Can lymphatic drainage be measured non-invasively in human limbs, using plethysmography?

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ABSTRACT

There is always rapid volume restitution of the accumulated interstitial fluid after a VCP (venous congestion plethysmography) protocol. It has been suggested that backward extrapolation of the relationship between applied hydrostatic pressure and fluid filtration may give a measure of tissue $J_{vl}$ (lymph flow); if so, this could be of immense value in pathophysiological investigations. We hypothesized that the congestion pressure decrease following the VCP protocol might be the stimulus for activating the observed rapid interstitial fluid removal mechanism. We investigated this hypothesis by using a cumulative small step VCP protocol to a maximum arterial diastolic pressure, followed by a mirror image of step pressure decreases. The increases and decreases in cuff pressure produced capillary filtration capacities that were not significantly different from one another [(3.8 ± 1.0) × 10^{-3} and (3.7 ± 1.2) × 10^{-3} ml · 100 ml^{-1} · min^{-1} · mmHg^{-1} respectively]. However, we did observe a significant 3-fold increase in estimated lymph flow between the up and ‘mirror’ down protocol. Moreover, the calculated supine control value, reflecting interstitial fluid removal ($J_{vl}$), of 0.03 ± 0.03 ml · 100 ml^{-1} · min^{-1} was within the range of lymph flows in human limbs described by other workers, as was the 3-fold increase to 0.09 ± 0.03 ml · 100 ml^{-1} · min^{-1} following the release of the venous congestion. These results support the notion that strain-gauge plethysmography might provide a non-invasive means of assessing peripheral lymph flow in human limbs.

INTRODUCTION

Failure to regulate extracellular fluid volume plays a major role in the pathophysiology of diseases complicated by oedema. Increased microvascular permeability, as seen in septic shock [1,2], diabetes [3,4], nephrotic syndrome [3,5] and ischaemia/reperfusion injury [6,7], as well as altered lymphatic drainage after lymphadenectomy [8], may both contribute to oedema as a result of imbalance between tissue fluid formation and its removal. The quantitative assessment of factors influencing lymph formation in human lower limbs is dominated by the work of Olszewski on chronically cannulated lymph vessels [9–11]. Nonetheless, this technique is invasive and it also suffers the disadvantage that, since the control rate of lymph flow in the adult supine subject is small, accurate assessment necessitates lengthy collection periods. However, invasive studies on the hindlimbs of sheep have shown that sympathetic chain stimulation, which gives rise to an increase in lymph flow from the preparation, is also associated with tissue volume reduction as measured plethysmographically [12].

Key words: capillary filtration capacity, human limb, isovolumetric venous pressure, lymphatic drainage, venous congestion plethysmography.

Abbreviations: CFC, capillary filtration capacity; $J_{v}$, fluid flux; $J_{vl}$, lymph flow; $P_{a}$, arterial blood pressure; $P_{cuff}$, congestion cuff pressure; $P_{v}$, venous pressure; $P_{vi}$, isovolumetric $P_{v}$; $Q_{a}$, total calf blood flow; $\pi_{p}$, plasma colloid osmotic pressure; $V_{a}$, vascular filling; VCP, venous congestion plethysmography.

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There is always clear evidence of marked accumulation of interstitial fluid during VCP (venous congestion plethysmography). Despite this, removal of the congesting pressure is invariably followed by rapid restitution of the tissue volume, which is attributable to accumulated filtered fluid. This observation suggests that the plethysmographic technique itself might offer a non-invasive means of assessing changes in the rate of lymph removal. The theoretical examination of plethysmographic data by Michel [13] suggested a method for examining this hypothesis.

