Microvascular filtration is increased in the forearms of patients with breast cancer–related lymphedema

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1Department of Clinical Physiology and Nuclear Medicine, Bispebjerg Hospital, University Hospital of Copenhagen, Copenhagen, Denmark; 2Department of Dermatology and Venereology, Bispebjerg Hospital, University Hospital of Copenhagen, Copenhagen, Denmark; and 3Department of Biomedical Sciences, The Panum Institute, University of Copenhagen, Copenhagen, Denmark

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Jensen MR, Simonsen L, Karlsmark T, Bülow J. Microvascular filtration is increased in the forearms of patients with breast cancer–related lymphedema. J Appl Physiol 114: 19–27, 2013. First published November 1, 2012; doi:10.1152/japplphysiol.01116.2012.—Breast cancer–related lymphedema (BCRL) is a frequent and debilitating complication of breast cancer treatment. The pathophysiology is complex and remains poorly understood; however, data suggest that changes in the peripheral circulation may contribute to edema formation. In 13 volunteers with unilateral BCRL, the following aspects of upper extremity peripheral circulation were examined: muscle relative microvascular volume; capillary filtration coefficient; central and local sympathetic vascular reflexes; skin blood flow; and forearm blood flow. These were studied via real-time, contrast-enhanced ultrasound; venous occlusion strain-gauge plethysmography; lower-body negative pressure; noninvasive blood pressure measurements; and skin 99mTc-pertechnetate clearance technique. Measurements were performed bilaterally and simultaneously in the forearms, enabling use of the nonedematous forearm as a control. Capillary filtration coefficients were additionally measured in healthy, age-matched controls. The capillary filtration coefficient was 7.98 ± 2.52 l·100 ml−1·mmHg−1·min−1 (mean ± SD) in edematous forearms and 6.09 ± 1.83 l·100 ml−1·mmHg−1·min−1 in nonedematous forearms in the patient group (P < 0.001). The capillary filtration coefficient was 3.32 ± 1.17 l·100 ml−1·mmHg−1·min−1 in the forearms of healthy controls; significantly less than the both the edematous and nonedematous forearms of the patient group (P < 0.001). No significant differences were found in muscle relative microvascular volume, forearm blood flow, skin blood flow, or central or local sympathetic vascular reflexes. Forearm microvascular filtration is increased in patients with BCRL, and more so in the edematous arm. The vascular sympathetic control mechanisms seem to be preserved. We propose that the increased capillary permeability may be due to low-grade inflammation promoted by reduced clearance of inflammatory mediators.

capillary filtration coefficient; sympathetic vascular reflexes; forearm blood flow; contrast-enhanced ultrasound

BREAST CANCER IS THE MOST COMMON cancer in women worldwide. Advances in diagnosis and treatment have in many developed countries resulted in a 5-yr survival rate close to or above 80% (9, 29). In relation to this, breast cancer–related lymphedema (BCRL), a chronic swelling of the ipsilateral upper extremity, is a frequent complication of breast cancer treatment that has a negative impact on quality of life and upper extremity function (1, 5, 16). Sixty-five percent of patients treated with a combination of axillary lymph node dissection, radiation therapy to the anterior thoracic wall, and chemotherapy subsequently report symptoms of BCRL (16). The pathophysiology of BCRL remains elusive even though other risk factors such as postsurgical seroma, younger age, and obesity have been identified (14, 16, 20).

The classic pathophysiological explanation for the development of BCRL is that an edema high in protein content develops secondary to decreased lymph drainage capacity caused by treatment-induced damages to the lymphatic system (11). However, this view fails to explain key puzzles in the clinical characteristics of BCRL such as 1) a substantial delay of months to years often exists from the initial breast cancer treatment to the development of BCRL symptoms; and 2) many patients do not develop BCRL despite axillary lymph node dissection and subsequent radiotherapy to the axilla, although others treated with less-invasive sentinel node biopsy do (38).

