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# Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia

ANDREW HASKELL, ETHAN R. NADEL, NINA S. STACHENFELD,  
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**Haskell, Andrew, Ethan R. Nadel, Nina S. Stachenfeld, Kei Nagashima, and Gary W. Mack.** Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. *J. Appl. Physiol.* 83(2): 407–413, 1997.—To test the hypotheses that plasma volume (PV) expansion 24 h after intense exercise is associated with reduced transcapillary escape rate of albumin ( $TER_{alb}$ ) and that local changes in transcapillary forces in the previously active tissues favor retention of protein in the vascular space, we measured PV,  $TER_{alb}$ , plasma colloid osmotic pressure ( $COP_p$ ), interstitial fluid hydrostatic pressure (Pi), and colloid osmotic pressure in leg muscle and skin and capillary filtration coefficient (CFC) in the arm and leg in seven men and women before and 24 h after intense upright cycle ergometer exercise. Exercise expanded PV by 6.4% at 24 h ( $43.9 \pm 0.8$  to  $46.8 \pm 1.2$  ml/kg,  $P < 0.05$ ) and decreased total protein concentration ( $6.5 \pm 0.1$  to  $6.3 \pm 0.1$  g/dl,  $P < 0.05$ ) and  $COP_p$  ( $26.1 \pm 0.8$  to  $24.3 \pm 0.9$  mmHg,  $P < 0.05$ ), although plasma albumin concentration was unchanged.  $TER_{alb}$  tended to decline ( $8.4 \pm 0.5$  to  $6.5 \pm 0.7\%/h$ ,  $P = 0.11$ ) and was correlated with the increase in PV ( $r = -0.69$ ,  $P < 0.05$ ). CFC increased in the leg ( $3.2 \pm 0.2$  to  $4.3 \pm 0.5$   $\mu\text{l} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ ,  $P < 0.05$ ), and Pi showed a trend to increase in the leg muscle ( $2.8 \pm 0.7$  to  $3.8 \pm 0.3$  mmHg,  $P = 0.08$ ). These data demonstrate that  $TER_{alb}$  is associated with PV regulation and that local transcapillary forces in the leg muscle may favor retention of albumin in the vascular space after exercise.

capillary filtration coefficient; Evans blue dye; interstitial fluid colloid osmotic pressure; interstitial fluid hydrostatic pressure; plasma volume

PLASMA VOLUME (PV) expansion is a well-described consequence of endurance exercise training (5–7). Plasma albumin content expansion has been hypothesized to facilitate PV expansion through albumin's colloid osmotic properties (5, 6). Convertino et al. (5) demonstrated a progressive expansion of plasma albumin content during 3 days of endurance exercise training, whereas Gillen et al. (6, 7) showed that albumin content expands by 1 h after exercise to the level it maintains for the next 40 h. An expansion of plasma albumin content without preceding exercise, however, does not lead to prolonged PV expansion (31). Therefore, conditions must favor the retention or restitution of albumin in the vascular space for up to 40 h after exercise.

Mechanisms that regulate plasma albumin content are the transcapillary escape rate of albumin ( $TER_{alb}$ ), lymphatic albumin return, and the albumin synthesis-to-degradation ratio.  $TER_{alb}$  measures the whole body rate at which albumin leaves the vascular space. It is controlled by macrovascular and microvascular factors

including plasma albumin concentration, plasma atrial natriuretic peptide (ANP) concentration, capillary filtration coefficient (CFC), capillary hydrostatic pressure, interstitial fluid hydrostatic pressure (Pi), interstitial fluid colloid osmotic pressure ( $COP_i$ ), and interstitial fluid albumin concentration ( $[Alb]_i$ ) (2, 16, 24, 25, 30, 31, 34). Pi and the transcapillary colloid osmotic pressure gradient are increased in previously active muscle within 14 min after intense exercise (16), but these microcirculatory forces remain to be characterized 24 h after exercise.

The purpose of this study was to investigate the contribution of reduced  $TER_{alb}$  to the general process of exercise-induced hypervolemia. In addition, we characterized the local microcirculatory forces, in the previously active muscle and overlying skin, that govern albumin flux with the purpose of correlating these forces with changes in  $TER_{alb}$ . We hypothesized that PV expansion 24 h after exercise would be associated with a decrease in  $TER_{alb}$ . We further hypothesized that Pi would increase and  $COP_i$  would decrease in the previously active muscle.