VCP protocols comprising small cumulative pressure steps enable assessment of the relationship between microvascular pressure and net fluid filtration [14]. It has been shown [14] that congestion pressures as great as mean arterial diastolic pressure can be achieved without activating the arteriolar constrictor mechanisms described by Henriksen [15], or altering limb blood flow [16]. The controlling mechanism for the sustained blood flow is believed to be endotheliually-dependent, driven by upstream metabolic demand [17]. Using this protocol, no steady state increase in measured tissue volume is observed until the $P_{\text{cuff}}$ (congestion cuff pressure) exceeds the dynamic equilibrium pressure in the supine subject. We call this pressure $P_{\text{vi}}$ (isovolumetric venous pressure). Up to this pressure the transmicrovascular (Starling) forces are in a state of dynamic equilibrium and we assume, like Michel [13], that any fluid filtering across the microvascular interface is being removed at an equivalent rate by lymphatic drainage. In his paper, Michel went on to say that “If we assume that lymph flow reaches its maximum at this level, we can make a rough estimate of its value.” [13]. Above $P_{\text{vi}}$, the linear relationship between steady-state volume change and pressure reflects the CFC (capillary filtration capacity). It is assumed that, at pressures lower than $P_{\text{vi}}$, there is a balance between fluid filtration and lymphatic drainage and, as a result, the tissue remains isovolumetric and no net flux can be measured. Michel [13] had reasoned that, since “lymph flow reaches a maximum before steady state rates of tissue swelling are observed” and went on to reason that “backward extrapolation of the relation between tissue swelling rate and $P_{\text{cuff}}$, might give a value equivalent to the lymph flow.” We used Michel’s reasoning to look for changes in the calculated values of lymph flow during a stepwise decompression protocol that we had added to the end of our normal small cumulative pressure step protocol. Using this procedure, we examined the hypothesis that the release of the congestion pressure at the end of the cumulative pressure protocol will cause an up-regulation of lymphatic drainage, giving rise to the observed rapid tissue volume restitution. If our hypothesis is upheld, the deflation protocol should show evidence of changes attributable to an up-regulation of lymphatic pumping.

**METHODS**

**Subjects**

Studies were performed on 13 young healthy volunteers (five male) aged $22 \pm 2$ years. None of the subjects were smokers and all were asked to refrain from eating or taking caffeine-containing beverages for at least 2 h prior to the study.

**Protocol**

All studies were performed on the calves of supine subjects, in a temperature-controlled environment ($24 \pm 1^\circ$C), using computer-assisted VCP [14]. The subjects lay on a tiltable bed, adjusted so that the mid-calf was at right atrial level, which was assumed to be one-third of the distance down from the sternal angle to the posterior surface.

**$P_a$ (arterial blood pressure)**

Measurements of $P_a$ were made, in triplicate, on the contralateral calf at the start of each study. The measurements were made with a Critikon Dinamap Vital Signs Monitor (type 8100; Critikon, Tampa, FL, U.S.A.), and mean values were calculated.

**VCP study protocol and analysis**

All microvascular assessments were derived from the changes in volume occurring in response to either increases or decreases in venous congestion pressure. The protocol involved application of a series of five to seven small (8–10 mmHg) cumulative congestion pressure steps, each of 5 min duration (inflation). After 5 min at the maximum pressure step, the pressure was decreased in identical steps (deflation) and the regression slope, representing fluid movement, was measured after 4 min (Figure 1A). The maximum pressure used never exceeded the subjects’ arterial diastolic pressure. Moreover, this protocol has been shown not to interfere with limb arterial blood flow [16] or to have a significant effect on either interstitial pressure or oxygenation [18]. Since illustrated descriptions of the analysis procedure have been given previously [14], a brief summary will suffice. Congestion pressures in excess of the ambient $P_a$ (venous pressure) cause a change in limb volume, attributable to $V_a$ (vascular filling). At pressures greater than the dynamic equilibrium pressure ($P_{\text{vi}}$), a slow steady-state volume change also occurs, this reflects the net increase in $f_v$ (fluid flux) at the applied congestion pressure (Figure 1B). The data analysis procedure enables differentiation between $V_a$ and $f_v$ components, as illustrated in Figure 1B [14], and was used in the analysis of the responses to both step increases and decreases in congestion pressure. Exceeding the Starling equilibrium pressure during the step pressure increases gives rise to a linear relationship between $P_{\text{cuff}}$ and $f_v$ co-ordinates (Figure 2).

The slope of this relationship represents what is frequently termed CFC (units = ml ⋅ 100 ml$^{-1}$ ⋅ min$^{-1}$).
mmHg\(^{-1}\)). This, in reality, is an index of the permeability of the whole exchange surface under examination, that is from the true capillaries, through to the post-capillary venules [14,19]. A similar relationship was observed as the P\(_{\text{cuff}}\) was decreased (Figure 2).