Edema formation is a result of an imbalance between the lymphatic drainage rate and the capillary filtration rate (2). In a prospective study using quantitative lymphoscintigraphy, Stanton et al. (39) found increased lymphatic drainage rates in the arms of patients who subsequently developed BCRL, and muscle lymphatic drainage rates remained high after edema development. Increased lymphatic drainage rates in arms with edema imply increased capillary filtration rates. Such an increase can be caused by several factors in the microcirculation: 1) increased capillary hydrostatic pressure due to decreased arteriolar tone; 2) increased capillary surface area; 3) increased capillary permeability; or 4) increased interstitial osmotic pressure due to increased interstitial protein concentration.

The aim of the present study was to elucidate the possible roles of some of these factors in the pathophysiology of BCRL using an integrative approach by measuring forearm blood flow rate (FBF), local skin blood flow rate, local and central sympathetic vascular reflexes, relative microvascular volumes, and the capillary filtration coefficient (CFC) in patients with unilateral BCRL.

METHODS

Ethical approval. The study conformed to the Declaration of Helsinki and was approved by the Regional Research Ethics Committees for Capitoli Region of Denmark (H-4-2011-005). Written, informed consent was obtained from all study participants.

Study populations. Thirteen otherwise healthy women with previously diagnosed unilateral BCRL participated in the study. BCRL was defined as a present or historic excess arm volume of ≥200 ml (compared with the nonedematous arm) that developed following treatment for unilateral, invasive breast carcinoma. All patients had stable edema. Patient history and treatment data are listed in Table 1.

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Table 1. Patient history and treatment data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Axillary lymph node dissection</td>
<td>100%</td>
</tr>
<tr>
<td>Adjuvant radiation therapy</td>
<td>85%</td>
</tr>
<tr>
<td>Adjuvant chemo therapy</td>
<td>77%</td>
</tr>
<tr>
<td>Endocrine therapy (anti-oestrogen)</td>
<td>85%</td>
</tr>
<tr>
<td>Compression sleeve</td>
<td>85%</td>
</tr>
<tr>
<td>Time to BCRL-debut (months)</td>
<td>8.7 ± 5.3</td>
</tr>
<tr>
<td>Duration of BCRL (months)</td>
<td>51.2 ± 41.9</td>
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Patients were not eligible for participation if one or more of the following factors were present: 1) active cancer; 2) ongoing treatment with radiation or chemotherapy; 3) other causes of edema (e.g., cardiac insufficiency, nephropathy, hypoalbuminemia, venous thrombosis, or local inflammation/erysipelas); 4) a history of apoplexia cerebri or stenosis of a. carotis communis; 5) treatment with β-blockers, calcium blockers, or diuretics; 6) contraindications to the use of the ultrasound contrast medium (SonoVue, Bracco, Milano, Italy), including ischemic heart disease, pulmonary hypertension, right-left shunt, severe arterial hypertension, unstable neurological disease, or severe pulmonary disease.

Because lymphatic drainage rates are increased in patients who have been treated for breast cancer and who later develop BCRL (39), CFC was additionally measured in an age-matched control group consisting of 14 healthy women. Anthropometric data for the two study groups are listed in Table 2.

Study settings. To achieve a steady extremity volume, compression-sleeve treatment was paused at least 12 h before measurements. Measurements were performed in a quiet examination room at constant room temperature (23–24°C) between 8:00 and 11:00 a.m. A flow chart of the study protocol with duration and temporal relation between measurements and supine resting periods is shown in Fig. 1. Due to well-known intravascular individual variations in blood pressure, sympathetic activation, and skin temperature, measurements of sympathetic vascular reflexes, relative microvascular volume, local skin blood flow, and FBF in the patient group were performed bilaterally and simultaneously, thus enabling use of the nonedematous arm as a control. To our knowledge, none of these factors are affected in the nonedematous arm of patients with BCRL. Noninvasive blood pressure measurements were performed unilaterally because of equipment limitations.

