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CONTRACTILITY OF HUMAN LEG LYMPHATICS DURING EXERCISE BEFORE AND AFTER INDOMETHACIN

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ABSTRACT

Subcutaneous lymphatics in the lower leg were catheterized in the retrograde direction in 6 healthy male subjects. The catheter was connected to a pressure transducer, and pressure was measured during three stages of exercise including standing, tip-toeing, and running in place. Before the third stage, indomethacin (50mg) was given i.v. Rhythmic pressure waves were registered in each subject. During the second stage, when the subjects were "warmed up," the frequency (min^{-1}) was 2.4±0.5 (mean±SEM). The corresponding values during tip-toeing and running were 5.8±0.7 ($p<0.05$) and 5.4±0.5 ($p<0.05$), respectively. The amplitudes (mean values between 3.2-4.7mmHg while standing) were not consistently altered during tip-toeing or running in any of the three stages. During standing there was a negative correlation between frequency and amplitude. No such correlation was found during tip-toeing or running, or after injection of indomethacin. Indomethacin did not significantly alter any of the measured parameters, but in two subjects the frequencies and amplitudes were decreased (about 50%) during standing, tip-toeing, and running.

Several investigations have demonstrated that intrinsic contractile activity is an important component in the regulation of lymph flow (1-4). Collecting lymphatics which contain smooth muscle cells undergo intrinsic rhythmic contractions and relaxations. Olszewski and Engeset have recorded rhythmic pressure waves in peripheral lymphatics in man (5,6). They suggest that intrinsic lymphatic contractility is a potent force in lymph propulsion, and when deranged, can be a major factor in disorders characterized by lymphatic dysfunction.

Knowledge about the mediation of intrinsic contractions in human lymphatics is limited. *In vitro* studies on human groin lymphatic ring-preparations have shown that acetylcholine, amines, prostaglandins E_2 and $F_{2\alpha}$ exert little or no contractile response (7). On the other hand, the prostaglandin-endoperoxide analogue U-44069, a thromboxane A_2 (TXA_2) mimetic, elicits strong contractions, with a threshold concentration in the nanomolar range.

The aim of the present investigation was to study the rhythmic intrinsic contractions in human lymphatics in the lower leg at rest and during different types of exercise, and to determine whether indomethacin (a cyclooxygenase inhibitor, which suppresses the synthesis of prostanoids, e.g. TXA_2), affects (diminishes) lymphatic contractility.

MATERIALS AND METHODS

Six healthy male subjects aged 24-55 years (mean 32 years) participated in the study, which was approved by the Ethics Committee of the University of Lund.

The method used for lymphatic catheterization has been described by Engeset

et al (8) and by Olszewski (9). In brief, under local anesthesia (Carbocain 5mg/ml), a 2cm long transverse incision is made just medial to the tibia, 15-20cm above the medial malleolus of the leg. When a proper lymphatic is found in the subcutaneous fat layer, a polyethylene catheter PE50 is tunneled through the tissue and inserted retrograde into the lymphatic. The procedure is facilitated by using a microscope with 40X magnification. The catheter tip is elongated to a diameter corresponding to the lymphatic vessel diameter. The lymphatic is tied over the catheter and catheter function and lymphatic patency are checked by injection of a small amount of saline that visibly distends the lymphatic. The wound is sutured, and the participant walks around the room (temperature held constant at 22°C) for about half an hour. The catheter is then connected to a Gould P23 pressure transducer (Gould Statham Instruments Inc., Puerto Rico) which has been calibrated to a water column with a pressure of 100mmHg. The pressure measurements are performed with the catheter tip at the same level as the opening of the stopcock used to obtain a zero pressure reading. When the leg is at the highest point during tip-toeing and running, the catheter tip is accordingly about 10cm above this level. This arrangement signifies that the mean and maximal pressures recorded during these periods are about 3 and 7mmHg too high, respectively. The signal is amplified by a Mingograf 4 (Siemens, FRG) and displayed on a Servogor 2 recorder (Goertz, Austria).

