respectively, p= 0.008). Our study had a greater sample size, and for upper body trained and untrained females, the resting clearance rates in the upper limb were 0.06% SD (0.03) and 0.06% (SD 0.07) respectively, but no significant difference was found during the one hour resting protocol. Possible explanations may be due to differences in adaptation within the lymphatic system based

on gravitational stresses, such that vessels within the lower limbs may show greater changes than in the upper limbs. Another explanation is the difference in the injection site. The trained runners received injections into their vastus lateralis, an area most likely to have enhanced vascularization with lower body training (16). Ideally, to find a difference between trained and untrained females, an injection into an area of high capillary density within the arm muscles would be ideal, but in this study injections occurred in the web space of the hand.

4.5 Pressure Gradient

There are multiple mechanisms that affect the movement of lymph, one being alterations in the various pressure gradients (12) at the capillary level or within the lymphatic vessel. High central venous pressure is directly linked to lymphatic outflow pressure which is linked to an attenuation in lymph flow velocity (49); however, whether there is a linear decrease in lymph flow with increased outflow pressure in surrounding lymph vessels remains to be clarified (35). Thus, any change in pressure at the initial lymphatics, within the collector vessels, or at the final destination of the right lymphatic or the thoracic duct, has potential to alter lymph flow. If the thoracic duct pressure is high, the lymphatic vessels leading into the duct will be competing not only against gravitational forces, but against a higher pressure gradient within its own system, possibly slowing overall lymph flow. It is possible that lymphatic outflow pressure reaches an upper limit due to a leveling off of central venous pressure, causing a maximal flow rate within the lymphatic system. Other human and animal studies infer the idea of a maximal flow rate, such that after the initial spike in clearance rate at the onset of exercise, a plateau in clearance rate is demonstrated, even with further increases in intensity (8, 15).

4.6 Respiration

Respiration has been shown to have variable influence on lymph flow in different animals due to changes in pleural pressure, affecting thoracic duct flow, consequently altering the pressure gradient throughout the lymphatic vessels (6, 35). To try to maintain a similar breathing pattern between subjects, a consistent rpm was used, but ventilation was not measured during the lymphoscintigraphy tests. At maximal exercise, rpms were

85 (SD3) and during interval exercise, rpm was held at 70. Other than avoiding an entrainment response with breathing, a secondary reason for consistency with rpms was to ensure no variation in contractile rhythm. Muscle lengthening allows for lymphatic filling, and the duration of the lymphatic refilling period within the vessels influences lymphatic flow (25). Thus, muscle movement was kept as constant as possible, so when the arm muscle lengthened lymphatic filling stayed consistent.

4.7 Active vs. Passive Transport Mechanisms

Gashev and colleagues have demonstrated a decrease in contractile frequency and amplitude within the lymphangion when there is an increase in lymph flow (13). The proposed explanation suggests that the lymphatic system attempts to conserve metabolic energy by creating a balance between the active and passive transport forces (12). Lymph flow is dominantly influenced by the lymphangion at rest, but with an instant increase in edema, due to exercise or an injection, the passive pumps such as the suctioning of the lungs, arteriole pulsations, pressure changes and the muscle pump become the dominant forces behind lymph transport (14). This balance between lymph transport mechanisms could limit lymph flow.

4.8 Adaptations with Training

The trained required less cardiac effort (a lower heart rate) than the untrained to attain an equivalent amount of AC and Ax, which suggests that alterations in blood flow mechanics and vascular adaptations occurred.

It has been documented that upper body trained individuals have increased capillary density of greater than 500 capillaries/mm^2 in the upper limbs depending on the sport and training level (7). This increase in capillary density and accompanying blood flow (1, 46), may increase potential fluid leakage into the interstitial space, causing the lymphatic system to adapt to the rising fluid demand to prevent edema. A potential adaptation by the lymphatic system to this chronic stress of increased fluid may be an augmentation in lymphatic vessel development. Upper body trained athletes may have a greater number of lymphatic vessels, compensating for the increased number of vascular capillaries.

If there is an upper limit for maximal flow, the increase in lymphatic vasculature may not change the rate of lymph flow. But, if no chronic increase in lymph flow is possible, perhaps an increase in lymphatic vasculature will inadvertently produce a safety net to prevent a decline in lymphatic function. If damage occurs to the lymphatic system (as in BCRL), trained individuals may compensate through the development of potential rerouting mechanisms with their extra number of lymphatic vessels, avoiding compromised lymphatic function.

4.9 Limitations

As a cross-sectional study, our major limitation was the inability to control for type, intensity, duration and frequency of training. As well, our definition of trained using a VO_{2max} value may have been flawed because it is well documented that VO_{2max} is also influenced by genetics. To get an accurate measurement of the effect of upper body exercise's influence on lymphatic function, a training study needs to occur.

One of the potential limitations to this study was changing the lymphoscintigraphy protocol from two injections into the second and fourth web spaces to one injection into the third web space of the hand. This study demonstrated similar clearance rate values to that of previous studies using two injections (21). Resting values had a clearance rate of -0.06% while intermittent submaximal exercise produced a clearance rate of -0.22% compared with two injection induced clearance rates of -0.08% and -0.24% (20) respectively. This finding will allow future studies using lymphoscintigraphy to decrease the number of injections per hand, potentially increasing compliance rate of the volunteers.

As previously discussed, understanding lymph node retention mechanisms needs to occur. The use of axillary acquisitions is required because clearance rates do not differ from the hands in women with compromised lymphatic function in the upper limb (20) showing a lack of sensitivity to defining lymphatic function efficiency. Axillary uptake in this study only gives an estimation of the lymph movement occurring within the upper

limb, but perhaps if multiple images were taken, time of first appearance may be a more sensitive measurement.

Our choice of submaximal intermittent exercise did not elicit equivalent workload intensities between groups. The wattage was calculated based on weight; calculating a load based on a percentage of VO_{2max}, or on equivalent cardiac output would be the next step to obtain more accurate comparisons in training differences.

Further, hormonal fluctuations were not controlled even though it is acknowledged that changes in hormone levels can affect various other physiological systems in the body.

We estimated our sample size using the averages in intensity differences (21), producing a sample size requirement of 10 in each group. Using differences in clearance rate slopes produced from this study, a sample size of 2321 may have detected differences between groups during rest. A sample size of 60 during submaximal exercise and a sample size of 119 at the end of maximal exercise were calculated to detect differences.