Objective measures of BCRL. To objectively quantify the edema characteristics of patients, three measures of edema were recorded. Upper extremity volumes were measured with opto-electric perometry (Perometer 1000M, Pero-System Messgeräte, Wuppertal, Germany) as described by others (12). Briefly, the measurements were performed with the upper extremity abducted to 90° and the tip of the index finger resting on a hand support. The extremity was measured from fingertips to the axilla. Subsequently, the volume of a standardized arm section (between 5.3 and 65.9 cm measured from the tip of the index finger) was calculated off-line using PeroPlus software. Additionally, forearm volumes were measured from the ulnar styloid process to the olecranon process. Excess volume is expressed as the ratio between the edematous and nonedematous arm volumes, in percent.

Tissue edema was quantified noninvasively by measuring the tissue dielectric constant using the MoistureMeterD Compact (Delfin Technologies, Kuopio, Finland). The tissue dielectric constant is a relative and dimensionless physical entity that is directly proportional to bulk tissue water ranging from 1 in a vacuum to 78.5 in pure water (31). The tissue dielectric constant was measured at a fixed, effective frequency of 9–3 MHz linear-array transducer placed perpendicular to the skin surface on the same sites as described above.

Real-time, contrast-enhanced ultrasound. Real-time, contrast-enhanced ultrasound enables noninvasive, real-time imaging of the microcirculation and measurements of relative microvascular blood volumes (42). Relative microvascular blood volume in a forearm extensor muscle was measured to estimate whether the microvascular surface area available for filtration is increased in the edematous arm compared with the nonedematous arm in patients with BCRL.

The ultrasound contrast agent consists of phospholipid-stabilized hexafluoride microbubbles. After intravenous bolus injection the microbubbles are mixed with the blood and confined to the circulation due to their size (mean 2.5 μm). Whenever exposed to a spectrum of ultrasound waves emitted from an ultrasound transducer, the microbubbles are excited into resonant, nonlinear oscillations that scatter the incident pulse. The scattered signal contains higher harmonics that are contrast agent-specific. These are received and processed by the ultrasound scanner, which enables imaging of the microcirculation. The signal intensity in a region of interest (ROI) is recorded following a bolus injection of the microbubbles resulting in a time-signal intensity curve. A representative example from the edematous forearm of a study patient appears in Fig. 2. Before bolus arrival the contrast signal intensity is constant, representing the background signal from the tissue in the ROI (A). When the bolus arrives in the microcirculation a sharp increase in signal intensity is followed by a plateau phase (B) in which the inflow and outflow of microbubbles in microcirculation is equal. In this steady state, the difference (C) between the signal intensity of the plateau phase and the signal intensity at baseline is a relative measure of the microvascular blood volume within the ROI because the increase in signal intensity stems solely from the microbubbles, and because the ROI was carefully placed to avoid large blood vessels (30).

The advantage of this method is that it enables noninvasive measurement of relative microvascular volumes in deep tissues with short acquisition times. However, there are some limitations to the method: 1) it does not enable absolute quantification of microvascular blood volume; 2) baseline signal intensity depends on the measured tissue; therefore, skin that has a high contrast signal cannot be measured concomitantly with deep tissues [we chose to measure muscle because muscle lymphatic drainage rates have been shown to be increased in BCRL (39)]; and 3) the signal intensity of the plateau phase is directly proportional to the number of microbubbles in the bolus (30). Even though contrast bolus volume is carefully administered, the concentration of microbubbles may vary considerably (34). This may cause large variations in the signal intensity of the plateau phase; however, in the present study, this problem was avoided because measurements on the forearms were made simultaneously on the same bolus.

Subjects were placed comfortably in the supine position with the arms extended along the sides of the body, and the cubital vein of the nonedematous arm was catheterized (18 G Venflon, Becton Dickinson Infusion Therapy, Helsingborg, Sweden). Two identical ultrasound scanners were used (Philips IU22, Philips Healthcare). Linear array transducers (9–3 MHz) were fixed vertically and longitudinally in custom-made holders over the area of the proximal forearm with maximal objective signs of edema and over the corresponding area of the control forearm. To avoid tissue compression, a thick layer of ultrasound gel was applied between the skin and the transducer.

