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Ramp acceleration and hydraulic conductivity (L_p) of living capillaries

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Abstract

Living mesenteric capillaries with either an intact or disrupted glycocalyx were challenged with ramp change in shear stress ($\Delta\tau$). Animals (*Rana pipiens*) were divided randomly into two experimental groups and two true capillaries (TC) per animal were investigated. The modified Landis technique was combined with intravital microscopy to view individual TC and assess hydraulic conductivity (L_p), an index of capillary function. Median L_p was 3.2×10^{-7} for control and 11.8×10^{-7} cm·s⁻¹·cm H₂O⁻¹ after mild, brief (1 min) pronase treatment ($P < 0.0001$). Analysis by stimulus component showed that L_p for untreated capillaries was related negatively to ramp acceleration ($R^2 = 0.46$, $P < 0.0001$, $n = 38$) and positively to $\Delta\tau$ magnitude ($R^2 = 0.28$, $P = 0.0006$, $n = 38$). Disrupting the capillary glycocalyx revealed a positive and previously unknown relationship between ramp acceleration and L_p ($R^2 = 0.44$, $P = 0.002$, $n = 19$) plus an upward shift (increased intercept) of the magnitude $\Delta\tau$ - L_p relationship compared to abrupt stimulation. These data suggest that bloodstream hemodynamics may impact capillary function. Further, an intact glycocalyx may protect capillaries when blood flow changes.

Keywords

cardiovascular; microcirculation; mechanotransducer; frog; mesentery; permeability; endothelial cell; edema; blood flow; perfusion

Introduction

Studies of blood vessel responses to fluid shear stress (τ) have focused primarily on large blood vessels where hemodynamic changes are related to early disease (Asakura and Karino, 1990; Friedman and Fry, 1993; Fry, 1968). Moving down the vascular tree, peripheral arterioles located within the microcirculation, although not atherogenic, also respond to τ (Bevan, 1995). Further, blood flow does correlate with vascular permeability *in vivo* (Björnberg, 1990; Mahany and Parsons, 1978; Rippe *et al.*, 1978; Shibata and Kamiya, 1992) suggesting that exchange vessels also sense τ . However, very little attention has been focused on individual, intact capillaries, the major exchange microvessels, and how τ influences their function.

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A positive, linear relationship between change in shear stress ($\Delta\tau$), the force associated with flowing blood, and capillary hydraulic conductivity (filtration coefficient, L_p) has been demonstrated in two species (rat, Kim *et al.*, 2005; frog, Williams, 1999; Williams, 2003; Williams, 2007;). Further, Sill *et al.* (1995) and Chang *et al.* (2000) reported that L_p responses of endothelial cell monolayers correlate with τ . These findings *in vitro* are consistent with those obtained from living capillaries, with one exception reported by Neal and Bates (2002).

Using a biophysical experimental approach, *in vivo* capillaries demonstrated blunted responses to ramp compared to square wave $\Delta\tau$ (Williams, 2003). Additionally, the slope of the linear relationship between $\Delta\tau$ and L_p was shifted left (greater sensitivity) when capillaries were challenged with square wave $\Delta\tau$ after treatment with pronase, an enzyme selected to target the entire luminal glycocalyx (Williams, 2007). Whether the $\Delta\tau$ - L_p relationship is maintained after ramp $\Delta\tau$ following pronase disruption of the glycocalyx has not been investigated.

The purpose of the present study was to assess L_p following ramp $\Delta\tau$ in capillaries where the glycocalyx was intact and in capillaries with a disrupted glycocalyx. Established biophysical and biochemical protocols for the fluid mechanical stimulus (Williams, 2003) and for the pronase treatment (Adamson, 1990; Williams, 2007), respectively, were utilized. It was hypothesized that both components of the $\Delta\tau$ stimulus, ramp acceleration and $\Delta\tau$ magnitude, would relate positively to L_p for control and pronase-treated capillaries. Results have been presented previously (abstracts, Williams and Flood, 2007; Williams and Flood, 2009).

Materials and Methods

North American leopard frogs (*Rana pipiens*, male, Alburg, VT and Nashville, TN; 6 to 10 per container) were housed for a minimum of 1 wk with access to fresh water and a dry area and fasted 4 days prior to experiments. Containers were maintained in a controlled environment (15°C, 12 hr light:12 hr dark) with gentamicin (1.3 mg·ml⁻¹) added to the bath water for the first 24 hrs in the animal care facility. Animal Care and Use Committees at the University of Missouri, Columbia, MO and Montana State University, Bozeman, MT approved all procedures.

Animal Preparation

A cerebral pith was performed on each frog and cotton was placed in the cranial cavity. Surgical and body fluid sampling procedures have been published previously (Williams, 1999; Williams, 2003; Williams, 2007). Briefly, a loop of intestine was exteriorized, kept moist and cool (frog Ringer's solution, 14 to 16°C, type-T thermocouple wire, Digi-Sense®, Cole Parmer, Vernon Hills, IL), and perfusion was maintained.

Capillary Identification

Tubes of endothelial cells with no surrounding vascular smooth muscle, no rolling or sticking white blood cells (WBC), and situated between branches were defined as individual capillaries. Only true capillaries (TC, flow diverging at one end and converging at the other end, Chambers and Zweifach, 1944) were used in this study.