The intercept of the slope on the abscissa represents the Starling equilibrium pressure, or P\(_{\text{vi}}\) (Figure 2). This is the pressure that needs to be exceeded to induce net, i.e. measurable, filtration [20].

The relationship between P\(_{\text{cuff}}\) and V\(_a\) is curvilinear and the intercept with the x-axis represents P\(_{v}\) at the level of the strain gauge [14,21].

Q\(_a\) (total calf blood flow) was measured in duplicate prior to the beginning of the VCP protocol. Q\(_a\) was determined from the initial rate of volume change following a 10 s duration 80 mmHg congestion pressure step [16].

**Calculation of estimated J\(_L\) (lymph flow)**

Some of the details of the way that the J\(_L\) can be calculated have been published previously [22]. In that paper, we used the Starling equation to assess J\(_v\) across the microvascular interface into the interstitium:

\[
J_v = \text{CFC}[(P_c - P_i) - \sigma(\pi_p - \pi_i)]
\]  

(1)

where P\(_c\) and P\(_i\) are the hydrostatic pressures in the exchange vessels and the tissue respectively, and \(\pi_p\) and \(\pi_i\) are plasma and interstitial colloid oncotic pressures respectively. \(\sigma\) is the osmotic reflection coefficient to plasma proteins. For the calculation of J\(_L\) several assumptions have to be made: (i) the fluid movement through the microvascular barrier, in excess of that being voided by the lymphatics, is zero at the capillary pressure in the control supine subject [13,22]; (ii) the interstitial hydrostatic pressure is unaltered by the congestion pressure protocol used and would be of the order of −1.0 mmHg [18]; (iii) \(\pi_p\) was not significantly influenced by the application of sub-diastolic pressures [21] (a value of \(\pi_p\) of 25 mmHg is appropriate for young healthy subjects [23]); (iv) the value of 0.95 was used for the reflection coefficient of the microvascular surface of musculature, under normal conditions, that is, with no inflammation [24]; and (v) \(\pi_i\) is assumed to be 15.7 mmHg [25].

To calculate J\(_L\) using the eqn (1), the values for all five variables have to be assumed, and this was the strategy used in the previous paper [22]. In the present study, we have chosen to use the method proposed by Michel [13].
and assess \( J_{vL} \) using the equation describing the regression line for CFC determination:

\[
J_{vL} = (\text{CFC} \times P_{v}) + c
\]

(2)

where \( c \) is the intercept on the ordinate of the linear regression equation for CFC in each subject. Since we assume that all Starling forces are in balance until \( P_{vL} \) is exceeded, we calculated \( J_{vL} \) by extrapolating the CFC slope to \( P_{v} \) in the calf in each subject. The values we obtained are negative and represent the rate at which the steady-state ‘insensible’ filtration by the supine control subject is being voided by the lymphatics. Insensible, because it is being voided from the tissue at the same rate as it is being formed, largely by lymphatic drainage. In Figure 2, the values of \( J_{vL} \) calculated for one subject using eqn (2) are depicted by symbols with a superimposed plus sign during both the inflation and deflation steps.

**Statistics**

All data were normally distributed (according to Kolomogorov–Smirnov test), and paired assessments were made using Student’s \( t \) test. The tests were performed using SigmaStat (Jandel Scientific, Erkrath, Germany). Significance was assumed when \( P < 0.05 \). Values are means ± S.D.

**Ethical approval**

Ethical approval was obtained from the Ethical Committee of Charing Cross and Westminster Medical School. The procedures, which were wholly non-invasive, were explained to the subjects, who gave informed consent.

**RESULTS**

\( Q_s, P_a \) and \( P_v \) and calf circumference at the level of the strain gauge are shown in the Table 1.

