Capillary filtration coefficient is independent of number of perfused capillaries in cat skeletal muscle

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Received 25 October 2000; accepted in final form 5 February 2001

Bentzer, Peter, Lis Kongstad, and Per-Olof Grände.
Capillary filtration coefficient is independent of number of perfused capillaries in cat skeletal muscle. Am J Physiol Heart Circ Physiol 280: H2697–H2706, 2001.—The capillary filtration coefficient (CFC) is assumed to reflect both microvascular hydraulic conductivity and the number of perfused capillaries at a given moment (precapillary sphincter activity). Estimation of hydraulic conductivity in vivo with the CFC method has therefore been performed under conditions of unchanged vascular tone and metabolic influence. There are studies, however, that did not show any change in CFC after changes in vascular tone and metabolic influence, and these studies indicate that CFC may not be influenced by alteration in the number of perfused capillaries. The present study reexamined to what extent CFC in a pressure-controlled preparation depends on the vascular tone and number of perfused capillaries by analyzing how CFC is influenced by 1) vasoconstriction, 2) increase in metabolic influence by decrease in arterial blood pressure, and 3) occlusion of precapillary microvessels by arterial infusion of microspheres. CFC was calculated from the filtration rate induced by a fixed decrease in tissue pressure. Vascular tone was increased in two steps by norepinephrine (n = 7) or angiotensin II (n = 6), causing a blood flow reduction from 7.2 ± 0.8 to at most 2.7 ± 0.2 ml·min⁻¹·100 g⁻¹ (P < 0.05). The decrease in arterial pressure reduced blood flow from 4.8 ± 0.4 to 1.40 ± 0.1 ml·min⁻¹·100 g⁻¹ (n = 6). Vascular resistance increased to 990 ± 260% of control after the infusion of microspheres (n = 6). CFC was not significantly altered from control after any of the experimental interventions. We conclude that CFC under these conditions is independent of the vascular tone and number of perfused capillaries and that variation in CFC reflects variation in microvascular hydraulic conductivity.

microvessels; microspheres; metabolic influence; norepinephrine; sympathetic stimulation

MICROVASCULAR PERMEABILITY is an important factor in the regulation of fluid homeostasis, and increased permeability may induce loss of plasma volume to the interstitial compartment, resulting in hypovolemia, interstitial edema, and increased tissue pressure. Increased permeability constitutes an important change in many pathophysiological conditions such as systemic inflammatory response syndrome, sepsis, and brain trauma and may be a factor of importance for outcome (12, 29). From a clinical point of view, the availability of methods to study mechanisms controlling microvascular permeability in vivo therefore could be of great importance.

In the Starling fluid equilibrium equation describing transcapillary fluid exchange (Jv) in a tissue, 

\[ J_v = L_p A (\Delta P - \sigma \Delta \pi) \]

where \( \Delta P \) is average transcapillary hydrostatic pressure, \( \Delta \pi \) is average transcapillary oncotic pressure, and \( \sigma \) is the net reflection coefficient for macromolecules. \( L_p A \) is the hydraulic conductance of a tissue, i.e., the product of hydraulic conductivity (Lp) and surface area available for fluid exchange (A) (41).

The latter is suggested to reflect number of perfused capillaries at a given moment, which in turn may be influenced by vascular tone (19, 40) and by variation in myogenic and metabolic stimulus on precapillary sphincter activity (vasomotion) (13, 19, 24, 28). The capillary filtration coefficient (CFC), defined as the change in transcapillary fluid exchange induced by a fixed change in transcapillary hydrostatic pressure (22, 33), is suggested to equal \( L_p A \) (30, 33). The notion that not only hydraulic conductivity but also the number of perfused capillaries may be reflected in CFC is based on observed alterations in CFC after changes in vascular tone (3, 8, 15) or changes in metabolic and myogenic influence on sphincter activity (3, 8, 20, 31).