Intravital Video Microscopy

Video Imaging—TC were transilluminated and identified using an inverted, compound microscope (UM 10x long working distance objective, 0.22 NA, Diavert, Leitz). Real time video recordings were captured for each experiment (Panasonic AG-6300, Matsushita Electric Industries, JN) with time (0.01 s, VTG-33, For-A, JN) superimposed onto the image of the TC (final magnification, 500x). Video clips were digitized (media converter DVMC-DA2, Sony) and archived (LaCie, Ltd.). Measurements were calibrated to a stage micrometer (0.01 mm,

Meiji Techno, JN). The microscope assembly and glass microtools used for cannulation have been described previously (Williams, 1999; Williams, 2003; Williams, 2007). A water manometer attached to the pipette assembly maintained pressure and flow.

Solutions

Frog Ringer's solution was prepared fresh daily from 5x concentrated stock (pH 7.4 at 15°C) and contained (in mM) NaCl (111.0), KCl (2.4), MgSO₄ (1.0), CaCl₂ (1.1), glucose (5.0), NaHCO₃ (2.0), and N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES)/Na-HEPES (5.0).

For the control solution, bovine serum albumin (BSA) was dialyzed (6,000 to 8,000 MWCO, Spectra/Por membrane, Spectrum, Houston, TX) and dissolved in frog Ringer's solution (10 mg·ml⁻¹; A-4378, Sigma Chemical Co., St. Louis, MO). The same BSA lot number was used for all experiments.

As described previously (Williams, 2007), a stock solution of pronase E (*Streptomyces griseus*, 1.0 mg·ml⁻¹; 4 U·mg⁻¹, P-5147, Sigma Chemical Co.) was made fresh daily and kept on ice until use. Similar to BSA, pronase E was from the same lot number for all experiments to eliminate variability between batches. Before each experiment the pronase stock was diluted to a final concentration of 0.1 mg·ml⁻¹ in 10 mg·ml⁻¹ BSA/frog Ringer's. hRBC were prepared and added to the solution as described previously (Williams, 2007).

Estimates of Ramp Acceleration and Fluid Shear Stress (τ)

Velocity (v)—Instantaneous RBC velocity (v^i) was measured directly (15 s intervals) on the video monitor as described previously (Williams, 1999; Williams, 2003; Williams, 2007):

$$v^i (\mu\text{m} \cdot \text{s}^{-1}) = dx/dt \quad (1)$$

v was calculated from v^i and a correction factor (CF, Michel *et al.*, 1974; hRBC radius, R , μm ; capillary radius, r μm) applied assuming a centered RBC within the vessel.

$$\text{CF} = 2 \left(1 - \left[(R)^2 / 2(r)^2 \right] \right) \quad (2)$$

$$v (\mu\text{m} \cdot \text{s}^{-1}) = v^i / \text{CF} \quad (3)$$

Acceleration (a) was calculated from v :

$$a (\mu\text{m} \cdot \text{s}^{-2}) = dv/dt \quad (4)$$

TC Diameter—Average diameter was obtained from video recordings at 3 sites $\approx 100 \mu\text{m}$ apart.

Apparent Viscosity (η^{apparent})—The lower limit of η^{apparent} was estimated from protein concentration (Chick and Lubrzynska, 1914) as,

$$\eta^{\text{apparent}} (\text{poise}) = \left(5.7 \times 10^{-5} [\text{protein, mg} \cdot \text{ml}^{-1}]^{37^\circ\text{C}} + 0.0089 \right), \quad (5)$$

and corrected to 15°C (Weast, 1970; Williams, 2007).

τ — τ was calculated from v , r , and shear rate (γ):

$$\gamma (\text{s}^{-1}) = 4v/r \quad (6)$$

$$\tau (\text{dynes} \cdot \text{cm}^{-2}) = (\gamma) (\eta^{\text{apparent}}). \quad (7)$$

Tube Hematocrit (thct)

Capillary tube hematocrit was calculated from number of fRBC per 500 μm length of capillary (RBC count) and r :

$$\text{thct} (\%) = (\text{RBC count} \times 294.4 \times 100) / (3.14 \times (r)^2 \times 500) \quad (8)$$

Measurement of Volume Flux (J_v) per Surface Area (S)

J_v —The modified Landis technique (Landis, 1927; Michel *et al.*, 1974) was used to measure J_v . An important advantage of this technique is that the capillary's downstream end remains intact during mechanical stimulation. The technique for the initial occlusion and time course has been described (Williams 1999; Williams, 2003; Williams, 2007). Visual inspection was used to determine a successful occlusion and valid measure of J_v .

S —Volume and S were calculated from radius (r , cm) assuming cylindrical geometry of capillaries. Three measures of diameter were obtained at 3 time points during each occlusion to verify that S remained constant.

J_v/S — J_v/S was calculated from hRBC velocity ($dx/dt^{\text{occlusion}}$, $\text{cm} \cdot \text{s}^{-1}$), TC length (x_0 , cm), and the volume to surface area ratio ($r/2$, cm) measured during the occlusion:

$$J_v/S (\text{cm} \cdot \text{s}^{-1}) = (dx/dt^{\text{occlusion}}) (1/x_0) (r/2). \quad (9)$$

To increase precision, $dx/dt^{\text{occlusion}}$ and x_0 were measured on 3 hRBC (spaced $\approx 50 \mu\text{m}$ apart) at 3 time points (2.0, 2.3, and 2.6 s) during the first occlusion.

