

DIURNAL AND LONG-TERM VARIATIONS OF LYMPH CAPILLARY PRESSURE IN HEALTHY SUBJECTS

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ABSTRACT

The variability of pressure in the cutaneous lymph capillaries on the forefoot was determined in 2 groups of healthy volunteers. In group A, including 12 healthy subjects (8 men, 4 women; mean age 28 years, range 22 to 37 years), measurements were performed in the morning and late afternoon of the same day. In group B (12 healthy subjects, 5 women, 7 men; mean age 53 years, range 23 to 72 years), measurements of lymph capillary pressure were repeated with an interval of 7 weeks. The superficial microlymphatics were visualized by intravital fluorescence microlymphography, cannulated with glass micropipettes, and the lymph capillary pressure was measured using a servo-nulling pressure system.

In group A, lymph capillary pressure measured in the morning (mean 7.5 ± 4.4 mmHg; range -4 to 16 mmHg) did not differ ($p > 0.05$) from the pressure in the late afternoon (mean value 5.6 ± 3.4 mmHg; range -1 to 13 mmHg).

In group B, initial lymph capillary pressure (mean 3.9 ± 2.9 mmHg, range -1.1 to 9.7 mmHg) was not different ($p > 0.05$) compared with the pressure after 7 weeks (2.9 ± 2.7 mmHg, range -1.0 to 6.8 mmHg).

We conclude that lymph capillary pressure in healthy subjects does not exhibit significant changes during the daytime or over the long term.

Recently we have developed a method to measure lymph capillary pressure (LCP) in human skin (1). LCP has been measured in healthy controls (1-4) as well as in patients with primary and secondary lymphedema (2,5). In both primary and secondary lymphedema, *microlymphatic hypertension* was demonstrated (2,5). The objective of the present study was to analyze the variability of LCP during the daytime and over an extended period of time in healthy individuals.

MATERIAL AND METHOD

Group A

In twelve healthy volunteers (8 males, 4 females, mean age 28 years, range 22 to 37 years) without clinical signs and history of lymphatic, arterial or venous disease, microlymphatic pressure (LCP) was measured to investigate diurnal variability. The subjects did not take any medication and the women were not on oral contraceptive drugs. LCP was measured in each individual in the morning between 8 and 10 a.m. and in the afternoon after 4 p.m. on the same day.

Group B

In 12 healthy subjects (5 women, 7 men) with a mean age of 53 years (range 23 to 72 years), LCP measurements were repeated after an interval of 7 weeks. LCP was measured in the afternoon.