**CFC and \( P_{vL} \)**

The studies on 13 subjects showed that the mean value of CFC obtained during the increase series (inflation, \( 3.8 \pm 1.0 \) × 10⁻³ ml · 100 ml⁻¹ · min⁻¹ · mmHg⁻¹]) was not significantly different (\( P = 0.73 \)) from that obtained during the decrease steps (deflation, \( 3.7 \pm 1.2 \) × 10⁻³ ml · 100 ml⁻¹ · min⁻¹ · mmHg⁻¹]). Moreover, the mean correlation coefficients for these slopes, which were based on 4–8 data points for the inflation series and 3–6 for the deflation, were 0.94 ± 0.04 and 0.90 ± 0.07 respectively. In order to test that fluid movement was sustained without decrement, during deflation, the steps were held for 10, rather than 5, min in four of the 13 studies. The values reflecting fluid movement (\( J_v \)) were measured at both 5 and 10 min. The resulting values of CFC [(3.6 ± 0.1) × 10⁻³ and (4.0 ± 0.7) × 10⁻³ ml · 100 ml⁻¹ · min⁻¹ · mmHg⁻¹] obtained at 5 and 10 min respectively) were not significantly different from one another (\( P = 0.3 \).

Although the values of CFC did not differ from one another during the increases and decreases in \( P_{vL} \), those of the intercept on the abscissa (\( P_{vL} \)) did (Figure 2). The mean value during the inflation (16.4 ± 5.6 mmHg) was significantly lower than that obtained during the deflation (33.4 ± 7.6 mmHg; \( P < 0.0001 \), as determined by a paired Student \( t \) test; Figure 3A). Thus, although the slopes had not altered, the deflation slopes exhibited a parallel shift to the right on the abscissa.

**Estimation of \( J_{vL} \)**

The mean value of \( J_{vL} \) obtained by extrapolation of the CFC regression slope to \( P_v \) in each study during the inflation procedure using eqn (2) was 0.03 ± 0.03 ml · 100 ml⁻¹ · min⁻¹. The mean value obtained during the deflation procedure, 0.09 ± 0.03 ml · 100 ml⁻¹ · min⁻¹, was 3-fold greater than that obtained in response to the preceding inflation sequence, the differences being highly significant (\( P < 0.0001 \), as determined by a paired Student \( t \) test). The data obtained using eqn (2) are shown in Figure 3(B).

**DISCUSSION**

Rapid restitution of accumulated interstitial fluid, following the removal of venous \( P_{vL} \) challenges, is a common observation in congestion plethysmography studies. We examined the possibility that the rapid change might reflect an increased rate of interstitial fluid removal largely by up-regulation of lymphatic pumping. We used a protocol comprising cumulative small pressure step increases, followed by a mirror image of pressure decreases. This protocol was derived to examine the suggestion that extrapolation of the regression line (CFC) to the value of ambient \( P_v \) in the supine control calf would give the rate of filtered fluid normally voided to the lymphatics (\( J_{vL} \)) in the supine subject [13]. If this is the case and \( J_{vL} \) is up-regulated by cuff deflation, then we might expect an increase in the value of \( J_{vL} \) in response to this procedure (Figure 2).

We found that the pressure decreases were associated with a 3-fold increase in the value \( J_{vL} \), suggesting a marked
Plethysmographic estimation of lymph flow

Changes of $P_{vi}$ and $J_{vL}$ during the inflation and deflation protocol

(A) Individual and mean $\pm$ S.D. $P_{vi}$ values obtained during the inflation and deflation protocol are shown. $P_{vi}$ was found to be increased in all subjects, and the mean values were significantly higher in response to the deflation protocol.

(B) Estimated $J_{vL}$ data obtained from 13 studies. All individual values and means $\pm$ S.D. are shown. *$P < 0.001$ compared with inflation, as determined by a paired Student t test.

Although we have focused on the up-regulation of lymphatic drainage to account for the rapid volume restitution, it would be inappropriate not to consider the possibility that upstream filtration is matched by a downstream sustained reabsorption. However, there is little contemporary evidence to support such a reabsorptive mechanism in all but specialized tissues, including the renal cortex, lymph nodes and intestinal mucosa. Any reabsorption into the microvasculature that does occur will rapidly give rise to a counter-acting increase in the ablumenal interstitial oncotic pressure, as discussed by Levick [24]. Should this happen and a significant volume of water be reabsorbed, the volume response during the pressure decrease would be alinear, probably exponential, reaching the asymptote when the ablumenal colloid concentration approached the value of $1 - \sigma$ [26]. Since there was no evidence of alinearity, particularly during the 10 min pressure step decreases, it suggests that any tissue fluid reabsorption that does occur represents a very small part of the measured volume decrease. We feel that this increases the likelihood that the protocol used enables the non-invasive assessment of $J_{vL}$. That post-capillary reabsorption may transiently be facilitated by cyclical increases and decreases in precapillary resistance, vasomotion or volumotion was examined by Intaglietta [27]. Indeed, the observation that profound cyclical variations in tissue volume are observed in patients with hypovolaemic shock [28] and ischaemia/reperfusion injury [29] supports this theory. However, there was no evidence of tissue volumotion during either the inflation or deflation stages in the present studies. Moreover, there was no significant difference in the slopes for CFC either during inflation and deflation or between the values for CFC obtained at 5 and 10 min during deflation in a limited number of studies. We believe these data suggest that downstream reabsorption plays a very limited role in the observed rapid volume restitution.