Calculation of Hydraulic Conductivity (L_p)

The Starling equation assumes a linear relationship between J_v/S and capillary pressure (P_c). The slope of the regression equation for J_v/S and P_c is L_p :

$$J_v/S = L_p [(P_c - P_i) - \sigma (\pi_c - \pi_i)] \quad (10)$$

where $(P_c - P_i)$ and $(\pi_c - \pi_i)$ are hydrostatic (P) and oncotic (π) pressure differences between capillary lumen (c) and interstitium (i). Sigma (σ) is the reflection coefficient of the capillary wall to protein (for assumptions see Williams, 2007). For the present study, L_p in $\text{cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$ was calculated from measures of J_v/S obtained at 30 cm H₂O. The 9 measures of J_v/S (see above) were calculated to L_p and then averaged to obtain a mean($\pm$ SE) L_p value for the first occlusion.

Under circumstances where a rapid change in flux occurs, Neal and Michel (1995) have used zero as the x-axis intercept ($\sigma(\pi_c - \pi_i)$), a procedure that conservatively underestimates L_p (Williams, 1999; Williams, 2003; Williams, 2007).

Experimental Protocol

Frogs were divided into Control and Test Groups and groups were experimented on in random order. L_p was assessed on two capillaries per frog. TC1 in both groups experienced the same mechanical stimulus as its capillary pair (TC2).

Control Group—TC1 was cannulated at 8 cm H₂O (5.9 mm Hg) and low flow (BSA/frog Ringer's solution) was established for 2 min (Steady State 1). A steady infusion pump (5 cc syringe, Model 355, Sage Instruments, Cambridge, MA) was used to produce the ramp $\Delta\tau$ stimulus by raising pressure to 30 cm H₂O (22.1 mm Hg) over a period of 2 or 5 min. Various downstream resistances of the microcirculation resulted in an acceleration range between 10 and 320 $\mu\text{m} \cdot \text{s}^{-2}$. The new level of flow was maintained for 2 min (Steady State 2) and J_v/S was measured (at 30 cm H₂O). TC2 was cannulated at low flow (1 min) with BSA/frog Ringer's-filled pipette, recannulated with the same pipette (low flow, 2 min), stimulated with ramp $\Delta\tau$, and J_v/S was measured after 2 min at Steady State 2.

Test Group—TC1 experienced the same protocol as TC1 in the Control Group. TC2, however, was cannulated at low flow (1 min) with a pronase-containing pipette. The pipette was removed and TC2 was recannulated with a BSA/frog Ringer's-containing pipette (2 min low flow, Steady State 1). TC2 was then stimulated with ramp $\Delta\tau$ and J_v/S was measured after 2 min at Steady State 2.

Plateaus for Steady States 1 and 2 were identified by visual inspection of τ curves and magnitude of the mechanical stimulus ($\Delta\tau$) was calculated from plateau values as:

$$\Delta\tau = \tau^{\text{Steady State 2}} - \tau^{\text{Steady State 1}}. \quad (11)$$

In general, TC2 was located in a separate section of the mesentery and it was assumed that filtration during occlusion reflected filtration at Steady State 2. The PI performed all experimental protocols and the laboratory technician performed videotapes measurements to minimize measurement error and bias.

Statistical Analyses

Normality of each data set was assessed using JMP software (SAS Institute, Inc.) and means ($\pm$ SE) or medians are reported as appropriate. Values > maximum from a box plot of each data set were identified as outliers. Clusters of data were determined using a combination of nonparametric density, quantile density contour, and mesh plots (JMP). Paired *t*-tests or analysis of variance were used to test for differences between means followed by Tukey post hoc tests. Differences between medians were determined using van der Waerden test. Relationships between acceleration, $\Delta\tau$, and L_p were determined using linear regression. Significance was set at $P \leq 0.05$ before initiating experiments.

Results

Frogs ($N=39$; body wt, 32.8 ± 0.9 g; heart rate, 63.6 ± 1.6 bpm after pith) were confirmed healthy by inspection. Average systemic values for blood and body fluids (Table 1) were within range of those reported previously for *Rana pipiens* (Hargrave *et al.*, 1995; Harris, 1972; Williams, 2007).

Physical/cellular contacts between TC and their surrounding native tissue were maintained throughout each experiment. Constituents of the lumen were left undisturbed and, overall, the microvessels remained intact prior to initiating the protocol. Characteristics of the individual capillaries are presented in Table 2. No capillaries were excluded due to technical problems.

Capillary L_p

Careful cannulation technique, establishing low flow prior to ramp $\Delta\tau$, and controlling the presentation of ramp $\Delta\tau$ contributed to similar ($P=0.29$) median L_p values for TC1, Control Group compared to TC1, Test Group (pooled median $L_p = 3.6 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$). Consistent technical procedures also were utilized during the pronase/ramp $\Delta\tau$ protocol where median L_p for TC2, Test Group (11.8×10^{-7}) was elevated ($P<0.0001$) 3-fold above L_p for TC1, Test Group ($3.2 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$). Figure 1 shows the frequency distributions for all four data sets. Statistical outliers were identified and excluded from further analyses.

