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

Andreas BAUER*, Frank CHRIST* and John GAMBLE†
*Clinic for Anaesthesiology, Ludwig-Maximilians University Munich, 81377 Munich, Germany, and †School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, U.K.

A B S T R A C T

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 [$(3.8 \pm 1.0) \times 10^{-3}$ and $(3.7 \pm 1.2) \times 10^{-3}$ 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; $\dot{Q}_a$, total calf blood flow; π_p, plasma colloid osmotic pressure; V_a, vascular filling; VCP, venous congestion plethysmography.

Correspondence: Dr John Gamble (e-mail john.gamble@virgin.net).

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 endothelially-dependent, driven by upstream metabolic demand [17]. Using this protocol, no steady state increase in measured tissue volume is observed until the P_{cuff} (congestion cuff pressure) exceeds the dynamic equilibrium pressure in the supine subject. We call this pressure P_{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_{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_{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_{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 decongestion 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 ± 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_v (venous pressure) cause a change in limb volume, attributable to V_a (vascular filling). At pressures greater than the dynamic equilibrium pressure (P_{vi}), a slow steady-state volume change also occurs, this reflects the net increase in J_v (fluid flux) at the applied congestion pressure (Figure 1B). The data analysis procedure enables differentiation between V_a and J_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_{cuff} and J_v co-ordinates (Figure 2).

The slope of this relationship represents what is frequently termed CFC (units $= ml \cdot 100\ ml^{-1} \cdot min^{-1} \cdot$

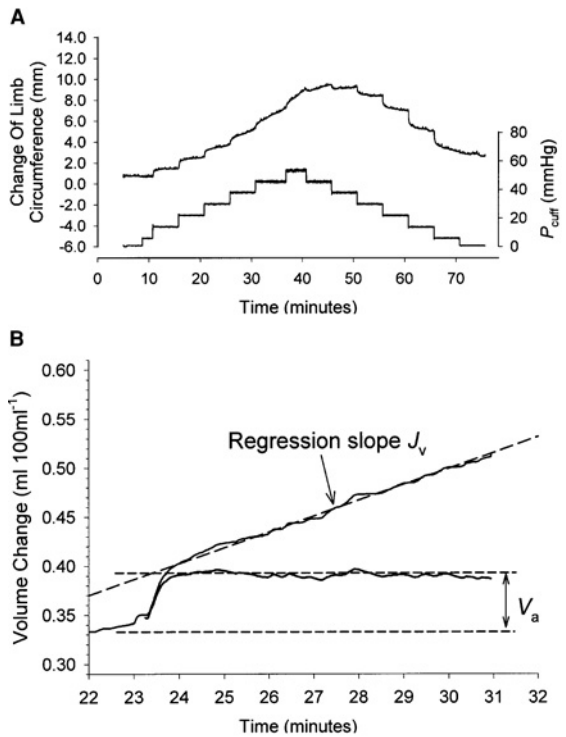

Figure 1 VCP pressure protocol and analysis procedure

(A) A record from one study showing the relationship between limb volume change (upper trace) and the step increases or decreases in venous P_{cuff} (lower trace) that were applied during the course of each study protocol. (B) Analysis routine for differentiation between the J_v component and the total volume response (upper trace) to a P_{cuff} step increase of 8 mmHg to 50 mmHg. The regression slope, representing J_v, is based upon the rate of volume changed obtained 3 min after the imposition of the small cumulative pressure step. This analysis routine was applied to the responses to both step increases and decreases in pressure.