## METHODS

**Subjects.** Seven men and two women (age  $29 \pm 2$  yr, body weight  $71.9 \pm 2.9$  kg) gave informed consent to participate in this protocol, which was approved by the Yale University School of Medicine Human Investigation Committee. Subjects had no history of cardiovascular or renal disease and were cleared for intense exercise by a physician. All testing on the women was completed during the early follicular phase of their menstrual cycle (*days 3–7*) to control for the effects of cyclic hormonal changes on transcapillary fluid dynamics (19, 32, 33). Before participation, subjects were familiarized with the testing environment during a separate orientation session, and each underwent a standard test of maximum oxygen consumption ( $\dot{V}O_{2max}$ ) on an upright bicycle ergometer.  $\dot{V}O_{2max}$  ranged from 33.0 to 56.6  $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  with a mean of  $42.4 \pm 2.3$   $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ .

Each subject was tested on 3 separate days: 2 data collection days and 1 exercise day. Data-collection days were timed such that one immediately followed the exercise day (postexercise), and the other was separated from the exercise day by at least 1 wk (control). The order of testing was randomized such that four subjects completed the control day before the postexercise day, and five subjects completed the opposite sequence. Subjects were asked to refrain from heavy physical activity for 2 days before any testing and from caffeine and alcohol for 1 day before testing. Subjects ate and drank only the food and beverage we provided, starting 15 h before testing (breakfast: 380 kcal, 2 g fat, 87 g carbohydrate, 7 g protein, 200 mg  $\text{Na}^+$ ; lunch: 940 kcal, 33 g fat, 111 g carbohydrate, 46 g protein, 1,800 mg  $\text{Na}^+$ ; dinner: 1,400 kcal, 35 g fat, 225 g carbohydrate, 48 g protein, 1,820 mg  $\text{Na}^+$ ),

which included 1 liter of water the night before each testing day, with additional water intake at home allowed ad libitum.

**Data-collection days.** The protocol on control and postexercise data-collection days was identical (Fig. 1). Except for brief periods of standing during urine collection, subjects rested supine in an environmental chamber ( $27 \pm 0.1^\circ\text{C}$ ,  $<40\%$  relative humidity). Supine posture was chosen for data-collection days so that measurement of the effects of exercise on transcappillary forces would not be confounded by the effects of postural compensatory mechanisms (17, 28). Additionally, supine posture allows direct comparison of CFC data collected on the upper and lower extremities without regard to the effect of posture on capillary hydrostatic pressure (28). Subjects hydrated with 7 ml/kg water over 10 min, after which catheter and strain-gauge placement took place. Measurements of CFC in the arm and leg,  $P_i$  in the leg muscle and skin,  $\text{COP}_i$  in the leg muscle and skin, and  $[\text{Alb}]_i$  in the leg muscle and skin were made from 10:30 AM to 12:00 PM, during which time heart rate and blood pressure were recorded every 5 min with an automated blood pressure cuff (STBP-780, Colin). Measurements of hematocrit, hemoglobin, plasma total protein concentration, plasma albumin concentration ( $[\text{Alb}]_p$ ), plasma osmolality, plasma colloid osmotic pressure ( $\text{COP}_p$ ), ANP, PV, and  $\text{TER}_{\text{alb}}$  were made from 12:00 PM to 1:15 PM. Blood samples were taken without stasis.

CFC was measured in the left forearm and leg by using venous occlusion plethysmography by relating the rate of change in limb girth to measured venous pressure on the assumption that increase in limb girth after cessation of venous filling is attributable to capillary fluid extravasation (10, 11, 29). Cuff pressures of 20 mmHg for 7 min, 30 mmHg for 8 min, and 40 mmHg for 9 min were applied, thereby

allowing for 4 min of data contributable to fluid extravasation after cessation of vascular filling (29). The sequence was repeated, and the order of cuff pressure application during each sequence was randomized. Mercury in Silastic strain gauges (Parks Medical Electronics) were applied over the left forearm and calf at the estimated point of maximal circumference and tensioned to 20 g. Strain-gauge calibration was performed on a hard plastic cylinder of approximately the same diameter as the subject's limb. Occlusion cuffs were placed around the left arm and thigh and covered with metal restraining bands. Care was taken to avoid influencing circulation to the limbs with the deflated cuffs. Venous pressure was measured by attaching left forearm and leg indwelling venous catheters (18 g; Jelco) to pressure transducers (P23XL, Visso-Spectramed), which were positioned such that zero pressure corresponded to the height of the respective catheter tip. Pressure transducers were calibrated by using a water-filled manometer. The left arm was hung with a foam sling around the hand, and with support at the elbow, so that the forearm was horizontal and at heart level without disturbing the strain gauge. The leg was similarly supported at the knee and ankle. Recordings of venous pressure and strain-gauge length were recorded twice per second by computer.