Time course data were obtained for 5 TC1 and 8 TC2 capillaries in the Test Group. Figure 2 displays the compiled data, which were representative of the individual capillary curves. L_p averaged $1.7(\pm 0.2) \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$ for the 5 min time course obtained for TC1, Test Group. For TC2, Test Group, L_p was higher initially compared to TC1, then declined at a rate of $3.4 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$ per min and plateaued within 3 min at $4.9(\pm 0.4) \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$, a value that was 2 to 3-fold above ($P<0.0001$) the 5 min average for TC1.

Components of the ramp $\Delta\tau$ mechanical stimulus and capillary L_p

Values for average ramp acceleration and magnitude of $\Delta\tau$ were similar between capillaries ($P>0.05$, Table 2). No relationship was detected between the two components of ramp $\Delta\tau$ for control ($R^2=0.03$, $P>0.05$) or pronase-treated capillaries ($R^2=0.01$, $P>0.05$). However, analyses of L_p as a function of components of the ramp $\Delta\tau$ stimulus did reveal relationships not recognized previously. Initial results ($N=12$) were verified in a second set of experiments ($N=18$) and verified a second time in a third set of experiments ($N=9$).

Ramp acceleration and L_p —Figure 3 illustrates a negative relationship for L_p as a function of ramp acceleration for untreated capillaries. These data demonstrate for the first time that a majority (72%) of individual, intact, true capillaries may have the capacity to adjust function in response to acceleration of a fluid stimulus. The relationship between ramp acceleration and L_p expands on a previous report (Williams, 2003) where L_p was higher after abrupt (square wave) versus slow (ramp) $\Delta\tau$ stimuli, again, suggesting that intact capillaries adjust function based on different rates of change in τ . Data here extend knowledge concerning the capillary response to rate of change in a fluid stimulus by quantifying fluid acceleration and examining more closely its relationship to L_p . Data in Figure 3 also suggest the existence of a threshold at $\approx 150 \mu\text{m} \cdot \text{s}^{-2}$ where L_p appeared to change direction and respond positively rather than negatively to acceleration. This observation should, however, be considered tentative since only 4 data points were $>150 \mu\text{m} \cdot \text{s}^{-2}$.

For pronase-treated capillaries, a ramp acceleration- L_p relationship also was discovered (Figure 3). However, in contrast to untreated capillaries, the relationship was reversed and

significantly positive. L_p values for 11 untreated capillaries fell within range of L_p for the pronase-treated capillaries.

$\Delta\tau$ Magnitude and L_p —Figure 4A illustrates the positive, linear $\Delta\tau$ - L_p relationship that has been repeated consistently for untreated capillaries in two different species (Kim *et al.*, 2005; Williams, 1999; Williams, 2003; Williams, 2007).

Initially, the $\Delta\tau$ - L_p relationship was not detected for TC pre-treated with pronase and stimulated with ramp $\Delta\tau$ (Figure 4A). In addition to being in direct contrast to the untreated capillaries, the initial observation with pronase also was inconsistent with previous results when an abrupt, square wave $\Delta\tau$ was used as the fluid mechanical stimulus (Williams, 2007).

To reconcile the inconsistency between the present and previous studies, data from the previous study for pronase-treated capillaries were superimposed on the present data. Both sets of data were collected under the same experimental conditions with rate of mechanical stimulation (abrupt versus slow presentation of $\Delta\tau$) as the only difference between the two experiments. The secondary analysis that is illustrated in Figure 4B revealed four data points from the present study that were beyond the threshold (magnitude of $\Delta\tau > 70$ dynes·cm⁻²) where L_p had plateaued in the previous study. Identifying the L_p plateau and organizing the data by diameter (as in the previous study) clarified the present data set and demonstrated a positive magnitude $\Delta\tau$ - L_p relationship for the pronase-treated capillaries, consistent with what has been observed in the past.

Additional insight into living capillaries, fluid $\Delta\tau$ challenges, and a disrupted glycocalyx was evident from the analysis pictured in Figure 4B. First, the slopes for the two lines that represented capillaries with diameters < 15 μm were similar numerically with no difference until the third decimal place suggesting that sensitivity to magnitude $\Delta\tau$ did not change with ramp versus abrupt mechanical stimulation. Second, the line for ramp $\Delta\tau$ -stimulated capillaries was shifted above that for the abrupt $\Delta\tau$ -stimulated capillaries by $\approx 10.0 \times 10^{-7}$ cm·s⁻¹·cm H₂O⁻¹, a value that is much larger than the error of the method ($\pm 1.5 \times 10^{-7}$ cm·s⁻¹·cm H₂O⁻¹) in our hands. The increase in the y-axis intercept with no change in slope indicates that ramp $\Delta\tau$ somehow increases capillary L_p uniformly for any given magnitude of $\Delta\tau$ compared to abrupt $\Delta\tau$ when the luminal glycocalyx is disrupted. Variability of the L_p data after ramp $\Delta\tau$ also was increased compared to abrupt $\Delta\tau$ stimulated capillaries. Two capillaries with diameter > 15 μm also tended to demonstrate the same left shift with ramp $\Delta\tau$, however, too few data fell into this subgroup preventing any firm conclusion.

Discussion

Two new findings are presented regarding mechanical, flow-induced force and capillary function *in vivo*. First, L_p assessed on untreated capillaries was related negatively to ramp acceleration. Second, pronase treatment before the ramp stimulus revealed a positive relationship between ramp acceleration and L_p and shifted the $\Delta\tau$ - L_p relationship above that for capillaries stimulated abruptly with $\Delta\tau$. Pronase capillaries retained functional capacity as demonstrated by a tightening of the barrier with time.