The injection location may be a limitation when trying to determine differences between groups. The amount of change in capillary density in the hand due to upper body training may be less than that found in the arm muscles. An injection into the forearm or upper arm instead of into the web spaces may be a more viable method to determine if there are differences with training.

4.10 Future Directions

This cross-sectional study investigated lymphatic function in women who were previously trained and physically healthy. A training study using sedentary subjects is the next step. This training study would involve a change in injection location, as well as investigating various exercise intensities to verify if there is a maximal flow limitation in healthy females. If a limitation is found, then a prospective training study in women with compromised lymphatic function would follow. If their lymphatic function cannot reach the defined upper limit of lymph flow, perhaps training could induce rerouting

mechanisms, and dissipate the effect of impaired lymph transport. The type of training, intensity, frequency and the duration that could generate alterations in lymphatic vasculature remains unknown, but measurements of not only clearance rate, but axillary uptake are necessary.

4.11 Conclusion

In summary, the results demonstrate that lymphatic function is similar between trained and untrained females. This introduces the concept of a maximal flow limitation within the lymphatic system in healthy females. On further examination this study proposes that lymphatic adaptations occurred in the trained females due to a decrease in cardiac requirement needed to produce equivalent lymphatic clearance rates and similar axillary uptake values.

References:

1. **Ahlborg G and Jensen-Urstad M**. Arm blood flow at rest and during arm exercise. *Journal of Applied Physiology* 70: 928-933, 1991.

2. **Aukland K and Reed RK**. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiological Reviews* 73: 1-78, 1993.

3. **Board J and Harlow W**. Lymphoedema 1: components and function of the lymphatic system. *British Journal of Nursing* 11: 304-309, 2002.

4. **Borg GA**. Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise* 14: 377, 1982.

5. **Bridenbaugh EA, Gashev AA, and Zawieja DC**. Lymphatic muscle: a review of contractile function. *Lymphatic Research and Biology* 1: 147-158, 2003.

6. **Browse NL, Rutt DR, Sizeland D, and Taylor A**. The velocity of lymph flow in the canine thoracic duct. *Journal of Physiology* 237: 401-413, 1974.

7. **Calbet JA, Holmberg HC, Rosdahl H, van Hall G, Jensen-Urstad M, and Saltin B**. Why do arms extract less oxygen than legs during exercise? *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 289: R1448-1458, 2005.

8. **Coates G, O'Brodovich H, and Goeree G**. Hindlimb and lung lymph flows during prolonged exercise. *Journal of Applied Physiology* 75: 633-638, 1993.

9. **Coates G, O'Brodovich H, Jefferies AL, and Gray GW**. Effects of exercise on lung lymph flow in sheep and goats during normoxia and hypoxia. *Journal of Clinical Investigation* 74: 133-141, 1984.

10. **Cooke CJ, Nanjee MN, Stepanova IP, Olszewski WL, and Miller NE**. Variations in lipid and apolipoprotein concentrations in human leg lymph: effects of posture and physical exercise. *Atherosclerosis* 173: 39-45, 2004.

11. **Duncker DJ and Merkus D**. Acute adaptations of the coronary circulation to exercise. *Cellular and Biochemical Biophysiology* 43: 17-35, 2005.

12. **Gashev AA**. Physiologic aspects of lymphatic contractile function: current perspectives. *Annals of the New York Academy of Sciences* 979: 178-187; discussion 188-196, 2002.

13. **Gashev AA, Davis MJ, Delp MD, and Zawieja DC**. Regional variations of contractile activity in isolated rat lymphatics. *Microcirculation* 11: 477-492, 2004.

14. **Gashev AA, Davis MJ, and Zawieja DC**. Inhibition of the active lymph pump by flow in rat mesenteric lymphatics and thoracic duct. *Journal of Physiology* 540: 1023-1037, 2002.

15. **Havas E, Lehtonen M, Vuorela J, Parviainen T, and Vihko V**. Albumin clearance from human skeletal muscle during prolonged steady-state running. *Experimental Physiology* 85: 863-868, 2000.

16. **Havas E, Parviainen T, Vuorela J, Toivanen J, Nikula T, and Vihko V**. Lymph flow dynamics in exercising human skeletal muscle as detected by scintography. *Journal of Physiology* 504: 233-239, 1997.

17. **Lane K, Dolan L, Worsley D, McKenzie D**. Symmetry in Right and Left Upper Extremity Lymphatic Function During Intermittent Arm Crank Exercise. *Canadian Journal of Applied Physiology* 30S: S47, 2005.

18. **Kramer EL**. Lymphoscintigraphy: Defining a clinical role. *Lymphatic Research and Biology* 2: 32-37, 2004.

19. **Krasnow AZ and Hellman RS**. Lymphoscintigraphy revistited: 1999. In: *Nuclear Medicine Annual*. Philadelphia: Lippincott, Williams & Wilkins, 1999, p. 17-97.

20. **Lane K**. *Development of a lymphatic stress test, PhD Dissertation*: UBC, 2006.

21. **Lane K, Dolan L, Worsley D, and 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* 4: 159-165, 2006.

22. **Lane K and McKenzie DC**. Cancer. In: *Exercise testing and exercise prescription for special cases: Theoretical basis and clinical application* (3rd ed ed.), edited by Skinner JS. Baltimtore: Lippincott, Williams, & Wilkins, 2005, p. 363-376.

23. **Lane K, Worsley D, and McKenzie D**. Lymphoscintigraphy to evaluate the effects of upper body dynamic exercise and handgrip exercise on radiopharmaceutical clearance from the hands of healthy females. *Lymphatic Research and Biology* 3: 16-24., 2005.

24. **Mazzoni MC, Skalak TC, and Schmid-Schonbein GW**. Effects of skeletal muscle fiber deformation on lymphatic volumes. *American Journal of Physiology* 259: H1860-1868, 1990.

25. **McGeown JG, McHale NG, and Thornbury KD**. The role of external compression and movement in lymph propulsion in the sheep hind limb. *Journal of Physiology* 387: 83-93, 1987.