Most previous studies (18, 25, 35, 36, 45) analyzing alterations in hydraulic conductivity with the CFC method have therefore been conducted with a maximally dilated vascular bed perfused at a constant flow to avoid concomitant changes in the number of perfused capillaries during the experiment. It is obvious that such an experimental design does not represent normal physiology with regard to arterial pressure, blood flow, and vascular tone, especially if constant flow is obtained by pump perfusion with a non-blood perfusate. No doubt these limitations have restricted the possibility to make quantitative estimations of alterations in hydraulic conductivity and have not allowed analysis of net effects on fluid balance. Thus a more physiological in vivo method to evaluate microvascular hydraulic permeability would be of value.

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There are studies, however, that do not support the concept that changes in the number of perfused capillaries (precapillary sphincter activity) in skeletal muscle influence CFC. Thus hypoxia, shown to cause capillary recruitment in vital microscopy studies (19, 24), did not alter CFC in skeletal muscle (42), and no change in CFC was observed in the lower leg muscles of humans after a myogenic stimulus induced by a change from the supine to the upright position (7, 38). There are also studies that could not demonstrate any effect on CFC at steady state (14) after strong metabolic inhibition of precapillary sphincter activity induced by muscle exercise (13, 19). Furthermore, under carefully controlled methodological conditions with regard to blood flow and arterial pressure, we (21) could not find any significant effects on CFC in cat skeletal muscle after either a pharmacologically induced marked decrease in vascular tone or after a moderate decrease in arterial pressure. Thus there are indications that CFC in skeletal muscle, under carefully controlled methodological conditions, is not influenced by alteration in the number of perfused capillaries, and, if this is so, then CFC may be a tool to analyze changes in hydraulic conductivity under more physiological conditions.

On the basis of these considerations, the present study aimed at reexamining the hypothesis that CFC is dependent on variation in the number of perfused capillaries. This aim was pursued by analyzing the effects on CFC of 1) an increase in vascular tone, 2) a reduced sphincter activity following a graded increase in metabolic dilatory influence obtained by a decrease in perfusion pressure, and 3) of a graded decrease in the number of perfused capillaries by local intraarterial infusions of microspheres.

**MATERIALS AND METHODS**

**Material and Anesthesia**

The study was approved by the Local Ethics Committee. Nineteen male cats from a local breeder were used (3.9–6 kg body wt). Anesthesia was induced by intravenous infusion of α-chloralose (50 mg/kg), urethan (10 mg/kg), and pentobarbital sodium (3–4 mg/kg) and continued with infusion of α-chloralose (4 mg h⁻¹ kg⁻¹). To counteract muscle shivering during the recordings, relaxation was maintained with an intravenous infusion of pancuronium bromide (0.15 mg h⁻¹ kg⁻¹; Pavulon, Organon Technica; Boxtel, Holland). Before the vessels were cannulated, blood coagulation was prevented by heparin at a dose of 1,000 U/kg supplemented by 100 U h⁻¹ kg⁻¹. A mixture of Ringer solution and 20% albumin (Pharmacia; Stockholm, Sweden) at a ratio of 8:1 was infused continuously as volume substitution at an infusion rate of 6 ml h⁻¹ kg⁻¹. The animals were mechanically ventilated with air (Servo 900, Siemens-Elema; Solna, Sweden) through a tracheal cannula, maintaining a normal endtidal PCO₂ of 4.8–5.5 kPa (Eliza CO₂ analyzer, Gambro Engström; Bromma, Sweden). Body temperature was kept at 37.5°C using a heating pad controlled by an esophageal thermistor. Blood gases, arterial pH, and hematocrit were monitored regularly to ensure normal state of the animal. After the experiment was terminated, the cats were killed with an intravenous injection of 3 M KCl.