Continuous pressure measurements were recorded during different exercise conditions before and after an intravenous injection of 50mg indomethacin (Confortid®, Dumex, Denmark). The subjects were: a) standing during 10 min, b) tip-toeing during 2 minutes (120 times according to a metronome), and c) running in place during 3 minutes (maximal speed for each subject). Between each period of exercise, each subject was allowed to rest for 5 min. The injections were given with the participants supine, a position in which they stayed for an additional 10 minutes before measurements were resumed. For accuracy, the control exercise protocol was repeated twice before drug administration. Before the second stage and before indomethacin, the catheter was opened to atmospheric pressure via the stopcock, so that each cycle began with similar intralymphatic pressure conditions.

Blood samples for determination of the amount of circulating indomethacin were taken from four participants before, and at different times during the first hour after injection. In three participants, lymph was collected in heparinized tubes after finishing the exercise protocol. Indomethacin in plasma and lymph was analyzed by high-pressure liquid chromatography.

Assessment of the curves

A contraction was defined from the curves as a pressure rise of ≥1mmHg reaching a maximum in ≤10s. Frequencies (min^{-1}) and amplitudes (mmHg) of the pressure waves were calculated. The recording for each exercise period (i.e., standing, tip-toeing, and running) was divided into 30s long segments, and the average pressure for each segment was determined graphically by drawing a line through the curve (equal areas between the line and the curve on both sides of the line). The mean of these pressures within the same exercise period was calculated and reduced by 3mmHg--the increase in hydrostatic pressure during tip-toeing (see above). The value obtained was taken as the "mean pressure." The "maximal pressure" depicts the highest pressure obtained within each exercise period. The values obtained from the curves were reduced by 7mmHg--the increase in hydrostatic pressure during running (see above).

Statistics

Wilcoxon signed rank test for paired data was used. Correlations were determined by using the method of least

squares. A probability of <0.05 was regarded as significant.

RESULTS

In most of the subjects it was possible to find 3-4 lymphatics, but in some, only one could be found. The catheterized lymphatics were 0.2-0.4mm in outer diameter. When several lymphatics were found in the same subject, most had diameters in the lower size-range, and ramifications among them were frequent. Minor blood vessels, vasa lymphorum, were clearly seen in the wall of the lymphatics. It was also possible to observe pumping activity, i.e., "peristaltic" contractions along the visible part of the lymphatic vessel.

The results of the lymphatic pressure measurements are shown in *Table 1-3.* Tip-toeing (compared with standing) increased the frequency of lymphatic pressure waves during the three stages of exercise, but the increase was statistically significant only during the second stage *(Table 2).* The amplitudes showed slight decreases during tip-toeing (not significant). The mean pressures of the first stage were significantly higher during tip-toeing, while the mean pressures during the second and third stages and the maximal pressures during all stages were not significantly increased *(Table 2).*

Running (compared with standing) also caused an increase in frequency during the three stages, but as during tip-toeing, the increase was significant only during the second stage *(Table 3).* The amplitudes were increased during the first and third stages, but the second stage showed a slight decrease. None of the altered amplitudes was statistically significant. The mean and maximal pressures were significantly higher during running in the three stages *(Table 3).*

Indomethacin did not significantly alter any of the parameters obtained by lymphatic pressure measurements, when the comparison was made to the first or the second stage of exercise *(Table 1-3).* However, after indomethacin injection in two participants, the frequencies and amplitudes during standing, tip-toeing, and running were decreased about 50% of the values of the second stage, whereas in the others there was no consistent tendency. *Fig. 1* shows lymphatic pressure tracings recorded before and after indomethacin.

Fig. 2 depicts the amplitudes plotted against the frequencies of the two stages of standing before drug administration. There was a negative correlation (corr.

Table 1
Lymphatic contractile and pressure measurements (mean ± SEM) before and after i.v. indomethacin (INDO) (50mg). The range (in brackets) is also shown (n=6).