$P_i$  was measured in the right vastus lateralis muscle and overlying subcutaneous tissue by using the slit catheter and wick catheter techniques, respectively (1, 2, 18, 21). These techniques measure hydrostatic pressure in free interstitial fluid at the end of a fluid-filled column connected to a pressure transducer (2, 18). Noddeland et al. (18) demonstrated duplicate measurements in thoracic subcutaneous tissue in eight and seven humans by using the wick catheter technique, resulting in mean  $P_i$  of  $-0.2 \pm 0.2$  and  $-0.6 \pm 0.4$  [not significant (NS)], which agree well with simultaneous measurements made by the wick-in-needle technique. Rapid dynamic measurement of  $P_i$  has been made by slit catheter in the human leg muscle and subcutaneous tissue during lower body negative pressure (1). Time control studies on four human subjects in our laboratory show the measurement of  $P_i$  by wick and slit catheter to be stable for up to 3 h with average standard deviation over time of 0.48 mmHg and between-subject variation of  $<9\%$ . Under sterile conditions and local anesthesia ( $\approx 0.6$  ml 1% lidocaine/site), 16-gauge catheter insertion units (Jelco) were inserted into the appropriate tissue spaces and the plastic sheaths were advanced 1–2 cm. The metal needles were removed from the sheaths, and the slit catheter and wick catheter, filled with sterile saline, were advanced into the muscle and subcutaneous tissue, respectively. The plastic sheaths were removed over the slit and wick catheter tubing, the catheters were held in place with a clear plastic dressing (Tegaderm, 3M), and the free ends were connected to pressure transducers (P23XL, Visso-Spectramed) positioned such that zero pressure corresponded to the height of the respective catheter tips. Proper catheter placement was verified by lightly tapping over the catheter tip (skin response) and by having the subject contract the anterior thigh muscles (muscle response). Catheters with poor dynamic response or that failed to show the appropriate response for their compartment were replaced. Slit catheters were made by cutting six 2-mm longitudinal slits into one end of a 30-cm length of PE-50 tubing (Becton-Dickenson) and attaching a 23-g Luer stub adapter (Becton-Dickenson). Wick catheters were made by pulling  $\sim 5$  mm of doubled 3–0 polyester fiber (Johnson & Johnson) with 6–0 polypropylene monofilament (Johnson & Johnson) into a

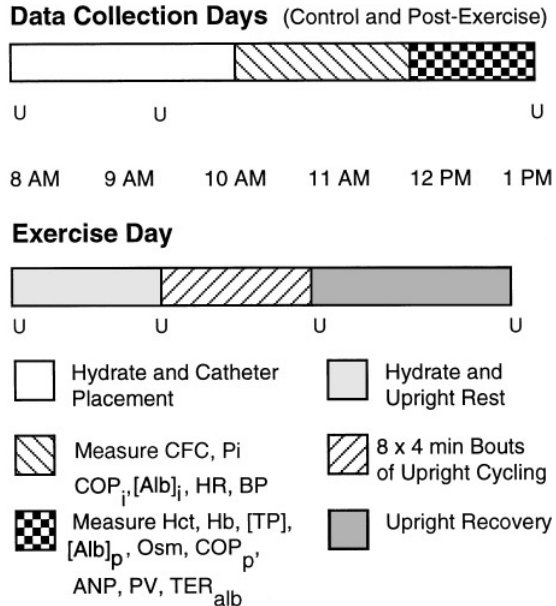


Fig. 1. Experimental protocol. Each subject completed 1 exercise day and 2 data-collection days: 1 immediately after exercise day (postexercise) and 1 separated by  $>1$  wk from exercise day (control). CFC, capillary filtration coefficient;  $P_i$ , interstitial fluid hydrostatic pressure;  $\text{COP}_i$ , interstitial fluid colloid osmotic pressure;  $[\text{Alb}]_i$ , interstitial fluid albumin concentration; HR, heart rate; BP, blood pressure; Hct, hematocrit; Hb, hemoglobin; [TP], plasma total protein concentration;  $[\text{Alb}]_p$ , plasma albumin concentration; Osm, plasma osmolality;  $\text{COP}_p$ , plasma colloid osmotic pressure; ANP, plasma atrial natriuretic peptide concentration; PV, plasma volume;  $\text{TER}_{\text{alb}}$ , transcappillary escape rate of albumin; U, urine collection and body weight measurement.

30-cm length of PE-50 tubing and attaching a 23-g Luer stub adapter.

Interstitial fluid samples for measurement of COP<sub>i</sub> and [Alb]<sub>i</sub> were collected from the right vastus lateralis muscle and overlying subcutaneous tissue by using the empty wick catheter technique (2, 8, 18). COP<sub>i</sub> measured after direct collection of interstitial fluid by this technique is comparable to that measured by interstitial fluid sampling by implanted nylon wicks and by an implanted colloid osmometer with a mean standard deviation of 1.7 mmHg, although variables such as amount of negative pressure and sample collection time must be controlled (18). Increasing amounts of negative pressure applied to the sample collection catheter decrease measured COP<sub>i</sub>, although the decrease is unlikely to be significant at pressures <10 mmHg (18). Catheter insertion units were placed into the appropriate tissue space as described above. Empty wick catheters were dipped into sterile saline and shaken to remove excess saline before insertion. Less than 10 mmHg of negative pressure applied to the skin wick catheter and periodic gentle massage of the skin overlying the catheter sites facilitated sample collection (2, 18). Interstitial fluid samples were grossly bloody in 2 of 18 muscle samples and in 4 of 18 skin samples. Data from these samples were excluded from the calculation of mean COP<sub>i</sub> and [Alb]<sub>i</sub> in both control and postexercise, leaving seven and five complete data sets in the leg muscle and skin, respectively.