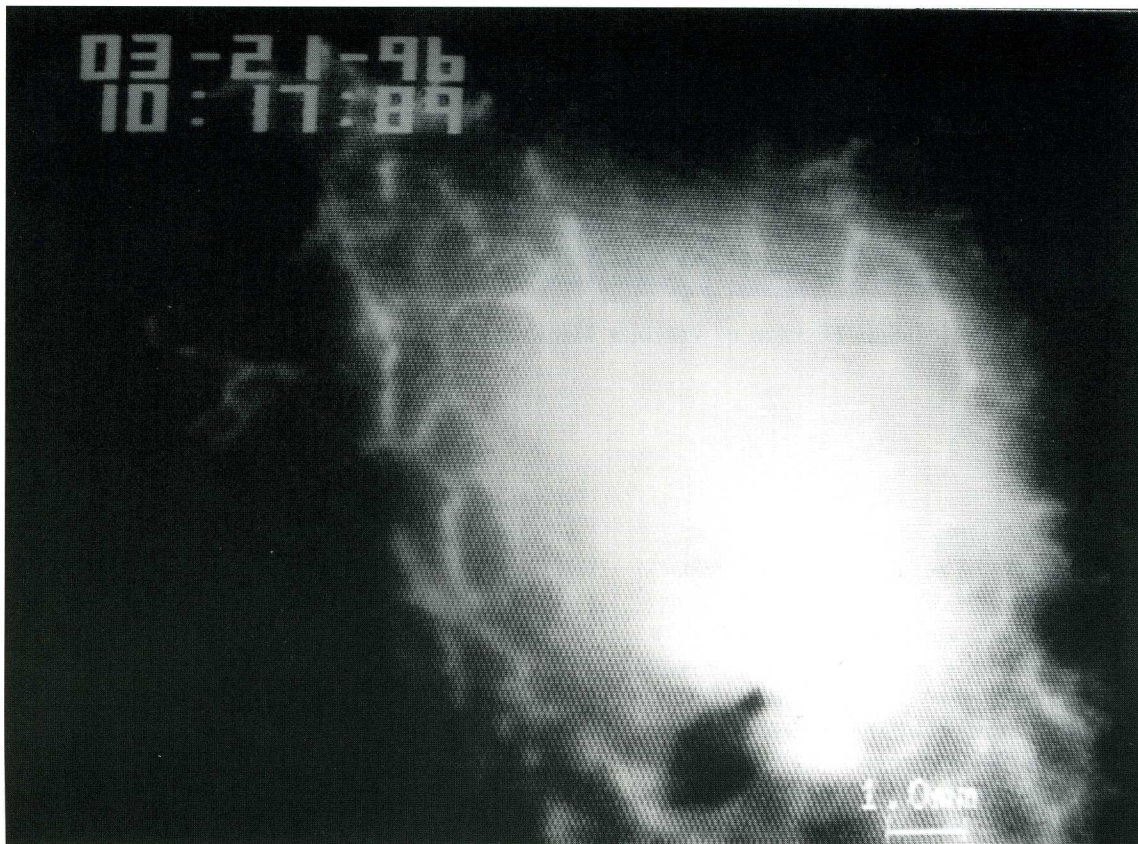


Fig. 1. Fluorescence microlymphography in a healthy subject. From the initial depot of the fluorescent contrast medium (0.01 ml) only a few lymph capillaries are filled because most of the fluorochrome is drained via precollectors to the lymph collectors (contrast with Fig. 2).

Oral informed consent was obtained from each individual. The study was approved by the Ethical Committee of the Department of Medicine, University Hospital, Zürich.

The site of the measurement in both groups was the right forefoot at the base between the first and second toe. The volunteers were examined after at least 20 minutes rest in the supine position. The foot was placed at heart level and fixed with a vacuum pillow to avoid movement artifacts.

Before the lymphatic capillaries can be punctured, they had to be visualized. We used a relatively atraumatic fluorescence microlymphography (FML) technique (6,7), which we have developed in our clinical microcirculation laboratory. The lymphatic

capillaries were visualized using FITC-dextran (fluorescein-isothiocyanate-labeled dextran 150,000; Sigma Chemical, St. Louis, MO, USA). 0.01 ml of FITC-dextran (25% w/v in sterile saline) was injected into the subepidermal layer of the skin using a steel microneedle (0.2 mm outer diameter; A. Bott, Zürich, Switzerland) connected to a microsyringe (Hamilton, Bonaduz, Switzerland). The microlymphatics became visible by FITC-dextran, which acted as a contrast medium, as it passed from the initial depot site along the lymphatic capillaries (Figs. 1,2). Video recordings were obtained using fluorescence video microscopy. The method for obtaining LCP measurements has been previously reported in detail (1). LCP was