STANDING	PRE-INDO		POST-INDO
	1st stage	2nd stage	3rd stage
Frequency (min^{-1})	3.6±1.0 (0.6-6.3)	2.4±0.5 (0.4-3.5)	3.2±1.0 (0.2-6.7)
Amplitude (mmHg)	3.2±0.8 (1.5-7.0)	4.7±0.7 (2.4-6.3)	3.4±0.6 (1.7-5.6)
Mean pressure (mmHg)	29±5 (11-46)	38±8 (19-63)	36±9 (19-80)
Max pressure (mmHg)	35±5 (24-52)	48±8 (26-69)	43±11 (22-95)

Table 2
Lymphatic contractile and pressure measurements (mean ± SEM) before and after i.v. indomethacin (INDO) (50mg). The range (in brackets) is also shown (n=6).

TIP-TOEING	PRE-INDO		POST-INDO
	1st stage	2nd stage	3rd stage
Frequency (min^{-1})	5.7±1.2 (1.6-9.0)	5.8±0.7* (4.5-8.4)	5.7±0.7 (4.0-7.5)
Amplitude (mmHg)	2.7±0.4 (1.9-4.0)	3.8±0.9 (1.6-6.8)	3.1±0.5 (2.0-4.4)
Mean pressure (mmHg)	38±7* (24-63)	54±16 (18-103)	48±16 (26-96)
Max pressure (mmHg)	42±12 (21-85)	58±18 (18-110)	50±16 (32-98)

*p<0.05 as compared with Standing values (see Table 1)

Table 3
Lymphatic contractile and pressure measurements (mean ± SEM) before and after i.v. indomethacin (INDO) (50mg). The range (in brackets) is also shown (n=6).

RUNNING	PRE-INDO		POST-INDO
	1st stage	2nd stage	3rd stage
Frequency (min^{-1})	5.4±0.7 (2.4-7.0)	5.4±0.5* (3.5-6.7)	5.3±1.0 (3.0-10.0)
Amplitude (mmHg)	4.9±0.8 (2.4-8.1)	4.4±0.4 (2.7-5.7)	4.1±0.7 (2.5-6.7)
Mean pressure (mmHg)	63±16* (28-115)	80±19* (44-137)	65±16* (35-128)
Max pressure (mmHg)	71±19* (28-139)	80±17* (46-145)	69±15* (39-140)

*p<0.05 as compared with Standing values (see Table 1)

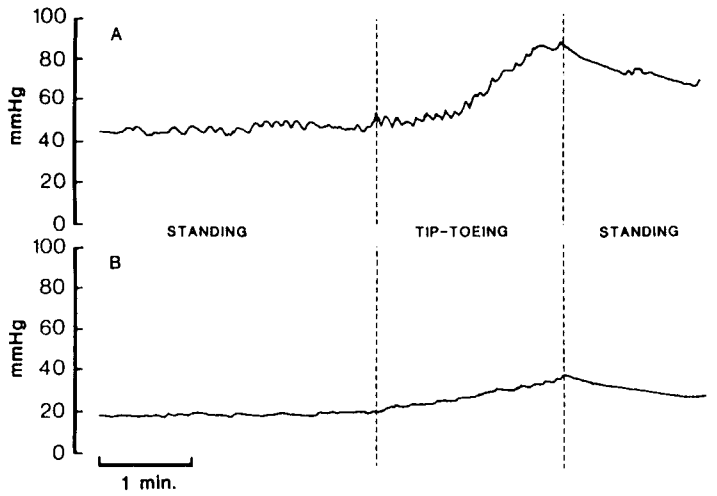

Fig. 1. Tracings from the recorded lymphatic pressure measurements in one subject before (A) and after (B) indomethacin.