Table 2. Anthropometric data

| Measurement | Patients (n = 13) | Controls (n = 14) | P  
<table>
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<tr>
<td>Age (yr)</td>
<td>54.1 ± 7.3</td>
<td>53.0 ± 7.6</td>
<td>0.705</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 ± 0.03</td>
<td>1.68 ± 0.07</td>
<td>0.351</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.9 ± 10.8</td>
<td>66.0 ± 9.2</td>
<td>0.138</td>
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</table>
Measuring depth was set to 3 cm and focus was adjusted to the entire depth. The gain was set to 97%. The mechanical index of the incident pulse was 0.06. Contrast harmonic signals were received at 8 MHz. A 4-ml bolus of SonoVue was injected intravenously and immediately followed by a flush of 10 ml 0.9% saline solution. Twenty-millisecond, simultaneous B-mode, and contrast images were captured consecutively for 2 min.

Data analysis was performed off-line using ultrasound quantification software (QLAB, version 8.2, Koninklijke Philips Electronics). The ROI was drawn in a forearm muscle avoiding large blood vessels, and the signal intensities at baseline and plateau were determined from the signal intensity curve generated by the software.

Examinations of two subjects had to be excluded from the data analysis due to air and motion artifacts.

Venous occlusion strain-gauge plethysmography. FBF and CFC were determined by venous occlusion strain-gauge plethysmography (19) using the programmable AI6 arterial inflow system (D.E. Hokanson, Bellevue, WA). Venous occlusion strain-gauge plethysmography is a noninvasive and well established method of measuring both FBF (43) and forearm capillary filtration rates (15). FBF was measured for two reasons: 1) to determine whether total resting FBF is increased in the edematous arm compared with the nonedematous arm in BCRL as would be expected if a decrease in vascular resistance had been induced by damages to the sympathetic innervation; and 2) to calculate changes in forearm vascular resistance induced by lower-body negative pressure (see below). CFC was measured to determine whether total capillary filtration is increased in the edematous arm compared with the nonedematous arm in patients with BCRL.

For both FBF and CFC measurements, subjects were placed comfortably in the supine position with the arms resting at heart level on padded supports. A pressure cuff was placed around the upper arm. A silastic mercury strain gauge was placed around the largest circumference of the forearm measured bilaterally. The length of the strain gauge was chosen to ensure skin contact with minimal tension to minimize pitting of the strain gauge during prolonged venous congestion.

FOREARM BLOOD FLOW RATE. The combination of rapid venous occlusion and unimpeded arterial inflow results in an increase in forearm volume. In the initial linear phase the volume increase rate is directly proportional to total forearm blood flow (18). Venous occlusion was induced and held for 7 s by rapid inflation of the pressure cuff to 45 mmHg. Relative forearm volume changes were measured by the strain gauge. A 30-s interval between each measurement allowed for venous emptying. FBF was determined from an average of five consecutive measurements. To avoid the influence of blood
shunting through finger arterio-venous anastomoses a wrist-cuff was inflated to and held 50 mmHg above the systolic blood pressure for 10 s before and during FBF measurements. FBF was calculated from the initial linear slope of the time-volume change (%) curve using the built-in software. Manual curve fitting was performed by eye to avoid the effect of cuff artifacts.