**Skeletal Muscle Preparation**

Observations were made on the denervated lower leg muscles of the cat right hindlimb (Fig. 1) (21). The gastrocnemius muscle was the largest muscle of the preparation, but the soleus and the tibialis muscles were also included. The paw and skin were removed, and the muscle preparation was isolated from the body, with the femur cut, the marrow cavity plugged, and the lymph vessels ligated. A bipolar probe for electrical stimulation was attached to the most proximal part of the cut sciatic nerve in the experiments analyzing the effects of sympathetic stimulation. Great care was taken with ligatures and electrocautery so as to achieve complete hemostasis. The muscle was autoperfused from the animal via an arterial shunt from the left femoral to the right popliteal artery. Venous blood was drained from the popliteal vein via a tube, with its outlet open to atmospheric pressure and placed above an open funnel connected to the right external jugular vein. The arterial pressure to the muscle was controlled and kept constant at the desired level by a screw clamp placed around the arterial shunt (Fig. 1). The screw clamp was adjusted by a servo pressure-controlled electric motor via a pressure transducer and set to keep the desired mean arterial pressure.

![Fig. 1. The experimental setup showing the cat and the cat calf muscles enclosed in the plethysmograph. Pᵥ, venous pressure; Pₐ, arterial pressure.](http://ajpheart.physiology.org/ by 10.220.33.3 on June 13, 2017)
Determination of Total Vascular Resistance

Arterial inflow pressure to the muscle and venous outflow pressure were monitored via T tubes in the shunts close to the cannulated popliteal artery and vein (Fig. 1). Blood flow to the muscle was recorded continuously using an ultrasonic flowmeter (T106, Transonic System; Ithaca, NY) in the arterial shunt, which was calibrated by measurement of venous blood flow with a graduated glass. Total vascular resistance was recorded continuously by electronically dividing signals representing arterial-venous pressure fall with blood flow. Vascular resistance is expressed in peripheral resistance units (PRU; in mmHg·ml⁻¹·min⁻¹·100 g muscle⁻¹). The weight of the skeletal muscle was calculated as 1.45% of the body weight for a 6.0-kg cat and 1.55% for a 4.0-kg cat, as deduced from previous experiments (21). The muscle weight for the other cats was obtained by linear interpolation. All parameters were recorded on a Grass Polygraph (Grass Instruments; Quincy, MA).

Determination of Muscle Volume Changes

The muscle preparation was enclosed in a temperature-controlled (37°C) plethysmograph sealed with a two-component silicon elastomer (Elastosil M4400, Wacker Chemie; Munich, Germany). The plethysmograph was filled with Ringer solution. Ciprofloxacin (Ciproxin, Bayer) at a dose of 125 mg was added to the Ringer solution to prevent bacterial growth in the plethysmograph. The plethysmograph was in fluid connection via a tube to an open water-filled reservoir (Fig. 1). The volume changes of the muscle were recorded continuously by monitoring the zero baseline of the plethysmographic pressure, which in turn was obtained by lowering of the reservoir (Fig. 1). The decreased tissue pressure caused an initial rapid large increase in tissue volume, mainly representing an increase in venous blood volume, followed by a slow increase in tissue volume. All CFC values were calculated as the average slope during the 3- to 4-min period after the tissue pressure decrease. It has previously been shown that the volume curve slope during this period represents only transcapillary fluid filtration (21, 44).

Experimental Protocol

The following parameters were recorded: arterial pressure, venous pressure, arterial blood flow, total vascular resistance, and tissue volume variations. CFC was analyzed in six experimental series, as defined below, of which series 1–4 (n = 7), series 5 (n = 6), and series 6 (n = 6) were performed in separate cats.

Series 1. Six consecutive CFC measurements were performed in seven cats to test the reproducibility of the CFC method.

Series 2. Intra-arterial infusion of norepinephrine (Apoteksbolaget; Umeå, Sweden) dissolved in isotonic saline was given in increasing doses (0.3–1.2 μg·min⁻¹·100 g⁻¹) with the aim of a two-step increase in vascular resistance to ~150 and 200% of the control value. In two of these cats, the infusion rate was further increased to evaluate the effects of a more marked increase in vascular tone.