26. **McHale NG and Adair TH**. Reflex modulation of lymphatic pumping in sheep. *Circulation Research* 64: 1165-1171, 1989.

27. **McHale NG and Roddie IC**. The effect of intravenous adrenaline and noradrenaline infusion of peripheral lymph flow in the sheep. *Journal of Physiology* 341: 517-526, 1983.

28. **McHale NG and Roddie IC**. The effects of catecholamines on pumping activity in isolated bovine mesenteric lymphatics. *Journal of Physiology* 338: 527-536, 1983.

29. **Naylor LH, O'Driscoll G, Fitzsimons M, Arnolda LF, and Green DJ**. Effects of training resumption on conduit arterial diameter in elite rowers. *Medicine and Science in Sports and Exercise* 38: 86-92, 2006.

30. **O'Brodovich H and Coates G**. Effect of exercise on lung lymph flow in unanesthetized sheep with increased pulmonary vascular permeability. *American Review of Respiratory Disease* 134: 862-866, 1986.

31. **O'Brodovich H and Coates G**. Effect of isoproterenol or exercise on pulmonary lymph flow and hemodynamics. *Journal of Applied Physiology* 60: 38-44, 1986.

32. **O'Mahony S, Rose SL, Chilvers AJ, Ballinger JR, Solanki CK, Barber RW, Mortimer PS, Purushotham AD, and Peters AM**. Finding an optimal method for imaging lymphatic vessels of the upper limb. *European Journal of Nuclear Medicine and Molecular Imaging* 31: 555-563, 2004.

33. **Olszewski W**. Contractility patterns of normal and pathologically changed human lymphatics. *Annals of the New York Academy of Sciences* 979: 52-63, 2002.

34. **Olszewski W, Engeset A, Jaeger PM, Sokolowski J, and Theodorsen L**. Flow and composition of leg lymph in normal men during venous stasis, muscular activity and local hyperthermia. *Acta Physiologica Scandinavica* 99: 149-155, 1977.

35. **Onizuka M, Flatebo T, and Nicolaysen G**. Lymph flow pattern in the intact thoracic duct in sheep. *Journal of Physiology* 503: 223-234, 1997.

36. **Pain SJ, Barber RW, Ballinger JR, Solanki CK, O'Mahony SO, Mortimer PS, Purushotham AD, and Peters AM**. Side-to-side symmetry of radioprotein transfer from tissue space to systemic vasculature following subcutaneous injection in normal subjects and subjects with breast cancer. *European Journal of Nuclear Medicine and Molecular Imaging* 30: 657-661, 2003.

37. **Pain SJ and Purushotham AD**. Lymphoedema following surgery for breast cancer. *British Journal of Surgery* 87: 1128-1141, 2000.

38. **Petersen SE, Wiesmann F, Hudsmith LE, Robson MD, Francis JM, Selvanayagam JB, Neubauer S, and Channon KM**. Functional and Structural Vascular Remodeling in Elite Rowers Assessed by Cardiovascular Magnetic Resonance. *Journal of the American College of Cardiology* 48: 790, 2006.

39. **Petrek JA, Senie RT, Peters M, and Rosen PP**. Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. *Cancer* 92: 1368-1377, 2001.

40. **Stanton AW, Holroyd B, Mortimer PS, and Levick JR**. Comparison of microvascular filtration in human arms with and without postmastectomy oedema. *Experimental Physiology* 84: 405-419, 1999.

41. **Stanton AW, Svensson WE, Mellor RH, Peters AM, Levick JR, and Mortimer PS**. Differences in lymph drainage between swollen and non-swollen regions in arms with breast-cancer-related lymphoedema.[comment]. *Clinical Science* 101: 131-140, 2001.

42. **Swartz MA**. The physiology of the lymphatic system. *Advanced Drug Delivery Reviews* 50: 3-20, 2001.

43. **Szuba A, Shin WS, Strauss HW, and Rockson S**. The third circulation: Radionuclide lymphoscintigraphy in the evaluation of lymphedema. *Journal of Nuclear Medicine* 44: 43-57, 2003.

44. **Tomczak H, Nyka W, and Lass P**. Lymphoedema: lymphoscintigraphy versus other diagnostic techniques--a clinician's point of view. *Nuclear Medicine Review* 8: 37-43, 2005.

45. **Valic Z, Buckwalter JB, and Clifford PS**. Muscle blood flow response to contraction: influence of venous pressure. *Journal of Applied Physiology* 98: 72-76, 2005.

46. **Volianitis S, Yoshiga CC, Nissen P, and Secher NH**. Effect of fitness on arm vascular and metabolic responses to upper body exercise. *American Journal of Physiology: Heart and Circulation Physiology* 286: H1736-1741, 2004.

47. **von der Weid PY and Zawieja DC**. Lymphatic smooth muscle: the motor unit of lymph drainage. *International Journal of Biochemistry and Cell Biology* 36: 1147-1153, 2004.

48. **Wakatsuki T, Schlessinger J, and Elson EL**. The biochemical response of the heart to hypertension and exercise. *Trends in Biochemical Sciences* 29: 609-617, 2004.

49. **Wu J and Mack GW**. Effect of lymphatic outflow pressure on lymphatic albumin transport in humans. *Journal of Applied Physiology: Respiratory, Environmental & Exercise Physiology* 91: 1223-1228, 2001.

50. **Zawieja D**. Lymphatic biology and the microcirculation: past, present and future. *Microcirculation* 12: 141-150, 2005.

APPENDIX I: LITERATURE REVIEW

Anatomy and Physiology:

The lymphatic vascular transport system is composed of: lymph, lymphatic capillaries, pre-collector vessels, collector vessels, lymphatic trunks, lymphatic ducts and lymph nodes. Lymph is a clear fluid made up of water, macromolecules, dying cells and other waste products from the interstitial fluid (3). The non-contractile lymphatic capillaries, or the initial lymphatic vessels, consist solely of endothelium and have anchoring filaments attached at right angles. Under increased interstitial stress, the filaments exert a radial tension on the lymphatic vessel, expanding the luminal volume and creating new gaps for fluid entrance (42). This expansion drops the luminal pressure, favoring the pressure gradient, driving the interstitial fluid through the openings between the filaments, forming lymph. The lymph then drains from the capillaries into the pre-collector vessels within the dermis. Towards the lower aspect of the dermis, the pre-collector vessels develop valves and a layer of smooth muscle. The functional units within the muscular lymphatic vessels are called lymphangions, and are separated by the lymphatic valves (5). The main function of the numerous pre-collector vessels is to propel the lymph from mid to lower dermis to the larger collector vessels. The collector vessels transport the lymph through the lymph nodes to the lymphatic trunks, where they return the lymph into the bloodstream via one of the two lymphatic ducts: the large thoracic duct or the right lymphatic duct.