The mean value of $J_{vL}$ obtained in response to the step increases in $P_{cuff}$ ($0.03 \pm 0.03$ ml·100 ml$^{-1}$·min$^{-1}$) was at the lower end of the range described by Michel [13]. It was also well within the range of values recorded by Olszewski et al. [10] from studies on chronically cannulated lower limb lymphatics. Although Olszewski et al. [10] described a decrease in $J_{vL}$ during venous congestion, the protocol involved the application of a single sustained 50 mmHg pressure step. Such a step should have caused a decrease in arterial inflow and, therefore, presumably interstitial fluid formation through activation of the mechanism described by Henriksen and Sejrsen [15]. Moreover, in the present studies, the parallel shift in the regression slope obtained in response to the stepwise decreases in congestion pressure gave rise to a 3-fold increase in the value $J_{vL}$ ($0.09 \pm 0.03$ ml·100 ml$^{-1}$·min$^{-1}$). If, as in our previous study [22], we calculate the value of $J_{vL}$ obtained from the Starling equation [eqn (1)], using the value of CFC for each subject and assuming the values for the five variables given in the Methods section, we get $0.05 \pm 0.02$ ml·100 ml$^{-1}$·min$^{-1}$.

Comparison of the values obtained using eqns (1) and
head-up tilt, the value of intravascular oncotic pressure was induced by a passive increase. It was demonstrated that, when an increase in local oncotic pressure exceeded \([13, 20, 30, 31]\). Indeed, in earlier studies \([19]\), workers showed a 3-fold increase in the value of \(J_{vl}\), which may imply a marked increase in the rate of lymphatic drainage from the limb \([13]\). That such changes are transient can be deduced from control observations in studies where two consecutive cumulative congestion protocols were separated by a rest interval of 30 min. In these studies, the data showed no differences in CFC, \(P_v\), and, therefore, the value of \(J_{vl}\). This implies that the tissue had regained its equilibrium state during the 30 min rest phase \([33]\).

More recently, we \([22]\) showed that, during a two-stage cumulative pressure step protocol, the first step increase, following a transient decrease in congestion pressure to 30 mmHg, resulted in a significantly decreased value of filtration. However, during subsequent step increases, the filtration responses progressively approached the pre-deflation values. We believed these data showed that, although the pressure decrease had caused transient up-regulation of the lymphatic pumping mechanism, this was partly reversed by the following stepped pressure increases. These suggestions are in keeping with the notion that the initiation of the lymphatic pumping mechanism is probably myogenic and that the stimulus for its initiation might be the suction force generated in the terminal lymphatics by the decrease in volume of adjacent veins following the \(P_{cuff}\) decrease \([32]\).

The validation of these, probably simplistic, explanations requires further investigations, possibly using similar protocols to those described in the present study. For example, bilateral assessments of the arms of patients after unilateral lymphadenectomy for breast cancer, following which only lymphatic drainage from the untreated side would remain unimpaired. In this context, it is tempting to speculate whether the plethysmographically measured decrease in tissue volume measured by McGeown et al. \([12]\) in response to sympathetic chain stimulation in sheep, actually reflected the increased lymphatic drainage they measured, rather than the “net uptake of fluid by the blood vessels” they attributed it to. In future work, our hypothesis could also be examined by using the present protocol in conjunction with the administration of calcium-channel blocking agents, such as nifedipine and diltiazem, which have been shown to inhibit lymphatic pumping at concentrations within the range used clinically \([34]\).
In conclusion, the possibility that further studies might lead to a non-invasive method of assessing peripheral lymphatic function, which would be of great clinical utility, would, we feel, make such studies a worthwhile objective.

REFERENCES


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