**CAPILLARY FILTRATION COEFFICIENT.** CFC is a measure of capillary permeability to water (32). Measurements of total forearm CFC may be increased by a) increased capillary permeability, or b) increased capillary surface area available for filtration, or both of these. Initially, an increase in venous pressure results in a fast, nonlinear increase in forearm volume due to venous distension with displacement of surrounding soft tissues. The duration of the venous distension phase is positively correlated with the venous pressure increment. When venous pressure surpasses cuff pressure, venous flow is re-established. The increased venous pressure results in increased capillary hydrostatic pressure, which augments capillary filtration rates. The result is a slow, linear increase in forearm volume due to an increase in interstitial fluid volume (edema formation) (15). Forearm CFC was measured using a custom, three-step, 10-min venous congestion protocol as described by others (22). First, the cuff was rapidly inflated to and held at 30 mmHg; at 4 min the pressure was increased to 50 mmHg; and finally, at 7 min, the pressure was increased to 70 mmHg for 3 min. The forearm volume changes were recorded continuously by the strain gauge. The capillary filtration rate ($\mu$100 ml$^{-1}$·min$^{-1}$) was measured as the slope of the time-volume change (%) curve at steady state at the end of each pressure phase. Measured sections with a minimum length of 30 s were fitted by eye. CFC ($\mu$100 ml$^{-1}$·mmHg$^{-1}$·min$^{-1}$) was calculated by linear regression of the determined capillary filtration rates for each pressure step. Several sources of error exist when CFC is measured by venous congestion strain-gauge plethysmography. Strain-gauge volume measurements are sensitive to changes in forearm volume caused by finger movement and deep respiration. Furthermore, prolonged measurements result in edema with pitting of the strain gauge in the skin. Pitting results in an underestimation of the increase in forearm volume (edema formation) (15). Forearm CFC was measured continuously and kept constant throughout the measurements. Mean arterial pressure, heart rate, cardiac output, and total peripheral resistance were recorded continuously and noninvasively, both at rest and during LBNP using a Finometer on the index finger of the edematous extremity (Finapres Medical Systems, Amsterdam, The Netherlands). An increase in total peripheral resistance was used to monitor reflex activation. FBF measurements by bilateral, simultaneous venous occlusion strain-gauge plethysmography were performed at rest during lower-body ambient pressure (LBAp) and during the 3–5 min phase of LBNP. Forearm vascular resistance (FVR) was calculated off-line using the following equation FVR = MAP/FBF. The central sympathetic reflex was quantified as the relative increase in FVR (%) induced by LBNP.

Local veno-arteriolar sympathetic axon reflex and local skin blood flow rate. $^{99m}$Tc-pertechnetate is a small, water-soluble molecule. The clearance rate of a skin $^{99m}$Tc-pertechnetate depot is directly proportional to skin blood flow (13). To determine whether skin sympathetic vascular control is compromised in the edematous arms of patients with BCRL, the local vascular sympathetic reflex (veno-arteriolar axon reflex) in the skin was induced by an increase in venous pressure and measured using a $^{99m}$Tc-pertechnetate clearance technique (8, 21). A depot consisting of $\sim$0.1 ml [25 MBq/ml] $^{99m}$Tc-pertechnetate was injected subdermally on the dorsum of the wrist bilaterally. Portable scintillation detectors (Mediscint, Oakfield, Oxford, UK) were fixed directly above the depots. Subjects were seated in a chair with feet resting on the floor, and padded supports were placed horizontally at heart level. Time-activity curves were generated in three consecutive 8-min steps: 1) with the depot at heart level; 2) with the depot dependent (mean vertical distance to heart level was $\sim$38 cm); and 3) with the depot at heart level again. Depot washout rate constants for each step were calculated using exponential regression. The reflex was quantified as the washout rate measured below heart level relative to the average washout rate at heart level.

Local skin blood flow rate was calculated from the average washout rate constants measured at heart level using the following equation: $f = k \lambda 100$, where $f$ is the perfusion coefficient (ml·g$^{-1}$·min$^{-1}$), $k$ is the washout rate constant (min$^{-1}$), and $\lambda$ is the tissue/blood partition coefficient (ml/g) [is 0.7 ml·g$^{-1}$ in skin and muscle (26)].

**Statistical analysis.** Results are presented as means ± SD unless specified otherwise. The paired $t$-test was used to determine whether statistically significant differences existed between measurements on the edematous and nonedematous arms in the patient group. The FBF data failed the normality assumption and results are therefore given as the median and range. For comparison of CFC values between the patient and control groups, the unpaired $t$-test was applied. Due to multiple comparisons ($n = 19$) and the associated risk of mass significance, we chose to apply a Bonferroni correction to the significance level. The significance level was therefore set to $P < 0.003$ ($P = 0.05/19$). The Pearson correlation test was used to determine whether a correlation existed between forearm capillary filtration coefficients and forearm soft tissue volume.