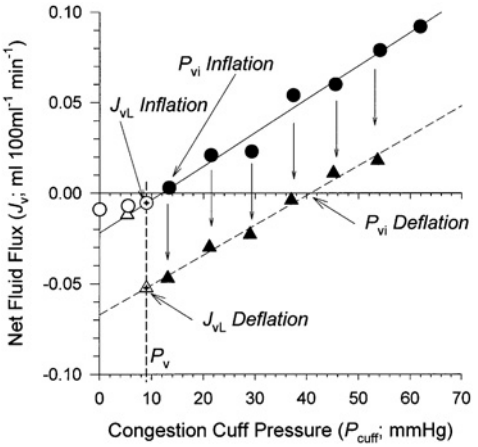

Figure 2 Relationship between net J_v and P_{cuff} obtained in the depicted study record in Figure 1(A) from one subject

The circles indicate the net J_v in response to step increases in pressure (inflation). It can be seen that, at pressures greater than P_{vi}, there is a net increase in J_v (●). The triangles depict the values of J_v obtained as P_{cuff} was decreased (deflation) stepwise. It is evident that, as the pressure is lowered, the value of net filtration is less than that obtained at the corresponding pressure during the part of the protocol when pressure was increased. The regression slopes are based on the co-ordinates depicted by the filled symbols. The vertical dotted line represents the value of P_v in the calf of this subject. The symbols with a superimposed '+' represent the values of J_{vL} calculated using eqn (2) at the resting P_v in this subject.

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_{cuff} was decreased (Figure 2).

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

The relationship between P_{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].

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

Calculation of estimated J_{vL} (lymph flow)

Some of the details of the way that the J_{vL} 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:

$$J_v = CFC[(P_c - P_i) - \sigma(\pi_p - \pi_i)] \quad (1)$$

where P_c and P_i are the hydrostatic pressures in the exchange vessels and the tissue respectively, and π_p and π_i are plasma and interstitial colloid osmotic (oncotic) pressures respectively. σ is the osmotic reflection coefficient to plasma proteins. For the calculation of J_{vL} 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) π_p was not significantly influenced by the application of sub-diastolic pressures [21] (a value of π_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) π_i is assumed to be 15.7 mmHg [25].

To calculate J_{vL} 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]

Table 1 Plethysmographic data and P_a

As a result of movement artefacts, $\dot{Q}_a$ could only be determined in nine of the subjects.

	Mean	S.D.	*n*
Mean P_a (mmHg)	78.10	8.50	13
Calf circumference (mm)	367.50	25.50	13
Calf P_v (mmHg)	7.70	2.90	13
$\dot{Q}_a$ (ml · 100 ml^{-1} · min^{-1})	2.45	1.31	9

and assess J_{vL} using the equation describing the regression line for CFC determination:

$$J_{vL} = (\text{CFC} \times P_v) + c \quad (2)$$

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_{vi} 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

$\dot{Q}_a$, P_a and P_v and calf circumference at the level of the strain gauge are shown in the Table 1.

CFC and P_{vi}

The studies on 13 subjects showed that the mean value of CFC obtained during the increase series [inflation, $(3.8 \pm 1.0) \times 10^{-3}$ ml · 100 ml^{-1} · min^{-1} · mmHg^{-1}] was not significantly different ($P = 0.73$) from that obtained during the decrease steps [deflation, $(3.7 \pm 1.2) \times 10^{-3}$ 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 [$(3.6 \pm 0.1) \times 10^{-3}$ and $(4.0 \pm 0.7) \times 10^{-3}$ 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_{cuff}, those of the intercept on the abscissa (P_{vi}) 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_{cuff} 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

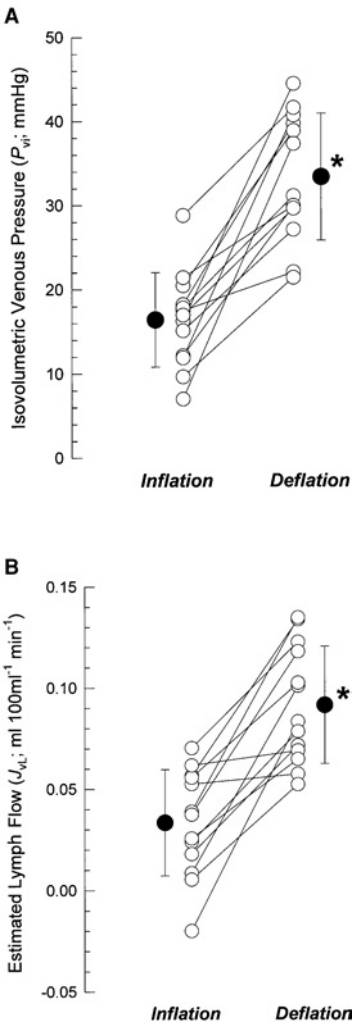

Figure 3 Changes of P_{vi} and J_{vL} during the inflation and deflation protocol