Endothelial cells line the cardiovascular system, form the large, capillary surface area, and provide the first defense against diseases of morbidity and mortality that are recognized to have mechanical origins (Ingber, 2003). Consequently, investigative attention has turned to mechanisms that control vascular function and τ using culture cell systems. Cultured cells possess an important advantage in that fluid force can be controlled precisely (Asakura and Karino, 1990; Chang *et al.*, 2000; Friedman and Fry, 1993; Fry, 1968; Sill *et al.*, 1995). To gain the advantage, however, the cells are removed from their position within the vessel,

network, and/or organ, leaving unknown their exact location and *in vivo* hemodynamic history. Use of intravital microscopy to view microvessels in perfused tissue and perform functional studies *in situ* fills an important experimental gap between the culture dish and whole organ by providing information about the network location for the microvessel as well as flow direction and magnitude. This distinct advantage is gained at the expense of some precision since fluid force due to τ is estimated.

It is important to note that ramp acceleration was related to L_p after brief exposure to pronase (Figure 3) and that the direction of that relationship was reversed compared to control. Based on what we now know it also appears that intact TC adjust their function in response to $\Delta\tau$ magnitude both with and without an intact glycocalyx, regardless of the rate of stimulation (Williams, 2007). A key point, however, is that the slow (ramp) fluid stimulus along with pronase treatment appears to increase L_p for any given value of $\Delta\tau$ as evidenced by an increased intercept and unchanged slope compared to abrupt fluid stimulation.

It is logical to speculate that a threshold exists above which faster flow triggers a positive response. Clearly the sensing mechanisms responsible for detecting ramp acceleration are dampened by an intact glycocalyx (Figure 3, control situation). However, the data do hint at a threshold that is at or near $150 \mu\text{m}\cdot\text{s}^{-2}$ where L_p begins to increase with higher ramp acceleration. It is possible that pronase treatment allowed us to “see” this increase with acceleration and that the reversed, positive relationship with pronase is merely an extension of the control curve with a left shift of the x-axis intercept due to glycocalyx disruption and increased sensitivity to the fluid stimulus.

In addition to data for capillaries presented here, different responses to rate of change and magnitude of τ have been reported for isolated 1A arterioles *in situ* (rat cremaster muscle, Butler *et al.*, 2000). Mechanisms governing the responses remain elusive for both capillaries and arterioles, in part, due to complexities that are inherent in living microvessels. For example, the surface glycocalyx has been proposed to transduce mechanical, flow-induced force in endothelial monolayers (Tarbell and Pahakis, 2006). However, the results reported here do not support that hypothesis in living capillaries for either ramp acceleration or $\Delta\tau$ magnitude due to the fact that their relationships with L_p were not eliminated by treatment with pronase. This new result draws mechanistic attention to the capillary wall. Following enzymatic disruption, cellular or subcellular sensing entities may coordinate an integrated response to ramp acceleration and $\Delta\tau$ magnitude that is hidden, buffered, and/or deactivated with an intact lumen. Alternatively, whole cell deformation, another mechanosensing mechanism used by endothelial cells (Ingber, 2003), may be blunted with an intact glycocalyx and accentuated upon disruption. Mass transport of vasoactive agonists such as ATP (Dull and Davies, 1992) also cannot be excluded from consideration.

The similar response patterns between pronase-treated capillaries and 20 to 30% of the untreated capillaries that did not cluster in Figures 3 and 4 are of potential significance. These capillaries were retained in the data set (as we have in the past) because they fell within range of published values (see below, **Limitations and Methodological Considerations**), did not fit the exclusion criteria, were not identified as statistical outliers, and had no visible signs of damage. As such, the most reasonable interpretation is and has been in the past that these capillaries simply are filtering at a higher rate. The capillary responses to pronase, however, provide, for the first time, some context for understanding these apparently uncooperative capillaries with higher filtration rates that do not fit neatly onto a single regression line. Absent damage, a second explanation for the higher L_p values is that approximately one quarter of the capillary bed is under-perfused *in vivo* (a reasonable expectation for gut and mesenteric tissue) and that the glycocalyx in these chronic low flow capillaries is thin, under-developed, or, in the case of the extreme outliers, absent. Without the protective layer, these capillaries

would be more sensitive to fluid stimuli even under control conditions. The striking similarities between responses to pronase and these high L_p capillaries provides some validity to this idea, which does require further investigation.

Limitations and Methodological Considerations

In the present study ramp $\Delta\tau$ was produced by increasing the pressure gradient. Consequently the stimulus, selected for its physiological relevance, was comprised of flow and pressure. Importantly, the downstream end of each capillary remained intact and unobstructed during the entire stimulus causing less overall disruption to continuity of the capillary network. Only one flow/pressure change occurred prior to the first assessment of L_p . However, following the first occlusion τ and wall tension did change with each occlusion.

Sensing of fluid mechanical force by intact TC suggests that an investigator could potentially produce different values for L_p simply by changing characteristics of a flow stimulus. Methods should include information about the flow stimulus when capillary L_p is reported.