**RESULTS**

Objective measures of BCRL. The results of upper extremity volume, skin tissue dielectric constant, and dermal thickness are listed in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Edematous</th>
<th>Nonedematous</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>Volume (mL)</td>
<td>3726 ± 835</td>
<td>3134 ± 522</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tissue dielectric constant</td>
<td>41.5 ± 6.5</td>
<td>27.0 ± 3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Skin thickness (mm)</td>
<td>2.26 ± 0.49</td>
<td>1.94 ± 0.42</td>
<td>0.006</td>
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</table>

**Table 3. Objective measures of BCRL**
forearms in the patient group were significantly larger compared to the pooled CFC values from the forearms in the control group. Furthermore, the CFC values from the edematous forearms were significantly larger compared to the CFC values from the nonedematous forearms within the patient group ($P < 0.001$).

Calculated forearm muscle relative microvascular volumes are shown in Fig. 5. No statistical difference ($P = 0.962$) was found between the relative microvascular volumes in the extensor muscles of edematous forearms (3.72 dB ± 1.82 dB) and nonedematous forearms (3.73 dB ± 1.91 dB). No correlation was found between forearm soft tissue volume and CFC in edematous arms ($r = −0.116, P = 0.750$).

Total forearm blood flow and local skin blood flow. Results of resting FBF measurements from the patient group are presented in Fig. 6. The median (range) FBF was 2.2 (0.8–4.8) ml·100 ml$^{-1}$·min$^{-1}$ in edematous forearms and 2.1 (0.9–4.4) ml·100 ml$^{-1}$·min$^{-1}$ in nonedematous forearms ($P = 0.104$). The local skin blood flow rate on the dorsum of the wrist was $4.5 ± 1.7$ ml·100 g$^{-1}$·min$^{-1}$ on edematous arms and $4.9 ± 1.5$ ml·100 g$^{-1}$·min$^{-1}$ on nonedematous arms with no significant difference ($P = 0.400$).

Central sympathetic vascular reflexes. The hemodynamic changes induced by 3 min of LBNP are illustrated in Fig. 7. Cardiac output was reduced on average by 14% ($P = 0.002$) and total peripheral resistance was increased on average by 26% ($P = 0.012$). Heart rate and mean arterial pressure were unchanged ($P > 0.05$). LBNP induced a similar relative increase in FVR (Fig. 8) in both edematous (1.30 ± 0.48) and nonedematous forearms (1.36 ± 0.55) with no significant difference ($P = 0.639$).
Local sympathetic veno-arteriolar axon reflex. The $^{99
u}$Tc-pertechnetate washout rate constants are illustrated in Fig. 9. Arm dependency induced a significant, 36% decrease in local skin blood flow in both edematous ($P < 0.002$) and nonedematous forearms ($P < 0.001$). The relative decrease in the washout rate induced by arm dependency was 0.64 ± 0.31 on the edematous side and 0.64 ± 0.24 on the nonedematous side ($P = 0.953$).

**DISCUSSION**

**Microvascular filtration.** A novel finding of this study is that the CFC is significantly increased in edematous forearms compared with nonedematous forearms in patients with unilateral BCRL. Two factors may cause such an increase: 1) increased surface area available for filtration; and 2) increased permeability of the microcirculation to water. Indeed, Mellor et al. (27) found evidence of dermal microvascular angiogenesis in BCRL. Using fluorescence video angiography, local dermal microvessel density was similar in edematous arms compared with nonedematous arms in patients with BCRL; however, following a correction for the difference in skin surface area, the total number of microvessels was 30% larger in the edematous arm. Our measurements of muscle relative microvascular volume support that the microvascular density in the edematous arm is unchanged. If microvascular density were increased in the soft tissues in BCRL we would have expected an increase in muscle relative microvascular volume. We agree with Mellor et al. that angiogenesis is likely to occur in BCRL to compensate for the expansion in soft tissue volume (7).