(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.

increase in the rate at which fluid was being removed from the tissue. In addition, we noted that the direction of P_{cuff} change had no influence on the slope of the relationship between J_v and P_{cuff} (CFC). These data, in conjunction with the similarity of the correlation coefficients for the CFC regression slopes, suggests that no events, other than pressure-related changes in fluid movement (J_v), occurred during the stepwise increments or decrements in P_{cuff}.

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 ± 0.03 ml $\cdot$ 100 ml^{-1} $\cdot$ 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 ± 0.03 ml $\cdot$ 100 ml^{-1} $\cdot$ 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 ± 0.02 ml $\cdot$ 100 ml^{-1} $\cdot$ min^{-1}. Comparison of the values obtained using eqns (1) and

(2) shows that the differences are small but significant ($P = 0.047$, as determined using a paired Student t test). However, since five of the variables had to be assumed for the Starling equation [eqn (1)] calculation and, by contrast, only the value of pre-post capillary resistance ratio needed to be assumed for the calculation of J_{vL} using eqn (2), we feel that this gives more weight to the use of eqn (2). In addition, the deflation protocol-induced changes in the Starling forces will be an integral part of the tissue's own response to the P_{cuff} change, expressed in the alterations in J_v in the CFC regression slope. Thus, using eqn (2), no further adjustments need to be made. Overall, we feel that the similarities obtained using these two types of calculation support the hypothesis.

Olszewski et al. [10] showed that, in studies on chronically cannulated lymph vessels, the J_{vL} rate was doubled after a sudden decrease in venous congestion pressure. However, we believe that one disadvantage of the protocol in this study [10] is that, unlike the cumulative step protocol, which is not associated with a decrease in blood flow [16], the application of a sustained 50 mmHg P_{cuff} step would be expected to invoke an increase in pre-capillary resistance [15], as stated previously. As a consequence, their protocol may well give rise to a decrease in the rate of fluid filtration, secondary to the decrease in capillary pressure after the step pressure had been applied. Furthermore, the subsequent removal of the congestion pressure step might be expected to give rise to post-occlusion hyperaemia, thereby exacerbating the post-occlusion J_{vL} seen by these workers.

There is a tacit assumption that J_{vL} reaches a maximum before steady rates of tissue swelling are observed [13]. During the cumulative pressure step protocol, no net change in tissue fluid formation is observed until P_{vi} is exceeded [13,20,30,31]. Indeed, in earlier studies [19], it was demonstrated that, when an increase in local intravascular oncotic pressure was induced by a passive head-up tilt, the value P_{vi} increased by an amount related to the change in local oncotic pressure in the dependent limb. In these studies [19], the values of CFC before and after the imposition of the tilt were the same, but the slopes had undergone a parallel shift not dissimilar to that observed in the present studies. However, the P_{vi} intercept during the pressure decrease in the present study, although also reflecting an isovolumetric state, i.e. an equilibrium state, is, by definition, the point where the regression line transects the ordinate. However, as the pressure was lowered further, the tissue volume continued to decrease, as illustrated in Figure 2, and volume decrease occurred in a steady-state manner for the duration of each step. The decreases in volume were by mechanisms distinct from the tissue's compliance function, since this was automatically subtracted during the analysis routine [14]. We believe that, although the parallel shift in the relationship between J_v and P_{cuff} seen in the tilt studies reflected changes in local plasma oncotic pressure, the shift in the present study resulted largely from changes in interstitial forces attributable to lymphatic pump up-regulation. Our reasoning for this is that up-regulation of lymphatic drainage would change the interstitial forces in the peri-lymphatic spaces, thereby providing a driving force for the increased rate of interstitial fluid removal [32].