Series 3. Intra-arterial infusion of angiotensin II (0.08–0.3 μg·min⁻¹·100 g⁻¹, Sigma; St. Louis, MO) dissolved in isotonic saline was given at increasing doses with the aim of a similar two-step increase in vascular resistance to ~150 and 200% of control.

Series 4. Sympathetic stimulation of the sciatic nerve using a Grass S88 stimulator with the aim of a two-step in-
crease in vascular resistance to ~150 and 200% of the control value.

Series 5. Arterial pressure reduction in two steps from 90 to 100 mmHg with the aim of the lowest blood flow in the range of 1–1.5 ml·min⁻¹·100 g⁻¹ with the purpose of increasing metabolic dilatory influence on precapillary sphincters. Series 6. Intra-arterial bolus infusions of doses of 6.4 × 10⁶, 6.4 × 10⁶, 3.2 × 10⁶, and 3.2 × 10⁵ microspheres/100 g (diameter 15 μm, Dynospheres, Dynoparticles; Lillestrøm, Norway) with the purpose of reducing the number of perfused capillaries. The microspheres were suspended in Ringer solution using an ultrasonic bath for 3 min. Before infusion, the solution was examined in a microscope to ensure that no aggregates were present.

After the preparation, which lasted for ~4 h, was completed, a stabilizing period of ~90 min passed before any measurements were made. To obtain accurate CFC recordings, no measurements were performed until the volume and vascular resistance registrations showed a strict steady state after the experimental interventions. CFC measurements were thus only performed under constant conditions with regard to vascular resistance and blood flow, which were most often achieved 10–20 min after start of each experimental intervention. At least 90 min of washout time elapsed between each series of experiments and a new control was established.

Statistics

All values are presented as means ± SE. After tests for normality and equal variance, the statistical analysis was performed with one-factor repeated measures analysis of variance. Statistically significant differences were isolated using the Student-Newman-Keuls test. Data from the norepinephrine, angiotensin II, and blood pressure experiments were fitted to a linear regression, and statistical comparisons were made using Student’s t-test with the null hypothesis of r = 0. Probability values <0.05 were considered significant.

RESULTS

Reproducibility of the CFC Method

Mean values of the six subsequent measurements of CFC in each of the seven cats in the reproducibility series are presented in Table 1. The mean CFC value for all measurements was 0.00796 ± 0.00004 ml·min⁻¹·mmHg⁻¹·100 g⁻¹ (n = 42). Blood flow in this series was 5.6 ± 0.4 ml·min⁻¹·100 g⁻¹.

Norepinephrine and CFC

Figure 2 shows an original registration of the CFC volume curve before and after the infusion of norepinephrine at a dose of 0.43 μg·min⁻¹·100 g⁻¹, by which vascular resistance increased from 11.6 to 40 PRU. The average volume curve slope relative to control during the 3- to 4-min period after the transmural pressure increase was close to identical at the two resistance levels. The compiled data for the norepinephrine experiments (n = 7) are presented in Fig. 3. CFC was measured at two vascular resistance levels of 158 ± 12 and 268 ± 30% compared with the control resistance of 11.9 ± 2.3 PRU (P < 0.05; Fig. 3A). Blood flows at these resistance levels were 61 ± 6.1 and 32 ± 4.0% compared with the control value of 8.0 ± 1.2 ml·min⁻¹·100 g⁻¹. The relative CFC values were 102 ± 2 and 101 ± 2% compared with the control value of 0.0079 ± 0.0004 ml·min⁻¹·mmHg⁻¹·100 g⁻¹ [not significant (NS); Fig. 3B]. In the two experiments in which the norepinephrine infusion rate was further increased, vascular resistance increased to 630 and 813% of control, and CFC remained unaltered. Figure 3C shows a scatterplot of the absolute CFC values as a function of vascular resistance and fitted to a linear regression, giving an r value for the regression line of 0.0797 (NS).