Measurements of TER<sub>alb</sub> and PV were made by using Evans blue dye (Ophthalmic Labs) washout and dilution, respectively. Exact dye mass (~0.05 mg dye/kg) was determined by syringe weight to ±0.0001 g (Mettler). Time for the TER<sub>alb</sub> measurement started when the dye was injected and the venous catheter tubing and injection syringe had been completely flushed of dye (≈3 min from the start of injection). Immediately before dye injection, a 20-ml blood sample was taken from the left forearm indwelling venous catheter used for venous pressure measurement. Post-dye injection blood samples of 2.5 ml were taken every 5 min for 1 h. This provided 8 min between dye injection and the first blood sample, allowing single-compartment analysis of TER<sub>alb</sub> (3). Test-retest reliability for measurement of TER<sub>alb</sub> by Evans blue dye washout is 85% and for measurement of PV by Evans blue dye dilution is 99%. TER<sub>alb</sub> measured by blue dye is comparable to that measured by <sup>131</sup>I-labeled albumin (22).

**Exercise day.** After initial hydration and a 90-min upright seated rest period, the subjects underwent eight bouts of upright bicycle exercise lasting 4 min at 85% of their VO<sub>2max</sub>, with 5-min rest periods between bouts. After exercise, subjects rested seated upright for 2 h, after which they drank a volume of water equal to the estimated water loss calculated by change in body weight.

**Analyses.** Interstitial fluid samples were diluted with 0.9% NaCl to achieve a volume of ~13 μl, with exact interstitial fluid sample dilution factors determined by weighing the samples before and after dilution to ±0.0001 g. Mean interstitial fluid sample sizes at control and 24 h postexercise were 4.5 ± 1.4 and 3.4 ± 0.6 μl (NS) for muscle and 1.7 ± 0.4 and 2.5 ± 0.5 μl (NS) for subcutaneous tissue, respectively. COP<sub>i</sub> was measured by using a sample volume of 10 μl with a small volume membrane colloid osmometer with pore restriction of 30,000 Da (PM30, Amicon). [Alb]<sub>i</sub> was measured by bromocresol purple (Sigma Chemical).

A small aliquot of each blood sample was used for immediate determination in quadruplicate of hematocrit and total protein concentration by microhematocrit and refractometry, respectively. Hemoglobin was measured in the predye sample by cyanomethemoglobin (Boehringer Mannheim). Five millili-

ters of the predye blood was aliquoted into an EDTA tube with aprotinin, whereas the remaining blood was aliquoted into potassium-heparin tubes, and all tubes were spun for 15 min at 1,500 *g* at 4°C. Plasma was aliquoted for immediate determination of [Alb]<sub>p</sub> colorimetrically with a bromocresol purple reaction (Sigma Chemical), plasma osmolality by freezing-point depression (Advanced Instruments), COP<sub>p</sub> by using a small-volume membrane colloid osmometer with pore restriction of 30,000 Da, and Evans blue dye concentration by spectrophotometry at 620 nm. Plasma was frozen at -70°C for later determination of ANP by radioimmunoassay (INCSTAR; intra-assay coefficient of variation of 2.6% for midrange standards) by utilizing one kit for all samples.

**Calculations.** CFC was calculated as below

$$\text{CFC} = \frac{2 \cdot 100 \cdot 1,000}{\text{limb girth} \cdot \frac{1}{1 + \Psi}} \cdot \frac{\Delta \text{ limb girth}}{\text{time} \cdot \Delta \text{ venous pressure}} \quad (\mu\text{l} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1})$$

where Ψ is the ratio of post- to precapillary resistance, taken to be constant at 0.16 (10), and Δ is change.

COP<sub>i</sub> was determined for the diluted interstitial fluid samples and converted to an equivalent concentration of albumin by using a COP-albumin standard curve. The interstitial fluid sample dilution factor was applied to this albumin concentration to obtain a corrected albumin concentration, which was converted back to COP. [Alb]<sub>i</sub> was directly corrected for the interstitial fluid sample dilution factor.