The primary objective of the present study was to investigate the rapid limb volume restitution following the release of venous congestion pressures. Our data 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_{vi} 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

1 Christ, F., Gamble, J., Gartside, I. B. and Kox, W. J. (1998) Increased microvascular water permeability in patients with septic shock, assessed with venous congestion plethysmography (VCP). Intensive Care Med. **24**, 18–27
2 Rackow, E. C. and Astiz, M. E. (1991) Pathophysiology and treatment of septic shock. JAMA, J. Am. Med. Assoc. **266**, 548–554
3 Jaap, A. J., Shore, A. C., Gartside, I. B., Gamble, J. and Tooke, J. E. (1993) Increased microvascular fluid permeability in young Type 1 (insulin-dependent) diabetic patients. Diabetologia **36**, 648–652
4 Oomen, P. H. M., Jager, J., Hoogenberg, K., Dullaart, R. P. F., Reitsma, W. D. and Smit, A. J. (1999) Capillary permeability is increased in normo- and microalbuminuric type I diabetic patients: amelioration by ACE-inhibition. Eur. J. Clin. Invest. **29**, 1035–1040
5 Lewis, D. M., Tooke, J. E., Beaman, M., Gamble, J. and Shore, A. C. (1998) Peripheral microvascular parameters in the nephrotic syndrome. Kidney Int. **54**, 1261–1266
6 Bertuglia, S., Colantuoni, A. and Intaglietta, M. (1993) Effect of leukocyte adhesion and microvascular permeability on capillary perfusion during ischemia-reperfusion injury in hamster cheek pouch. Int. J. Microcirc. Clin. Exp. **13**, 13–26
7 Christ, F., Gamble, J., Raithel, P., Steckmeier, B. and Messmer, K. (1999) Preoperative changes in fluid filtration capacity in patients undergoing vascular surgery. Anaesthesist **48**, 9–18
8 Pain, S. J. and Purushotham, A. D. (2000) Lymphoedema following surgery for breast cancer. Br. J. Surg. **87**, 1128–1141
9 Engeset, A., Olszewski, W., Jaeger, P. M., Sokolowski, J. and Theodorsen, L. (1977) Twenty-four hour variation in flow and composition of leg lymph in normal men. Acta Physiol. Scand. **99**, 140–148
10 Olszewski, W., Engeset, A., Jaeger, P. M., Sokolowski, J. and Theodorsen, L. (1977) Flow and composition of leg lymph in normal men during venous stasis, muscular activity and local hyperthermia. Acta Physiol. Scand. **99**, 149–155
11 Olszewski, W. L. (1977) Collection and physiological measurements of peripheral lymph and interstitial fluid in man. Lymphology **10**, 137–145
12 McGeown, J. G., McHale, N. G. and Thornbury, K. D. (1987) The effect of electrical stimulation of the sympathetic chain on peripheral lymph flow in the anaesthetized sheep. J. Physiol. (Cambridge, U.K.) **393**, 123–133
13 Michel, C. C. (1989) Microvascular permeability, venous stasis and oedema. Int. Angiol. **8**, 9–13
14 Gamble, J., Gartside, I. B. and Christ, F. (1993) A reassessment of mercury in silastic strain gauge plethysmography for microvascular permeability assessment in man. J. Physiol. (Cambridge, U.K.) **464**, 407–422
15 Henriksen, O. and Sejrsen, P. (1977) Local reflex in microcirculation in human skeletal muscle. Acta Physiol. Scand. **99**, 19–26
16 Gamble, J., Christ, F. and Gartside, I. B. (1998) Human calf precapillary resistance decreases in response to small cumulative increases in venous congestion pressure. J. Physiol. (Cambridge, U.K.) **507**, 611–617
17 Cohen, K. D., Berg, B. R. and Sarelius, I. H. (2000) Remote arteriolar dilations in response to muscle contraction under capillaries. Am. J. Physiol. Heart Circ. Physiol. **278**, H1916–H1923
