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# Postural Effects on Interstitial Fluid Pressure in Humans

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**Key Words**
Edema · Fluorescence microlymphography · Interstitial fluid pressure · Postural changes · Servonulling micropressure system

**Abstract**
***Background:*** Direct assessment of the effect of postural changes on interstitial fluid pressure (IFP) in the human skin under physiological conditions is important for the understanding of mechanisms involved in diseases resulting in lower limb edema. Previous techniques to measure IFP had limitations of being invasive, and acute measurements were not possible. Here we describe the effect of postural changes on IFP in the skin of the foot using the minimally invasive servonulling technique. ***Results:*** Measurements were performed in 12 healthy subjects. IFP (means ± SD) was significantly higher in the sitting (5.1 ± 2.9 mm Hg) than in the supine position (–0.3 ± 3.6 mm Hg, p = 0.04) when measured in the sitting position first. The difference between the sitting and the supine position was not significant when measurements were taken in the supine position first [from 1.0 ± 4.3 (supine) to 3.6 ± 6.7 mm Hg (sitting), p = 0.46]. Spontaneous low-frequency pressure fluctuations occurred in 58% of the recordings during sitting, which was almost twice as frequent as in the supine position (33%; p = 0.001), while no effects on lymphatic capillary network extension were observed (p = 0.12). ***Conclusion:*** Using the servonulling micropressure system, postural effects on IFP can be directly assessed. IFP is higher in the sitting position, but differences are influenced by the time in the upright position.



## Introduction

Postural homeostasis of interstitial fluid pressure (IFP) is regulated by a variety of mechanisms. A rise in arterial and/or venous pressure, resulting in increased capillary pressure and net filtration, is counteracted primarily and directly by colloid osmotic forces opposing filtration across the endothelial glycocalyx [1]. Moreover, indirect effects mediated through changes in the 'Starling forces', including hydrostatic and colloid osmotic pressures of capillary blood and interstitial fluid, play a role [2]. The 'edema-preventing mechanisms' are thought to consist of various regulatory factors [3]. These include (i) the veno-arteriolar response, a local autoregulatory precapillary vasoconstriction in response to the upright position that decreases capillary net filtration [4, 5]; (ii) increases in colloid osmotic pressure of blood and reduced interstitial fluid colloid osmotic pressure [6]; (iii) a greater lymphatic density [7], and (iv) increased lymphatic contractility [8,

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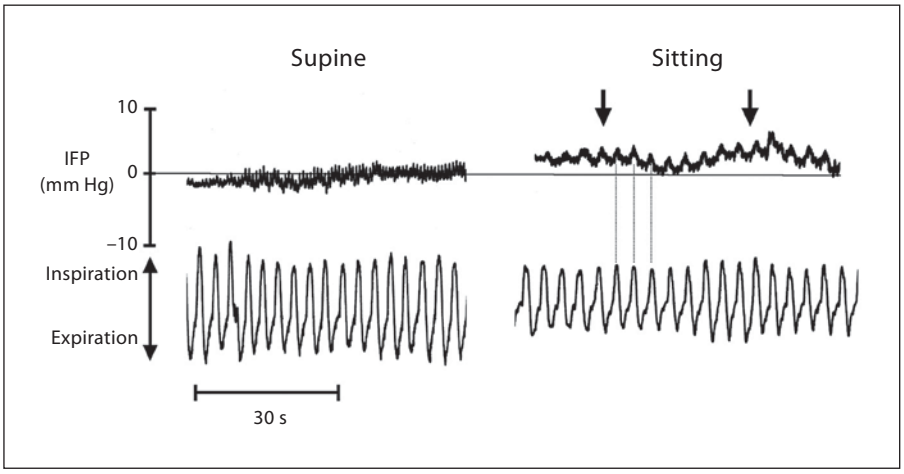


**Fig. 1.** Representative original recordings of IFP measurements of the human skin at the dorsum of the foot (above) and respiration changes (below) obtained in a healthy volunteer in the supine (left) and the sitting (right) position. In the supine position, no spontaneous low-frequency fluctuations and only small respiration-related changes were seen compared to the sitting position (dotted lines indicate respiration-related fluctuations, and arrows low-frequency fluctuations).