Each day approximately 16-18 L of interstitial fluid develops and 2-4 liters are drained back into the lymphatic system, maintaining the necessary homeostatic balance (3). Active pumps, passive pumps and valves are used to form and move this newly formed lymph against gravitational forces, a hydrostatic pressure gradient and a protein concentration gradient (5). Active pumps, or intrinsic forces are coordinated contractions of the lymphangions with the lymphatic valves (12). Passive pumps, or extrinsic driving forces, consist of lymph formation, arterial pulsations, changes in venous pressures, skeletal muscle contractions, and respiration (12).

The lymphatic muscle plays an important role in lymphatic function because it influences the generation and regulation of lymph flow (5). Lymphatic smooth muscle produces

phasic and tonic contractions of its vessels to modulate lymphatic flow (47). Tonic contractions change the diameter of the lymphatic vessels, which alters lymph outflow resistance, therefore manipulating lymph flow (5). Phasic contractions, which are similar to those seen in cardiomyocytes, are brief yet intense, and effectively open and close lymphatic valves, providing energy to produce lymph flow (5).

Chemically, acetylcholine inhibition, β-adrenoreceptor activation, and humoral agents inhibit lymphatic contractions by increasing cGMY and cAMP production (5) while nitric oxide can reduce the action potentials in the pacemaker muscle, reducing contractions (43). The intravenous administration of adrenaline and noradrenaline has been shown to increase the force and frequency of lymphatic contractions in popliteal, prefemoral and mesenteric lymphatic vessels (27). Too much administration of noradrenaline depresses lymphatic flow, perhaps due to the decrease in stroke volume with the increase in contractile frequency (28).

Filtration forces are responsible for maintaining the equilibrium of fluid exchange between the circulatory and the lymphatic systems. Hydrostatic pressure is based on the pressure-volume relationship, whereas colloid osmotic pressure is based on the changing protein concentration within the interstitial fluid (2). Hydrostatic and osmotic colloid pressures are forces that influence the transmural pressure, which can influence the pacemaking activity within the lymphangions, resulting in a change in lymph flow (12). A rise in transmural pressure causes distension of the lymphatic wall, this may increase the number and the force of lymphatic muscular contractions, which in turn enhances flow (12, 47). A rise in lymph flow can provoke the pacemaking potential of the smooth muscle cells by increasing their spontaneous transient depolarization, while a reduction in lymph flow decreases the activity of the spontaneous transient depolariztions (47). These depolariztions can occur in the absence of innervation or endothelieum, thus provide possible evidence of a myogenic effect (47). Although contrary to this lymphatic myogenic hypothesis, too much luminal pressure can diminish the lymphatic pumping action and when there is no distension due to a lack of transmural pressure, the

lymphatics still contract (47). This suggests that distension of the lymphatic wall is influential, not mandatory, in altering the pacemaker activity in the lymphatics (12).

Research has demonstrated that increases in flow cause a decrease in contractile frequency and amplitude (14). This may be due to an overriding regulatory factor in the lymphatic system, and at high levels of lymph formation, the passive lymph pump becomes a greater force than the active lymph pump. The flow inhibition response may occur to save metabolic energy by decreasing the contractions and outflow resistance because other forces are providing a strong enough drive to propel the lymph (14).

Respiratory movements, muscle contractions and arterial pulsations are considered secondary mechanisms that influence lymphatic dynamics over the intrinsic contractions of the lymphatics (33). However, there is a building opinion that the muscle pump makes a strong contribution to the increase in lymphatic flow (16, 23). The lymphatics transport fluid centrally against a hydrostatic pressure gradient and against a protein concentration gradient (47); hence, mechanisms which aid in altering pressure gradients, can help improve lymph flow. When the muscle is stretched, the cross-sectional area of the muscle fibers decreases and radially pulls on the adjoining connective tissue, this in turn pulls open the initial lymphatics (24). This opening lowers the pressure within the lymphatic vessel, allowing it to fill with interstitial fluid. When the muscle shortens to its minimal length, the contractile tissues compress the surrounding lymphatic vessels to propel the lymph forward (16). The contraction and elongation of the muscles drive the fluid along the lymphatic network, demonstrating a key relationship between exercising skeletal muscle and lymphatic flow.

Lymphatic vessels are adjacent to the arterioles in skeletal muscle, potentially making arteriolar pulsations and vasomotion mechanisms for lymph formation (24). Arteriolar constriction and dilation may provoke the movement of interstitial fluid toward the lymphatic capillaries, while the changing diameter may be responsible for the opening and closing of lymphatics, causing lymph formation and flow (24).

The contractions of the lymphatic system have been compared to the muscular contractions of the heart over those in the vascular system (5). Perhaps, with the development similarities between cardiomyocytes and lymphatic muscle cells (5), there could be similar beneficial exercise effects on lymphangions, as there are on cardiomyocytes (11, 48).

Exercise and lymphatic function:

Various studies have used exercise stress to investigate the functional components of the lymphatic system. In sheep, a rise in cardiac output with short term exercise increased lymph flow due to the recruitment of previously unperfused microvascular beds (30, 31). Exercise time in these studies averaged approximately 40 minutes, thus lymphatics may not have reached a steady state condition (30, 31). This led to another study by Coates et al., (8) which looked at a longer bout of exercise and it's effect on lung and hindlimb lymph flow. Twelve sheep were trained on a treadmill for 2-4 weeks prior to onset of testing. As Havas showed in humans (15), the sheep had a 5 fold increase in lymph flow at the onset of exercise, but then with continued exercise, it tapered off to 130% above baseline values. The authors believe the marked increase resulted from the rise in tissue pressure, and the gradual reduction (but still above baseline values) may be contributed to the reduced volume in the compartment drained by the lymph. With exercise, it was noted that the combination of both increased vascular surface area and hydrostatic pressure aided in the steady state increase in lymph flow. The exercise induced a drop in systemic vascular resistance secondary to the recruitment and dilation of the microvascular beds in the exercising muscles, therefore increasing microvascular surface area for fluid exchange (8).