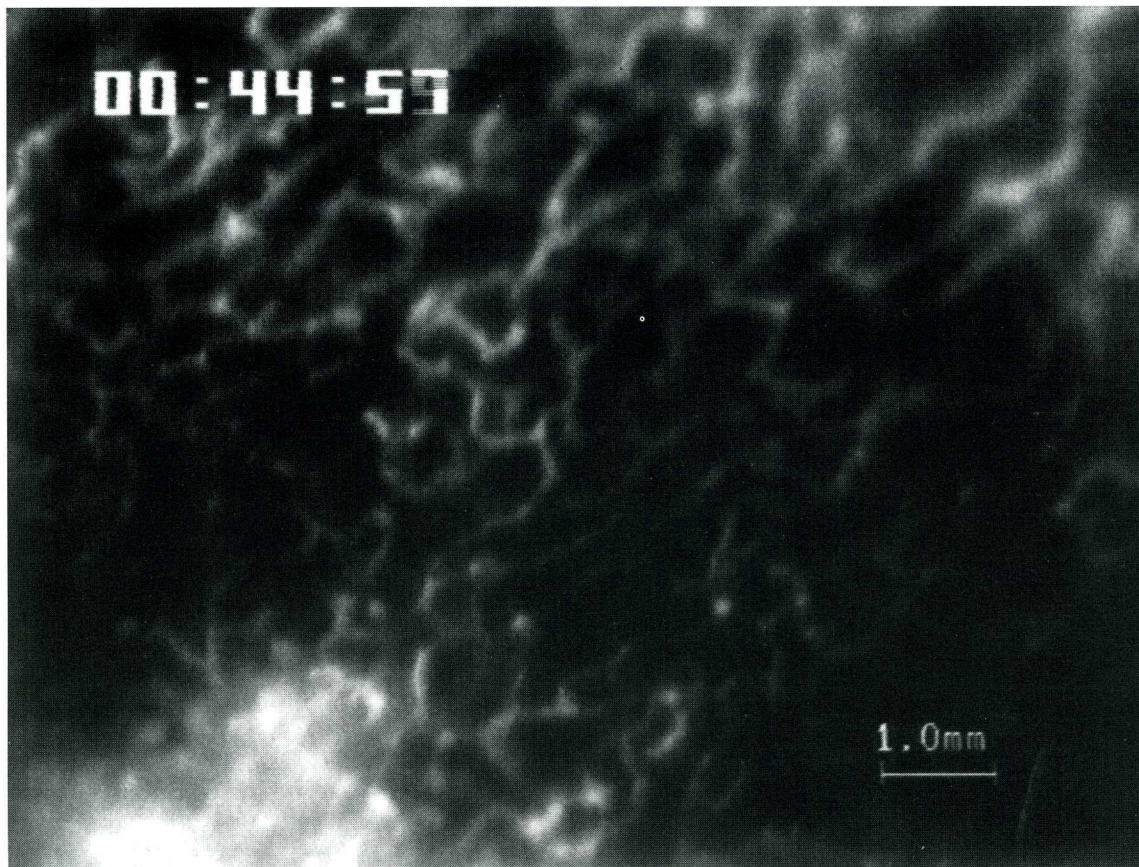


Fig. 2. Fluorescence microlymphography in a patient with primary lymphedema. In contrast to normal (see Fig. 1), the spread of the fluorescent contrast medium in the lymph capillary network in lymphedema is greatly enhanced because of impaired lymphatic drainage through the larger lymph vessels. The superficial microlymphatics are acting as a collateral network.

measured using the servo-nulling pressure system (Model 5A, IPM, San Diego, CA, USA), a counterpressure pump (Type 203; Lung Dynamics Systems, Royston, UK), a pressure transducer (Statham, Spectra Med P 23 XL, USA) and a pressure amplifier (Gould Biophysical Universal Amplifier 13-4615-58, Gould, Cleveland, OH, USA). A glass micropipette with a tip diameter of 7-9 μm was inserted into a well-delineated lymphatic capillary by means of a micromanipulator (Leica, Glattbrugg, Switzerland). Intralymphatic pressure was measured after connection to the servo-nulling system (8). The capillaries selected for micropuncture

were at least 2.5 mm away from the initial FITC-dextran depot. Mean LCP was calculated from measurements in at least 2 capillaries per subject. The correct intralymphatic position of the micropipette was checked by the servo-nulling system itself: the feedback gain of the servo-controlled counter pressure system could be varied without changing the recorded pressure (1,9).

STATISTICS

Statistical analysis of the pressure values was performed on a personal computer (Apple Macintosh II CX) using a statistical