9]. Disturbances in these regulatory factors result in fluid accumulation and edema via increases in interstitial fluid volume and IFP [3]. Although changes in IFP have been investigated in humans and only little changes in IFP assessed by the 'wick-in-needle' method have been shown, it is still unclear whether IFP may function as a preload of the lymphatic system [10]. Data on measurements of the effects of acute postural changes on IFP in humans are not available, and it is not known whether low-frequency fluctuations occur in interstitial fluid. Therefore, we studied these parameters with the servonulling micropressure system in combination with microlymphography in healthy volunteers.

## Methods

*Human Subjects*

The study comprised 12 healthy volunteers (8 women and 4 men; mean age: 32 years, range 21–59 years). None of the subjects had a history or clinical findings of venous, lymphatic, arterial or inflammatory diseases of the lower extremities or any other systemic diseases. Subjects were nonsmokers and not taking any medication including oral contraceptives. Each individual gave written informed consent to the study, which was approved by the Ethics Committee of the University Hospital Zurich.

*Microlymphography*

All measurements were performed between 8 and 11 a.m. Intervals from rising to measurement could vary. Volunteers were asked to rest at least 8 h at night before the examination. The participants of the study were asked to limit physical activities before the examinations, i.e. to use public transport or a car (instead of a bicycle) as well as to refrain from working 12 h before the examination started. Before the interstitial tissue could be punctured, the lymphatic capillary network of the skin had to be visualized by the fluorescence microlymphography (FML) technique to avoid puncturing of microlymphatics [11]. The lymphatic capillaries were filled using fluorescein isothiocyanate (FITC)-Iabeled dextran (molecular weight: 150,000; Sigma, St. Louis, Mo., USA); 0.01 ml of FITC-labeled dextran (25% w/v in sterile saline) were injected into the upper dermis of the skin using a steel microneedle (0.2 mm outer diameter; Bott, Zurich, Switzerland). The microlymphatics were filled with FITC-labeled dextran, which acted as a contrast medium as it passed from the initial depot site to the lymphatic capillaries. Due to the draining of the dye in the lymphatic capillaries and the time difference from the sitting to the supine position or reverse, FML measurements had to be repeated in the second position. The second injection site was in the same foot at a maximum distance of 2–3 cm from the previous injection site. Video recordings were obtained using fluorescence video microscopy.

The maximal visible extension distance of the fluorochrome in the microlymphatic network was measured during the examination, 10 min after dye injection [11]. The reference point for the network extension was the border of the dye depot.

*Measurements of Interstitial Fluid Pressure*

The method for obtaining IFP measurements has been previously reported in detail for lymphatic capillary pressure measurements [8, 12] and for IFP [13, 14]. IFP was measured using the servonulling pressure system (model 5A; IPM, San Diego, Calif., USA), a counter-pressure pump (type 203; Lung Dynamics Systems, Royston, UK), a pressure transducer (Spectra Med P 23 XL, Oxnard, Calif., USA) and a pressure amplifier (Biophysical Universal Amplifier 13-4615-58; Gould, Cleveland, Ohio, USA). A glass micropipette with a tip diameter of 7–9 μm was inserted into a lymphatic capillary-free area, which was well delimited by lymphatic capillaries by means of a micromanipulator (Leica, Glattbrugg, Switzerland). IFP was measured after connection to the servonulling system [13, 14]. The interstitial areas selected for micropuncturing were at least 2.5 mm away from the initial FITC-labeled dextran depot to avoid artifacts. The needle was inserted into the interstitial space in areas with regular and intact meshes of well-depicted lymphatic capillaries at an approximate depth of 200 μm. Due to the counter-pressure effects there are small high-frequency oscillations in the recordings (fig. 1).