This raises the question: is the larger forearm CFC found in the present study caused by an increase in the total number of forearm soft tissue microvessels? Calculation of filtration rates is based on the percentage change in volume on the section of the arm directly beneath the strain gauge. Because a volume difference exists between edematous and nonedematous arms, a larger volume of soft tissue and hence a larger microvessel volume is measured in the edematous arm. To elucidate the effect of this we estimated the total forearm soft tissue CFC of edematous and nonedematous forearms (36). Total forearm soft tissue CFC is estimated by multiplying CFC with forearm soft tissue volume. Forearm soft tissue volume is calculated by subtracting the forearm bone volume, which normally comprises approximately 14% of the total forearm volume (10). It is assumed that the CFC does not change significantly along the forearm. This results in a mean forearm CFC that is 59 ± 27% larger in the edematous forearm than in the nonedematous forearm. The difference in soft tissue volume is 23 ± 15%. Because microvessel density seems to be unchanged, it is reasonable to assume that the total increase in microvascular volume is proportional to the volume increase. Therefore, the increase in CFC on edematous forearms is larger than what can be expected solely by an increase in total number of microvessels due to forearm soft tissue expansion. The finding that no...
correlation existed between forearm soft tissue volume and CFC on the edematous forearm supports this. We therefore propose that the most likely explanation for the larger CFC is an increase in the microvascular permeability to water concomitant with an increased microvascular surface area. To support this, others have published indirect evidence of increased microvascular filtration in BCRL:

1) Stanton et al. (39) found a bilateral increase in lymphatic drainage rates in both muscle and skin in patients with breast cancer who subsequently developed unilateral BCRL compared with those who did not, and muscle lymphatic drainage rates remained high in the affected arm in the initial phase of lymphedema development; and

2) Bates et al. found increased interstitial hydrostatic pressure (4) and decreased interstitial total protein concentration (3) in the edematous arm compared with the nonedematous arm.

An unexpected finding was that CFC in the nonedematous forearms of the patient group was significantly higher than it was in the forearms of the age-matched control group. The CFC values found in the present control group are similar to values reported by others (15, 24). This is in agreement with the finding that BCRL development occurs in patients with high peripheral lymph flow rates (39).

Microvascular filtration rates in patients with BCRL has to our knowledge been measured only in one previous study with the same experimental technique as applied in the present study (36). In that study, the same CFC values were found in edematous and nonedematous forearms, and CFC values were significantly lower than those found in the present study. This discrepancy may be explained by differences in both study protocols and populations. Stanton et al. applied a two-step protocol with a duration of each step of 10–15 min. Cuff pressure was 22 mmHg during the first step and 44 mmHg during the second step. Each arm was measured separately, resulting in a total examination time of at least 40 min. Readings from 10 of 22 patients had to be discarded due to erratic curves.

In the present study, a much shorter, three-step-protocol with higher occlusion pressures and simultaneous bilateral measurements was applied. The aim was to reduce sources of error such as vasomotion, sudden volume changes (deep respiration and muscle activity), and pitting of the strain gauge on the skin, all of which may be augmented by long venous congestion protocols. A point of concern was whether the venous distention phase had been completed to allow for a reliable measurement of the capillary filtration rate before the next increment in cuff pressure. If not, capillary filtration rates would be overestimated. However, CFC values from the control group are in accord with forearm values reported by others (15, 23). Furthermore, inspection of the time-volume curves ensured that a linear volume increase ensued the initial nonlinear phase on average after 1–2 min in the first phase (30 mmHg) and faster in the subsequent phases. Comparison of filtration rates early and late in the first phase showed no significant differences (data not shown). Measurement of capillary filtration in three steps confirmed a positive linear relationship between cuff pressure and filtration rate (Fig. 3). Pitting of the strain gauge was not avoided in the edematous arm and was especially observed toward the end of the last phase. Pitting results in an underestimation of the capillary filtration rate and may explain why the relationship between cuff pressure and filtration rate is not as linear in edematous forearms. The use of plastic strips to spread the load of the strain gauge as applied by Stanton et al. (36) may have prevented this.