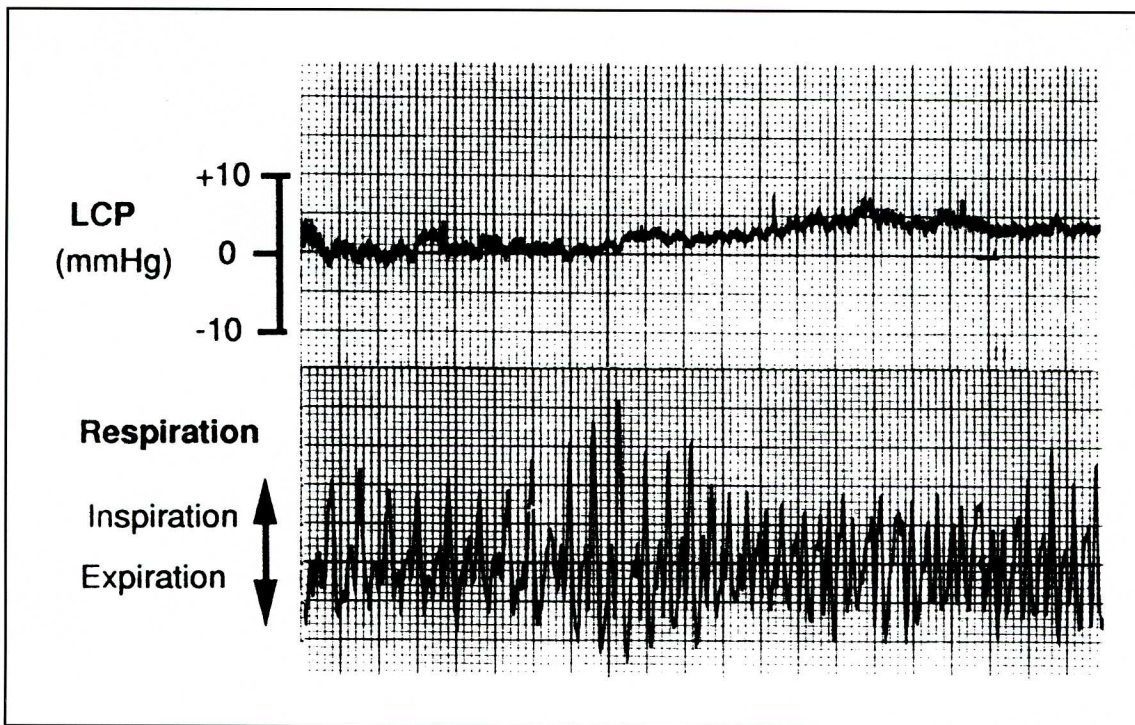


Fig. 3. Original recording of the lymphatic capillary pressure (LCP) in a healthy subject. The pressure fluctuates between -1 mmHg and + 6 mmHg. Note the pressure fluctuation with respiration.

program (StatView II™, Abacus Concepts). Diurnal and long-term reproducibility were assessed using single factor ANOVA for repeated measurements.

RESULTS

Diurnal Variability (Group A)

In 12 volunteers, 33 different capillaries were cannulated both in the morning and in the afternoon and LCP measurements were performed. Fig. 3 shows an original characteristic recording of the lymph capillary pressure.

Mean pressure in the morning was 7.5 ± 4.4 mmHg (range -4 to 16 mmHg) and in the late afternoon was 5.6 ± 3.4 mmHg (range -1 to 13 mmHg). The difference between the two sets of pressure data was not significant ($p > 0.05$; ANOVA). Fig. 4 shows the LCP in the morning and afternoon in box plots.

Mean recording time of LCP in the morning was 96 seconds, and in the late afternoon 104 seconds.

Long-Term Reproducibility (Group B)

In 12 volunteers, initial mean LCP was 3.9 ± 2.9 mmHg (range -1.1 to 9.7 mmHg). Three capillaries per subject were cannulated on both occasions. After 7 weeks, the mean LCP of 2.9 ± 2.7 mmHg with a range from -1.0 to 6.8 mmHg was not different ($p > 0.05$) compared with the earlier pressures. The recording times were 110 seconds and 115 seconds, respectively. Fig. 5 shows the results of the long-term LCP measurements in box plots.

DISCUSSION

The results demonstrate low variability and consistency in the short (same day) and

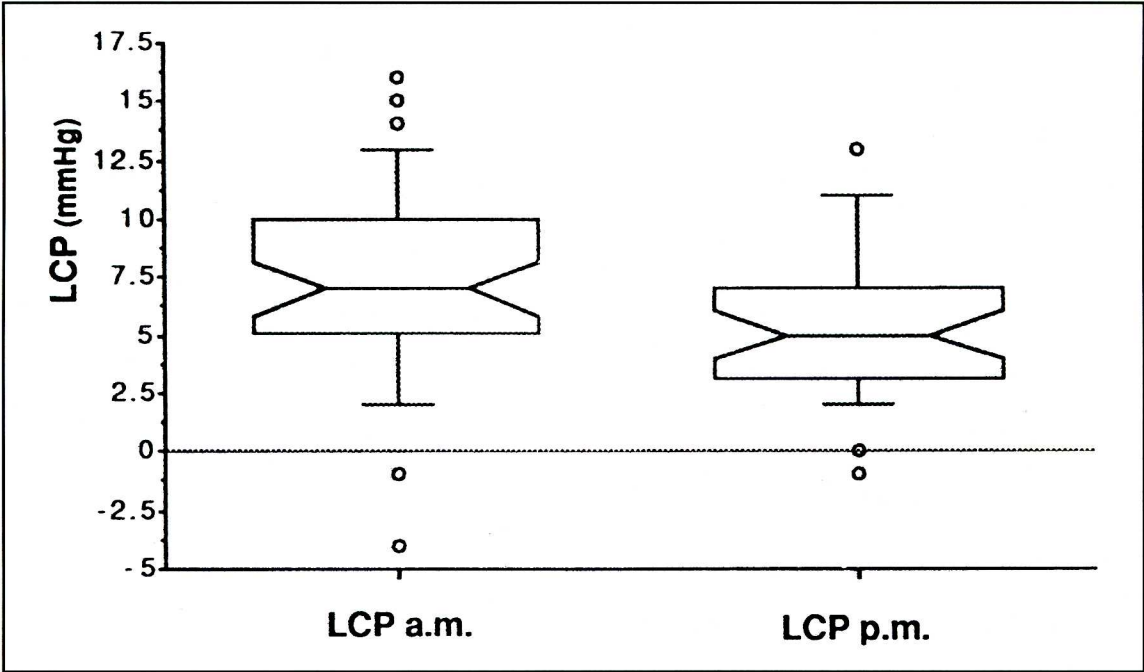


Fig. 4. Lymph capillary pressures (LCP) in 12 healthy volunteers (group A) measured in the morning (a.m.) and in the afternoon (p.m.). The notches of the box plots display the 95% confidence bands about the median value.