Pronase, selected here to target the entire glycocalyx, may have acted on receptor proteins. While this possibility does exist, it is important to note that the protocol for pronase used on *in vivo* capillaries was established previously (Adamson, 1990). Disrupting proteins in other cellular preparations is often accomplished by incubation periods of 24 hrs as compared to the 1 min, short duration exposure used here. Additional evidence that capillaries retained their ability to function is found in Figure 2 where the capillary barrier tightened 3-fold in 3 min, evidence of minimal to no damage of cellular function. The plateau value of 4.9×10^{-7} $\text{cm}\cdot\text{s}^{-1}\cdot\text{cm H}_2\text{O}^{-1}$ presented here, remarkably, matches exactly with the value reported in the past (Adamson, 1990) for capillaries following pronase when the glycocalyx, specifically, had been disrupted as verified by electron microscopy.

In the past (Williams, 1999; Williams, 2003; Williams, 2007) and in the present study, we have not used ratios for L_p responses believing that the absolute values reveal much more about function of capillaries *in vivo*. Values for L_p outliers also are included as a reminder of the biological variability that does exist in living capillaries. Michel's original L_p data collected on frog capillaries (Michel *et al.*, 1974) ranged from 1.0 to 13.0×10^{-7} (at 14 to 16°C) and 1.0 to 35.0×10^{-7} (at 22 to 26°C) $\text{cm}\cdot\text{s}^{-1}\cdot\text{cm H}_2\text{O}^{-1}$, quite comparable to the data presented here, 35 years later. The capabilities of the capillary wall for controlling fluid between the vascular and interstitial spaces remain remarkable as we continue to discover the vast and sensitive array that exists.

In conclusion, L_p was related to acceleration and magnitude components of a ramp $\Delta\tau$ stimulus when assessed on untreated capillaries. Capillary L_p also was related to acceleration and $\Delta\tau$ magnitude following pronase treatment with an increase in the y-axis intercept after slow (ramp) compared to abrupt mechanical stimulation. L_p decreased with time after pronase and ramp $\Delta\tau$ and was shifted left of data obtained under similar conditions following abrupt $\Delta\tau$. Fluid mechanical responses of endothelial cells are well documented in culture systems, but are less so in living capillaries. Relationships with acceleration and magnitude of $\Delta\tau$ that vary with the conditions of the inner capillary lining demonstrate sophisticated mechanisms present in intact capillaries for coordinating function in concert with bloodstream fluid dynamics. Intact constituents of the lumen appear to be essential for maintaining control of capillary function and for protecting it when blood flow changes.

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References

- Adamson RH. Permeability of frog mesenteric capillaries after partial pronase digestion of the endothelial glycocalyx. *J. Physiol* 1990;428:1–13. [PubMed: 2231409]
- Asakura T, Karino T. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ. Res* 1990;66:1045–1066. [PubMed: 2317887]
- Bevan, JA.; Kaley, G.; Rubanyi, GM. *Flow-dependent Regulation of Vascular Function*. Oxford University Press; New York: 1995.
- Björnberg J. Forces involved in transcapillary fluid movement in exercising cat skeletal muscle. *Acta Physiol. Scand* 1990;140:221–236. [PubMed: 2267951]
- Butler PF, Weinbaum S, Chien S, Lemons DE. Endothelium-dependent, shear-induced vasodilation is rate-sensitive. *Microcirculation* 2000;7:53–65. [PubMed: 10708337]
- Chambers R, Zweifach BW. Topography and function of the mesenteric capillary circulation. *Am. J. Anat* 1944;75:173–205.
- Chang YS, Yaccino JA, Lakshminarayanan S, Frangos JA, Tarbell JM. Shear-induced increase in hydraulic conductivity in endothelial cells is mediated by a nitric oxide-dependent mechanism. *Arterioscler. Thromb. Vasc. Biol* 2000;20:35–42. [PubMed: 10634798]
- Chick H, Lubrzynska E. The viscosity of some protein solutions. *Biochem. J* 1914;8:59–69. [PubMed: 16742290]
- Dull RO, Davies PF. Mechanisms of flow-mediated signal transduction in endothelial cells: kinetics of ATP surface concentrations. *J. Vasc. Res* 1992;20:410–419. [PubMed: 1489886]
- Friedman MH, Fry DL. Arterial permeability dynamics and vascular disease. *Atherosclerosis* 1993;104:189–194. [PubMed: 8141842]
- Fry DL. Acute vascular endothelial changes associated with increased blood velocity gradients. *Circ. Res* 1968;22:165–197. [PubMed: 5639037]
- Hargrave RW, Huxley VH, Thipakorn B. Seasonal variations of capillary hydraulic conductivity and volume status. *Am. J. Physiol. (Regulatory Integrative Comp. Physiol.)* 1995;37:R468–R474.
- Harris JA. Seasonal variation in some hematological characteristics of *Rana pipiens*. *Comp. Biochem. Physiol* 1972;43A:975–989.
- Ingber DE. Mechanobiology and diseases of mechanotransduction. *Ann. Med* 2003;35:564–577. [PubMed: 14708967]
- Kim M, Harris NR, Tarbell JM. Regulation of capillary hydraulic conductivity in response to an acute change in shear. *Am. J. Physiol. – Heart* 2005;289:2126–2135.
- Landis EM. Micro-injection studies of capillary permeability. II. The relation between capillary pressure and the rate at which fluid passes through the walls of single capillaries. *Am. J. Physiol* 1927;82:217–238.
- Mahany TM, Parsons RH. Circulatory effects on osmotic water exchange in *Rana pipiens*. *Am. J. Physiol* 1978;234:R172–R177. [PubMed: 306202]
- Michel CC, Mason JC, Curry FE, Tooke JE, Hunter PJ. A development of the Landis technique for measuring the filtration coefficient of individual capillaries in the frog mesentery. *Quart. J. Exp. Physiol* 1974;59:283–309. [PubMed: 4548540]
- Neal CR, Bates DO. Measurement of hydraulic conductivity of single perfused *Rana* mesenteric microvessels between periods of controlled shear stress. *J. Physiol* 2002;543:947–957. [PubMed: 12231650]
- Neal CR, Michel CC. Transcellular gaps in microvascular walls of frog and rat when permeability is increased by perfusion with the ionophore A23187. *J. Physiol* 1995;488(2):427–437. [PubMed: 8568681]
- Rippe B, Kamiya A, Folkow B. Simultaneous measurements of capillary diffusion and filtration exchange during shifts in filtration-absorption and at graded alterations in the capillary permeability surface area product (PS). *Acta Physiol. Scand* 1978;104:318–336. [PubMed: 102112]
- Shibata M, Kamiya A. Blood flow dependence of local capillary permeability of Cr-EDTA in the rabbit skeletal muscle. *Jap. J. Physiol* 1992;42:631–639. [PubMed: 1474680]