In male humans, lymphatic clearance rate increased while walking (34), while performing specified muscular contractions (16) and during prolonged running (15). Based on the concept of increased arterial pulsations and muscle fiber deformations, Havas et al., (16) looked at the removal rate of injected albumin from the gastrocnemius and tibialis anterior muscles. Compared to resting values, greater clearance rates were observed during isometric flexion, isometric extension and concentric contractions. The

greatest clearance rates were seen in isometric extension and concentric contractions, proposing that the most efficient phase in lymph propulsion occurs when the muscle shortens to its minimal length. This study also looked at the difference between resting clearance rates in trained and untrained runners. The trained runners had greater resting clearance rates due to potential adaptations of exercise induced increases in vascularity in skeletal muscle and perhaps enhanced adaptations within the lymphatic system (16).

Trained runners, undergoing prolonged running, had similar effects (15) to those found in sheep (8). There was a 5 fold increase in lymphatic clearance rate at the onset of exercise, and then it dropped to twice the rate of resting levels (15). This rapid increase was proposed to be associated with an overshoot in capillary filtration due to an increase in blood delivery and a rise in intramuscular osmotic pressure, secondary to muscle contractions. This effect may be due to the inability of the lymphatic system to match fluid accumulation demand, however, other studies have shown that the lymphatic system responds rapidly to the exercise stress and limits the accumulation of interstitial fluid in the muscles (16). This study demonstrates the capability of the lymphatic system to handle the stress of prolonged exercise, however the muscle contractions, as well as the arteriole pulsations, remained constant throughout the exercise, nonetheless the estimated lymphatic flow did not.

Lymphatic clearance rates during isometric handgrip exercise have been compared with dynamic arm cranking exercise in healthy females (23). Arm cranking produced higher clearance rates (-28% per min), while handgrip exercise produced comparable results to the resting control clearance rates (-18% per min, -14% per min respectively). The dynamic exercise was thought to increase the lymphatic clearance rates through multiple changes in lymphatic transport mechanisms. Exercise increased the subjects cardiac output, inducing vasodilation in the working muscles which augmented muscle blood flow. This rise in muscle blood flow promoted capillary filtration, which increased interstitial pressure, leading to greater lymph formation and lymph flow (23).

Central and peripheral changes with chronic exercise include increased capillary density, increased sympathetic tone, and increased muscular adaptations, which are mechanisms linked to improving lymphatic function (46). Sympathetic nervous system activity increases with dynamic exercise which may enhance the frequency and force of smooth muscle contraction in larger contractile lymph vessels promoting lymph propulsion (23, 27). McHale and Adair suggest that the reflex activation of the sympathetic nervous system could promote lymph movement due to a rise in circulating catecholamines (26), while Valic and colleagues have demonstrated that vasodilation plays a greater a role in lymph propulsion than previously thought (45). If upper body trained individuals have greater upper body sympathetic tone, increased capillary beds within the trained muscles and increased metabolic activities (46), the rise in vasodilation and vascular conductance may increase lymph flow in these individuals.

Imaging Techniques

Lymphoscintigraphy is a highly reproducible method used to quantify lymphatic function (44). An injected amount of a radiopharmaceutical is monitored through a gamma camera, and quantitative and qualitative data can be observed. The radiation dose received is low and is less invasive compared to the past technique of lymphography which involved the cannulation of lymphatic vessels (44). Unlike the old method, lymphoscintigraphy cannot directly measure lymph flow, because the total volume of lymph remains uncertain, but one can infer lymph flow by the clearance rate of the radioisotope from the injection site (41). There is no standardized procedure for lymphoscintigraphy, and variations include the selected radiotracer, the site of injection (32), whether an exercise stress is used and different acquisition times (43, 44).

Because there is the possibility of a disturbance in homeostasis with the injection, due to increases in blood flow and swelling (42), there has been questionable results in the first 20 minutes after the injection (16). To encourage the disappearance of impurities and provoke uptake into the lymphatic capillaries after injection, patients are encouraged to massage the site of injection (43) or in one study, subjects walked for 10 min, then rested for 15 minutes before imaging began (15).

Sites for injection vary from intradermal, intermuscular, subdermal to subcutaneous locations. Intradermal injections produce rapid visualization of the dermal lymphatics, where subdermal and subcutaneous injections seem to have a slower uptake into the lymphatics (18). The dermis has a higher concentration of lymphatics, which provides greater surface area for lymphatic uptake (32). One study looked at various radiotracers and their site of injection, and showed that intradermal injections produced the clearest images of lymphatic vessels under 30 minutes, whereas subcutaneous injections took longer for a quality image of the lymphatics to be produced (32). In general, both limbs are injected, with two injections per limb (43). Injections vary between the webspaces of the hands, to the dorsum of the foot, to areas around the styloid process or at the malleolous in the lower extremity (18) .

Technetium-99m sulphur colloid is the most commonly used radiotracer because it is inexpensive with a high safety profile. Imaging is always performed on a high-resolution collimater (44) and should be recorded with a 20% window centered on the 140-keV photopeak of ^{99m}-Tc using a scan speed of 10cm/min (43).

Size is an important factor when determining uptake and retention in the lymphatics. Molecules smaller than 10nm are preferentially absorbed into the circulatory system, whereas molecules between 10nm and 100nm are ideal for lymphatic uptake (42). The larger the size, the greater preference to lymphatic uptake, yet the rate of uptake is slower (42).

Breast Cancer Related Lymphedema (BCRL):

The etiology and pathophysiology of breast cancer related lymphedema is multi-factorial and requires greater understanding (37). Breast cancer treatment frequently involves surgical mastectomy, chemotherapy and radiation in order to increase survival rates following diagnosis. However, these treatments have strong associations with the development of lymphedema of the upper extremity (40). Other risk factors are pathological nodal status, obesity and tumor stage (39). There is a lack of evidence-based

medical information on the management of lymphedema; furthermore, there is no standardized test to evaluate the functional aspect of the lymphatic system in women with BCRL (20). Understanding the mechanisms that influence lymph flow in the upper extremities may help to prevent or alleviate post-mastectomy lymphedema in breast cancer survivors. With the combined technique of lymphoscintigraphy and potential lymphatic training adaptations (as previously discussed), the concept of rerouting mechanisms and increased efficiency in lymph transport may help women suffering from BCRL.