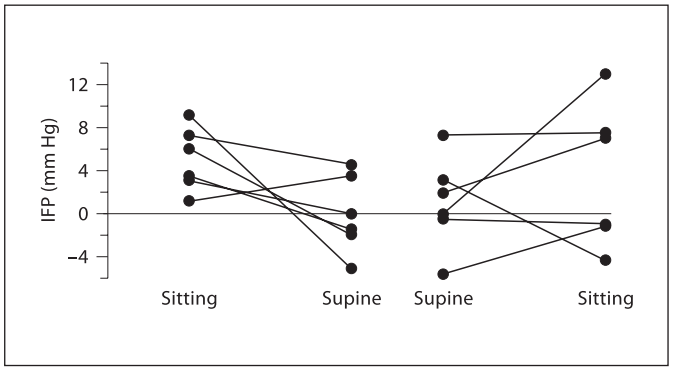


**Fig. 2.** Individual changes in IFP from the sitting to the supine position (left, p = 0.04) and from the supine to the sitting position (right, p = 0.46) measured at the ankle in 12 healthy volunteers.

IFP measurements were performed with the subjects in supine and sitting positions after a resting period of at least 20 min in each position; the volunteers actively adopted the new posture. Subsequently, pressure measurements were carried out and could last up to 1 h. Maximum time in one position was therefore 90 min (mean 60 ± 30 min). All investigations were performed in a temperature-controlled room with a mean temperature of 22–24°C.

The starting position (either sitting or supine) was chosen at random. In 6 subjects, IFP measurements were taken first in the sitting and subsequently in the supine position; in the remaining 6, the order was reversed (first supine and then sitting). The site of pressure measurements was the distal forefoot. The dorsal aspect of the leg was embedded in a vacuum pillow (Wiedmer, Rüschlikon, Switzerland) to avoid movement artifacts. For measurements in the sitting position with the leg dependent, the subject was seated on a chair placed on a table, which provided comfortable working conditions for micropuncturing. During sitting the angle between calf and thigh was about 100° and there was no tourniquet effect on the thigh. The correct interstitial position of the micropipette was checked by the servonulling system itself: the feedback gain of the servo-controlled counter-pressure system could be varied without changing the recorded pressure provided that the tip of the micropipette was located in a liquid-filled space [12, 14]. All measurements were performed under microscopic control. If the skin surface was out of the planar focus due to movements, records were discarded. Only stable measurements of a minimum period of 20 s were included. Mean IFP was calculated from measurements in at least two different areas per subject in each position. The values of each individual for each position were averaged (fig. 2).

Two patterns of IFP fluctuations were detected: respiration-related pressure changes and spontaneous low-frequency fluctuations [15]. Low-frequency fluctuations are considered IFP changes in amplitude >3 mm Hg compared to baseline lasting >5 s (fig. 1). For each recording, two different persons without knowing the body position visually analyzed the recordings for the presence or absence of respiration-related and spontaneous low-frequency fluctuations. Incidence and frequency of IFP fluctuations were determined by counting the respective pressure changes on hard copies of the chart recorder. Respiratory movements of the chest were recorded by photocells, and IFP and respiration rates were simultaneously registered (chart speed 1 mm/s) on a four-channel recorder (Brush 2600S Recorder; Gould). Systemic blood pressure was measured on the upper arm in both postures, using the Riva-Rocci technique.

*Statistics*

Results are presented as means ± SD. Statistical analyses were performed using a statistical program (StatView 4.57, Abacus Concepts, Berkley, Calif., USA). Data obtained from different positions per group were compared using the Wilcoxon signed rank test. Intergroup (supine-sitting vs. sitting-supine) comparisons were done using the Mann-Whitney U test. Differences in the occurrence of IFP fluctuations were examined using the $\chi^2$ test. A p value <0.05 was considered significant.

## Results

IFP was calculated from measurements obtained in 12 volunteers. The mean IFP was calculated from 33 interstitial areas in the supine position (2 areas in 6 subjects, 3 areas in 3 subjects and 4 areas in 3 subjects) and 31 areas in the sitting position (2 areas in 7 subjects, 3 areas in 3 subjects and 4 areas in 2 subjects). Duration of IFP measurements was 164 ± 75 (supine position) and 156 ± 82 s (sitting position). The maximal visible transport distance of the fluorochrome in the lymphatic capillary network was independent of the starting condition and without differences in the upright or supine position (p = 0.89, network extension, table 1). Representative examples of measurements in the sitting and supine position recorded in 1 study subject are shown in figure 1.