Angiotensin II and CFC

The compiled data for the angiotensin II experiments (n = 6) are presented in Fig. 4. CFC was measured at two vascular resistance levels of 130 ± 6% (P < 0.05) and 206 ± 21% (P < 0.05) compared with the control resistance of 14.9 ± 2.7 PRU (Fig. 4A). Blood flows at these resistance levels were 70 ± 4 and 48 ± 4% of the control value of 6.4 ± 1.0 ml·min⁻¹·100 g⁻¹. The relative CFC values at the corresponding resistance levels were 100 ± 4 and 102 ± 2% of the control value of 0.0076 ± 0.0003 ml·min⁻¹·mmHg⁻¹·100 g⁻¹ (NS; Fig. 4B). Figure 4C shows a scatterplot of the absolute CFC values as a function of vascular resistance and fitted to linear regression, giving a r value of 0.0106 (NS). In one of the cats, it proved impossible to obtain a steady state with regard to vascular resistance after infusion of angiotensin II, and this part of the experiment was excluded from the study.

Sympathetic Stimulation and CFC

Vascular resistance was increased through electrical stimulation of the sciatic nerve at a frequency of 9–25 Hz in seven experiments. During these experiments, it proved difficult to obtain a steady state in vascular resistance, most likely due to sympathetic fatigue (33), and the stimulation frequency had to be increased during the experiment to maintain vascular resistance at a constant level. Even so, the increased vascular resistance could be maintained at a constant level for a time span long enough for an adequate CFC measurement in only two experiments, in which mean vascular

Table 1. Reproducibility of CFC measurements as tested by six consecutive measurements in each of seven cats

<table>
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<th>Cat</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Mean CFC, ml·min⁻¹·mmHg⁻¹·100 g⁻¹</td>
<td>0.00704</td>
<td>0.00774</td>
<td>0.00771</td>
<td>0.00797</td>
<td>0.00814</td>
<td>0.00907</td>
<td>0.00801</td>
</tr>
<tr>
<td>Coefficient of variation</td>
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<td>3.7</td>
<td>3.6</td>
<td>2.6</td>
<td>3.4</td>
<td>3.0</td>
<td>2.9</td>
</tr>
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Values are means ± SE; n = 6 consecutive measurements of the mean capillary filtration coefficient (CFC).
resistance was increased to 164 and 269% compared with the mean control value of 11.2 PRU. The obtained relative CFC values were 101 and 100% of the mean control value of 0.0078 ml·min⁻¹·mmHg⁻¹·100 g⁻¹.

Perfusion pressure and CFC

By decreasing arterial pressure in two steps from a mean control value of 93 ± 3.6 mmHg to 45 ± 5.6 and 28 ± 1.8 mmHg, blood flow was reduced to 57 ± 5 and 33 ± 3% of the control blood flow of 4.8 ± 0.4 ml·min⁻¹·100 g⁻¹ (n = 6, P < 0.05; Fig. 5A). The relative CFC values after reductions in blood flow were 99 ± 1% at the middle blood flow level and 100 ± 2% at the lowest blood flow level compared with a control CFC value of 0.0071 ± 0.0003 ml·min⁻¹·mmHg⁻¹·100 g⁻¹ (NS; Fig. 5B). Vascular resistance decreased after the reduction in arterial pressure to 78 ± 2 and 77 ± 5% compared with the control resistance of 18.1 ± 2.9 PRU. Figure 5C shows a scatterplot of the absolute CFC values as a function of absolute blood flow and fitted to linear regression, giving an r value 0.0852 (NS).
Microvessel Occlusion and CFC