APPENDIX 2- RAW DATA

Table 5. Individual descriptive data

	Age years	Ht cm	Wt kg	BMI $kg \cdot m^{-2}$	VO2 $ml \cdot kg \cdot min^{-1}$	VO2 $L \cdot min^{-1}$	Dominant arm	Elbow nodes
Trained								
CC	33	156.5	59.8	24.4	30.3	1.81	R	N
AS	29	180.8	75.8	23.2	36.5	2.77	R	R
WW	35	150.7	46.8	20.6	34.6	1.62	R	N
KI	28	151.7	49.7	21.6	35.8	1.78	R	B
SHu	26	176.0	85.0	27.4	32.0	2.72	R	R
Shi	22	161.4	65.6	25.2	33.8	2.22	R	L
LG	24	155.3	60.8	25.2	40.7	2.48	R	B
JD	26	174.4	64.9	21.3	39.3	2.55	R	N
AW	23	175.0	62.0	20.2	33.8	2.09	R	R
KP	23	167.8	50.6	18.0	33.6	1.70	R	N
Untrained								
JM	26	165.3	63.6	23.3	22.9	1.46	R	R
CM	25	160.9	55.6	21.5	28.1	1.56	R	L
AL	32	164.0	50.0	18.6	25.5	1.28	R	L
SHa	38	159.0	85.3	33.7	16.7	1.42	R	B
LH	25	164.8	52.2	19.2	29.8	1.55	R	B
MM	33	177.0	61.6	19.7	23.7	1.46	R	B
RL	35	173.0	91.6	30.6	17.5	1.60	R	B
JH	22	178.2	76.8	24.2	19.6	1.50	R	N
MMc	35	171.3	79.5	27.1	22.5	1.79	L	B
SB	39	167.7	79.0	28.1	15.4	1.22	R	L

R= Right Arm, L= Left arm, B = Both arms, N= None.

Table 6. Individual Data for Power Output, Heart Rate and RPE

	Time M1	PO M1	HR M1	Time M2	PO M2	HR M2	PO SM	HR SM	HR Rest	RPE M1	RPE M2
Trained											
CC	11.20	110	189	10.00	100	187	36	123	62	10	10
AS	17.20	170	185	16.00	160	181	45	95	44	10	10
WW	11.25	110	184	12.00	110	184	28	120	58	10	10
KI	11.25	110	177	10.00	100	180	30	93	60	10	10
SHu	17.20	170	202	15.00	150	192	51	124	52	9.5	10
Shi	13.20	130	181	11.00	110	173	39	100	66	10	10
LG	14.00	140	186	11.00	110	165	36	91	60	10	10
JD	14.00	140	175	14.00	140	174	39	89	52	10	10
AW	10.75	110	190	11.00	110	192	37	104	62	10	10
KP	10.00	100	171	9.00	100	176	30	96.4	60	10	10
Untrained											
JM	8.00	80	174	8.00	80	172	38	130	78	10	10
CM	8.25	80	178	8.00	80	178	33	115	88	10	10
AL	7.00	70	167	6.00	60	161	30	118	77	9	10
SHa	7.20	70	167	7.00	70	162	50	143	69	10	10
LH	9.00	90	181	8.00	90	179	31	111	75	10	10
MM	7.20	80	178	7.00	80	182	37	132	67	10	10
RL	8.00	90	188	8.00	80	188	50	119	84	10	10
JH	7.50	80	173	8.00	80	177	45	134	64	10	10
MMc	9.75	100	178	8.00	80	184	45	119	78	10	10
SB	7.00	80	150	7.00	90	151	44	111	78	10	10

Time = min, PO = wattage, HR = beats·min^{-1}. M1 = maximal test 1; M2 = maximal lymphatic stress test; SM = submaximal intermittent protocol at 0.6W·kg^{-1}; RPE = Rating of perceived exertion at end of maximal test (0 = no effort to 10 = maximal effort).

Table 7: Individual data for axillary uptake

		Maximal				Submax				Rest			
	time	R-18	L-18	R-64	L-64	R-18	L-18	R-64	L-64	R-18	L-18	R-64	L-64
Trained													
AS	22	0.48	0.97	1.23	1.78	0.62	0.34	1.74	1.44	0.09	0.1	0.20	0.35
CC	17	0.62	0.10	2.13	0.86	2.90	0.22	4.52	1.42	0.04	0.04	0.89	0.26
SHU	25	0.70	0.23	1.39	0.57	0.53	0.93	0.94	2.76	0.12	0.08	1.00	0.55
WW	23	0.37	0.10	1.17	0.55	0.14	0.11	1.09	1.72	0.02	0.01	0.12	0.21
SHI	25	0.61	0.19	1.41	0.83	0.45	0.45	1.67	1.55	0.09	0.04	0.82	0.68
KI	25	0.83	0.18	1.63	0.47	0.38	0.05	0.71	0.54	0.02	0.02	0.29	0.05
LG	19	0.68	0.24	1.82	1.11	0.59	0.20	1.96	1.66	0.04	0.04	0.27	0.16
JD	25	1.14	1.19	1.81	2.16	0.10	0.14	1.01	0.48	0.03	0.01	0.02	0.07
AW	15	1.23	0.99	3.10	3.89	0.30	0.93	1.62	4.29	0.02	0.01	1.62	4.29
KP	15	0.34	0.12	1.87	1.43	0.20	0.01	2.98	1.3	0.02	0.08	0.28	2.00
Untrained													
AL	13	0.13	0.02	2.81	1.45	0.09	0.28	4.45	3.4	0.03	0.03	2.21	0.52
JM	14	0.11	0.07	2.92	1.59	0.13	0.12	2.40	2.1	0.02	0.04	0.17	0.16
CM	17	0.09	0.08	0.81	0.91	0.58	0.10	3.01	1.4	0.03	0.10	0.13	1.27
SH	21	0.47	0.15	1.29	0.43	0.32	0.03	1.39	0.5	0.00	0.00	0.16	0.13
MM	13	0.03	0.08	0.12	1.01	0.03	0.03	0.17	1.0	0.02	0.02	0.05	0.42
LH	14	0.05	0.04	0.63	0.51	0.03	0.03	0.62	0.6	0.01	0.03	0.01	0.02
RL	15	0.81	0.5	1.23	1.13	0.48	0.11	1.52	0.7	0.03	0.03	1.37	0.69
MMc	14	0.31	0.01	0.74	0.34	0.29	0.02	0.58	0.7	0.29	0.02	0.58	0.65
SB	13	0.18	0.04	1.82	1.43	0.35	0.07	1.66	2.3	0.02	0.02	0.11	0.08
JH	14	0.93	0.08	3.41	1.6	0.28	0.14	2.00	1.8	0.27	0.02	1.72	0.97