TER<sub>alb</sub> was calculated from the initial slope of the exponential curve fit through the optical density of post-dye injection plasma samples at 620 nm. PV was calculated from the known mass of dye injected and an estimate of the initial plasma concentration of dye taken from the extrapolated exponential curve. Percent change in PV was also calculated by using hematocrit and hemoglobin (3). Plasma protein and osmolar content were calculated by multiplying the corresponding concentration by PV. PV, protein, and osmolar contents are reported as the respective value divided by control body weight.

**Statistics.** Comparisons between control and postexercise data were analyzed by using a paired *t*-test. Pearson's product-moment correlations assessed the relationship between ΔTER<sub>alb</sub> and ΔPV. Confidence level for statistical significance was set at *P* < 0.05. All values are reported as means ± SE.

## RESULTS

**Whole body parameters and plasma constituents.** Data from control and 24 h postexercise are listed in Table 1. Exercise induced a PV expansion of 6.4% [mean change 2.8 ± 0.8 (SE) ml/kg] at 24 h associated with a tendency for mean TER<sub>alb</sub> to decrease by 1.9 ± 1.0%/h (Fig. 2). PV increased in all nine subjects, whereas TER<sub>alb</sub> decreased in six of nine subjects. Additionally, the postexercise increase in PV was related to the postexercise decrease in TER<sub>alb</sub> (ΔPV = -0.54 · ΔTER<sub>alb</sub> + 1.8; *r* = -0.69, *P* < 0.05) (Fig. 3). PV expansion was associated with a dilution of slowly changing plasma and blood constituents, including a decrease in hematocrit of 4.3%, plasma total protein concentration of 3.1%, and COP<sub>p</sub> of 6.9%. However, [Alb]<sub>p</sub> was unchanged, so albumin content showed a trend to increase by 5.9% (Fig. 2). COP<sub>p</sub> decreased by

Table 1. *Body weight and blood data from control and 24 h postexercise*

	Control	Postexercise	<i>P</i> Value
BW, kg	71.9 ± 2.9	72.0 ± 2.9	
Hct, %	42.3 ± 0.9	40.5 ± 1.1	<0.05
Hb, g/dl	14.2 ± 0.4	13.9 ± 0.4	<0.05
Osm, mosmol/kgH <sub>2</sub> O	283 ± 1	285 ± 1	<0.05
Osm content, mosmol/kg	11.5 ± 0.2	12.3 ± 0.3	<0.05
[Alb] <sub>p</sub> , g/dl	4.2 ± 0.1	4.2 ± 0.1	
Alb content, g/kg	1.86 ± 0.04	1.97 ± 0.04	0.11
[TP], g/dl	6.5 ± 0.1	6.3 ± 0.1	<0.05
TP content, g/kg	2.87 ± 0.06	2.94 ± 0.08	
COP <sub>p</sub> , mmHg	26.1 ± 0.8	24.3 ± 0.9	<0.05
PV (EB), ml/kg	43.9 ± 0.8	46.8 ± 1.2	<0.05
PV (Hct and Hb), %Δ		5.5 ± 1.2	
BV, ml/kg	76.2 ± 1.2	78.7 ± 2.1	0.07
TER <sub>alb</sub> , %/h	8.4 ± 0.5	6.5 ± 0.7	0.11
[ANP], pg/ml	49.6 ± 0.8	52.0 ± 5.6	

Values are means ± SE; *n* = 9 subjects. BW, body weight; Hct, hematocrit; Hb, hemoglobin; Osm, plasma osmolality; Osm content, plasma osmolar content; [Alb]<sub>p</sub>, plasma albumin concentration; Alb content, plasma albumin content; [TP], plasma total protein concentration; TP content, plasma total protein content; COP<sub>p</sub>, plasma colloid osmotic pressure; PV (EB), plasma volume calculated by Evans blue dye dilution; PV (Hct and Hb), %change (%Δ) plasma volume calculated by change in hematocrit and hemoglobin; BV, blood volume; TER<sub>alb</sub>, transcapillary escape rate of albumin; [ANP], plasma atrial natriuretic peptide concentration.

1.8 ± 0.7 mmHg (Fig. 4), whereas plasma osmolality increased by 1.9 ± 0.8 mosmol/kgH<sub>2</sub>O. Plasma ANP concentration was unchanged between control and postexercise. Measured cardiovascular parameters were similar between control and postexercise. Control and postexercise heart rates were 65 ± 4 and 62 ± 2 beats/min, respectively, systolic blood pressures were 117 ± 3 and 118 ± 3 mmHg, respectively, and diastolic blood pressures were 67 ± 1 and 68 ± 2 mmHg, respectively.