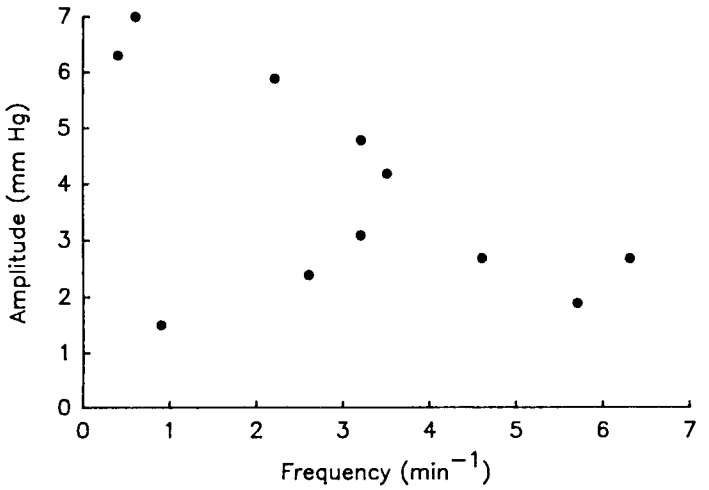

Fig. 2. The amplitude plotted against the frequency during standing. Measurements were made during two stages of standing in six volunteers. One data point is missing due to a technical failure.

coeff. -0.53; not significant) between frequency and amplitude. No such correlation was found during tip-toeing and running, or after indomethacin administration.

Seven minutes after injection, the mean concentration of indomethacin in plasma was 7.8±1.2μg/ml (n=4). Forty-five minutes after injection, the value was 2.5±0.4μg/ml. The concentration of indomethacin in lymph collection during 2-3h after the experiment was finished was 0.27±0.02μg/ml (n=3). The participants felt slight dizziness during and immediately after injection of indomethacin. The systolic blood pressure (obtained by a sphygmomanometer cuff) was determined two minutes after injection, and showed an increase from a preinjection value of 118±5 to 128±5mmHg. The diastolic pressure was also increased from 80±5 to 92±6mmHg.

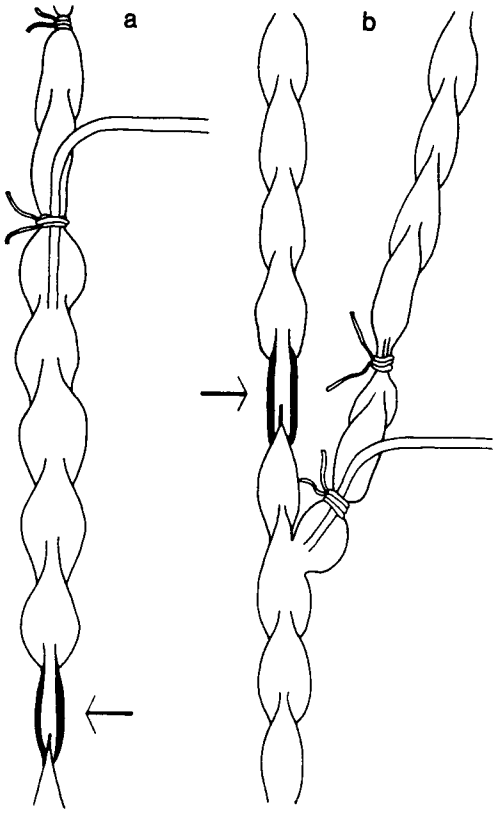

*Fig. 3. Schematic drawing of catheterized lymphatics. (**a**) If there are no ramifications in the vicinity of the catheter tip, an "end pressure" is measured, and (**b**) if the tip of the catheter is placed in close connection to a ramification, a "lateral pressure" is measured. The arrows indicate lymphangions in a contracted state.*

DISCUSSION

By means of an indwelling lymphatic catheter inserted against the direction of flow, an "end [lymphatic] pressure" is measured if there are no lymphatic ramifications near the tip of the catheter *(Fig. 3a)*. If the tip of the catheter is placed close to a branch vessel and if there are no valves between the tip an the ramification, a "lateral [lymphatic] pressure" is measured *(Fig. 3b)*. When a lymphatic is catheterized using the present method, it is not known with certainty where the catheter tip resides in relation to lymphatic branches.