The initial microsphere infusion \((6.4 \times 10^6\) microspheres/100 g) invariably caused a rapid increase followed by a gradual decrease in resistance to \(76 \pm 6\%\) at steady state compared with a control value of \(9.7 \pm 2.0\) PRU (NS; Fig. 6B). After the second, third, and fourth infusion of microspheres (resulting in a total dose of \(12.8, 16.0,\) and \(19.2 \times 10^6\) microspheres/100 g), vascular resistance increased to \(160 \pm 30, 550 \pm 200,\) and \(990 \pm 290\%\) of control, respectively (Fig. 6A). Mean blood flow at control was \(9.5 \pm 1.6\) and decreased to \(1.3 \pm 0.4\) ml·min\(^{-1}\)·100 g\(^{-1}\) at the highest dose of microspheres. The mean CFC value before the infusion was \(0.0082 \pm 0.0003\) ml·min\(^{-1}\)·mmHg\(^{-1}\)·100 g\(^{-1}\), and the corresponding CFC values after the graded infusion of microspheres were \(100 \pm 2, 95 \pm 3, 97 \pm 3,\) and \(89 \pm 8\%\) of control (NS; Fig. 6B). Figure 6C shows a scatterplot of the absolute CFC values as a function of the total number of infused microspheres.

**DISCUSSION**

The present study performed on a pressure-controlled and autoperfused cat skeletal muscle failed to...
demonstrate a statistically significant change on CFC after increases in vascular tone up to at least 40 PRU, as analyzed for three different vasoconstrictor mechanisms. In addition, CFC values were not significantly altered after decreases in arterial pressure, giving blood flow values in the range of those obtained during the vasoconstriction, or by a reduction in the number of perfused capillaries, accomplished by microsphere infusions.

Reported effects on CFC of norepinephrine, angiotensin II, and sympathetic stimulation have varied considerably in previous studies, perhaps reflecting the technical difficulties and the plethora of methods used in the study of this hemodynamic parameter. Thus infusion of norepinephrine was reported to cause either an increase in CFC (14), a decrease in CFC (9, 10, 35, 37, 43), or an unchanged CFC (15). The vasoconstrictor angiotensin II has been reported both to increase (16) and decrease CFC (15). The increase in vascular resistance induced by sympathetic nerve stimulation was, in one study (3), shown to cause a transient decrease in CFC, followed by an increase back to control, and in another study (26), sympathetic stimulation increased CFC markedly compared with the control.

Some comments regarding the methodological approach used in this study may help to explain the results obtained and strengthen their relevance when compared with results from some previous studies. An externally applied negative tissue pressure, as used in the present study, has been shown to be transmitted almost fully to the tissue regardless of the depth at which the pressure is recorded (27). All capillaries in the organ will, therefore, be exposed to a similar large decrease in interstitial hydrostatic pressure. Even if there is a slightly uneven distribution of the applied negative pressure within the tissue, this will not influence our results because our conclusions are based on relative variations in CFC. Furthermore, by decreasing the interstitial pressure during the CFC procedure by an externally applied reduction in tissue pressure, the capillary will be exposed to the same pressure change along its whole length. Like other techniques to increase transvascular pressure, this technique may, however, induce an active and/or passive change in post-capillary resistances, influencing the hydrostatic capillary pressure. If present, such an influence on recorded CFC must be small when using a transmural pressure change as low as 5 mmHg (21). This means that, when using the tissue pressure technique, the induced transcapillary pressure increase can be considered to be independent of vascular tone and the postcapillary-to-precapillary resistance ratio. This is in contrast to the situation when the transcapillary pressure is increased by a fixed increase in venous pressure (3, 8, 15, 16, 20, 26, 43), at which the magnitude of the retrogradely transferred pressure to the exchange vessels will vary with the postcapillary-to-precapillary resistance ratio and significantly influence CFC (21). In this respect, it can be calculated from Ohm’s Law that not only alterations in the postcapillary-to-precapillary resistance ratio due to active changes in vascular tone are of importance but also the small passive decrease in venous resistance (~4–5%) induced by the venous pressure increase per se.

The early increase in tissue volume during the CFC procedure represents an increase in both tissue blood volume and transcapillary filtration. Because vascular volume changes cannot be separated from the induced transcapillary filtration during the initial phase of the CFC procedure, CFC must be measured when the intravascular volume changes are completed. We (21) have previously shown that this has not occurred until 2.5–3 min after the pressure change, which suggests that in studies (3, 9, 10, 15, 26, 35, 37) in which CFC was measured at an earlier time point, vascular volume changes may have influenced the recorded CFC value.