**Microvascular parameters.** Measured microvascular data from control and 24 h postexercise are listed in Table 2. CFC increased by 34% in the leg at 24 h postexercise (Fig. 4), whereas CFC in the arm was unchanged. Pi in the leg muscle showed a trend to rise after intense exercise by 1.1 ± 0.5 mmHg (Fig. 4). No change was observed in Pi in the skin, or COP<sub>i</sub> in either the muscle or skin. [Alb]<sub>i</sub> showed a small but significant increase in the leg muscle of 0.6 ± 0.2 g/dl, with no change in the skin.

## DISCUSSION

Plasma albumin content expansion during hypervolemia and the role of albumin as the major contributor to COP<sub>p</sub> have led to the hypothesis that albumin content expansion is a driving force for PV expansion (5, 6). We induced a PV expansion of 6.4% with a trend for albumin content expansion of 5.9%. Convertino et al. (5) demonstrated a serial increase in plasma albumin content with little change in globulin content during 8 days of exercise training. Gillen et al. (6, 7) showed that albumin content expands by 1 h and remains expanded for up to 40 h after exercise.

This study represents the first attempt to simultaneously measure the TER<sub>alb</sub> and microcirculatory variables during PV expansion in humans. A significant new finding of this study is the association between decreased TER<sub>alb</sub> and PV expansion. A similar negative correlation between TER<sub>alb</sub> and PV has been demonstrated during hypervolemia induced by plasmapheresis in rats (36). While these results may suggest that TER<sub>alb</sub> contributes to PV expansion after exercise, an equally plausible interpretation is that PV regulates TER<sub>alb</sub> or that a third common factor contributes to both PV expansion and decrease of TER<sub>alb</sub>. In addition, the association is not strong. Some subjects displayed PV expansion without change in TER<sub>alb</sub>, suggesting that multiple overlapping mechanisms contribute to postexercise PV and albumin content expansion.

One such mechanism may be increased lymphatic return of albumin, with a subsequent redistribution of interstitial fluid albumin to the vascular space. To estimate protein movement, we converted TER<sub>alb</sub> to grams albumin per kilogram per hour, which yielded an 18% decrease in whole body transcapillary albumin flux after exercise. If TER<sub>alb</sub> were decreased to this

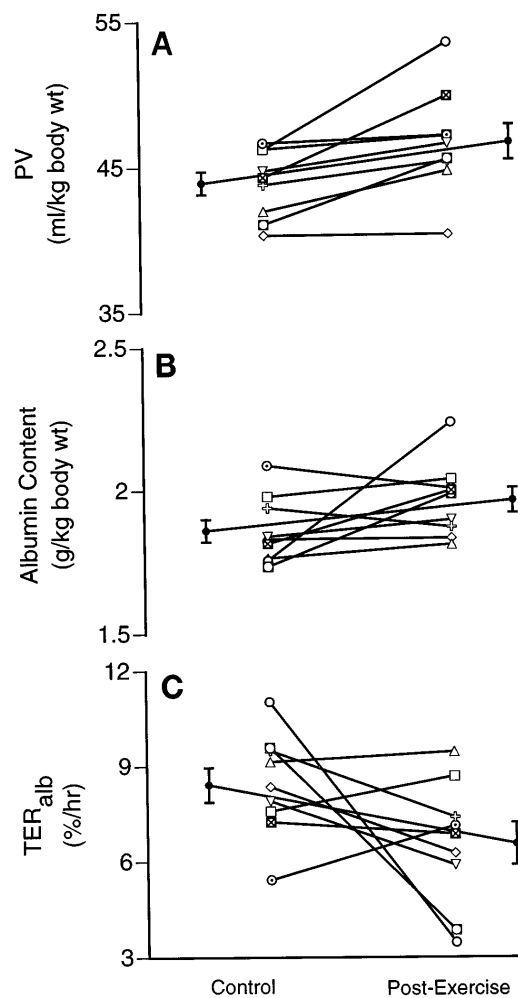


Fig. 2. PV (A), albumin content (B), and TER<sub>alb</sub> (C) at control and 24 h postexercise. Values are means ± SE; *n* = 9 subjects. Each subject is represented by a different symbol. ●, Means; error bars, SE.

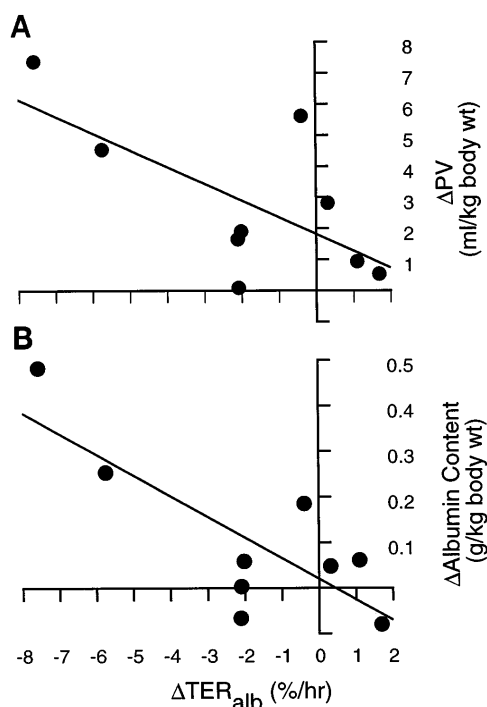


Fig. 3. Relationship between change ( $\Delta$ ) in TER<sub>alb</sub> and in both PV (A) and plasma albumin content (B). Values are individual measurements for 9 subjects.