The effects of postural differences on IFP were only significant when measurements were started in the sitting position [from 5.1 ± 2.9 (sitting) to –0.3 ± 3.6 mm Hg (recumbent); p = 0.04; n = 6] but not when IFP was measured in the recumbent position first [from 1.0 ± 4.3 (recumbent) to 3.6 ± 6.7 mm Hg (sitting); p = 0.46; n = 6; fig. 2]. In the supine-to-sitting group, 3 subjects showed an increase in IFP in the sitting position, whereas the 3 other subjects of the same group demonstrated a lower IFP. In the sitting-to-supine group, only 1 subject had a lower IFP in the sitting compared to the supine position. There was no significant difference in IFP between the two groups regarding the sitting position (supine-sitting vs. sitting-supine: 3.6 ± 6.7 vs. 5.1 ± 2.9 mm Hg, p = 0.63), nor in the supine position (supine-sitting vs. sitting-supine: 1.0 ± 4.3 vs. –0.3 ± 3.6 mm Hg, p = 0.68).

Two IFP fluctuation patterns were analyzed as present or absent in both positions. Respiration-related fluctuations in IFP in the sitting position were observed in 20 of

**Table 1.** Effect of postural changes from the supine to the sitting position and vice versa (12 healthy volunteers)

| | IFP, mm Hg | | Network extension[a] mm | Respiration rate breaths/min | Respiration-related fluctuation | | Spontaneous fluctuations | | Blood pressure, mm Hg | |
|---|---|---|---|---|---|---|---|---|---|---|
| | sitting-supine (n = 6) | supine-sitting (n = 6) | | | frequency cycles $min^{-1}$ | amplitude mm Hg | frequency $cycles^{-1}$ | amplitude mm Hg | systolic | diastolic |
| Supine | –0.3 ± 3.6 | 1.0 ± 4.3 | 6.3 ± 2.3 | 17 ± 5 | 14.2 ± 6.6 | 5.9 ± 3.3 | 1.5 ± 0.6 | 5.5 ± 5.1 | 108 ± 13 | 70 ± 10 |
| P value | 0.04 | 0.46 | 0.12 | 0.75 | 0.39 | 0.39 | 0.27 | 0.28 | 0.28 | 0.13 |
| Sitting | 5.1 ± 2.9 | 3.6 ± 6.7 | 8.3 ± 5.4 | 16 ± 6 | 12.7 ± 7.7 | 5.0 ± 3.1 | 1.6 ± 0.8 | 5.7 ± 3.2 | 110 ± 8 | 74 ± 8 |

Means ± SD.
[a] Maximal extension of lymphatic capillary network (10 min after dye injection).

the 31 measurements (60%), with a tendency to decrease while being supine (35%), however, this decrease was not statistically significant (p = 0.09). By contrast, the incidence of these low-frequency fluctuations during sitting was significantly higher than in the supine position (58 vs. 33%, p = 0.001), whereas the mean frequency of spontaneous pressure fluctuations showed no changes from the supine to the sitting position (1.5 ± 0.6 to 1.6 ± 0.8 cycles $min^{-1}$, respectively, p = 0.27; table 1). The mean amplitude of low-frequency fluctuations was 5.5 ± 5.1 and 5.7 ± 3.2 mm Hg in the supine and sitting position, respectively (p = 0.28, table 1).

The mean respiratory frequency was 17 ± 5 breaths/min in the supine and 16 ± 6 breaths/min in the sitting position. The mean difference between the heart level and the site of the measurement in the sitting position was 92 ± 4 cm (range 82–100 cm). Skin temperature at the measurement site averaged at 31.6 ± 1.8°C and did not significantly differ between sitting and supine positions.

## Discussion

This study demonstrates that in humans acute postural changes affect IFP measured by microlymphography and the servonulling micropressure system. Mean IFP changes ranged from 2.6 to 5.4 mm Hg and showed great interindividual differences. More prominently, the occurrence of IFP fluctuations depended on the posture.