Continuous recording of vascular resistance allowed us to measure CFC at a state of constant resistance. This means that no blood volume variations caused by changes in vascular resistance, will disturb the volume recording. The sympathetic experiments in the present study, in which sympathetic fatigue (33) led to a gradual decrease in vascular resistance, may illustrate the difficulties in measuring CFC when strict criteria on a constant vascular resistance are applied. In addition, the electronically controlled screw clamp around the arterial shunt (see MATERIALS AND METHODS and Figs. 1 and 2) assures a constant mean arterial pressure also during the CFC procedure. This is of great importance for obtaining adequate CFC values, because even small variations in arterial pressure will influence the volume curve slope due to changes in intravascular blood volume. Our strict control of vascular resistance and arterial pressure, and the fact that CFC is measured after the blood volume phase has passed, signify a good accuracy of the CFC measurement and may partly explain why we could not reproduce results from previous studies (3, 15, 26, 31) showing a change in CFC during variation in vascular tone and metabolic influence.

The induced filtration during the CFC procedure will cause a flow-dependent increase in plasma oncotic pressure and a flow-independent decrease in interstitial oncotic pressure, both causing an underestimation of the true CFC. The impact of these factors is dependent on the magnitude of the induced transvascular pressure change. It has been suggested that the former effect is a potential source of error when using the CFC method for evaluation of changes in hydraulic conductance during variation in blood flow (33). This flow-dependent decrease in CFC, however, can be calculated to be only 3% when decreasing blood flow from 3 to 1 ml·min⁻¹·100 g⁻¹ using a filtration rate of 0.008 ml·min⁻¹·mmHg⁻¹·100 g⁻¹ and a transvascular pressure change of 5 mmHg. Our findings of an unchanged CFC after marked reductions in blood flow support this calculation and suggest that this source of error has a limited impact on recorded CFC. The flow-independent decrease in CFC caused by the filtration-induced decrease in interstitial oncotic pressure can be calculated in a similar way to be ~3% when measured 3–4 min
after a pressure change of 5 mmHg and using a distribution volume of 5 ml/100 g (1). To minimize this effect, CFC should be measured as soon as the blood volume alterations are completed, i.e., 3–4 min after the pressure change.

Our finding that CFC did not change after an increase in vascular resistance might be explained by the fact that the vasoconstrictor effect on precapillary sphincters, which may decrease number of perfused capillaries, is outbalanced by a simultaneous metabolic inhibition of sphincter activity, resulting in unchanged number of perfused capillaries. If so, such an outbalancing effect must occur to exactly the same extent for all three vasoconstrictor mechanisms used, which is quite an unlikely coincidence. Furthermore, there are intravital microscopy studies (19, 28) showing that the vasoconstrictor stimuli used in the present study induced a decrease in the number of perfused capillaries.

It could also be argued that the unaltered CFC after vasoconstriction is a consequence of alteration in the hydraulic conductivity counteracting a change in the number of perfused capillaries. For example, it has been discussed that strong β-receptor stimulation may decrease microvascular permeability (11, 36). It is very unlikely, however, that the small β-stimulating effect of norepinephrine at the doses used in the present study had any significant effect on hydraulic conductivity (32). On the basis of observations in single capillaries, it has also been discussed that an increase in shear stress may decrease hydraulic conductivity (46). Our results of an unchanged CFC both when surface area was increased and decreased during a reduction in blood flow indicate that variation in shear stress cannot explain our results.

It can be argued that all capillaries may be open to flow already from the start of the experiment because of a significantly depressed myogenic sensitivity and precapillary sphincter activity (vasomotion) in this preparation. If this is so, an arterial pressure reduction should not change the number of perfused capillaries, and CFC should remain unchanged. This is most unlikely, however, because myogenic reactivity and autoregulatory response are previously shown to be well preserved in this preparation (2), a fact confirmed in the present study by the recorded decrease in resistance after arterial pressure reductions.