Ax, in % relative to initial amount injected at time zero, acquired at 18 (SD 5) and 64 minutes during maximal, submaximal and resting lymphatic stress tests for the right (R), and left (L) sides.

Table 8. Individual clearance rate equations

		Maximal CR		Intermittent Submaximal CR		Resting CR	
Trained							
KP	y =	-3.326 Ln(x) + 100.78	R^2 = .90	-0.243x + 100.43	R^2 = .98	-0.095x + 98.799	R^2 = .77
SHi	y =	-2.328 Ln(x) + 100.01	R^2 = .96	-0.186x + 100.55	R^2 = .98	-0.082x +100.92	R^2 =. 86
CC	y =	-2.224 Ln(x) + 99.97	R^2 = .96	-0.210x + 97.05	R^2 = .86	-0.020x + 100.51	R^2 = .36
SHu	y =	-2.727 Ln(x) + 100.47	R^2 = .92	-0.274x + 98.83	R^2 = .96	-0.105x + 100.4	R^2 = .92
LG	y =	-2.104 Ln(x) + 100.17	R^2 = .99	-0.225x + 99.52	R^2 = .98	-0.020x + 99.78	R^2 = .20
JD	y =	-2.885 Ln(x) + 100.41	R^2 = .97	-0.104x + 99.85	R^2 = .97	-0.064x + 100.48	R^2 = .87
WW	y =	-1.859 Ln (x) + 100.00	R^2 = .91	-0.161x + 101.03	R^2 = .94	-0.043x +100.48	R^2 = .63
AW	y =	-4.394 Ln (x) + 100.48	R^2 = .98	-0.281x + 98.53	R^2 = .95	-0.040x + 101.42	R^2 = .30
KI	y =	-3.451 Ln (x) + 99.73	R^2 = .98	-0.203x + 96.50	R^2 = .77	-0.060x + 99.83	R^2 = .75
AS	y =	-3.332 Ln (x) + 99.83	R^2 = .98	-0.195x + 101.00	R^2 = .94	-0.037x + 100.27	R^2 = .82
Untrained							
RL	y =	-3.689 Ln (x) + 99.63	R^2 = .97	-0.268x + 98.10	R^2 = .87	-0.183x + 101.49	R^2 = .85
JM	y =	-2.757 Ln (x) + 100.85	R^2 = .93	-0.250x + 100.49	R^2 = .98	-0.010x + 99.98	R^2 = .23
CM	y =	-1.687 Ln (x) + 100.22	R^2 = .82	-0.267x + 99.57	R^2 = .96	-0.042x + 99.55	R^2 = .55
AL	y =	-2.719 Ln (x) + 100.12	R^2 = .95	-0.283x + 98.72	R^2 = .94	-0.125x + 100.74	R^2 = .96
SHa	y =	-2.488 Ln (x) + 99.64	R^2 = .91	-0.229x + 97.21	R^2 = .84	-0.046x + 101.25	R^2 = .46
MM	y =	-2.330 Ln (x) + 100.54	R^2 = .96	-0.167x + 99.11	R^2 = .93	-0.001x + 98.79	R^2 = .01
MMc	y =	-2.943 Ln (x) + 100.41	R^2 = .88	-0.194x + 97.03	R^2 = .84	-0.036x + 98.86	R^2 = .48
LH	y =	-1.450 Ln (x) + 99.64	R^2 = .88	-0.146x + 100.31	R^2 = .98	0.041x + 99.74	R^2 = .39
SB	y =	-3.443 Ln (x) + 100.94	R^2 = .92	-0.315x + 95.79	R^2 = .86	-0.023x + 99.307	R^2 = .38
JH	y =	-3.490 Ln (x) + 100.48	R^2 = 97	-0.268x + 99.44	R^2 = .98	-0.150x + 98.855	R^2 = .87

Equations and R^2 values for maximal, intermittent and resting clearance rates. y = the amount cleared (%) for x time (min).