During tip-toeing and running, the frequency of the lymphatic pressure waves increased, while no consistent change was registered concerning the amplitudes. These findings are in agreement with earlier ones by Olszewski and Engeset (6). In the present study, it was found that tip-toeing promoted an increase in mean and maximal pressures, but the increase was significant only for the mean pressure during the first stage. On the other hand, running caused significant increases in both mean and maximal pressures. These data possibly relate to a higher arterial perfusion pressure and increased blood flow with greater plasma protein leakage and, in turn, thereby greater leg lymph flow during running where major muscle groups in the leg are activated, compared with tip-toeing where only minor muscle groups are used. Lymph flow depends on both lymph formation and lymphatic contractility, and there is a positive correlation between contractile frequency and lymph flow (6).

The present study shows variation in the contraction pattern regarding both frequency and amplitude during standing. This pattern is not necessarily related to a variation in lymph formation, but may represent an intrinsic regulatory mechanism by which the lymphangions (the lymphatic segment between two valves) adjusts to diminished lymphatic pulse frequency with an increased pulse amplitude (filling of the lymphangions and/or enhancement of contraction force). *Fig. 2* depicts the relation between the amplitude and frequency during standing. However, the correlation is not statistically significant. In one instance, the catheter was connected to the pressure transducer immediately after suturing the wound. The first recording in this subject showed a very low frequency and amplitude, and if the point representing this recording (0.9 in frequency and 1.5 in amplitude) is omitted from *Fig. 2*, the correlation would be statistically significant ($p<0.01$), with a slope of the regression line of -0.78 and a correlation coefficient of -0.83. No such correlation was found during tip-toeing or running, nor during any of the different exercise conditions after indomethacin

administration. The reason for this lack of correlation may be that by using "end pressure" measurements, the activity of the lymphangions was seriously disturbed by the increase in lymph flow volume during work, and that indomethacin adversely affected the intrinsic regulatory mechanism.

The mean and maximal pressures showed higher values, although statistically not significant, during the second stage of study compared with the first stage, during all exercise conditions. The reason for these pressure findings may be that the participants were "warmed up" during tip-toeing and running in the first stage, causing a rise in lymph production which persisted for some time thereby influencing pressure in the subsequent steps, despite the fact that the indwelling catheter was opened to the atmosphere in between each stage.

Statistically, cyclooxygenase inhibition had no effect on any of the measured parameters, but in two participants a decrease in frequency and amplitude consistently occurred. The dose of administered indomethacin (50mg) has been effective in the treatment of ureteral colic (10). The concentrations of indomethacin in plasma in the present study, correlated well to those found by others (11). Moreover, indomethacin was detected in lymph, suggesting that this agent can reach the lymphatic smooth muscle cells in two ways--first, by the vasa lymphorum (12), and second by passage from the blood capillaries via the interstitial fluid into the lymphatics. Nonetheless, it is possible that the concentration of indomethacin was too low to have noticeable effects in all cases in the present study. Furthermore, when giving drugs systemically it is difficult to discriminate between alterations in lymph load due to an increase or decrease in extravasation of fluid causing alterations of lymphatic contractility, or a direct drug-induced effect on the lymphatics. It has been previously shown that indomethacin (20-30mg) given systemically increases mean blood pressure and systemic vascular resistance, but not pulmonary vascular resistance in healthy volunteers (13). Injection of indomethacin in the present study similarly raised systemic blood pressure, which may be related to a reduction of prostacyclin.

We have added indomethacin 10^{-8} - 10^{-4}M to lymphatics from the same region, suspended in organ baths (for method, see 7). In these *in vitro* studies, indomethacin failed to alter the applied basal tension. When it was added to a TXA_2 or $PGF_{2\alpha}$ precontracted lymphatic vessel, a reduction in tone occurred only with the highest concentrations (10^{-5} and 10^{-4}M).

Thus, the present investigation suggests that the lymphangions possess an intrinsic regulatory mechanism, by which lymphatics adjust to a decreased lymphatic pulse frequency with an increase in pulse amplitude and thereby maintain the constancy of lymph fluid transport. Activation of major muscle groups in the leg promote significant increases in frequency, as well as mean and maximal pressures of the lymphatics. Cyclooxygenase inhibition by injection of 50mg indomethacin does not notably affect lymphatic contractility, but may decrease lymphatic activity.

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