Fig. 8. Increase in forearm vascular resistance in edematous and nonedematous forearms of the patient group induced by 3–5 min of mild, lower-body negative pressure. Results are displayed as means with SEM error bars.

Fig. 9. Change in wrist skin 99mTc-pertechnetate washout rate constants in edematous and nonedematous arms of the patient group induced by arm dependency. Values are means with SEM error bars.
Differences in study population characteristics may also explain the discrepancy between the study results. The population in the study by Stanton et al. (36) was older (mean age 66 yr vs. 54 yr), but the most important difference was the degree of swelling. In that study (36), the mean forearm volume difference was 56% compared with 23% in the present study. This difference in excess volume may affect CFC values in at least two ways: 1) a positive correlation has been shown between the sum of pressures (the interstitial hydrostatic pressure and the colloid osmotic pressure difference) opposing the capillary hydrostatic pressure and the excess volume of the edematous arm (4), thus capillary filtration rates may decrease with increasing swelling in BCRL; and 2) as lymphedema progresses, it is characterized by progressive soft tissue remodeling with subcutaneous adipose tissue expansion, adipose tissue fibrosis, and skin thickening (33), which may reduce soft tissue compliance.

**Sympathetic vascular reflexes.** We found no evidence of compromised sympathetic vascular reflexes. We were able to induce both central and local reflexes to the same extent in edematous and nonedematous forearms. Furthermore, we could not demonstrate a significant increase in resting FBF, which would be expected if there were a major loss of vascular resistance due to reduced sympathetic nervous tone. Therefore, these findings do not support the hypothesis that sympathetic nerve damage elicits loss of vascular control leading to edema formation. The present findings are in accord with those described by others (6, 37).

**Pathophysiological considerations.** A possible mechanism inducing the increase in microvascular hydraulic conductance is low-grade inflammation promoted by reduced clearance of inflammatory mediators from the arm tissues. Murine models of secondary lymphedema consistently show evidence of marked subcutaneous inflammation, adipose tissue expansion, and fibrosis (25, 40, 45, 46). Furthermore, large-scale transcriptional profiling in secondary murine lymphedema has shown a significant up-regulation of genes related to acute inflammation, immune response, and fibrosis (40). Another source of inflammatory mediators is adipose tissue. Many adipokines act as proinflammatory mediators, and adipose tissue inflammation has been suggested to play a role in the pathophysiology of secondary lymphedema (44). On the basis of these results, we hypothesize that in chronic lymphedema the sojourn time of large inflammatory substances secreted by immune cells and adipocytes is increased in lymphedematous tissue due to remodeling of the extracellular matrix. This will promote the inflammatory reaction and result in an increase in microvascular hydraulic conductivity.

An unexpected finding in the present study is that CFC values in nonedematous forearms of patients are about twofold higher than those of control subjects. One could speculate that patients with BRCL have a genuinely high CFC. As proposed by Stanton et al. (38), this could predispose subjects to edema development if the lymphatic drainage capacity is reduced (e.g., due to surgical procedures). Another possible explanation could be that patients with BCRL develop a systemic, low-inflammatory state in relation to a regional inflammation in the affected arm. A positive correlation between the molecular radius of adipokines and lymphatic transport has been reported (28). Therefore, small inflammatory mediators (e.g., interleukin-6 and interleukin-8) may escape to the systemic circulation from the inflamed lymphedematous tissue via the capillary routes and cause systemic, low-grade inflammation, giving rise to relatively high CFC values in the nonedematous forearms compared with the control forearms of healthy women. Both these possibilities need to be studied in additional experiments.

In conclusion, the vascular sympathetic control mechanisms seem to be preserved in BCRL. Capillary filtration is increased in both the edematous and nonedematous forearms of patients with BCRL, but more so in edematous forearms.

**REFERENCES**