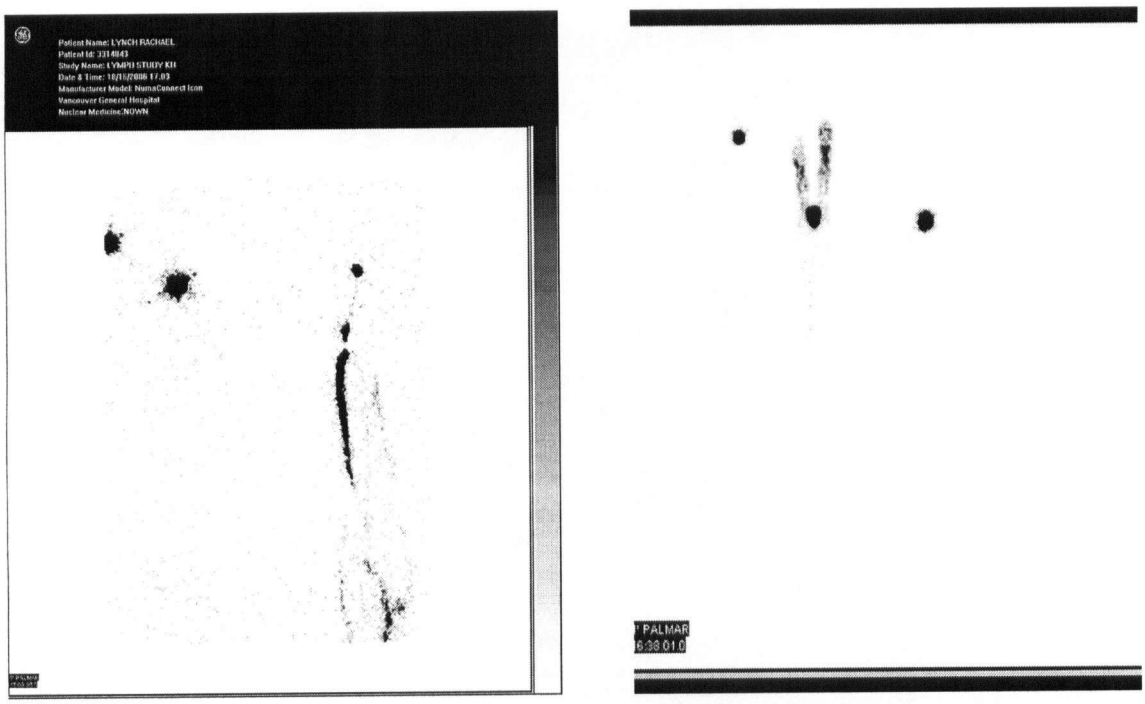

Figure 7. Two scintigraphs at initial injection. These scintigraphs visually demonstrate what occurs if the ^{99m}Tc is injected into the circulatory system, preventing any further testing.

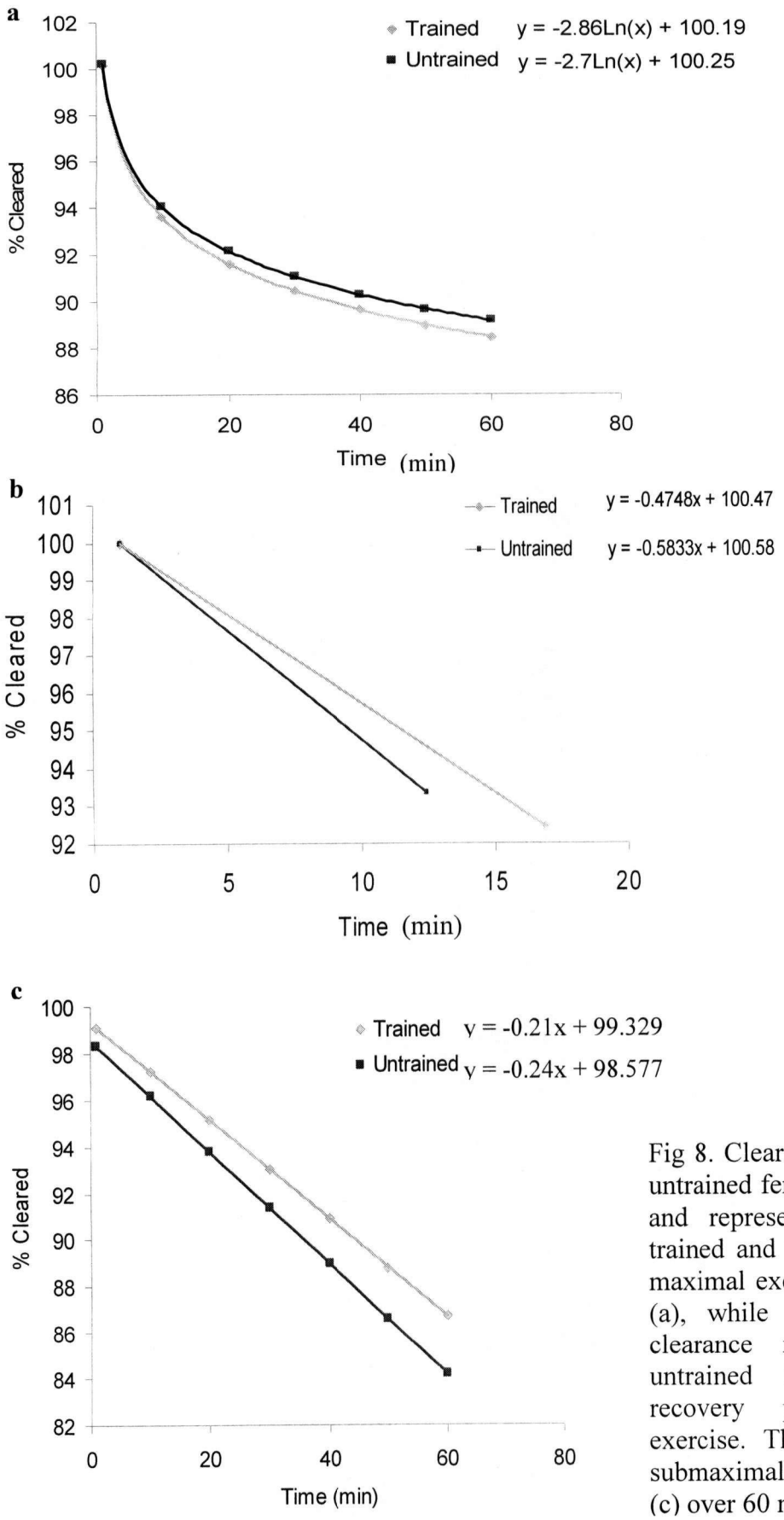

Fig 8. Clearance rates for trained and untrained females. Data was averaged and represents clearance rates for trained and untrained females during maximal exercise over 60 minutes in (a), while (b) represents maximal clearance rates for trained and untrained females excluding the recovery period post maximal exercise. The clearance rate during submaximal exercise is represented in (c) over 60 minutes.

Table 9. Amount cleared immediately post-maximal exercise.

Trained	Time	AC	Untrained	Time	AC
AS	19	10.85	JM	11	4.47
CC	13	5.41	CM	11	4.41
SHu	20	6.72	AL	10	6.05
WW	19	5.47	SH	10	7.90
Shi	13	6.88	MM	10	3.68
KI	15	10.2	LH	12	4.62
LG	16	5.13	JH	16	8.92
JD	19	7.02	RL	11	10.79
AW	16	10.46	SB	16	7.26
KP	19	7.36	MMc	17	8.40

Amount Cleared (AC), as a % relative to the initial injection, of ^{99m}Tc from the web spaces of the hand. Time (min) represents the time of the hand acquisition immediately post-maximal exercise.

APPENDIX 3 – CREB's CERTIFICATE OF APPROVAL