- Sill HW, Chang YS, Artman JR, Frangos JA, Hollis TM, Tarbell JM. Shear stress increases hydraulic conductivity of cultured endothelial monolayers. *Am. J. Physiol* 1995;268:H535–H543. [PubMed: 7532373]
- Tarbell JM, Pahakis MY. Mechanotransduction and the glycocalyx. *J. Int. Med* 2006;259:339–350.
- Weast, RC. Handbook of Chemistry and Physics. fifty-first ed. Chemical Rubber Co.; Cleveland: 1970.
- Williams DA. Network assessment of capillary hydraulic conductivity after abrupt changes in fluid shear stress. *Microvasc. Res* 1999;57:107–117. [PubMed: 10049659]
- Williams DA. Intact capillaries sensitive to rate, magnitude, and pattern of shear stress stimuli as assessed by hydraulic conductivity (L_p). *Microvasc. Res* 2003;66:147–158. [PubMed: 12935773]
- Williams DA. Change in shear stress ($\Delta\tau$)/hydraulic conductivity (L_p) relationship after pronase treatment of individual capillaries *in situ*. *Microvasc. Res* 2007;73:48–57. [PubMed: 17030043]
- Williams DA, Flood MH. Pronase treatment reveals a relationship between rate of change in shear stress ($\Delta\tau$) and hydraulic conductivity (L_p) for intact capillaries. *FASEB J* 2007;21:A490.
- Williams DA, Flood MH. Component analysis of change in fluid shear stress ($\Delta\tau$) stimulus and basal capillary hydraulic conductivity (L_p). *FASEB J* 2009;23

Figure 1.

Frequency distributions of the initial assessments of capillary hydraulic conductivity (L_p) after ramp $\Delta\tau$. TC – true capillary. Gray bars – treated with pronase before ramp stimulus and L_p assessment. Black solid arrows are medians; gray solid arrows are means. Number of statistical outliers: Control Group, TC1 – 0; Control Group, TC2 – 1 (146.3×10^{-7}); Test Group, TC1 – 2 (bold bars); Test Group, TC2 – 4 (46.2, 82.0, 407.4, $69.6 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{cm H}_2\text{O}^{-1}$).

Figure 2.

Representative time (t) course for hydraulic conductivity (L_p) following ramp $\Delta\tau$ for Test Group capillaries. TC – true capillary. TC1, untreated (n=5); TC2, pronase-treated (n=8). Data are mean ($\pm$ SE) for each time point (n). Solid line indicates period of decline in L_p ($L_p = -3.4(t) + 16.5$). *TC2>TC1.

Figure 3.

Hydraulic conductivity (L_p) plotted as a function of ramp acceleration (a) for untreated and pronase-treated capillaries. Data are mean ($\pm$ SE) of initial L_p assessment after ramp $\Delta\tau$. TC – true capillary. Solid line: regression for pooled, clustered data obtained from untreated capillaries ($L_p = -0.037(\pm 0.007)(a) + 6.6(\pm 0.6)$, $R^2=0.46$, $P<0.0001$, $n=38$; data for 11 (21%) untreated capillaries (small symbols) fell above nonparametric density cluster). Dashed line: regression for pronase-treated capillaries ($L_p = 0.112(\pm 0.031)(a) + 5.4(\pm 2.4)$, $R^2=0.44$, $P=0.002$, $n=19$; data for 1 (5%) pronase-treated capillary (small open triangle) fell outside nonparametric density cluster).

Figure 4A.

Hydraulic conductivity (L_p) plotted as a function of magnitude of change in shear stress ($\Delta\tau$) for untreated and pronase-treated capillaries. Data are mean ($\pm$ SE) of initial L_p assessment following ramp $\Delta\tau$. TC – true capillary. Solid line: regression for pooled, clustered data obtained from untreated capillaries ($L_p = 0.055(\pm 0.015)(\Delta\tau) + 0.6(\pm 0.7)$, $R^2=0.28$, $P=0.0006$, $n=38$; intercept not different from 0, $P=0.36$; data for 15 (28%) untreated capillaries (small symbols) fell above nonparametric density cluster). No relationship existed for TC2, Test Group ($R^2=0.02$, $P=0.56$).