It has been suggested that IFP acts as a lymphatic preload contributing to lymphatic contractile activity [16]. IFP Measurements have been performed using either the 'wick-in-needle' or the servonulling micropressure system [10, 17]. However, the effect of body posture on human skin IFP has been investigated solely by the 'wick-in-needle' method, and the occurrence of low-frequency oscillations has not been reported [10, 18]. In these studies, IFP averaged at –1.3 ± 1.6 mm Hg on the thorax and –0.4 ± 2.5 mm Hg at the ankle, and postural differences were only small.

We combined FML and the servonulling micropressure technique, because the combination offers the advantage of visualizing initial lymphatics and defining the interstitial space from well-delimited areas of complete lymphatic meshes. In addition, the servonulling micropressure system allows acute measurements that are less invasive than those using the 'wick-in-needle' method. Although absolute IFP values might be overestimated due to the injection of FITC-labeled dextran into the interstitial space, the IFP values found in our study were slightly higher but still comparable to those reported by Noddeland [10]. As we injected exactly the same volume in both positions, the relative changes in IFP were unlikely to be affected by the dye, although removal of the dye might differ between the two positions due to dependency and therefore increased lymphatic contractility. A second surprising finding of these experiments was that the order of measurements (supine-to-sitting vs. sitting-to-supine) influenced the postural effects on IFP. These changes were greater in the sitting-to-supine order. The small number in each group (n = 6) and the rather great interindividual variance does not allow to draw a conclusion about that time period in the upright position before the resting period may affect the adjustment of a stable homeostasis between microvasculature and interstitial space. Acute changes in IFP are small compared to hydrostatic pressure changes in the circulating blood, and it remains to be determined which mechanisms are involved, as some changes take much more time to adapt, i.e. changes in colloid osmotic pressure. Different mechanisms that contribute in temporally different sequences might explain in part why the differences occurred be-

tween the two sequences of measurements. Although there was a slight but significant difference in the sitting-to-supine order, we were overall not able to demonstrate a significant difference due to postural changes. Besides the already mentioned fact that different mechanisms regulating postural IFP hemostasis act at different times, a larger study cohort (and therefore larger subgroups) may be required to detect minor differences.

Servonulling micropressure and FML measurements have added new insights into lymphatic function in both health and disease [11, 12, 19]. Lymph capillary pressure has been demonstrated to be increased in patients with lymphedema (15.0 ± 5.1 mm Hg) [19] compared to healthy subjects (mean 4.0 ± 4.5 mm Hg) [12]. Moreover, the effects of postural changes on human lymphatic capillary pressure showed that lymphatic pressure significantly increased from the supine (3.9 ± 4.2 mm Hg) to the sitting position (9.9 ± 3.0 mm Hg) [8]. In the same study our group found an increased occurrence of intrinsic contractile activity in the sitting position with 89% compared to 54% in the supine position [8]. In the present study, spontaneous low-frequency fluctuations were also present in the interstitial space and they were dependent on the posture, although their occurrence was lower for both positions for IFP compared with previous measurements in lymphatic pressure. It is reasonable to speculate that the greater lymphatic density in the lower extremities compared to the upper extremities [7] as well as increased postural lymphatic contractility [8, 9] might contribute to the interstitial fluid balance in the sitting position.

The occurrence of spontaneous low-frequency fluctuations in the interstitial space could be explained by the anatomy and physiology of the interstitium and microlymphatics. Lymphatic endothelial cells are tightly connected to the surrounding interstitial matrix by fine strands of elastic fibers [20] and tether the endothelium to adjacent collagen fibers [21]. As IFP increases and swelling of the interstitial space occurs, anchoring filaments increase the vessel lumen diameter and pull open the intercellular junctions to permit passage of fluids and particles into the lymphatic vessel. After the transfer of fluids and particles into the initial lymphatics, IFP decreases and the junctions close and prevent retrograde flow into the interstitial space [16]. Our recordings of the spontaneous low-frequency fluctuations in the interstitial space support this concept, suggesting that the interstitial space may possibly function as an extension of lymphatic vessels or vice versa [16]. To elucidate the exact mechanisms underlying our observations, further studies are required, since a major limitation of this study is that very small movement artifacts, which may escape the method used, can interfere with IFP recordings and mimic low-frequency fluctuations. The question of whether respiration-related fluctuations may be caused through small movement artifacts or transmitted via the venous-lymphatic connection is still unsolved. Future studies are also needed to investigate the influence of time in a body position on IFP in lower extremity tissue as well as diurnal changes in IFP.