degree for any length of time, whole body lymphatic return of albumin would also have to decrease to account for the measured change in plasma albumin content. However, lymphatic return of albumin from the leg is likely unchanged or increased because CFC and Pi were increased (2). Prenodal lymphatic albumin return in the leg has been shown to increase immediately after exercise, although not for 24 h (20). This implies that a decrease in whole body lymphatic albumin return would be due to a change in transcapillary forces elsewhere in the body. For example, alterations in blood flow to the splanchnic region may account for this discrepancy, owing to its large capacity for fluid and protein movement (9, 26). However, plasmapheresis in anesthetized rabbits increases extravascular uptake of labeled protein, suggesting that increased lymph flow accounts for restoration of plasma albumin content (35), and increased lymph flow after plasmapheresis in sheep contributes to the maintenance of  $[Alb]_p$  despite depleted whole body albumin stores (23). No study we know of has measured both TER<sub>alb</sub> and lymph flow simultaneously.

Additionally, continued upregulation of protein synthesis or downregulation of protein degradation could affect albumin content expansion after exercise. Factors affecting albumin synthetic rate include hormone balance, nutrition state, and systemic stresses (27). Colloid osmotic pressure also plays an important role in both the synthesis and degradation of plasma albumin (27). Infusion of colloids other than albumin results in decreased albumin synthesis, and infusion of excess albumin results in increased albumin degradation. The mechanisms of colloid osmotic regulation of albumin

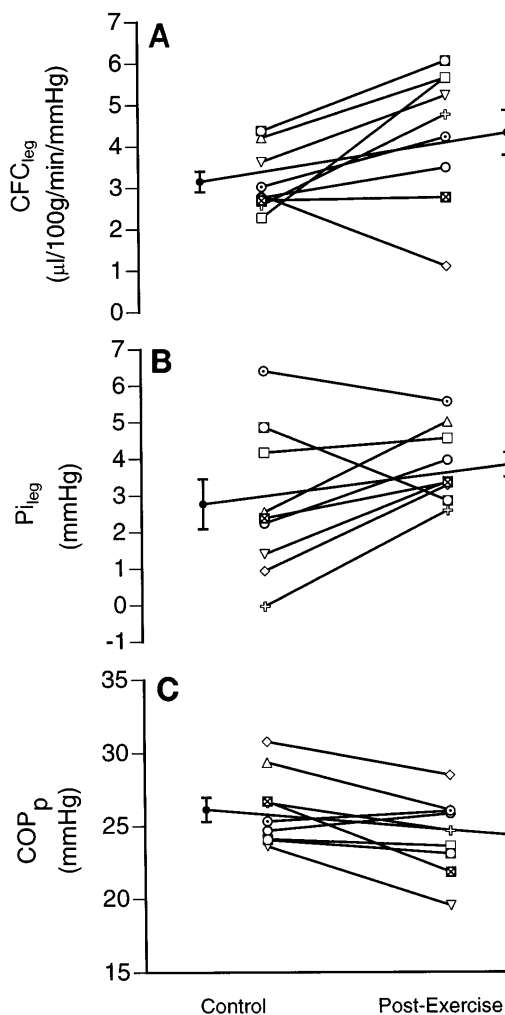


Fig. 4. Leg capillary filtration coefficient ( $CFC_{leg}$ ; A), leg muscle interstitial fluid hydrostatic pressure ( $Pi_{leg}$ ; B), and plasma colloid osmotic pressure ( $COP_p$ ; C) at control and 24 h postexercise. Values are means  $\pm$  SE;  $n = 9$  subjects. Each subject is represented by a different symbol. ●, Means; error bars, SE.