Figure 4B.

Hydraulic conductivity (L_p) plotted as a function of the magnitude of change in shear stress ($\Delta\tau$) for pronase-treated capillaries. “Slow Stimulus”-labeled data are from pronase-treated capillaries that were stimulated with ramp $\Delta\tau$ (Figure 4a, present study, note change in abscissa). “Abrupt Stimulus”-labeled data were obtained from pronase-treated capillaries that were stimulated with square wave $\Delta\tau$ and published previously (Figure 6, Williams, 2007). Dashed regression line for data below L_p plateau: slope = $0.254(\pm 0.041)$, intercept = $-4.46(\pm 1.80)$, $R^2=0.83$, $P=0.0002$, $n=10$. Bold-dashed regression line for data below L_p plateau (bold, open triangles): $L_p = 0.247(\pm 0.082)(\Delta\tau) + 5.22(\pm 2.93)$, $R^2=0.40$, $P=0.009$, $n=16$. Bracket 1 indicates shift of intercept for ramp-versus abrupt-stimulated capillaries with diameters $<15\ \mu\text{m}$. Dashed-dot regression line: slope = $0.566(\pm 0.063)$, intercept = $2.98(\pm 2.82)$, $R^2=0.93$, $P=0.0001$, $n=8$. Two data points from the present study fell within range of the dashed-dot line and two points fell to the left (bold dashed-dot line; gray-filled triangles). Bracket 2 indicates possible shift of intercept for ramp-stimulated capillaries with diameters $>15\ \mu\text{m}$ relative to those stimulated with abrupt $\Delta\tau$. No statistics were performed due to the low number of capillaries in this subset of the present data.

Table 1Systemic blood and body cavity fluid values for *Rana pipiens* (N).

	Control Group (15)	Test Group (24)
Hematocrit (%)	24.8 (± 1.9)	24.4 (± 1.4)
RBC concentration (cells·mm ⁻³)	303,067 ($\pm 22,982$)	276,417 ($\pm 16,990$)
Hb concentration (g·dl ⁻¹)	9.6 (± 1.1)	6.5 (± 0.4) [*]
Mean corpuscular Hb concentration (%)	38.7 (± 3.7)	28.1 (± 2.4) [*]
WBC concentration (cells·mm ⁻³)	6,157 (± 599)	5,742 (± 411)
Plasma protein (mg·ml ⁻¹)	28.2 (± 1.7)	22.9 (± 0.9) [*]
Abdominal cavity fluid protein (mg·ml ⁻¹)	12.6 (± 1.8) (9)	11.2 (± 1.3) (18)
Skin lymph fluid protein (mg·ml ⁻¹)	12.9 (± 1.8) (5)	10.5 (± 1.5) (11)

Data are mean ($\pm$ SE); RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell^{*} $P < 0.05$

Table 2Capillary data organized by experimental group (*N*).

	Control Group (15)		Test Group (24)	
	TC1	TC2	TC1	TC2
Diameter (μm)	11.6 $\pm$ 0.5	12.9 $\pm$ 0.7	13.2 $\pm$ 0.6	12.6 $\pm$ 0.6
Mean velocity ($\mu\text{m}\cdot\text{s}^{-1}$)	403.8 $\pm$ 81.4	577.9 $\pm$ 99.2	543.2 $\pm$ 112.8	510.0 $\pm$ 64.3 (23)
γ (s^{-1})	275.1 $\pm$ 51.2	317.0 $\pm$ 44.9	285.8 $\pm$ 49.7	346.2 $\pm$ 49.4 (23)
Estimated τ ($\text{dynes}\cdot\text{cm}^{-2}$)	4.7 $\pm$ 0.9	6.1 $\pm$ 1.0	5.5 $\pm$ 1.1	7.2 $\pm$ 0.9 (23)
Tube hematocrit (%)	10.0 $\pm$ 1.2	9.3 $\pm$ 1.6	8.8 $\pm$ 1.6	8.7 $\pm$ 1.1 ²⁴
Flow ($\text{nl}\cdot\text{s}^{-1}$)	48.1 $\pm$ 13.2	87.9 $\pm$ 18.9	71.8 $\pm$ 13.4	69.2 $\pm$ 12.5 (23)
Pressure ($\text{cm H}_2\text{O}$)	8.8 $\pm$ 0.7	8.8 $\pm$ 0.6	9.3 $\pm$ 0.5	9.6 $\pm$ 0.4
Ramp acceleration ($\mu\text{m}\cdot\text{s}^{-2}$)	130.9 $\pm$ 18.9	129.7 $\pm$ 28.1	82.3 $\pm$ 5.4	92.5 $\pm$ 11.5
$\Delta\tau$ magnitude ($\text{dynes}\cdot\text{cm}^{-2}$)	49.9 $\pm$ 4.6	40.1 $\pm$ 4.0	45.3 $\pm$ 3.0	46.1 $\pm$ 5.6

Data are mean $\pm$ SE; TC, true capillary; γ , shear rate; τ , shear stress