In conclusion, using servonulling micropressure measurements in combination with FML, higher IFP levels were found in the sitting position, and the spontaneous IFP fluctuations noted in our subjects depended on the posture.

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## References

1 Adamson RH, Lenz JF, Zhang X, Adamson GN, Weinbaum S, Curry FE: Oncotic pressures opposing filtration across non-fenestrated rat microvessels. J Physiol 2004;557:889–907.
2 Aukland K: Distribution of body fluids: local mechanisms guarding interstitial fluid volume. J Physiol (Paris) 1984;79:395–400.
3 Guyton AC: Compliance of the interstitial space and the measurement of tissue pressure. Pflugers Arch 1972:suppl:1–20.
4 Hassan AA, Tooke JE: Mechanism of the postural vasoconstrictor response in the human foot. Clin Sci (Lond) 1988;75:379–387.
5 Crandall CG, Shibasaki M, Yen TC: Evidence that the human cutaneous venoarteriolar response is not mediated by adrenergic mechanisms. J Physiol 2002;538:599–605.
6 Rayman G, Williams SA, Gamble J, Tooke JE: A study of factors governing fluid filtration in the diabetic foot. Eur J Clin Invest 1994;24:830–836.
7 Stanton AW, Patel HS, Levick JR, Mortimer PS: Increased dermal lymphatic density in the human leg compared with the forearm. Microvasc Res 1999;57:320–328.
8 Franzeck UK, Fischer M, Costanzo U, Herrig I, Bollinger A: Effect of postural changes on human lymphatic capillary pressure of the skin. J Physiol 1996;494(pt 2):595–600.
9 Olszewski WL, Engeset A: Intrinsic contractility of prenodal lymph vessels and lymph flow in human leg. Am J Physiol 1980;239:H775–H783.

10 Noddeland H: Influence of body posture on transcapillary pressures in human subcutaneous tissue. Scand J Clin Lab Invest 1982;42:131–138.
11 Bollinger A, Jager K, Sgier F, Seglias J: Fluorescence microlymphography. Circulation 1981;64:1195–1200.
12 Spiegel M, Vesti B, Shore A, Franzeck UK, Becker F, Bollinger A: Pressure of lymphatic capillaries in human skin. Am J Physiol 1992;262:H1208–H1210.
13 Intaglietta M, Pawula RF, Tompkins WR: Pressure measurements in the mammalian microvasculature. Microvasc Res 1970;2:212–220.
14 Wiederhielm CA, Weston BV: Microvascular, lymphatic, and tissue pressures in the unanesthetized mammal. Am J Physiol 1973;225:992–996.
15 Wen S, Dorffler-Melly J, Herrig I, Schiesser M, Franzeck UK, Bollinger A: Fluctuation of skin lymphatic capillary pressure in controls and in patients with primary lymphedema. Int J Microcirc Clin Exp 1994;14:139–143.
16 Aukland K, Reed RK: Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol Rev 1993;73:1–78.
17 Wiig H, Noddeland H: Interstitial fluid pressure in human skin measured by micropuncture and wick-in-needle. Scand J Clin Lab Invest 1983;43:255–260.
18 Noddeland H, Omvik P, Lund-Johansen P, Ofstad J, Aukland K: Interstitial colloid osmotic and hydrostatic pressures in human subcutaneous tissue during early stages of heart failure. Clin Physiol 1984;4:283–297.
19 Zaugg-Vesti B, Dorffler-Melly J, Spiegel M, Wen S, Franzeck UK, Bollinger A: Lymphatic capillary pressure in patients with primary lymphedema. Microvasc Res 1993;46:128–134.
20 Gerli R, Ibba L, Fruschelli C: Ultrastructural cytochemistry of anchoring filaments of human lymphatic capillaries and their relation to elastic fibers. Lymphology 1991;24:105–112.
21 Leak LV, Burke JF: Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am J Anat 1966;118:785–809.