18 Christ, F., Dellian, M., Goetz, A. E., Gamble, J. and Messmer, K. (1997) Changes in subcutaneous interstitial fluid pressure, tissue oxygenation, and skin red cell flux during venous congestion plethysmography in men. Microcirculation **4**, 75–81
19 Gamble, J., Christ, F. and Gartside, I. B. (1997) The effect of passive tilting on microvascular parameters in the human calf: a strain gauge plethysmography study. J. Physiol. (Cambridge, U.K.) **498**, 541–552
20 Pappenheimer, J. R. and Soto-Rivera, A. (1948) Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. Am. J. Physiol. **152**, 471–491
21 Christ, F., Gamble, J., Baschnegger, H. and Gartside, I. B. (1997) Relationship between venous pressure and tissue volume during venous congestion plethysmography in man. J. Physiol. (Cambridge, U.K.) **503**, 463–467
22 Bauer, A., Bruegger, D., Gamble, J. and Christ, F. (2002) Influence of different cuff inflation protocols on capillary filtration capacity in human calves: a congestion plethysmography study. J. Physiol. (Cambridge, U.K.) **543**, 1025–1031
23 Rayman, G., Williams, S. A., Gamble, J. and Tooke, J. E. (1994) A study of factors governing fluid filtration in the diabetic foot. Eur. J. Clin. Invest. **24**, 830–836
24 Levick, J. R. (1991) Capillary filtration-absorption balance reconsidered in light of dynamic extravascular factors. Exp. Physiol. **76**, 825–857
25 Bates, D. O., Levick, J. R. and Mortimer, P. S. (1993) Change in macromolecular composition of interstitial fluid from swollen arms after breast cancer treatment, and its implication. Clin. Sci. **86**, 737–746
26 Hu, X., Adamson, R. H., Liu, B., Curry, F. E. and Weinbaum, S. (2000) Starling forces that oppose filtration after tissue oncotic pressure is increased. Am. J. Physiol. Heart Circ. Physiol. **279**, H1724–H1736
27 Intaglietta, M. (1981) Vasomotor activity, time-dependent fluid exchange and tissue pressure. Microvasc. Res. **21**, 153–164
28 Bethell, D. B., Gamble, J., Loc, P. P. et al. (2001) Noninvasive measurement of microvascular leakage in patients with Dengue hemorrhagic fever. Clin. Infect. Dis. **32**, 243–253
29 Christ, F., Moser, C. M., Niklas, M. et al. (1995) Prevalence of cyclic changes in limb volume (volumotion) of male patients with knee injury and the effects of ischemia/reperfusion due to tourniquet. Int. J. Microcirc. Clin. Exp. **15**, 14–20
30 Drake, R. E., Smith, J. H. and Gabel, J. C. (1980) Estimation of the filtration coefficient in intact dog lungs. Am. J. Physiol. **238**, H430–H438
31 Gamble, J., Smaje, L. H. and Spencer, P. D. (1988) Filtration coefficient and osmotic reflection coefficient to albumin in rabbit submandibular gland capillaries. J. Physiol. (Cambridge, U.K.) **398**, 15–32
32 Aukland, K. and Reed, R. K. (1993) Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol. Rev. **73**, 1–78
33 Gamble, J., Grewal, P. S. and Gartside, I. B. (2000) Vitamin C modifies the cardiovascular and microvascular responses to cigarette smoke inhalation in man. Clin. Sci. **98**, 455–460
34 Atchison, D. J. and Johnston, M. G. (1997) Role of extra- and intracellular Ca^{2+} in the lymphatic myogenic response. Am. J. Physiol. **272**, R326–R333

Received 22 September 2003/20 January 2004; accepted 5 February 2004
Published as Immediate Publication 5 February 2004, DOI 10.1042/CS20030314