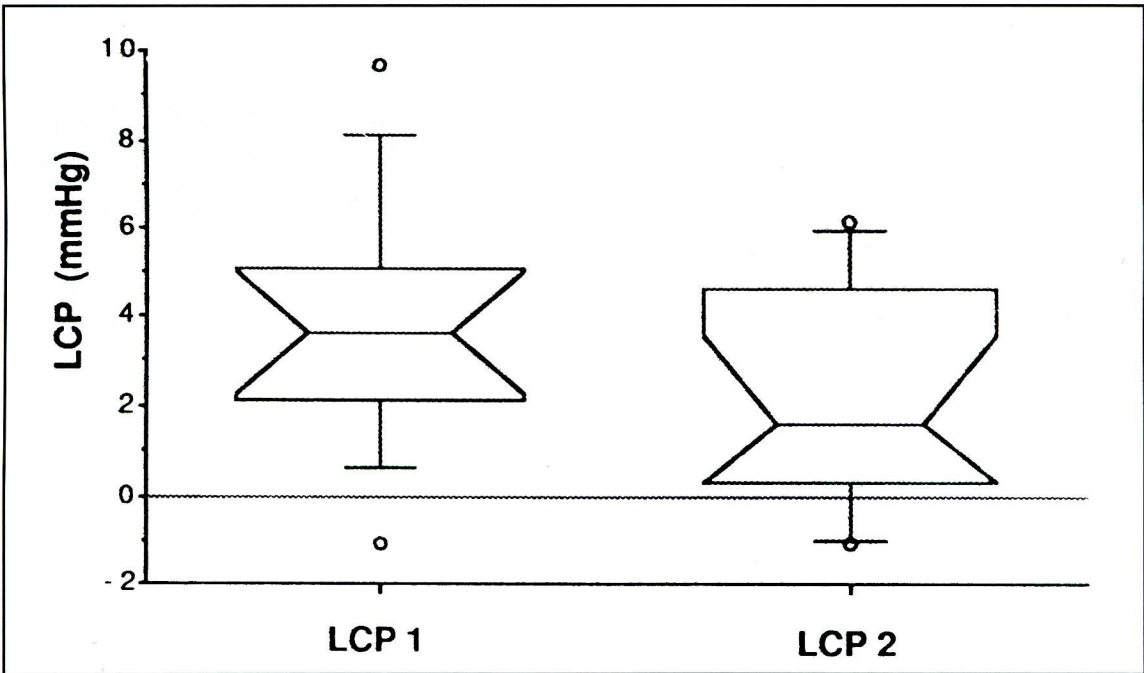


Fig. 5. Repeated lymph capillary pressures (LCP) in 12 healthy subjects (group B) measured at an interval of 7 weeks. LCP 1 (early); LCP 2 (late). The notches of the box plots display the 95% confidence bands about the median value.

long-term (over 7 weeks) of lymph capillary pressure in the human skin at the forefoot. In contrast to our expectations, the microlymphatic pressure showed a slight tendency towards lower values in the evening than in the morning. We initially had expected higher pressure in the evening as a result of potential mild edema formation during the day. However, slightly higher pressure values ($p > 0.05$) in the morning may relate to less vigorous muscle pumping of the leg. During the day, healthy persons who do not work predominantly in a standing position, likely maintain lymph capillary pressure relatively constant by activating the muscle pump, which may serve to expand and compress the initial lymphatics and lymph collectors when walking (10). Therefore, any increase in transcapillary filtration rate during the day is offset by a putative increase in lymph flow with constancy of lymph capillary pressure. Because in healthy subjects no obstruction in the lymphatic system exists, no significant change in microlymphatic pressure occurs. In addition, neither the investigators nor the volunteers observed any edema formation at the ankle or foot at the time of the second lymph capillary pressure measurement.

The long-term consistency of lymph capillary pressure in healthy subjects is demonstrated. Indeed, the mean difference of 0.9 mmHg on an interval of seven weeks parallels pressure fluctuations during a single measurement (3). The mean microlymphatic pressure in group B showed a slight trend towards lower values than in group A, which possibly resulted from the higher mean age in group B. Further studies including a larger number of subjects are required to verify whether a correlation exists between microlymphatic pressure and age.

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