Variations in capillary blood flow due to spontaneous sphincter activity (vasomotion) have been verified using intravital microscopic techniques (4, 19, 24). This means that the perfusion of the capillaries at a given moment varies with the intensity of vasomotion (6, 30), which is in turn influenced by vasoconstriction and by metabolites produced in the tissue (13, 19). On the basis of the present finding, that CFC is independent of vascular tone and metabolic activity, the question therefore must be raised of whether a transiently "closed" capillary also contributes to the filtration induced by the decrease in tissue pressure during the CFC procedure. It has been argued that filtration during the CFC procedure from the closed capillary is self-limiting due to a rapid increase in capillary colloid osmotic pressure and will therefore not fully contribute to fluid exchange (6). On the other hand, if the transiently closed capillary is open again within a few seconds (17, 39), the colloid osmotic pressure of the stagnant fluid column will not be increased to any significant extent, and the capillary may almost fully contribute to the induced filtration. In addition, capillaries closed off from the arterial end may be refilled through the existing network of interconnecting capillaries (5) and/or retrogradely from the venous side and thereby contribute to filtration induced by the CFC procedure (23). This idea is supported by our result that occlusion of precapillary microvessels by microspheres did not influence CFC. The microsphere infusion must have caused a marked reduction in the number of perfused capillaries, because it caused a significant increase in total vascular resistance.

If CFC is independent of the number of perfused capillaries, as suggested from these results, the question must also be raised as to whether also $L_pA$ in the Starling formula is independent of the number of perfused capillaries. The present study does not allow us to draw any conclusions on this issue. It is not certain that CFC equals $L_pA$ because CFC is measured by application of a negative tissue pressure, at which all capillaries are exposed to the same transmural pressure along their whole length, a condition not directly comparable with the physiological in vivo situation.

It is reasonable to believe, however, that the surface area available for diffusion, in contrast to the surface area available for CFC-induced filtration, is influenced by variation in vascular tone and precapillary sphincter activity (number of open capillaries). This finds support in a study (37) in which it was shown that the permeability-surface area product, reflecting the diffusion capacity, decreased much more than CFC after occlusion with microspheres (37).

Even though our findings of an unaffected CFC during variation in vascular tone and number of perfused capillaries are controversial and contrary to the common opinion, they find support in some previous studies. Thus they are in accordance with the fact that CFC did not change after a decrease in vascular resistance induced by hypoxia (42) in cat skeletal muscle and the finding that an increase in precapillary resistance after a postural change did not alter CFC in human calf muscles (7, 38). Furthermore, they agree with our own previous results showing that CFC did not change when vascular resistance was decreased pharmacologically or when there was a moderate decrease in arterial pressure (21). It is interesting to note that also in other tissues such as in the rat lung, vasoconstriction by norepinephrine and serotonin had no effect on CFC (34).

In conclusion, in our analysis under carefully controlled methodological conditions, we could not demonstrate a change in CFC after variation in vascular tone, precapillary sphincter activity, or in the number of perfused capillaries in pressure-controlled autoperfused cat skeletal muscle. These results, and our previous results investigating effects of vasodilation on
CFC, suggest that the surface area available for the CFC-induced fluid filtration is constant and that variation in CFC reflects a corresponding variation in hydraulic conductivity. The explanation may be that the transiently closed capillary is refilled through intercapillary connections and/or retrogradely from the venous side during the occlusion period and contributes to the CFC-induced filtration. The presently used CFC method therefore can be used for evaluation of changes in microvascular hydraulic conductivity in vivo also when vascular tone and blood flow are altered during the experiment. This may arouse interest in the CFC method as a tool to estimate variation in microvascular hydraulic conductivity in various physiological and pathophysiological conditions.

We sincerely thank Helén Davidsson and Christine Wikstrand for skilled technical assistance.

This study was supported by Swedish Medical Research Council Grant 11581, by the Knut and Alice Wallenberg Foundation, and by skilled technical assistance.

REFERENCES