Table 2. Microcirculatory variables from control and 24 h postexercise

	<i>n</i>	Control	Postexercise	<i>P</i> Value
$Pi$ , mmHg				
muscle	9	2.8 $\pm$ 0.7	3.8 $\pm$ 0.3	0.08
skin	9	1.1 $\pm$ 0.3	1.6 $\pm$ 0.5	
$COP_i$ , mmHg				
muscle	7	5.7 $\pm$ 0.8	6.4 $\pm$ 1.4	
skin	5	5.1 $\pm$ 0.7	5.0 $\pm$ 2.0	
$[Alb]_i$ , g/dl				
muscle	7	1.0 $\pm$ 0.3	1.6 $\pm$ 0.3	<0.05
skin	5	1.5 $\pm$ 0.4	1.2 $\pm$ 0.4	
$CFC$ , $\mu l \cdot 100 g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$				
arm	9	3.6 $\pm$ 0.4	4.0 $\pm$ 0.5	<0.05
leg	9	3.2 $\pm$ 0.2	4.3 $\pm$ 0.5	

Values are means  $\pm$  SE;  $n =$  no. of subjects.  $Pi$ , interstitial fluid hydrostatic pressure;  $COP_i$ , interstitial fluid colloid osmotic pressure;  $[Alb]_i$ , interstitial fluid albumin concentration; CFC, capillary filtration coefficient.

are unknown (27). An attenuation of volume-regulating mechanisms after exercise (7) could allow the plasma to become hyposmotic, effectively signaling the liver to increase albumin production. However, albumin synthesis has been shown not to increase after moderate aerobic exercise (4). In addition, chronic infusion of albumin resulting in hyperproteinemia in splenectomized dogs did not result in expanded blood volume despite an increase in plasma protein concentration, an increase in total extracellular fluid volume, and an increase in COP<sub>p</sub> (14). This may be because increased interstitial fluid protein concentration balances the increase in plasma protein concentration and maintains the capillary colloid osmotic pressure gradient, implying that increased synthesis of albumin would not lead to increased blood volume unless the albumin could be preferentially retained in the vascular space. Further studies are needed to assess the role of albumin synthesis after intense exercise.

The increase in CFC in the leg, and the decrease in COP<sub>p</sub>, both stimulate transcapillary fluid flux (2, 26). Changes in CFC may represent changes in total capillary surface area and/or capillary hydraulic conductivity. Alternatively, changes in measured CFC may reflect artifacts resulting from the large cuff pressures used to measure CFC. These pressures may activate smooth muscle constriction and/or alter capillary exchange (13). However, CFC also increases in maximally dilated rat hindquarters (30) after 6–10 wk of exercise training, a condition that eliminates the effect of vascular smooth muscle constriction, as well as during fluid loading in dogs (11) and in humans exposed to lower body negative pressure (12).

Increased transcapillary fluid filtration resulting from increased CFC and decreased COP<sub>p</sub> is expected to be offset by changes in Pi and COP<sub>i</sub>, thereby preventing edema in the legs (2). Indeed, Pi in the leg muscle tended to increase after exercise in seven of nine subjects. Pi has been shown to remain elevated in human leg muscle for at least 15 min after 3 min of intense exercise (16). Our data extend these findings to 24 h after exercise. COP<sub>i</sub> was expected to decrease after exercise, given an anticipated interstitial fluid albumin wash down (26); however, we measured no change in the leg skin or muscle. Similarly, COP<sub>i</sub> has been shown to remain unchanged in the neck muscle and subcutaneous tissue during head-down tilt despite facial edema (21).

Our estimates of microcirculatory forces that control muscle transcapillary albumin flux during exercise-induced hypervolemia change in a direction that supports reduced transcapillary albumin flux. The two-pore model of transcapillary solute transport describes convective and diffusive albumin flux through large and small pores. Under resting conditions, large pores account for ~80% of transcapillary albumin clearance, which is entirely convective and controlled by transcapillary hydrostatic pressure gradients (26). The remaining 20% of transcapillary albumin clearance is through small pores and is predominantly diffusive (26). In the present study, Pi tended to increase, which would limit

convective albumin flux through large pores. Although capillary hydrostatic pressure was not measured in our study, Michel et al. (15) demonstrated that, during steady state, changes in capillary pressure are compensated for by changes in the composition of the transcapillary filtrate, thus preserving capillary pressure. Additionally, because [Alb]<sub>i</sub> increased in the leg muscle with constant [Alb]<sub>p</sub>, the diffusive component of small-pore albumin clearance should decrease. On the other hand, the small but significant increase in [Alb]<sub>i</sub> in the leg muscle may reflect an increase in small-pore convective albumin flux resulting from increased CFC and decreased COP<sub>p</sub> because typical small pores allow passage of albumin but not immunoglobulins or fibrinogen (26). However, the small-pore convective component of transcapillary albumin transport represents <5% of total albumin clearance (26).

To summarize, we have measured TER<sub>alb</sub> and transcapillary forces in the leg muscle and skin before and 24 h after intense exercise in humans. Increased leg CFC and decreased COP<sub>p</sub> are compensated for by a tendency for increased muscle Pi and increased muscle [Alb]<sub>i</sub>. Thus the transcapillary clearance of albumin in previously active muscle may be decreased. Additionally, TER<sub>alb</sub> shows a trend to decrease after exercise and is negatively correlated with PV expansion.

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